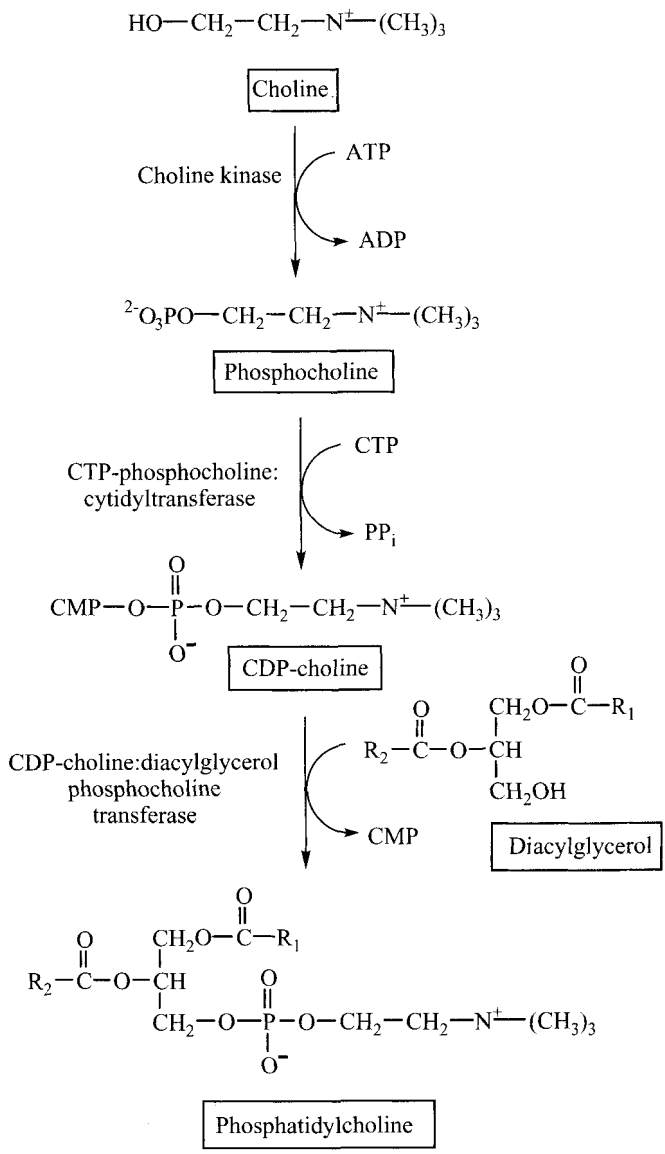

FIGURE 14-6 Synthesis of phosphatidylcholine by the salvage pathway.

include the brain and testis. The strategy of activating the diglyceride domain is also followed in the synthesis of phosphatidylglycerol and cardiolipin.

14.3.2.3 Phosphatidylserine Synthesis. Unlike the pathways described above that produce the other glycerophospholipids, human cells do not synthesize phosphatidylserine (PS) by condensation of the polar head group serine with the

diacylglycerol backbone. Instead, PS is produced by a novel mechanism that involves polar head group exchange between phosphatidylethanolamine and free serine in a reaction catalyzed by phosphatidylserine synthase:

$$\text{phosphatidylethanolamine} + \text{serine} \rightleftharpoons \text{phosphatidylserine} + \text{ethanolamine}$$

Since there is no net change in the number or types of bonds in the substrates and products, this reaction is reversible and does not require ATP or the involvement of any other high-energy compound. The ethanolamine that is released in this reaction is reincorporated into phosphatidylethanolamine by the CDP-ethanolamine pathway described above. A second isozyme of phosphatidylserine synthase preferentially utilizes phosphatidylcholine in place of phosphatidylethanolamine for synthesis of PS.

14.3.2.4 Synthesis of Sphingomyelin. Like PS, sphingomyelin is not synthesized by condensation of its polar head group (phosphocholine) with the lipid domain (ceramide). Instead, synthesis involves the transfer of the polar head group from phosphatidylcholine to ceramide:

$$\text{ceramide} + \text{phosphatidylcholine} \rightarrow \text{sphingomyelin} + \text{diacylglycerol}$$

14.3.3 Phospholipases

Membrane phospholipids are dynamic molecules that undergo rapid turnover and extensive modification reactions characterized by hydrolysis and resynthesis. The enzymes that catalyze phospholipid hydrolysis are called *phospholipases* and are grouped into classes that designate which bond is cleaved (Fig. 14-7).

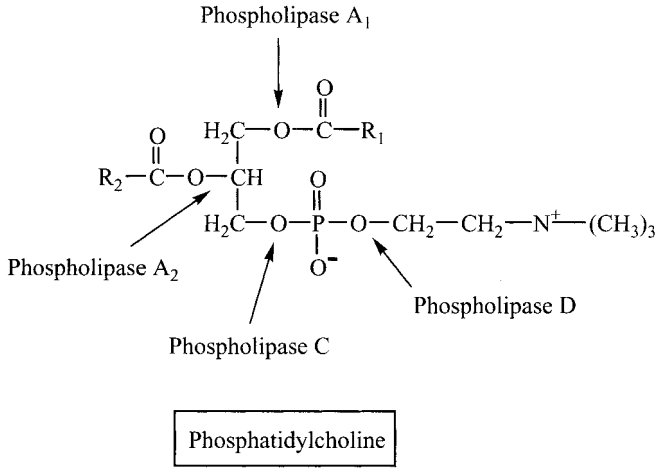

FIGURE 14-7 Sites of action of phospholipases.

14.3.3.1 Phospholipase A_1. Phospholipase A_1 is an enzyme that hydrolyzes the fatty acid in the 1-position of glycerophospholipids. Some lipases that act primarily as triacylglycerol lipases, such as hepatic lipase and lipoprotein lipase, also have phospholipase A_1 activity.

14.3.3.2 Phospholipase A_2 (PLA_2). Phospholipases A_2 release the fatty acid in the 2-position of phospholipids. These phospholipases are much more abundant and widely distributed than phospholipases A_1, possibly because the structure of phospholipids in membranes and micelles renders the acyl bond in the 2-position more accessible to the enzyme. There are several intracellular PLA_2's, including a cytosolic phospholipase A_2 that is specific for arachidonic acid, activated by micromolar Ca^{2+} concentrations, and involved primarily in the regulation of agonist-stimulated prostaglandin and leukotriene biosynthesis. Extracellular PLA_2's include pancreatic PLA_2, which hydrolyzes phospholipids in the small intestine and the secretory PLA_2's, which are small extracellular esterases that participate in the inflammatory response.

14.3.3.3 Phospholipase B. Phospholipase B has lysophospholipase as well as phospholipase activity and is able to remove both fatty acids from glycerophospholipids.

14.3.3.4 Phospholipase C. Phospholipase C is a phosphodiesterase that cleaves phospholipids so as to separate the phosphorylated polar head group from the diacylglycerol moiety. One especially important phospholipase C is specific for phosphatidylinositol bisphosphate (PIP_2) and catalyzes the following reaction (Fig. 14-8):

phosphatidylinositol bisphosphate + H_2O → inositol trisphosphate + diacylglycerol

Although PIP_2 represents only a small fraction of the inositol phospholipids, it plays a major role in signal transduction in that hydrolysis of PIP_2 generates two products, both of which are active as intracellular second messengers. Inositol trisphosphate (IP_3) triggers the release of calcium from the endoplasmic reticulum, and diacylglycerol activates protein kinase C.

14.3.3.5 Phospholipase D. Phospholipase D is a phosphodiesterase that cleaves on the polar head group side of the phosphodiester bond of phospholipids; for example:

phosphatidylcholine + H_2O → phosphatidic acid + choline

In some cells (e.g., neutrophils) a phospholipase D generates phosphatidic acid during agonist-stimulated cellular activation. Phosphatidic acid then regulates secretory and degranulation responses, possibly by recruiting other proteins to the

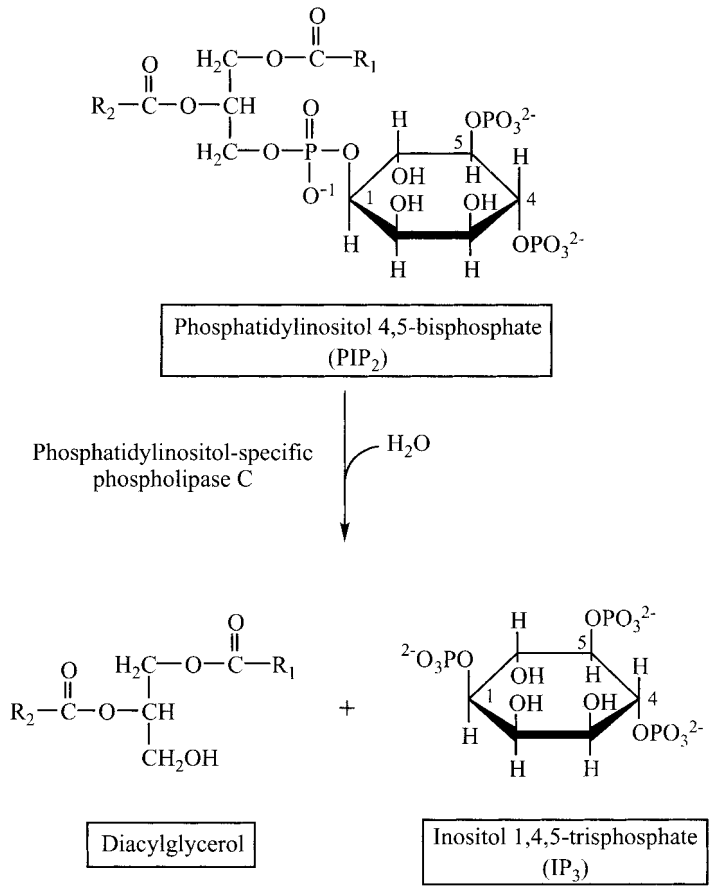

FIGURE 14-8 Phosphatidylinositol 4,5-bisphosphate as a precursor to intracellular second messengers.

membrane. Phosphatidic acid can also be hydrolyzed further to generate additional diacylglycerol for protein kinase C activation or to generate lysophosphatidic acid, which is released from the cell as an extracellular signaling molecule. Extracellular C- and D-type phospholipases cleave glycosylphosphatidylinositol anchors (see Fig. 14-2), releasing this particular class of membrane-bound proteins from the cell surface.

14.3.3.6 Sphingomyelinase. Sphingomyelinase is analogous to phospholipase C in that it catalyzes reactions that cleave sphingomyelin on the lipid side of the phosphodiester bridge:

$$\text{sphingomyelin} + H_2O \rightarrow \text{ceramide} + \text{phosphocholine}$$

Mammalian cells contain multiple sphingomyelinases that generate ceramide in response to a wide spectrum of agents, including 1,25-dihydroxyvitamin D_3, cytokines, and corticosteroids. Ceramide activates several phosphoprotein phosphatases as well as a protein kinase; increased concentrations of ceramide can lead to growth inhibition, differentiation, and in some cases, apoptosis.

14.3.4 Phospholipid Remodeling Reactions

Membrane phospholipids undergo extensive modification reactions. These reactions serve to convert the phospholipids generated by the biosynthetic pathways described above to the mixture of specific phospholipid structures required by the cell.

14.3.4.1 Deacylation/Reacylation Reactions. In many cases, the initially synthesized phospholipids do not have the appropriate fatty acids in the 1- and 2-positions of the glycerol backbone. As noted earlier, phosphatidic acid usually has palmitate or stearate in the 1-position and linoleate or oleate in the 2-position. Remodeling reactions are therefore required to introduce arachidonic acid into the 2-position of PC, PE, and PI, where it is then available for mobilization to initiate eicosanoid synthesis. In the lung, type II pneumocytes utilize remodeling reactions to generate dipalmitoylphosphatidylcholine (dipalmitoyllecithin), the major lipid component of pulmonary surfactant.

Remodeling is initiated by the action of a phospholipase A_2, which removes the fatty acid from the 2-position of the phospholipid, generating the corresponding lysophospholipid. There are two mechanisms for reacylating the lysophospholipid: direct acylation, which uses fatty acyl-CoA as the fatty acid donor, and transacylation, in which the fatty acid that acylates the lysophospholipid is taken from another phospholipid in a transesterification reaction.

Synthesis of Platelet-Activating Factor (PAF). An example of a set of deacylation/reacylation reactions is the pathway for the synthesis of PAF (Fig. 14-9). PAF is an alkyl lipid with a phosphocholine head group. Unlike typical glycerophospholipids, PAF contains an acetyl moiety in the 2-position instead of a long-chain fatty acid. PAF is synthesized by inflammatory cells, such as neutrophils and macrophages, and is a potent mediator of hypersensitivity, acute inflammatory reactions, and allergic reactions. Synthesis of PAF involves phospholipase A_2–mediated removal of the long-chain fatty acid from the 2-position of the ether-linked phospholipid followed by acetylation with acetyl-CoA. Since most of the long-chain fatty acid that is released is arachidonate, PAF production is often accompanied by eicosanoid synthesis. Inactivation of PAF involves hydrolysis to remove the acetyl moiety followed by reacylation to regenerate an ether-linked membrane phospholipid.

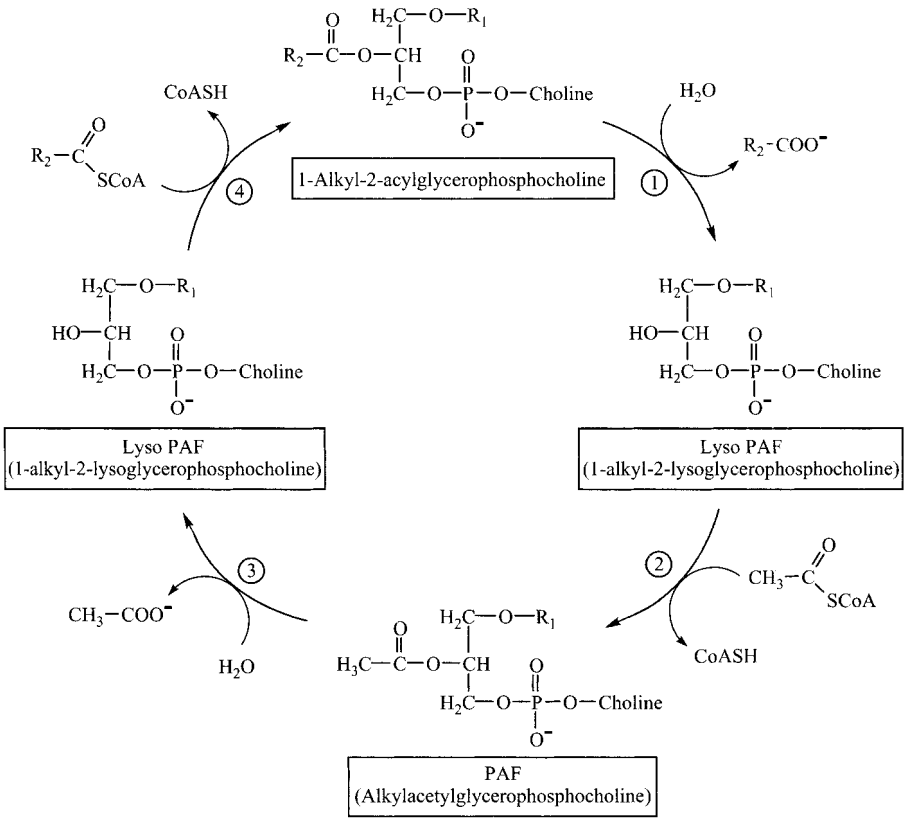

FIGURE 14-9 Role of remodeling reactions in the synthesis and inactivation of platelet activating factor (PAF), 1-alkyl-2-acetyl-glycerophosphocholine. The enzymatic steps are catalyzed by ①, phospholipase A_2; ② acetyl-CoA:alkyllysoglycerophosphate acetyltransferase; ③. PAF acetylhydrolase; ④. acyltransferase. Alternative transacylation mechanisms exist for the both the deacylation ① and reacylation ④ steps.

14.3.4.2* De Novo *Synthesis of Choline and Ethanolamine. Although inositol is synthesized from glucose 6-phosphate, there is no direct pathway for synthesizing either ethanolamine or choline. Instead, the ethanolamine and choline moieties of phospholipids are synthesized from the serine moiety of phosphatidylserine (PS). The generation of PE by decarboxylation of PS occurs in the mitochondria of many cell types (Fig. 14-10):

$$\text{phosphatidylserine} \rightarrow \text{phosphatidylethanolamine} + CO_2$$

By contrast, the conversion of PE to PC occurs only in hepatocytes. The pathway, which is catalyzed by a single enzyme, phosphatidylethanolamine

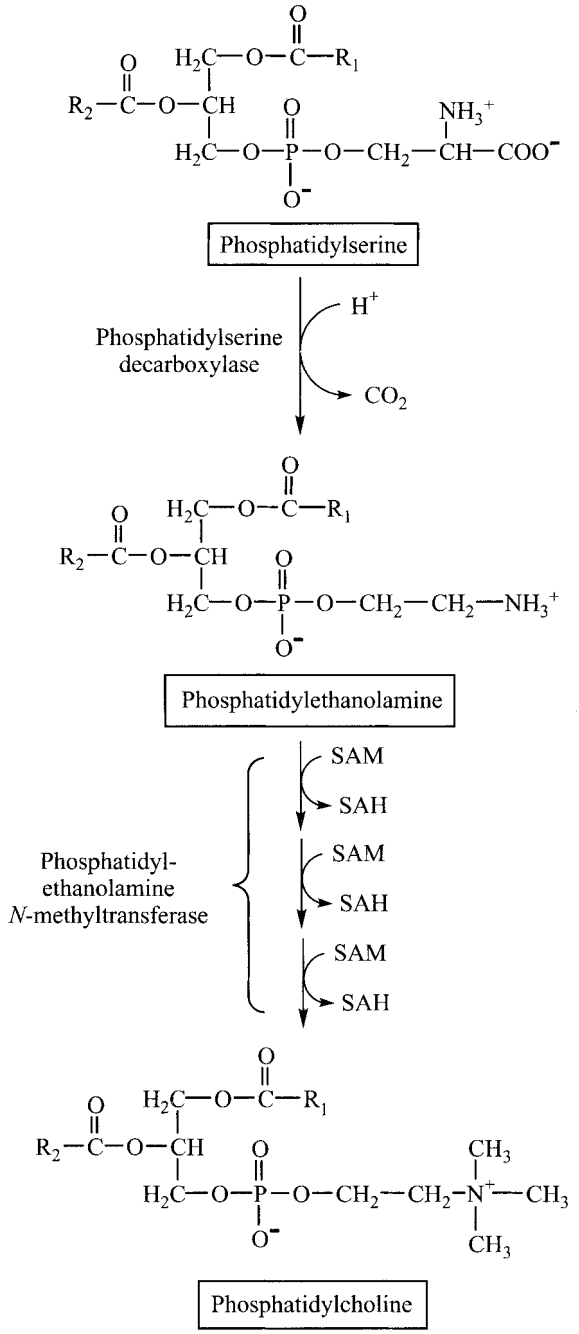

FIGURE 14-10 Synthesis of phosphatidylcholine from phosphatidylserine. SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine.

N-methyltransferase, uses three molecules of *S*-adenosylmethionine (SAM) to transfer three methyl groups successively to the nitrogen of phosphatidylethanolamine (Fig. 14-10):

$$\text{phosphatidylethanolamine} + 3S\text{-adenosylmethionine} \rightarrow \text{phosphatidylcholine} + 3S\text{-adenosylhomocysteine}$$

The subsequent metabolism of *S*-adenosylhomocysteine is discussed in Chapter 21.

As described above, the activation of choline to CDP-choline provides a pathway for incorporating free choline into phospholipids. The CDP-choline pathway captures and reutilizes choline released during the catabolism of PC, sphingomyelin, and the neurotransmitter acetylcholine, as well as choline derived from the diet. The pathway for incorporating choline into PC is therefore commonly referred to as a *salvage pathway*. Similarly, the CDP-ethanolamine pathway is used to salvage free ethanolamine released during PE turnover, as well as the ethanolamine released from PE during the synthesis of PS by base exchange and ethanolamine derived from the diet.

If humans can utilize the salvage pathway to incorporate dietary choline into glycerophospholipids, why is there still a need for de novo choline synthesis via phosphatidylethanolamine *N*-methyltransferase (PEMT)? It appears that the PEMT pathway has a special role in the secretion of VLDL by the liver, and may play a role in regulating plasma homocysteine levels.

14.3.4.3 Phosphorylation of Phosphatidylinositol. Another example of phospholipid synthesis occurring by modification of a preexisting phospholipid is the phosphorylation of the inositol moiety of phosphatidylinositol. The major products of this pathway are phosphatidylinositol 4,5-bisphosphate (PI-4,5-P_2), which is the substrate for phospholipase C, and phosphatidylinositol 3-phosphate (PI-3-P), which is involved in endosomal trafficking.

14.4 REGULATION OF PHOSPHOLIPID METABOLISM

The regulation of phospholipid synthesis is complex and incompletely understood. The rate-limiting step in the salvage pathway for phosphatidylcholine synthesis is the CTP : phosphocholine cytidylyltransferase (CCT) reaction. Translocation of CCT from the cytosol to the endoplasmic reticulum in response to physiological signals is associated with a large increase in CCT activity.

14.5 DISEASES INVOLVING PHOSPHOLIPIDS

14.5.1 Neonatal Respiratory Distress Syndrome

Around the twenty-sixth week of gestation, type II cells in the fetal lung begin synthesizing the components of surfactant, including dipalmitoylphosphatidylcholine. Failure to produce adequate amounts of surfactant during the third trimester results in *respiratory distress syndrome* (RDS) in the neonate (a.k.a., *hyaline membrane disease*), which is a major cause of infant mortality worldwide, particularly in preterm infants. The maturity of the fetal lung can be assessed by an increased phosphatidylcholine/sphingomyelin (P/S) ratio in amniotic fluid. Most cases of RDS can be prevented if mothers who go into preterm labor are given glucocorticoids, which stimulate surfactant production in the fetus. Respiratory failure due to insufficiency of surfactant can also occur in adults when their type II cells have been destroyed by severe infections or as an adverse side effect of chemotherapeutic drugs (e.g., bleomycin).

14.5.2 Choline Deficiency

Although choline synthesis in the liver does contribute to the choline pool, it is not always sufficient to satisfy a person's daily choline requirement. Choline is now recognized as an essential nutrient, especially for people with low dietary intakes of protein whose methionine pool may be inadequate. Choline deficiency compromises hepatic VLDL synthesis and secretion and can therefore result in fatty liver. There is evidence that choline deficiency may also compromise brain development, memory function, and cardiovascular health. Meats, eggs, and vegetables (e.g., cauliflower, lettuce) are good sources of choline.

14.5.3 Lupus

Systemic lupus erythematous (SLE) is an autoimmune disorder that affects the kidneys and the cardiovascular and central nervous systems. The hallmark of the disease is the production of antibodies directed against antigens of the host's own cells and tissues, which may be membrane phospholipids, usually phosphatidylserine, as well as nuclear proteins (e.g., histones) or even double-stranded DNA. Phosphatidylserine is normally concentrated on the cytosolic face of the plasma membrane; cellular damage may result in inappropriate phosphatidylserine exposure on the cell surface, giving rise to antibody formation. In lupus, antibodies are sometimes also directed against cardiolipin, another acidic phospholipid, which is normally localized to the inner mitochondrial membrane (Fig. 14-11).

14.5.4 Barth Syndrome

Barth syndrome is a genetic cardiomyopathy caused by mutations in the tafazzin gene, which is involved in the remodeling of the fatty acids in mitochondrial cardiolipin. The

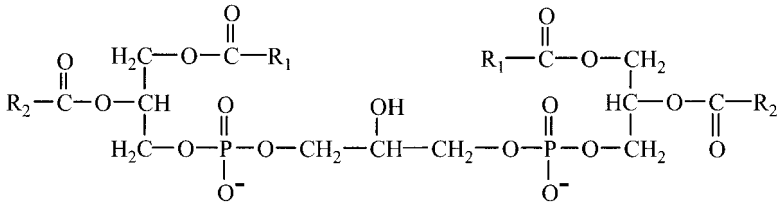

FIGURE 14-11 Structure of cardiolipin.

protein tafazzin transfers specific fatty acids from phosphatidylcholine to cardiolipin and is essential to the synthesis of the symmetrical cardiolipin molecules, such as tetralinoleoylcardiolipin, which is essential for normal mitochondrial function.

14.5.5 Niemann–Pick Disease, Types A and B

Mutations in the gene for lysosomal sphingomyelinase result in sphingomyelin accumulation in cells, particularly those of the reticuloendothelial system, and cause hepatosplenomegaly, jaundice, and neurologic disturbances. Type A Niemann–Pick disease is more severe, with early death resulting from excess deposition of sphingomyelin in the central nervous system. Patients with type B Niemann–Pick have more residual enzyme activity than those with type A Niemann–Pick disease, generally do not exhibit neurologic involvement, and usually survive into adulthood. Types A and B Niemann–Pick, should not be confused with type C_1 or C_2 Niemann–Pick Disease, in which the underlying molecular defect involves mutation in the gene *NPC1*, which is required for the transport of cholesterol and other lipids out of the lysosome.

CHAPTER 15

EICOSANOIDS

15.1 FUNCTIONS OF EICOSANOIDS

The eicosanoids are a complex family of bioactive lipid messengers generated by oxygenation of 20-carbon polyunsaturated fatty acids, primarily arachidonic acid (Fig. 15-1). Eicosanoids are local-acting autocrine and paracrine hormones that stimulate cells adjacent to their site of synthesis. In general, eicosanoids have a short half-life, usually on the order of minutes. They are not stored in cells but instead are released as soon as they are synthesized.

Eicosanoids fall into two main classes: (1) prostanoids that have a ring structure, including prostaglandins, thromboxanes, and prostacyclins, and (2) linear eicosanoids consisting of leukotrienes, lipoxins, and hydroxyeicosatetraenoic acids (HETEs).

15.1.1 Prostaglandins Are Eicosanoids with Ring Structures

The term *prostaglandin* reflects the original isolation of these molecules from seminal fluid, into which they are secreted by the seminal glands (rather than the prostate). Prostaglandins act to modulate many physiological functions, including blood pressure, uterine contraction, and the production of pain and fever. Prostaglandins are designated PGA, PGD, PGE, or PGF, based on the functional groups on the cyclopentane ring that is comprised of carbons 8 through 12 (Fig. 15-2). For example, PGE_2 contains a 9-keto and an 11-hydroxy group, while $PGF_{2\alpha}$ contains two hydroxy groups; α designates the stereochemistry of the C9 hydroxyl group. All of the

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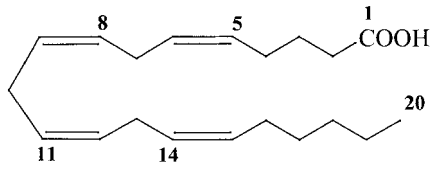


Arachidonic acid

FIGURE 15-1 Structure of arachidonic acid.

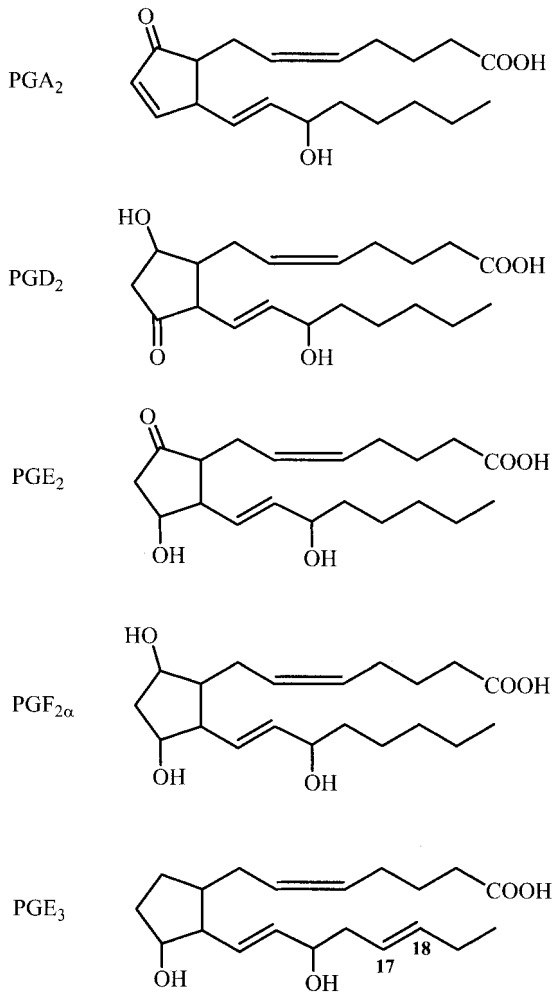


FIGURE 15-2 Structures of some common prostaglandins.

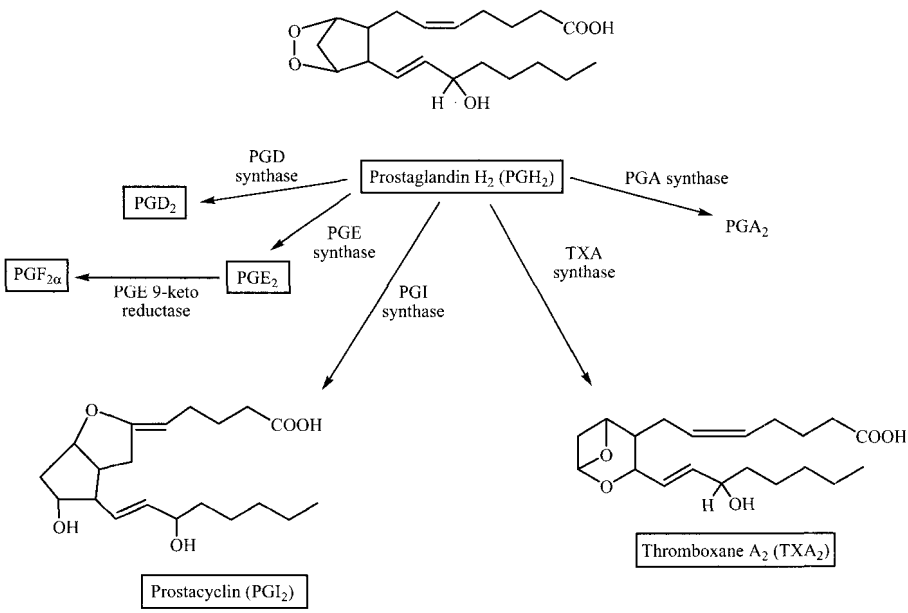


FIGURE 15-3 Synthesis of the different prostaglandins from their common precursor, prostaglandin H_2.

prostanoids that are derived from arachidonic acid have numeral 2 as a subscript, referring to the number of carbon–carbon double bonds in the two side chains.

Prostacyclin (PGI_2) and thromboxane (TX_2) are two prostanoids that have somewhat unusual ring structures. Relative to PGE_2 for example, prostacyclin has an additional oxygen-containing ring between C6 and C9. Thromboxanes have a six-membered oxygen-containing ring instead of the cyclopentane ring of classic prostaglandins (Fig. 15-3). The term *thromboxane* refers to the platelet-aggregating activity, which has thrombus-forming potential.

15.1.2 Leukotrienes and Lipoxins are Linear Eicosanoids

Unlike the prostanoids, which contain a ring element in their structure, the leukotrienes are linear molecules (Fig. 15-4). The term leukotriene derives from their cell of origin (leukocytes) and the fact that their structures contain three carbon–carbon double bonds in conjugation. The most important leukotrienes in humans are LTA_4 and LTB_4 and their cysteinyl derivatives, LTC_4, LTD_4, and LTE_4; all are derived from arachidonic acid and contain four double bonds. The cysteinyl-leukotrienes constitute the slow-reacting substance of anaphylaxis (SRS-A) and promote smooth muscle contraction, constriction of pulmonary airways, trachea and intestine, and increases in capillary permeability (edema).

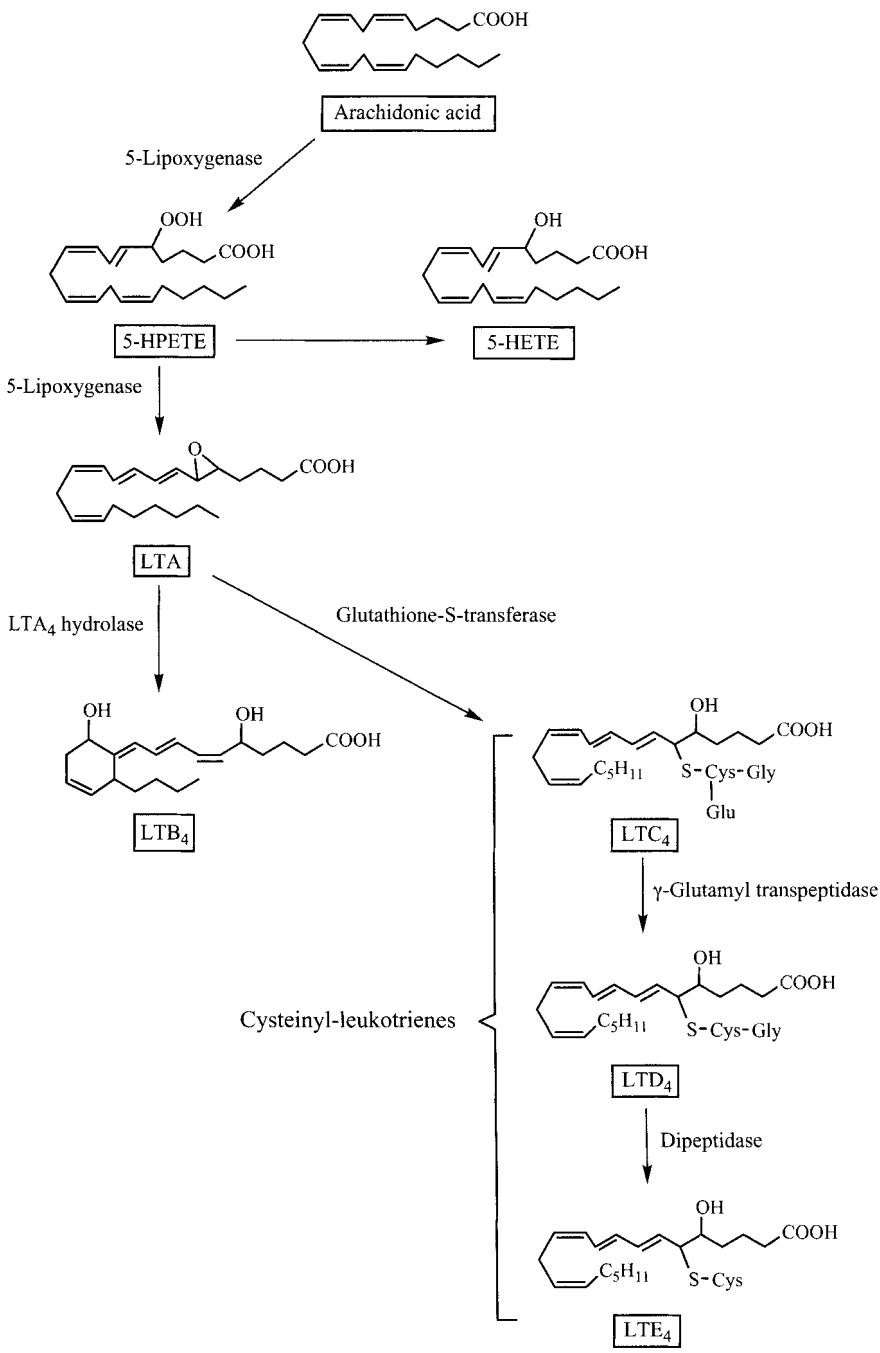


FIGURE 15-4 Structures and synthesis of some common leukotrienes.

Hydroxyeicosatetraenoic acids (HETEs) are closely related to noncysteinyl leukotrienes but lack the conjugated series of three double bonds (Fig. 15-4). 5-HETE and LTB_4 regulate neutrophil and eosinophil function; specifically, they mediate chemotaxis, stimulate adenylyl cyclase activity, and induce polymorphonuclear granulocytes to degranulate and release hydrolytic enzymes from lysosomes.

The lipoxins are another class of linear eicosanoids that are derived from arachidonic acid. Their structures are distinct from those of the leukotrienes and HETEs in that they contain three hydroxyl groups and a conjugated tetraene system (Fig. 15-5). LXA_4 and LTB_4 have many physiological functions, including antiangiogenic properties, enhanced clearance of exudates resulting from pulmonary edema, and protection from reperfusion injury.

15.1.3 Eicosanoid Receptors

Eicosanoids initiate their physiologic effects by binding to G-protein-coupled receptors on the plasma membranes of target cells. The receptors are named IP, EP, and so on, to designate the specific ligand they bind: EP1 and EP2 designate multiple receptors for the same prostaglandin. Various prostaglandin receptors signal through G_s-mediated increases in cAMP, G_q-mediated increases in intracellular free calcium, or G_i-mediated decreases in cAMP.

There are two classes of leukotriene receptors. Binding of LTB_4 or related hydroxyacids (i.e., HETEs) to B-LT receptors elicits chemotactic responses in leukocytes. The cysteinyl-leukotrienes bind to cys-LT receptors and stimulate contraction of smooth muscle cells. HETEs may also be incorporated into the phospholipids of membranes of target cells, where the presence of fatty acyl chains containing a polar hydroxyl group modulates lipid packing and thus the normal structure and function of the membrane.

15.1.4 Eicosanoids from Dihomo-γ-Linolenic Acid and Eicosapentaenoic Acid

The major precursor for the synthesis of human eicosanoids is arachidonic acid, which gives rise to the 2-series of prostaglandins (two of the arachidonate double bonds are removed as part of the cyclization reaction) and the 4-series of leukotrienes. Although eicosanoids can also be synthesized from dihomo-γ-linolenic acid (20:3ω6), the immediate precursor of arachidonic acid, there is little synthesis of 1-series prostaglandins and 3-series leukotrienes in humans. PGE_1 has, however, been utilized extensively as a pharmacological agent; one application of its vasodilatory effects has been in the diagnosis and treatment of erectile dysfunction.

Eicosapentaenoic acid (EPA, 20:5ω3) is the other physiologically significant precursor of human eicosanoids in humans. The main dietary source of EPA is fish oil, particularly oil from cold-water marine fish; some EPA is also synthesized from

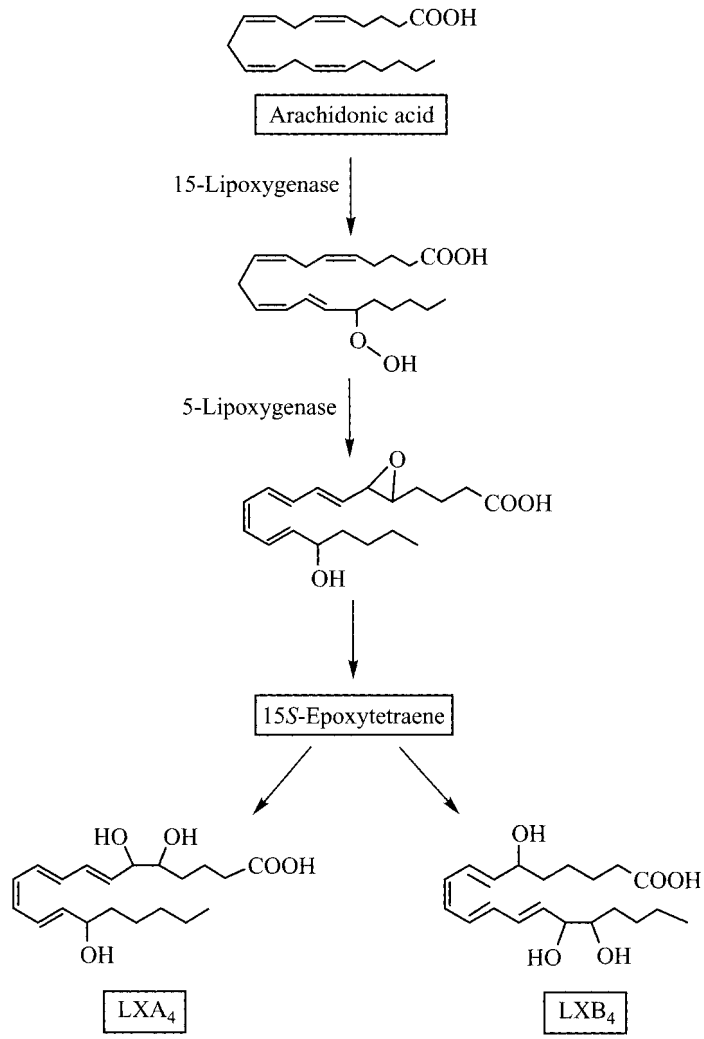


FIGURE 15-5 Structures and synthesis of lipoxins.

dietary α-linolenic acid (18:3ω3). EPA is the precursor to a family of eicosanoids, each of which has one more double bond than the corresponding eicosanoid derived from arachidonate (i.e., PGE_3, TXA_3, LTC_5) (Fig. 15-2). An increased dietary intake of fish oil can raise the ratio of membrane phospholipid EPA to arachidonate from the average of less than 0.1 to as high as 0.5. Although dietary fish oils have been shown to be cardioprotective, anti-inflammatory, and anticarcinogenic, it is still unclear how much these benefits are due to the partial replacement of arachidonate-derived eicosanoids with those synthesized from EPA.

15.2 SITES OF SYNTHESIS OF PROSTAGLANDINS AND LEUKOTRIENES

With the exception of red blood cells, prostaglandins are produced and released by nearly all human cells and act on adjacent cells of the same organ. The cyclooxygenases and related enzymes in the eicosanoid synthesis pathways are localized to the cytoplasmic surfaces of the nuclear envelope and endoplasmic reticulum. The pattern of both eicosanoid production and response are cell-type specific. The following are selected examples.

15.2.1 Gastrointestinal Tract

Prostaglandins serve a cytoprotective role in the stomach. PGE_2 is synthesized by epithelial and smooth muscle cells in the stomach, where it reduces gastric acid secretion while stimulating the production of protective mucus. For this reason, synthetic prostaglandins are helpful in promoting the healing of gastric ulcers.

15.2.2 Cardiovascular System

In blood vessels, different prostaglandins have opposing effects. For example, platelets produce thromboxane A_2 (TXA_2), which promotes platelet aggregation, whereas vascular endothelial cells produce prostacyclin (PGI_2), which inhibits platelet aggregation. Both PGE_2 and PGI_2 are vasodilators that lower systemic arterial pressure, thereby increasing local blood flow and decreasing peripheral resistance. By contrast, both TXA_2 and $PGF_{2\alpha}$ (produced by vascular smooth muscle) are vasoconstrictors.

15.2.3 Kidney

PGE_2 is the major prostaglandin in the kidney, and the collecting ducts are the main site of its production. The kidney also produces $PGF_{2\alpha}$, PGD_2, and thromboxane A_2. PGE_2 dilates renal blood vessels and increases blood flow through the kidney. PGE_2 is also an important stimulator of renin release, thus contributing to the regulation of sodium excretion and the glomerular filtration rate.

15.2.4 The Lungs

Monocytes and neutrophils in the lungs produce LTB_4, 5-HETE, and the cysteinyl-leukotrienes (LTC_4, LTD_4, and LTE_4), which are bronchoconstrictors. LTC_4 is more potent than histamine in contracting nonvascular smooth muscles of bronchi.

15.2.5 Female Reproductive Tract

PGE_2 within the ovarian follicle is essential for ovulation. During parturition, prostaglandins soften tissues in the cervix and stimulate uterine contractions to expel the fetus.

15.3 CONDITIONS WHEN EICOSANOID SYNTHESIS IS UP-REGULATED

Prostaglandin and leukotriene synthesis in human cells and tissues is often triggered by hormonal or neural excitation, or muscular activity. For example, histamine increases prostaglandin production in the gastric mucosa. Also, prostaglandins are released during labor and after cellular injury (e.g., platelets exposed to thrombin, lungs irritated by dust).

15.3.1 Inflammation

Prostaglandins, PGE_2 in particular, are mediators of the edema, erythema (redness of the skin), and the fever and pain associated with inflammation. Inflammatory reactions most often involve the joints (e.g., rheumatoid arthritis), skin (e.g., psoriasis), and eyes and are usually treated with corticosteroids that inhibit prostaglandin synthesis. PGE_2, generated in immune cells (e.g., macrophages, mast cells, B cells), evokes chemotaxis of T cells. It is thought that pyrogens (fever-inducing agents) activate the prostaglandin synthesis pathway with release of PGE_2 in the hypothalamus, where body temperature is regulated.

15.3.2 Activation of Neutrophils, Monocytes, and Macrophages

Synthesis of leukotrienes and hydroxyeicosatetraenoic acids (HETEs) is up-regulated under conditions of allergy and inflammation. Binding of IgE antibodies to membrane receptors stimulates mast cells to release HETEs, which then activate other cells. Similarly, HETEs (especially 5-HETE) and LTB_4 produced by activated leukocytes induce degranulation of neutrophils and eosinophils.

15.4 METABOLISM OF EICOSANOIDS

15.4.1 Release of Arachidonic Acid

Synthesis of eicosanoids is initiated by release of arachidonic acid, primarily by the action of phospholipase A_2's (PLA_2), which hydrolyze the fatty acid from the *sn*-2 position of membrane phospholipids:

$$\text{phospholipid} + H_2O \rightarrow \text{1-acyllysophospholipid} + \text{arachidonate}$$

Several PLA_2 enzymes are responsible for arachidonate mobilization. One is a cytosolic enzyme that is activated in response to signal-transduction cascades. There are also several small secretory PLA_2's which are active as extracellular enzymes; they are produced in response to sepsis and inflammation and found in high concentrations in the synovial fluid of patients with arthritis.

15.4.2 Synthesis of Prostaglandins

15.4.2.1 Prostaglandin G/H Synthase. The key enzyme of prostaglandin biosynthesis is the bifunctional enzyme prostaglandin G/H synthase (PGS). PGS is a single-polypeptide-chain enzyme that has two catalytic sites. One catalytic site has cyclooxygenase activity which catalyzes the addition of two molecules of molecular oxygen to arachidonate to form the initial prostaglandin, which is PGG_2 (Fig. 15-6). The second catalytic site is a glutathione-dependent, heme-containing

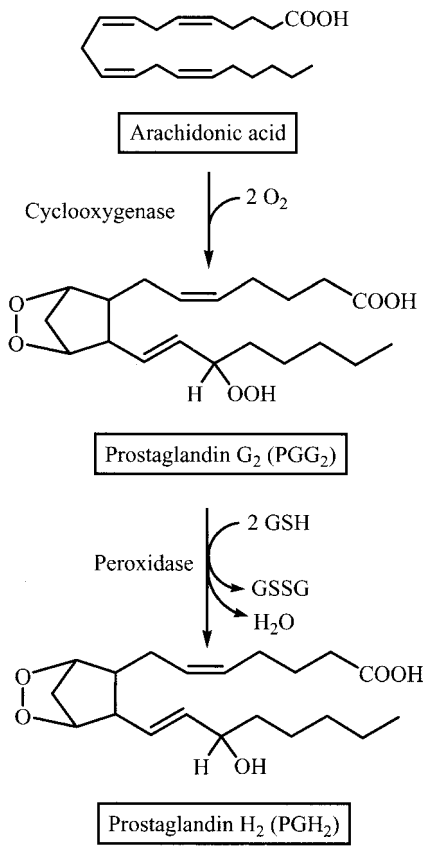


FIGURE 15-6 Synthesis of prostaglandin H_2.

peroxidase that converts the hydroperoxide group (—OOH) on carbon 15 of PGG_2 to a hydroxyl group.

There are two major isozymes of prostaglandin G/H synthase or cyclooxygenase (COX), commonly designated COX-1 and COX-2. COX-1 is primarily a constitutive enzyme of gastric mucosa, platelets, vascular endothelium, and kidney, whereas COX-2 is inducible and expressed in activated macrophages and monocytes as well as smooth muscle and epithelial cells.

Recent studies have identified a third PGS isozyme, COX-3, which is made from the COX-1 gene but retains intron 1 in its mRNA. COX-3 is expressed in the cerebral cortex and is inhibited by analgesic and antipyretic drugs, such as acetaminophen (Tylenol), that do not inhibit COX-1 and COX-2.

15.4.2.2 Synthesis of Prostaglandins. A family of prostaglandin synthases (i.e., PGD synthase, PGE synthase, PGI synthase) convert PGH_2 to the various prostaglandins (Fig. 15-3). PGH_2 is also the precursor of thromboxane A_2, in which the cyclopentane ring is replaced by a six-membered oxygen-containing ring. The one prostaglandin that is not synthesized directly from PGH_2 is $PGF_{2\alpha}$, which is synthesized from PGE_2 by PGE 9-keto reductase.

15.4.3 Synthesis and Catabolism of Linear Eicosanoids

15.4.3.1 Lipoxygenases. Lipoxygenases are dioxygenases that attach both atoms of molecular oxygen to a particular carbon atom of arachidonic acid (e.g., position 5, 12, or 15). In humans, the most important leukotrienes are the 5-lipoxygenase (5-LOX) products, which mediate inflammatory disorders; their synthesis is initiated by addition of a hydroperoxy group to arachidonic acid to produce 5-hydroperoxyeicosatetraenoic acid (5-HPETE) (Fig. 15-4). 5-LOX is activated by an accessory protein called *5-lipoxygenase-activating protein* (FLAP), an arachidonic acid transfer protein that presents the fatty acid substrate to the 5-LOX enzyme.

HPETE-hydroperoxides are highly reactive, unstable metabolic intermediates. The reduction of hydroxyeicosatetraenoic acids (HPETEs) to the hydroxyeicosatetraenoic acid (e.g., 5-HETE) occurs spontaneously or is catalyzed by peroxidases.

15.4.3.2 Leukotriene Synthesis. 5-LOX contains two enzymatic activities: the dioxygenase activity that converts arachidonic acid to 5-HPETE and a dehydrase activity that transforms 5-HPETE to the epoxide leukotriene A_4 (LTA_4) (Fig. 15-4). LTA_4 is an important branch point in the pathway of leukotriene synthesis. It can be converted to leukotriene B_4 by LTA_4 hydrolase, which opens up the epoxide ring. Alternatively, LTA_4 can be converted to LTC_4 by LTC_4 synthase, which catalyzes the conjugation of LTA_4 with glutathione. Sequential removal of glutamate and glycine residues by specific peptidases yields the leukotrienes LTD_4 and LTE_4, respectively.

15.4.3.3 Lipoxin Synthesis. Lipoxins are synthesized by multicellular processes involving the sequential actions of two lipoxygenases. One such sequence involves synthesis of LTA_4 by 5-lipoxygenase in granulocytes followed by

15-hydroxylation of LTA_4 by the 12/15-lipoxygenase in platelets and hydrolysis of the epoxide ring to generate LXA_4 (Fig. 15-5).

15.4.3.4 Cytochrome P450 Epoxygenase Pathway for Eicosanoid Synthesis. In addition to the lipoxygenase family of enzymes, linear eicosanoids are also synthesized by cytochrome P450 epoxygenases. The resulting epoxyeicosatrienoic acids (EETs) are converted into a variety of hydroxyeicosatrienoic acids and dihydroxyeicosatrienoic acids. The products differ from those of the lipoxygenase pathway in that they contain three rather than four double bonds.

15.4.4 Catabolism of Eicosanoids

Most eicosanoids are extremely unstable molecules. Thromboxane A_2, for example, has a half-life of about 30 seconds in water and is rapidly transformed into inactive thromboxane B_2 (TXB_2). Similarly, prostacyclin I_2 (PGI_2) spontaneously breaks down to 6-keto $PGF_{1\alpha}$. Within the cell, prostaglandins are often inactivated by the action of a prostaglandin 15-hydroxydehydrogenase. The 15-keto prostaglandins are then oxidized further by oxidation from the omega (ω) end as well as by β-oxidation from the carboxy end, and the resulting more polar molecules are excreted by the kidney.

15.5 REGULATION OF EICOSANOID SYNTHESIS AND ACTIVITY

15.5.1 Regulation of Arachidonate Mobilization

Activation of phospholipase A_2 is crucial for the release of arachidonic acid, which serves as a substrate for eicosanoid synthesis. Cytosolic phospholipase A_2 ($cPLA_2$) is activated in a cell-specific manner by a variety of agonists (e.g., by thrombin in platelets). Steroidal anti-inflammatory drugs such as prednisone and betamethasone block prostaglandin release in part by inducing the synthesis of members of the phospholipase A_2-inhibitory family of proteins called *lipocortins* or *annexins*. Glucocorticoids may also directly suppress transcription of the genes for $cPLA_2$ and for secretory PLA_2.

15.5.2 Regulation of Prostaglandin G/H Synthase

Prostaglandin G/H synthase represents the committed step in prostaglandin and thromboxane synthesis. Whereas COX-1 is constitutive in many cell types, synthesis of COX-2 in various cells is induced by a variety of cytokines and lipid mediators (e.g., sphingosine 1-phosphate). Although glucocorticoids had long been assumed to block prostaglandin synthesis at the level of phospholipase A_2, there is increasing evidence that they also suppress induction of COX-2 in many cell types (e.g., airway smooth muscle cells).

Prostaglandin synthase, a major target for pharmacological intervention, is inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin (acetylsalicylic acid), ibuprofen, indomethacin, and phenylbutazone. Most NSAIDs act as reversible inhibitors of the cyclooxygenase component of PGH_2. Aspirin acts differently in that it irreversibly inhibits PGS by acetylating the hydroxyl group of a particular serine hydroxyl at the active site of cyclooxygenase. Low-dose aspirin regimens are often used to decrease the risk of thrombosis and coronary heart disease in older persons. This therapy is effective because circulating platelets are unable to synthesize more prostaglandin synthase to replace that which has been inactivated.

Recent pharmaceutical efforts have focused on the development of selective COX-2 inhibitors such as Celebrex (celecoxib), with the goal of developing anti-inflammatory and pain-blocking drugs less likely to cause the gastric toxicity associated with chronic use of NSAIDs that block COX-1. Both Celebrex and Vioxx (now withdrawn from the market) have been associated with increased adverse cardiovascular events, possibly related to decreased production of antithrombotic PGI_2 while not inhibiting COX-1-mediated synthesis of thromboxane A_2 in platelets.

15.5.3 Regulation of Leukotriene Metabolism

Current therapies for asthma include use of 5-lipoxygenase inhibitors such as zileuton, and cysteinyl-leukotriene (cysLT) receptor antagonists such as montelukast.

15.6 DISEASES INVOLVING EICOSANOIDS

15.6.1 Aspirin-Intolerant Asthma

People with aspirin-intolerant asthma develop bronchoconstriction in response to aspirin and other NSAIDs, which, by inhibiting cyclooxygenase activity, leave more arachidonic acid available to enter the linear eicosanoid pathways. Aspirin-sensitive persons appear to have polymorphisms in one or more genes related to cysteinyl-leukotriene production, such as LTC_4 synthase itself or the EP2 receptor through which PGE_2 inhibits leukotriene synthesis.

15.6.2 Ulcers

Because prostaglandins have a protective effect on the lining of the stomach, long-term inhibition of COX-1 with NSAIDs can promote gastrointestinal bleeding.

15.6.3 Increased Tendency for Bleeding

Whereas the omega-3 fatty acids in fish oils have beneficial effects on cardiovascular health because they modify the ratio of prothrombotic and antithrombotic prostanoid activities, high doses (greater than 3 g/day) have been associated with increased bleeding times, cardiovascular disease, and stroke.

15.6.4 Cancer

COX-2 expression is high in many cancers, including those of the prostate, breast, and colon. High concentrations of PGE_2 promote survival of tumor cells by inhibiting apoptosis, stimulating cell proliferation, and promoting angiogenesis.

15.6.5 Therapeutic Uses of Eicosanoids

Exogenous prostaglandins have a number of therapeutic uses. For example, in the fetus, PGE_2 maintains the patency of the ductus arteriosus prior to birth. In infants born with congenital abnormalities that can be corrected surgically, infusion of PGE_2 will maintain blood flow through the ductus until surgery is performed. Conversely, if the ductus remains open after birth in an otherwise normal infant, closure can be hastened by the cyclooxygenase inhibitor indomethacin. PGE_2 has also been used to induce cervical ripening and uterine contractions, leading to parturition.

Misoprostol is a synthetic PGE_1 analog used to prevent NSAID-induced ulcers. In many countries, misoprostol is also used in combination with the synthetic steroid RU486 to block the action of progesterone and induce medical (as opposed to surgical) abortions.

CHAPTER 16

GLYCOLIPIDS AND GLYCOPROTEINS

16.1 FUNCTIONS OF GLYCOCONJUGATES

Glycoconjugates are a diverse group of molecules that contain one or more sugars covalently attached to protein or lipid. The carbohydrate domains of glycoconjugates often contain modified amino or acidic sugar derivatives that contribute to their strongly hydrophilic nature and structural specificity (Fig. 16-1). The glycoconjugates can be grouped into three major classes, described below.

16.1.1 Glycolipids

Glycolipids are membrane sphingolipids that contain one or more sugar moieties attached to the hydrophobic ceramide domain. The major glycolipids are cerebrosides, globosides, and gangliosides. Glycosphingolipids in the plasma membrane tend to concentrate along with sphingomyelin, cholesterol, and phosphatidylinositol-anchored proteins in lipid domains called *rafts*, which are involved in receptor-mediated plasma-membrane signal transduction and cell–cell adhesion.

16.1.1.1 Cerebrosides. Cerebrosides are ceramide monohexosides, the most common of which is *galactocerebroside*, also called *galactolipid* (Fig. 16-2). Most galactocerebroside is found in the brain, where it is a major component of the myelin sheath.

Medical Biochemistry: Human Metabolism in Health and Disease By Miriam D. Rosenthal and Robert H. Glew

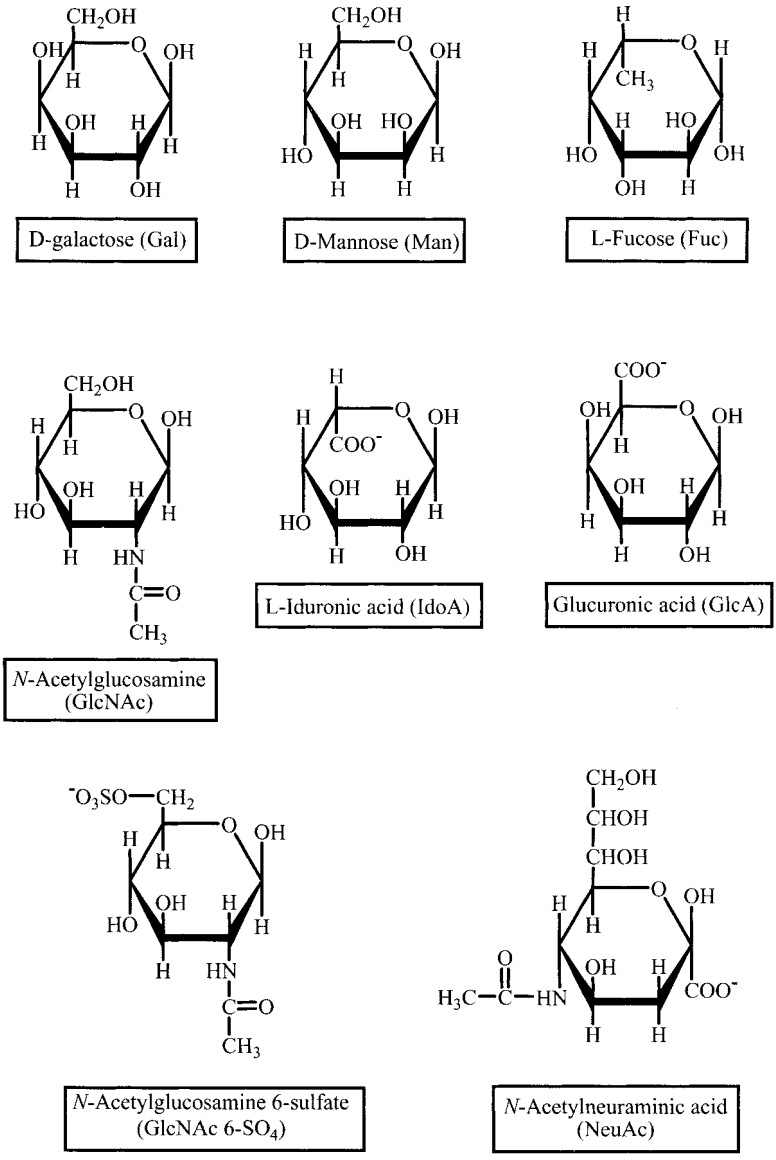
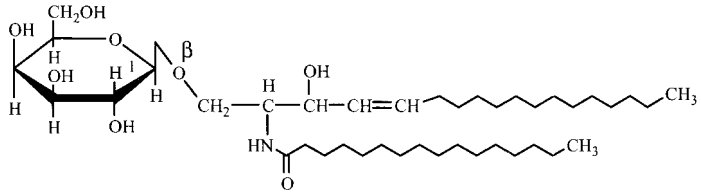
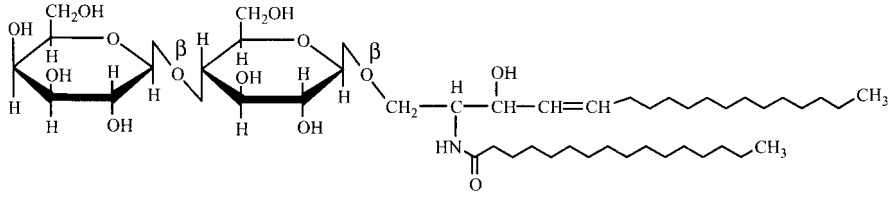


FIGURE 16-1 Structures of some of the sugars and sugar derivatives found in glycoconjugates.

16.1.1.2 Globosides. Globosides are sphingolipids that contain two or more sugar residues, usually a combination of galactose, glucose, and *N*-acetylgalactosamine. The oligosaccharides of globosides are uncharged and contain no free amino groups. Prominent globosides include lactosylceramide [ceramide-β-glc(4-1)-β-gal], which is present in the erythrocyte membrane, and ceramide



Galactocerebroside



Lactosylceramide

FIGURE 16-2 Structures of a cerebroside (galactocerebroside) and a globoside (lactosylceramide).

galactosyllactoside [ceramide-β-glc(4-1)-β-gal-(4-1)-α-gal], which is prominent in membranes of the nervous system (Fig. 16-2). Still other globosides contain carbohydrate domains that provide the ABO antigenic determinants (Fig. 16-3) on the surfaces of cells, particularly erythrocytes. ABO carbohydrate antigens are also found on membrane glycolipids. In some persons, the ABO carbohydrates are also found in plasma in soluble form associated with secreted glycoproteins or on free oligosaccharides.

16.1.1.3 Gangliosides. Gangliosides are sialic acid–containing glycosphingolipids which are highly concentrated in ganglion cells of the central nervous system, particularly in the nerve endings (Fig. 16-4). Lesser amounts of gangliosides are present in the plasma membrane of cells of most extraneural tissues. Sialic acid, which is found in glycoproteins and mucins as well as gangliosides, is the *N*-acetyl derivative of the nine-carbon amino sugar neuraminic acid (Neu) (Fig. 16-1). The carbohydrate domains of the various gangliosides serve as receptors for many different classes of ligands, including cytokines, microbial toxins (e.g., *Vibrio cholera* toxin), microbes, viruses, and hormones. Gangliosides play roles in diverse cellular processes, such as cell–cell recognition, cell homing and adhesion, and growth regulation and differentiation.

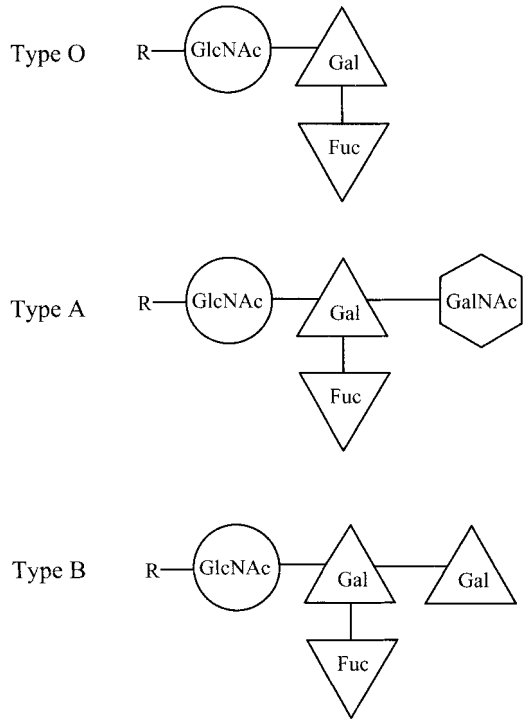


FIGURE 16-3 ABO antigens. R, ceramide or glycoprotein.

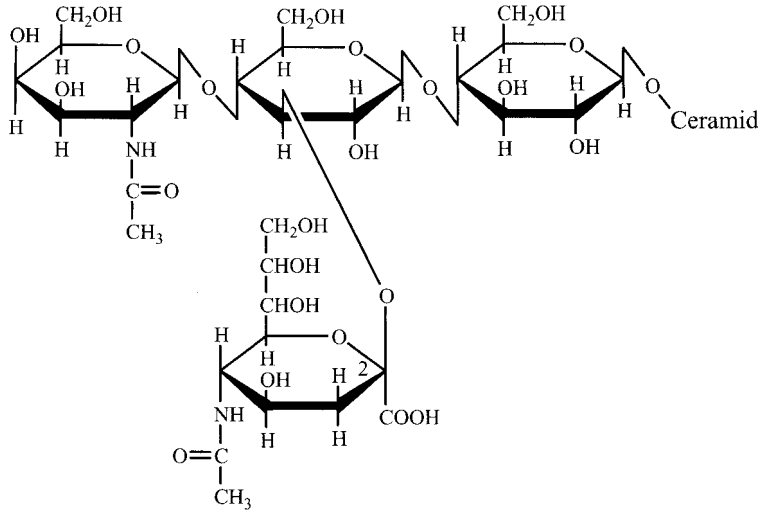


FIGURE 16-4 Ganglioside GM_2, which accumulates in Tay–Sachs disease.

16.1.2 Glycoproteins

Proteins with covalently attached carbohydrate chains (oligosaccharides) are found on the outer surface of the plasma membrane of cells, as part of the extracellular matrix, and in the blood; indeed, except for albumin, nearly all of the proteins in plasma are glycoproteins. Cell-associated glycoproteins include receptors (e.g., the LDL receptor) and ion exchangers in membranes (e.g., the chloride–bicarbonate exchanger known as band 3 of erythrocytes). Glycoproteins also play important roles in cell growth and development, and in the communication that occurs between cells.

A particular glycoprotein can have one or as many as 30 oligosaccharide chains, with the carbohydrate accounting for as little as 1% to as much as 70% of the mass of the glycoprotein. The oligosaccharide chains of a particular glycoprotein usually influence one or more of its biological properties, including intracellular transport, solubility, viscosity, susceptibility to inactivation (by heat, extremes of pH, and proteolysis), and the tendency to aggregate.

The carbohydrate chains of glycoproteins are grouped into two classes, depending on how they are linked to the protein. O-linked oligosaccharides are linked to protein through a glycosidic bond to the hydroxyl group of a threonine or serine residue, whereas N-linked oligosaccharides are attached to the amide nitrogen of an asparagine residue in the protein. Many proteins, including the LDL receptor, contain both N- and O-linked oligosaccharide domains. Figure 16-5 illustrates two types of *N*-asparagine-linked oligosaccharide chains commonly found in mammalian glycoproteins: the high-mannose type and the complex type.

16.1.2.1 Mucins. Mucins are a subclass of glycoproteins that are abundantly glycosylated. They are high molecular weight (200 to 10,000 kDa) glycoproteins that contain dozens to several hundred oligosaccharides O-linked to serine or threonine residues. Oligosaccharides usually account for 50 to 90% of the total mass of the mucin molecule. The sugars that comprise a particular oligosaccharide chain of a mucin may include *N*-acetylgalactosamine (GalNAc), galactose (Gal), *N*-acetylglucosamine (GlcNAc), fucose, and sialic acid.

Secreted mucins (such as salivary mucin) aggregate into oligomeric gels which form a protective layer over the digestive, respiratory, and reproductive tracts and provide lubrication as well as a barrier against pathogens and toxins. Other mucins are integral membrane proteins and remain cell-associated. Cancer cells often synthesize abnormal mucins, whose structures can perturb the normal function of a cell, including its immunologic and adhesive properties and its potential to invade and metastasize.

16.1.3 Proteoglycans

Proteoglycans (formerly called *mucopolysaccharides*) are a class of highly acidic molecules that function as lubricants and structural components of connective tissue. They also mediate adhesion of cells to the extracellular matrix. Proteoglycans are characterized by long glycosaminoglycan (GAG) chains that are attached covalently to a core protein. Most of the structure of the GAG chains of proteoglycans

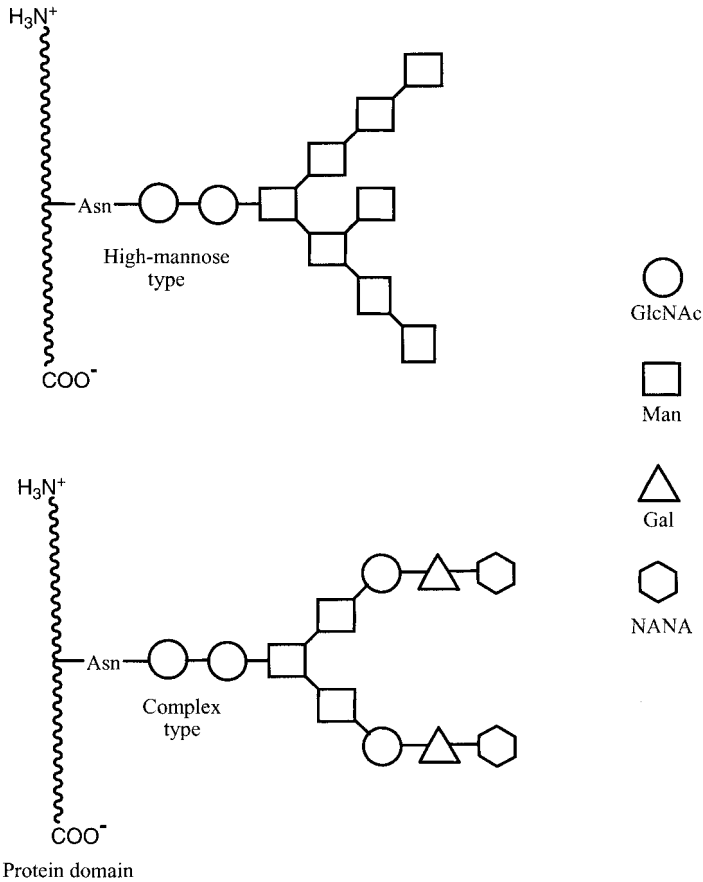
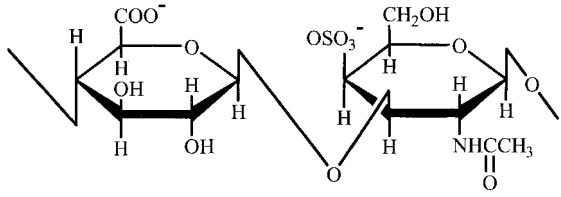


FIGURE 16-5 Structures of some common N-linked oligosaccharides.

is made up of disaccharide repeat units (Fig. 16-6). For example, in heparan sulfate the disaccharide repeat unit glucuronic acid–*N*-acetylglucosamine. In general, one of the two sugars of the disaccharide repeating unit of a GAG is an amino sugar (*N*-acetylglucosamine or *N*-acetylgalactosamine); the other is an acidic sugar (glucuronic acid or iduronic acid). Many of the sugars of GAGs contain sulfate in either O- or N-sulfate linkage.

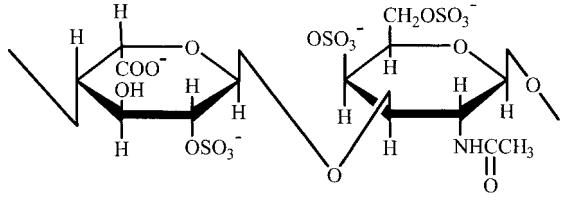
The core protein of a proteoglycan can contain as many as several hundred GAG chains per molecule. The high density of negative charges due to the carboxyl-containing sugars (e.g., glucuronic acid or iduronic acid) and the sulfate residues in the GAG chains cause proteoglycans to form an extended linear structure that resembles a bottle brush.

Hyaluronic acid is a glycosaminoglycan that is not a proteoglycan. Instead, the extremely long GAG chain of hyaluronic acid is secreted into the extracellular matrix as a polysaccharide that is not attached covalently to a core protein. Unlike the proteoglycans, the sugar residues of hyaluronic acid are not sulfated.



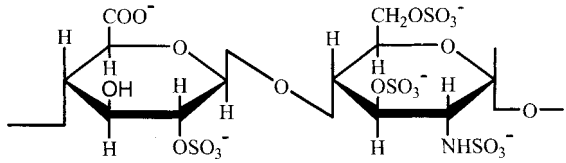
D-Glucuronic acid (GlcA) sulfate *N*-Acetyl-D-galactosamine (GalNAc) sulfate

Chondroitin 4-sulfate



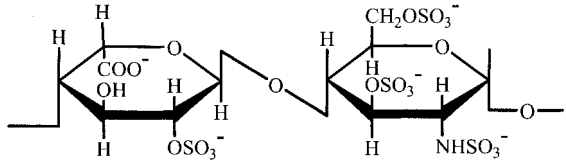
L-Iduronic acid (IdoA) sulfate *N*-Acetyl-D-galactosamine (GalNAc) sulfate

Dermatan sulfate



D-Glucuronic acid (GlcA) sulfate D-Glucosamine ($GlcNH_2$) sulfate

Heparan sulfate



L-Iduronic acid (IdoA) sulfate D-Glucosamine ($GlcNH_2$) sulfate

Heparin

FIGURE 16-6 Structures of repeating disaccharide units in proteoglycans.

16.2 WHERE ARE GLYCOCONJUGATES FOUND IN THE BODY?

16.2.1 Glycocalyx

Carbohydrates are a major component of the external surface of cell membranes. Both glycolipids and glycoproteins are integral components of the asymmetrical plasma membrane, with the carbohydrate moieties facing outward. Other glycoproteins and proteoglycans are adsorbed onto the extracellular surface of the membrane. One such proteoglycan is heparan sulfate (Fig. 16-6), which is the receptor for various growth factors, including vascular endothelial growth factor (VEGF). Heparan sulfate proteoglycan also serves as the ligand that binds lipoprotein lipase to the luminal surface of the capillary endothelium. Heparin, an anticoagulant, is more heavily sulfated than is heparan sulfate.

16.2.2 Extracellular Matrix

Proteoglycans are major components of the extracellular matrix where different classes of proteoglycans confer specific chemical and physical properties on the tissue. For example, chondrocytes (cartilage cells) secrete a variety of proteoglycans, of which a major component is a particular chondroitin sulfate named *aggrecan*. Following its secretion, aggrecan molecules aggregate spontaneously to form a supramolecular structure known as *hyaluronan* which endows cartilage with its load-bearing properties. Hyaluronic acid is a component of the extracellular matrix of skin and connective tissue where its viscous and elastic nature allows it to function as a lubricant and shock absorber in the synovial fluid of joints.

16.2.3 Plasma Proteins

As noted above, nearly all plasma proteins are glycoproteins, including many with enzymatic activity, such as lecithin : cholesterol acyltransferase (LCAT) and ceruloplasmin.

16.2.4 Lysosomal Proteins

Although most glycosylated proteins are secreted from cells, others are targeted to lysosomes. These include the lysosomal-associated membrane glycoproteins as well as lysosomal acid hydrolases.

16.3 METABOLISM OF GLYCOCONJUGATES

16.3.1 Synthesis of Sugar Residues

Glycoconjugates contain several sugar residues and sugar derivatives that have not been described in previous chapters (Fig. 16-1). These include mannose, L-fucose, glucuronic acid, iduronic acid, and sialic acid, as well as amino sugars such as

glucosamine. As is the case with the synthesis of glycogen, synthesis of glycoconjugates requires activated sugars, usually in the form of their UDP derivatives (e.g., UDP-glucose, UDP-xylose; UDP-*N*-acetylgalactosamine). Exceptions include mannose and L-fucose, which are activated as GDP sugars, and sialic acid, which is activated as the CMP derivative.

Mannose 6-phosphate is formed by aldose–ketose isomerization of fructose 6-phosphate and then activated by GTP to GDP-mannose. GDP-fucose, the activated form of the L-deoxysugar, is synthesized by NADH-dependent reduction of GDP-mannose. UDP-glucuronate is a sugar acid formed by oxidization of UDP-glucose. Following its incorporation into glycosaminoglycans, glucuronate may be isomerized to iduronate. UDP-glucuronate can also be converted to UDP-xylose.

Synthesis of amino sugars is initiated by glutamine : fructose 6-phosphate amidotransferase, which catalyzes the synthesis of glucosamine 6-phosphate. The amino groups of amino sugars are generally acetylated using acetyl-CoA. Sialic acid (*N*-acetylneuraminic acid) is synthesized by condensation of the carbon backbone of phosphoenolpyruvate with *N*-acetylmannosamine 6-phosphate.

16.3.2 Synthesis of Sphingolipids

16.3.2.1 Cerebrosides. Galactocerebroside and glucocerebroside are usually synthesized from ceramide and UDP-galactose or UDP-glucose, respectively, by galactosyl and glucosyl transferases that are associated with the endoplasmic reticulum:

$$\text{ceramide} + \text{UDP-galactose} \rightarrow \text{galactocerebroside} + \text{UDP}$$

Alternatively, in some tissues, synthesis of glucocerebroside proceeds by glucosylation of sphingosine by glucosyltransferase:

$$\text{sphingosine} + \text{UDP-glucose} \rightarrow \text{glucosylsphingosine} + \text{UDP}$$

followed by fatty acylation:

$$\text{glucosylsphingosine} + \text{acyl-CoA} \rightarrow \text{glucocerebroside} + \text{CoASH}$$

Unlike sphingomyelin, whose *N*-acyl group is usually stearate, glycolipids contain behenic acid, a saturated 22-carbon fatty acid or some other very long-chain saturated fatty acid. Galactocerebroside is the major cerebroside found in membranes; by contrast, glucocerebroside is primarily an intermediate in the synthesis of more complex sphingolipids.

16.3.2.2 Sulfatides. Galactocerebroside 3-sulfate, a sulfuric acid ester of galactocerebroside, is the major sulfolipid of brain, accounting for about 15% of the lipids of white matter. It is synthesized from galactocerebroside and 3′-phosphoadenosine

5′-phosphosulfate (PAPS; see Chapter 21) in a reaction is catalyzed by sulfotransferase:

galactocerebroside + PAPS →
galactocerebroside 3-sulfate + phosphoadenosinephosphate

16.3.2.3 Globosides. Globosides and gangliosides are synthesized in the Golgi apparatus by enzymes that transfer sugars sequentially onto a cerebroside. One such example is the synthesis of the glycosphingolipids containing A, B, and O blood group antigens (Fig. 16-3). The core structure of the O (or H) oligosaccharide is formed by sequential addition of *N*-acetylglucosamine, galactose, and fucose to galactocerebroside. Persons with a gene for the type A transferase are able to transfer *N*-acetylgalactosamine to the core structure to synthesize the A antigen from the O core structure, while those with the type B transferase transfer galactose to synthesize the B antigen. Some people have genes for both the A and B transferases and therefore synthesize both A and B antigens. The "O gene" is actually a mutation which results in premature termination of translation so that no active transferase A or B is formed; persons homozygous for the gene for the O blood group therefore synthesize only the core O antigen.

16.3.2.4 Gangliosides. Most gangliosides are built on lactosylceramide (Gal-β1,4-Glc-β1,1′-Cer), which is formed by the transfer of galactose from UDP-galactose to glucosylceramide (glucocerebroside). Additional sugars, including sialic acid, are then added stepwise to the growing glycan chain. Humans contain at least five different sialyltransferases, each with a different specificity with regard to the acceptor.

16.3.3 Synthesis of Glycoproteins

The oligosaccharide chains of glycoproteins can either be O-linked (to serine or threonine residues of the protein) or N-linked (to the amide nitrogen in the side chain of asparagine). O-linked glycosylation of proteins, like that of sphingolipids, occurs by stepwise glycosylation in the Golgi complex. By contrast, the synthesis of N-linked oligosaccharide chains involves a more complex sequence of reactions which is initiated in the endoplasmic reticulum and continues in the Golgi complex.

The first phase of N-linked oligosaccharide synthesis takes place on an isoprene lipid, dolichol pyrophosphate (Fig. 16-7). The core oligosaccharide, containing two *N*-acetylglucosamine, nine mannose, and three glucose residues, is assembled on dolichol pyrophosphate by the successive transfer of glycosyl residues from their respective nucleoside diphosphate sugar donors (e.g., GDP-mannose, UDP-glucose). The transfer of the two *N*-acetylglucosamine and five of the mannose residues occurs in the rough endoplasmic reticulum (ER). The dolichol moiety with its attached

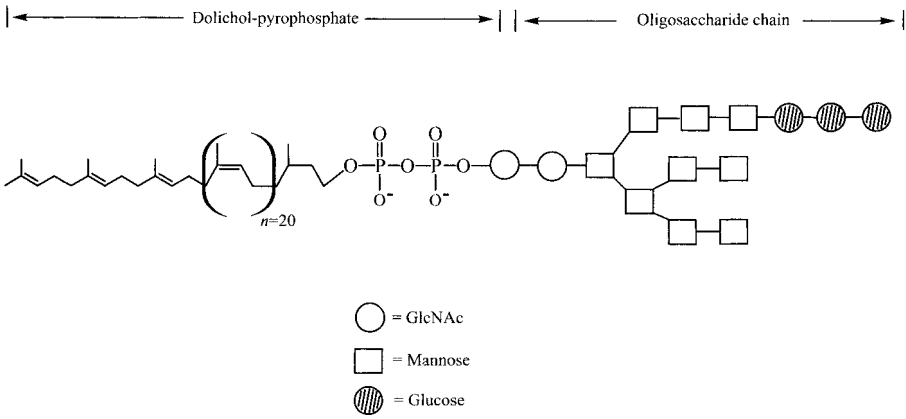


FIGURE 16-7 Structure of dolichol attached to a fully assembled oligosaccharide.

oligosaccharide chain is then flipped across the ER membrane into the lumen of the ER, where the four additional mannose residues plus the three glucose residues are then added. Once the target polypeptide is transported through a channel in the ER membrane and its signal sequence has been cleaved, the core oligosaccharide is then transferred en bloc from the dolichylpyrophosphate to the polypeptide. The asparagine that accepts the core oligosaccharide always occurs in the sequence Asn-X-Thr/Ser, where X is any amino acid except proline.

The next phase of N-linked oligosaccharide-chain processing involves α-glucosidase-catalyzed stepwise removal of the three glucose residues and one mannose from the core oligosaccharide. Two ER proteins, calnexin and calreticulin, assure the correct folding of the glycoprotein. The properly folded glycoprotein then moves by vesicular transport to the Golgi complex, where it undergoes a variety of additional posttranslational modifications, including the removal of additional mannose residues and the sequential addition of single residues each of *N*-acetylglucosamine, galactose, fucose, and sialic acid.

16.3.4 Synthesis of Proteoglycans

The initial synthesis of proteoglycans resembles that of O-linked glycoproteins in that sugars are added to the protein one at a time in the lumen of the endoplasmic reticulum. A xylose residue is attached to the hydroxyl group of a serine, followed by addition of two galactose residues to form what is called the *link trisaccharide*. Two glycosyltransferases enzymes then alternate adding sugar residues to generate the repeating disaccharide units (i.e., glucuronic acid and glucosamine) when heparin is being synthesized. Sulfation of sugar residues occurs after the sugars have been attached to the growing oligosaccharide chain, and as with the sulfation of galactosylceramide, PAPS is the donor of the activated sulfate moiety.

16.3.5 Catabolism of Glycoconjugates

Phagocytic cells, particularly the macrophages of the reticuloendothelial system located primarily in liver, spleen, and bone marrow, are especially active in glycosphingolipid catabolism. The glycosphingolipids are catabolized in lysosomes by sequential, irreversible removal of the carbohydrate residues—one at a time—followed by the hydrolysis of ceramide to sphingosine and a free fatty acid. This pathway requires enzymes that cleave specific bonds, including α- and β-galactosidases, a β-glucosidase, a neuraminidase, a hexosaminidase, a sphingomyelinase, a sulfatase, and a ceramide-specific amidase (ceramidase).

Lysosomes are also responsible for the stepwise degradation of the glycosaminoglycan (GAG) chains of proteoglycans. The lysosomal enzymes are all acid hydrolases with pH optima in the range 3.5 to 5.5. The enzymes that hydrolyze glycosphingolipids often require sphingolipid activator proteins, which promote interaction between these enzymes and their water-insoluble lipid substrates.

16.4 DISEASES OF GLYCOCONJUGATE METABOLISM

16.4.1 Sphingolipidoses

There are about one dozen different life-threatening human disorders that result from genetically-based deficiencies of lysosomal hydrolases which degrade glycolipids; collectively, they are referred to as the *sphingolipidoses*. Sphingolipid catabolism normally functions smoothly, all of the glycosphingolipids and sphingomyelin being degraded to their constituents. However, when the activity of one enzyme in the pathway is markedly reduced due to a genetic error, the substrate for that defective enzyme accumulates within the lysosomes of the tissue in which catabolism of that sphingolipid normally occurs. Examples of sphingolipidoses are described below.

16.4.1.1 Gaucher Disease. Gaucher disease is caused by a genetic deficiency of lysosomal glucocerebrosidase. The accumulation of glucocerebroside, primarily in macrophages of the reticuloendothelial system, results in hepatomegaly, splenomegaly, anemia, and bone pain. Gaucher disease is now treated effectively by enzyme replacement therapy using recombinant glucocerebrosidase, which is produced in human cells so as to obtain appropriate glycosylation of the enzyme with oligosaccharide chains terminating in mannose residues. Mannose receptors on the surface of macrophages bind the mannose-terminated enzymes and through a process of endocytosis deliver them into lysosomes, where they degrade the accumulated lipid, glucocerebroside.

16.4.1.2 Fabry Disease. Deficiency of lysosomal α-galactosidase A results in Fabry disease and accumulation of globotriaosylceramide (Cer → β-Glu → β-Glu → α-Gal) in tissues, mainly the walls of blood vessels. Unlike the other sphingolipidoses, which are autosomal recessive diseases, Fabry disease is X-linked. Enzyme replacement therapy is now also available for Fabry disease.

16.4.1.3 Tay–Sachs Disease. Tay–Sachs disease is a gangliosidosis caused by the absence of β-hexosaminidase A and results in neural accumulation of the ganglioside G_{M2} (Fig. 16-2). The disease is characterized by mental retardation, a cherry-red spot on the macula which reflects ganglioside accumulation in retinal ganglia, blindness, and for the most severe, infantile form, death before age 3. Because of the primary involvement of ganglion cells of the central nervous system, effective enzyme replacement therapy has not proven feasible.

16.4.2 Mucopolysaccharidoses

Deficiencies in lysosomal enzymes that are normally responsible for degrading the glycosaminoglycan (GAG) chains of proteoglycans result in accumulation of undegraded GAGs. Since there is some digestion of the oligosaccharide chains by lysosomal endoglycosidases, urinary excretion of shorter oligosaccharides is often diagnostic. The mucopolysaccharidoses are classified according to the substrate that accumulates (Table 16-1). Treatment of patients with MPS I (Hurler disease) with recombinant human α-L-iduronidase appears to reduce lysosomal storage in the liver and ameliorate some clinical manifestations of the disease.

16.4.3 Oligosaccharidoses

The genetic diseases that result from defects in the lysosomal pathway that degrades the oligosaccharide chains of glycoproteins are called *oligosaccharidoses*. They can result from deficiencies in any one of a number of enzymes, including α-mannosidase, β-mannosidase, α-fucosidase, and α-sialidase. Oligosaccharidoses are usually named for the deficient enzyme; for example, a deficiency in α-mannosidase is called α-mannosidosis.

TABLE 16-1 Selected Mucopolysaccharidoses and the Associated Enzyme Defect

Disease	Enzyme Defect	Accumulated Substance
Hurler, Scheie (MPS I)	α-Iduronidase	Dermatan sulfate, heparan sulfate
Hunter (MPS II)	Iduronate sulfatase	Dermatan sulfate, heparan sulfate
Sanfilippo A (MPS IIIA)	Heparan *N*-sulfatase	Heparan sulfate
Sanfilippo B (MPS-B)	*N*-Acetylglucosaminidase	Heparan sulfate
Morquio A (MPS IVA)	*N*-Acetylgalactosamine-6-sulfatase	Keratin sulfate
Maroteaux–Lamy (MPS VI)	*N*-Acetylgalactosamine-4-sulfatase	Dermatan sulfate

MPS, mucopolysaccharadosis.

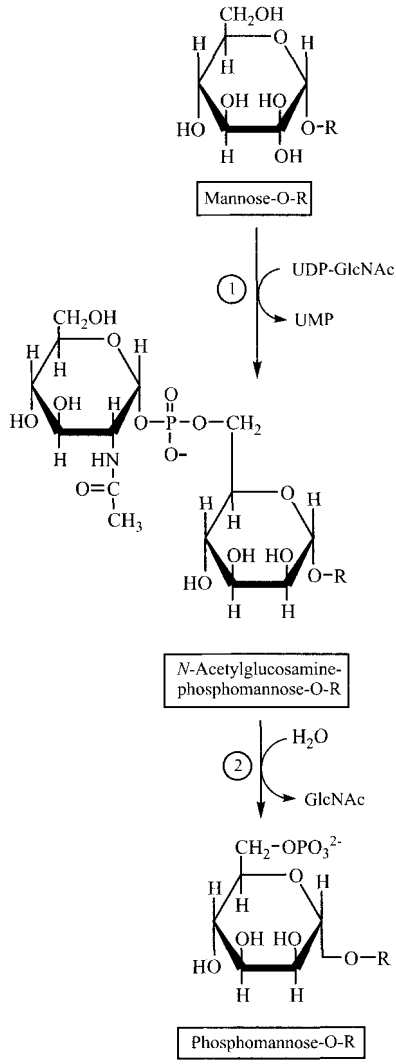


FIGURE 16-8 Generation of mannose 6-phosphate tag on the oligosaccharide chains of lysosomal enzymes: ① UDP-*N*-acetylglucosamine-1-phosphotransferase; ② *N*-acetylglucosamine-1-phosphodiester-α-*N*-acetylglucosaminidase. R, lysosomal enzyme.

16.4.4 I-Cell Disease

I-cell disease (mucolipidosis II) is a rare autosomal lysosomal storage disorder characterized by the accumulation of mucopolysaccharides, sphingolipids, and glycolipids inside lysosomes of visceral and mesenchymal cells. Patients with I-cell disease secrete large amounts of many different lysosomal enzymes into body fluids but have deficient enzyme activity within the lysosomes.

The trafficking of lysosomal enzymes, which contain *N*-asparagine-linked oligosaccharides, to the lysosome requires the addition of a mannose 6-phosphate recognition marker. Two enzymes are required to attach a phosphate group to a mannose moiety of oligosaccharide chains of lysosomal enzymes (Fig. 16-8). The first reaction is catalyzed by UDP-*N*-acetylglucosamine-1-phosphotransferase, commonly called *phosphotransferase*. The second enzyme in the pathway, *N*-acetylglucosamine-1-phosphodiester-α-*N*-acetylglucosaminidase, removes the terminal α-*N*-acetylglucosamine residue, leaving the phosphate group attached to the underlying mannose residue.

Lysosomal enzymes bearing the mannose 6-phosphate marker bind to the mannose 6-phosphate receptor in the trans Golgi, are packaged into clathrin-coated vesicles, and are transported to late endosomes, where the low pH causes the lysosomal enzymes to dissociate from the receptors. Patients with I-cell disease have a deficiency of the phosphotransferase, which impairs targeting of enzymes to the lysozyme.

16.4.5 Abnormal Glycosylation

Congenital disorders of glycosylation (CDGs) include abnormalities in either the glycosyltransferases, which elongate, or the glycosidases, which process the oligosaccharide chains of N-linked glycoproteins, O-linked glycoproteins, or both. The CDG I family comprises defects in the assembly of the dolichol-linked glycan and its transfer to the protein, whereas the CDG II family includes defects in the processing of the protein-bound glycans. CDG disorders affect many different glycoconjugates, including clotting factors, collagen, red cell membrane glycophorin, and α_1-antitrypsin.

16.4.6 Cholera

A ganglioside on intestinal mucosal cells binds cholera toxin, an 84-kDa protein secreted by the pathogen *Vibrio cholerae*. The toxin consists of one A subunit and five B subunits. The *choleragenoid domain*, as the B subunits are called, binds to the ganglioside G_{M1}. The A subunit then enters the cell and acts as an ADP-ribosyltransferase that transfers ADP-ribose of NAD onto the $G_{\alpha s}$ subunit of a G protein. This leads to activation of adenylyl cyclase, which stimulates secretion of chloride ion and produces diarrhea. Gangliosides may also bind other toxins (e.g., tetanus toxin) and viruses, such as the influenza viruses.

CHAPTER 17

CHOLESTEROL SYNTHESIS AND TRANSPORT

17.1 FUNCTIONS OF CHOLESTEROL

Cholesterol is the major sterol in humans. The sterol structure of cholesterol (Fig. 17-1) consists of four fused rings, three six-carbon and one five-carbon, designated A to D. Cholesterol has a hydroxyl group at C3, a C5–C6 carbon–carbon double bond, and two methyl groups, attached at positions C10 and C13 of the sterol ring. In addition, cholesterol has a branched eight-carbon hydrocarbon chain attached to the D ring at C17.

17.1.1 Cholesterol Is a Structural Component of Cellular Membranes

Cholesterol is a ubiquitous and essential component of mammalian cell membranes. It is also present in small amounts in the outer membrane of mitochondria. Cholesterol is especially abundant in myelinated structures of the central nervous system, with 25% of the body's cholesterol located in the brain. In contrast to plasma, where most of the circulating cholesterol exists esterified to a fatty acid, most cholesterol in cellular membranes is present in the free (unesterified) form. The fluidity of membranes is regulated in part by changing their cholesterol content.

17.1.2 Cholesterol Is a Major Component of Bile

Cholesterol is abundant in bile (normal concentration is about 15 mg per 100 mL, only 4% of which is esterified). The solubilization of free cholesterol in bile is achieved

Medical Biochemistry: Human Metabolism in Health and Disease By Miriam D. Rosenthal and Robert H. Glew

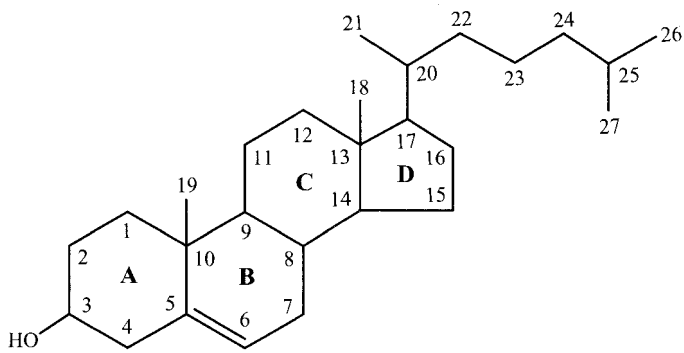


FIGURE 17-1 Structure of cholesterol.

in part by the detergent property of phosphatidylcholine, which is produced in liver and secreted into bile. Bile acids, which are metabolites of cholesterol, also aid in solubilizing cholesterol in bile. Increased biliary secretion of cholesterol or decreased secretion of phospholipids or bile acids into bile may lead to deposition of cholesterol-rich gallstones. Indeed, the name *cholesterol* was derived some 200 years ago from the Greek words *chole* (bile) + *stereos* (solid). Cholesterol and phospholipids in bile protect gallbladder membranes from potentially irritating or harmful effects of bile salts. In the absence of dietary intake of cholesterol (i.e., vegan or low-fat diets), the cholesterol in bile also provides enterocytes with a source of cholesterol for chylomicron synthesis.

17.1.3 The Cholesterol Synthesis Pathway Provides a Mechanism for Increasing the Hydrophobicity of Proteins

The activated prenyl groups farnesyl pyrophosphate (15 carbons) (Fig. 17-2) and geranylgeranyl pyrophosphate (20 carbons) can donate their hydrophobic domains to many proteins involved in cell signaling, including the γ-subunits of trimeric G proteins, Ras, and the nuclear lamins A and B. Posttranslational prenylation of these proteins increases their tendency to associate with membranes, and prenylation is usually required for their full activity. Covalent attachment of cholesterol to protein is required for the membrane tethering that is necessary for the activation of the Hedgehog family of proteins, which are essential to embryonic patterning; the cholesterol moiety is attached via an ester bond to the C-terminal glycine during autocatalytic cleavage and maturation of the initially soluble protein.

17.1.4 The Cholesterol Synthesis Pathway Provides Key Metabolic Intermediates

Cholesterol is the precursor of the various steroid hormones and bile acids. 7-Dehydrocholesterol, the immediate precursor of cholesterol, is converted to vitamin D_3 when skin is exposed to ultraviolet light. Isoprene units, generated as

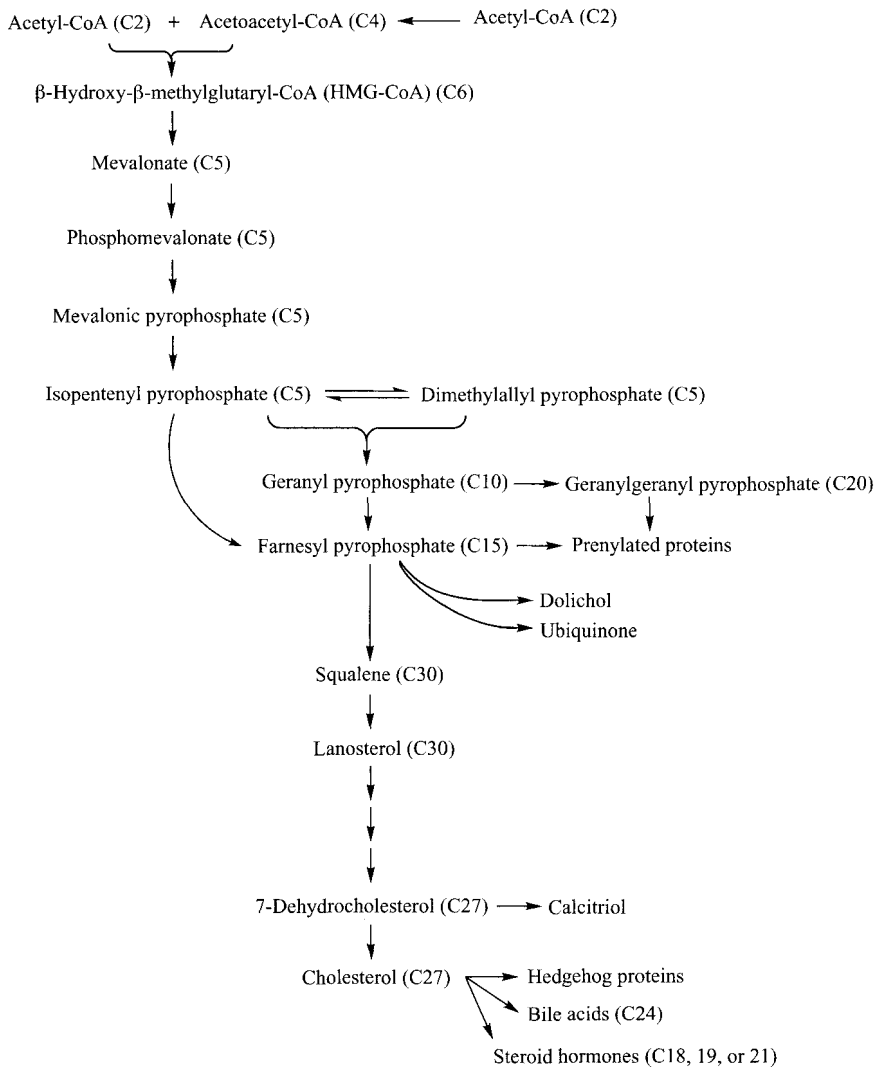


FIGURE 17-2 Substrates and intermediates in the cholesterol synthesis pathway. The numbers in parentheses indicate the number of carbon atoms in each molecule.

intermediates in the pathway of cholesterol synthesis, are also precursors in the synthesis of larger molecules, including heme A, dolichol pyrophosphate, and coenzyme Q (ubiquinone).

17.2 CHOLESTEROL HOMEOSTASIS

Cholesterol is both derived from the diet and synthesized de novo in the body. Humans cannot metabolize cholesterol to CO_2 and water. Excretion of cholesterol

and of bile acids synthesized from cholesterol occurs by way of the liver, gallbladder, and intestine.

17.2.1 Synthesis of Cholesterol

Although synthesis of cholesterol occurs to some extent in virtually all cells (except red blood cells), in adults this capacity is greatest in liver, intestine, adrenal cortex, and reproductive tissues, including ovaries, testes, and placenta. The brain is the most cholesterol-rich organ of the body; however, since plasma lipoproteins that transport cholesterol in the circulation do not cross the blood–brain barrier, all cholesterol in the brain must be synthesized within the central nervous system. Cholesterol synthesis in the brain occurs at a high rate during the period of active myelination and declines substantially thereafter. Within the cell, cholesterol synthesis takes place in the cytosol and endoplasmic reticulum.

17.2.2 Intestinal Absorption and Excretion of Cholesterol

The average adult who consumes a Western diet takes in about 500 mg of dietary cholesterol. Cholesterol also enters the small intestine as a component of bile (Fig. 17-3). Although there is wide individual variation, on average about half of the cholesterol that enters the small intestine is absorbed into the body. Animal products contain both cholesterol and cholesteryl esters; the latter are hydrolyzed in the small intestine by pancreatic cholesteryl esterase:

$$\text{cholesteryl ester} + H_2O \rightarrow \text{cholesterol} + \text{fatty acid}$$

Cholesterol is absorbed by the cells of the intestinal mucosa and incorporated into the surface of chylomicrons. Cholesterol in excess of that required for the chylomicron surface is esterified to cholesteryl esters and incorporated into the triacylglycerol-rich chylomicron core. Both free and esterified cholesterol are delivered to the liver as components of chylomicron remnants.

Cholesterol that is not secreted from the intestinal mucosa in chylomicrons is returned to the intestinal lumen as a component of sloughed mucosal cells and excreted. The fecal sterols are a mixture of cholesterol and cholesterol metabolites, such as cholestanol and coprostanol, generated by intestinal bacteria (Fig. 17-3).

17.3 PATHWAY OF CHOLESTEROL SYNTHESIS

The pathway of cholesterol synthesis is summarized in Figure 17-2. All of the carbon atoms of cholesterol are derived from acetyl-CoA, which can be obtained from several

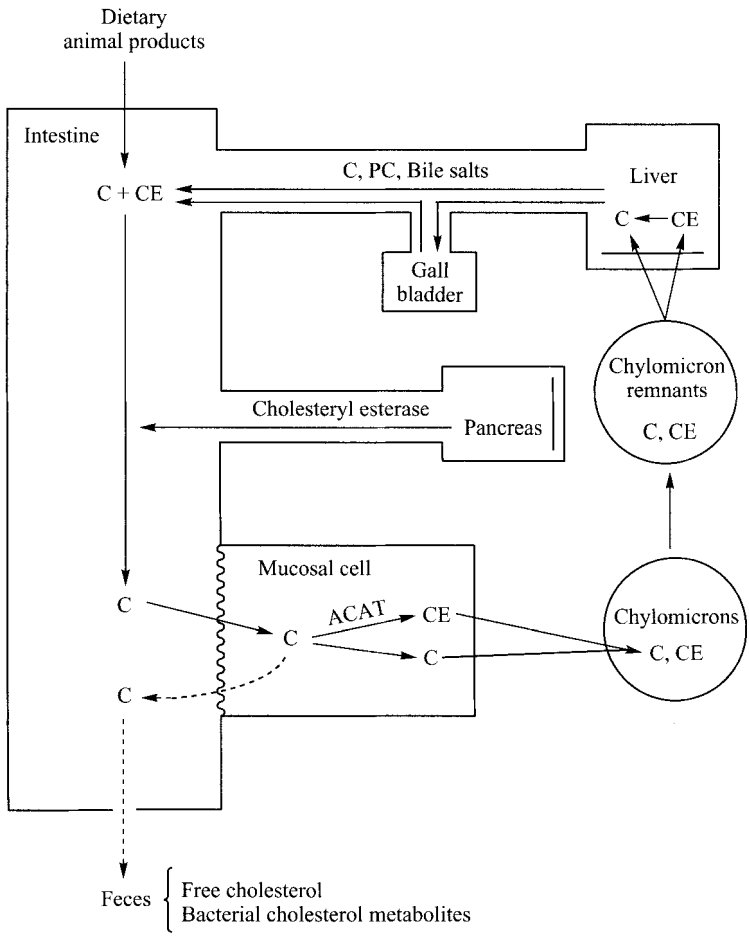


FIGURE 17-3 Intestinal absorption and excretion of cholesterol. ACAT; acyl-CoA–cholesterol acyltransferase; C, cholesterol; CE, cholesteryl ester; PC, phosphatidylcholine.

sources, including the pyruvate dehydrogenase reaction, β-oxidation of fatty acids, oxidation of amino acids, and ethanol. As with fatty acid synthesis, nearly all of the acetyl-CoA used for cholesterogenesis is generated in the mitochondrion; the acetyl moieties are transported to the cytosol in the form of citrate. Reducing power in the form of NADPH is required for cholesterogenesis and it is provided mainly by glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of the hexose monophosphate pathway and by the malic enzyme. Cholesterol synthesis is driven largely by hydrolysis of the high-energy thioester bonds of acetyl-CoA and the phosphoanhydride bonds of ATP. The synthesis of one molecule of cholesterol consumes 18 molecules of acetyl-CoA, 16 molecules of NADPH, and 36 molecules of ATP.

17.3.1 Synthesis of β-Hydroxy-β-methylglutaryl-CoA

As is the case in the pathway that produces ketone bodies, synthesis of cholesterol involves β-hydroxy-β-methylglutaryl-CoA as an intermediate. Synthesis starts with the condensation of two molecules of acetyl-CoA to form acetoacetyl-CoA in a reaction catalyzed by acetoacetyl-CoA thiolase (acetyl-CoA:acetyl-CoA acetyltransferase) (Fig. 17-4):

$$2\,\text{acetyl-CoA} \rightarrow \text{acetoacetyl-CoA} + \text{CoASH}$$

A third molecule of acetyl-CoA is then used to form the branched-chain compound β-hydroxy-β-methylglutaryl-CoA (HMG-CoA). This reaction is catalyzed by HMG-CoA synthase (Fig. 17-4):

$$\text{acetyl-CoA} + \text{acetoacetyl-CoA} \rightarrow \text{β-hydroxy-β-methylglutaryl-CoA} + \text{CoASH}$$

Hepatocytes contain two distinct HMG-CoA synthases: a cytosolic enzyme that is involved in cholesterol synthesis, and a mitochondrial enzyme which functions in the pathway of ketone body synthesis.

17.3.2 Synthesis of Mevalonic Acid

The first compound unique to cholesterol synthesis is mevalonic acid. Mevalonic acid is formed from HMG-CoA by the enzyme HMG-CoA reductase, which is an intrinsic protein of the endoplasmic reticulum whose catalytic C-terminal domain extends into the cytosol. The NADPH-dependent reaction catalyzed by HMG-CoA reductase is irreversible and represents the rate-limiting step in cholesterol biosynthesis. This reaction consumes two molecules of NADPH, results in hydrolysis of the thioester bond of HMG-CoA, and generates the primary alcohol group of mevalonate (Fig. 17-4):

$$\text{HMG-CoA} + 2\text{NADPH} \rightarrow \text{mevalonate} + \text{CoASH} + 2\text{NADP}^+ + 2\text{H}^+$$

17.3.3 Synthesis of Isoprene Pyrophosphates

Mevalonate is converted to 5-pyrophosphomevalonate by the stepwise transfer of the terminal phosphate group of two molecules of ATP. The two reactions are catalyzed by mevalonate kinase and phosphomevalonate kinase, respectively (Fig. 17-5):

$$\text{mevalonate} + \text{ATP} \rightarrow \text{5-phosphomevalonate} + \text{ADP}$$

$$\text{5-phosphomevalonate} + \text{ATP} \rightarrow \text{5-pyrophosphomevalonate} + \text{ADP}$$

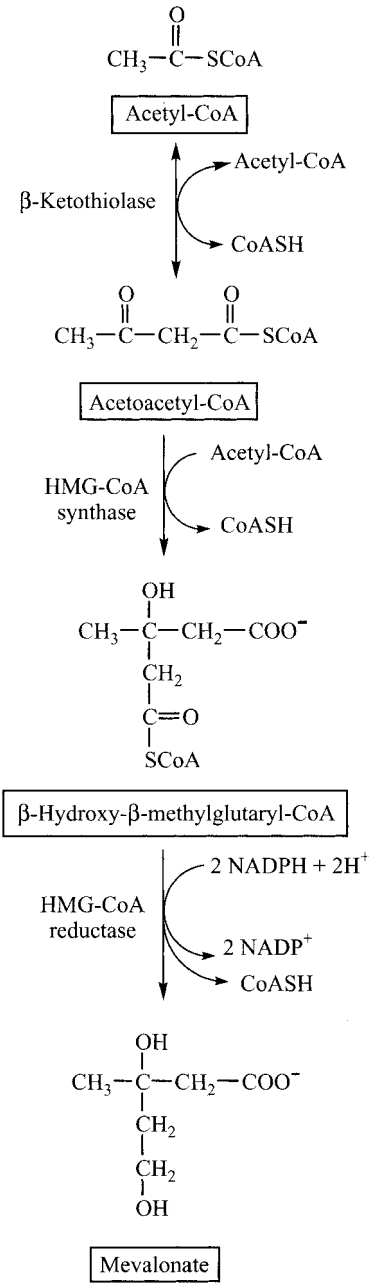


FIGURE 17-4 Synthesis of mevalonate.

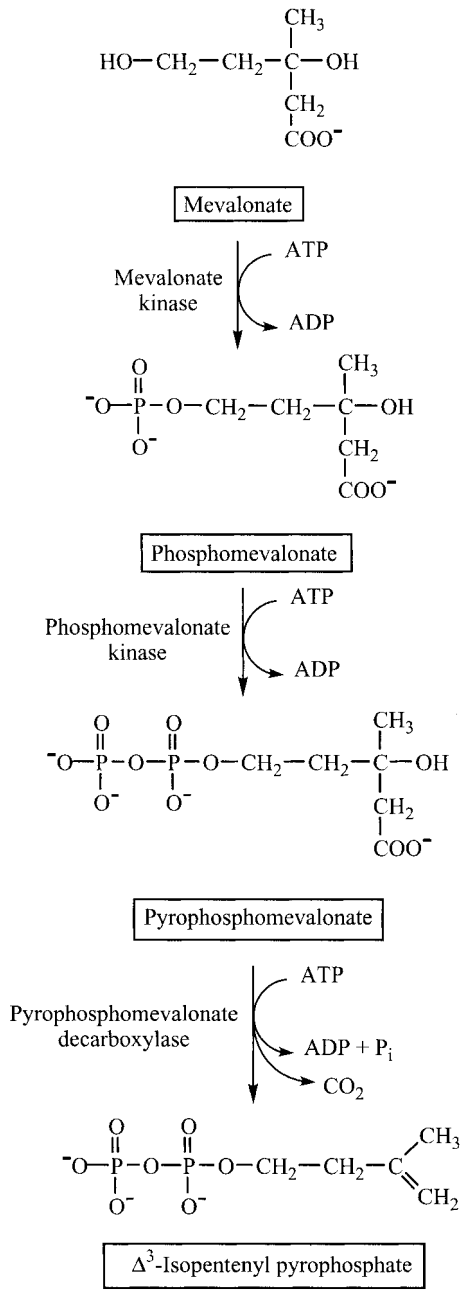


FIGURE 17-5 Synthesis of isopentenyl pyrophosphate.

5-Pyrophosphomevalonate is then decarboxylated by pyrophosphomevalonate decarboxylase to generate Δ^3-sopentenyl pyrophosphate.

$$\text{5-prophosphomevalonate} + \text{ATP} \rightarrow \Delta^3\text{-isopentenyl pyrophosphate} + CO_2 + \text{ADP} + P_i$$

Isopentenyl pyrophosphate is converted to its allylic isomer 3,3-dimethylallyl pyrophosphate by isopentenyl pyrophosphate isomerase.

17.3.4 Condensation of Isoprene Pyrophosphates

Next, six isoprene pyrophosphate molecules condense to form the 30-carbon polyprene molecule squalene, which is devoid of oxygen atoms (Fig. 17-6). First, prenyltransferase condenses two isoprene pyrophosphate molecules, 3,3-dimethylallyl pyrophosphate and 3-isopentenyl pyrophosphate (in a head-to-tail manner), to release one molecule of pyrophosphate (PP_i) and form the 10-carbon molecule geranyl pyrophosphate. A second prenyltransferase then adds another 3-isopentenyl pyrophosphate unit to form the 15-carbon intermediate, farnesyl pyrophosphate, with the release of a second molecule of pyrophosphate. The last condensation step in cholesterol synthesis involves head-to-head fusion of two molecules of farnesyl pyrophosphate to form squalene. The reaction, catalyzed by squalene synthase, occurs on the endoplasmic reticulum, requires NADPH, and releases two pyrophosphate groups.

17.3.5 Synthesis of Lanosterol and Its Conversion to Cholesterol

As seen in Figure 17-7, rotation about carbon–carbon bonds permits squalene to assume an overall shape resembling that of cholesterol. Cholesterol synthesis from squalene proceeds through the intermediate lanosterol, which contains the fused tetracyclic ring system and an eight-carbon side chain. The endoplasmic reticulum enzyme that catalyzes this cyclization reaction is bifunctional and has both squalene epoxidase and lanosterol cyclase activity. Cyclization is initiated by epoxide formation between the future C2 and C3 of cholesterol, the epoxide being formed at the expense of NADPH:

$$\text{squalene} + O_2 + \text{NADPH} + H^+ \rightarrow \text{squalene 2,3-epoxide} + H_2O + NADP^+$$

The hydroxylation at C3 triggers the subsequent cyclization of squalene to lanosterol, with many carbon–carbon bonds being formed in a concerted fashion (Fig. 17-7). This reaction sequence requires the addition of an OH group to C3, the shifting of two methyl groups, and the elimination of a proton. The OH group of lanosterol projects above the plane of the A ring (i.e., in the β-orientation).

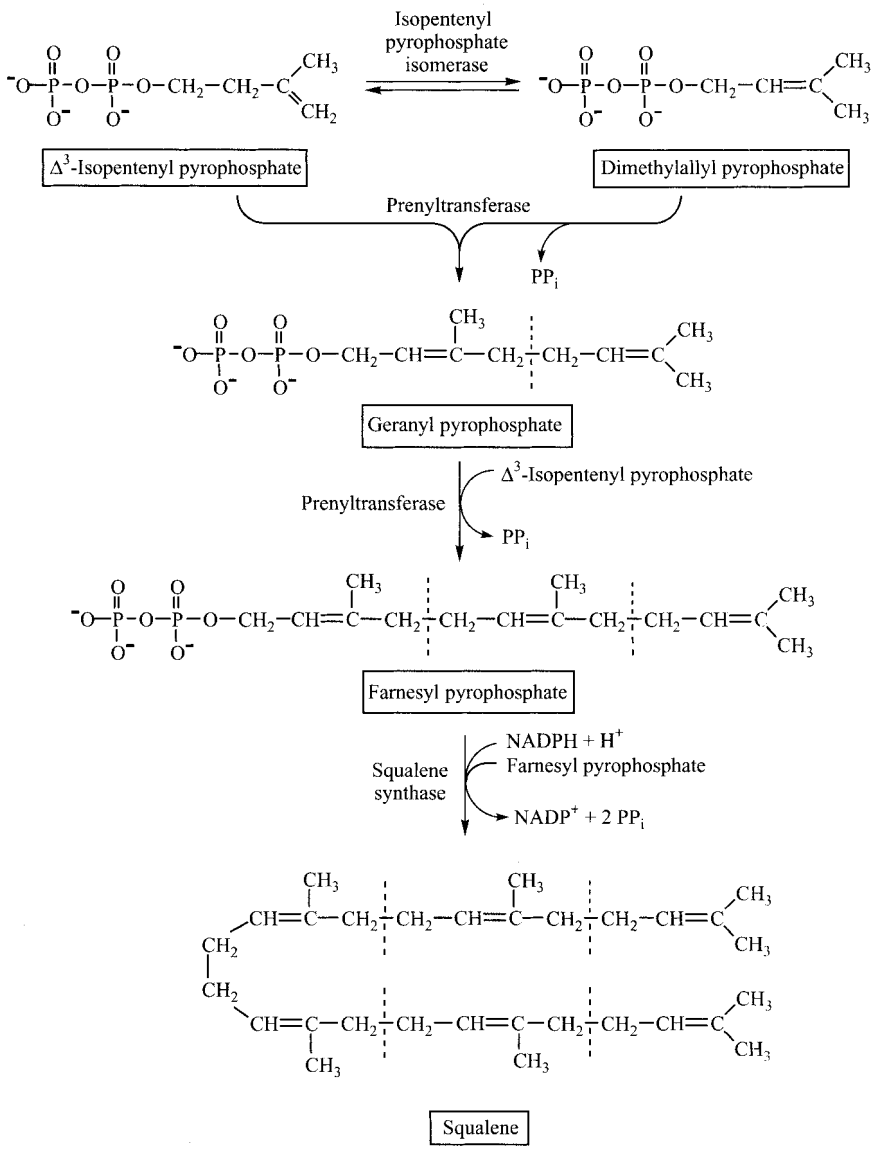


FIGURE 17-6 Condensation of isoprene pyrophosphate units generates squalene.

Transformation of the 30-carbon lanosterol to the 27-carbon cholesterol molecule requires at least eight different enzymes which catalyze removal of the methyl group at C14, removal of two methyl groups at C4, migration of the double bond from C8 to C5, and reduction of the double bond between C24 and C25 in the side chain (Fig. 17-7).

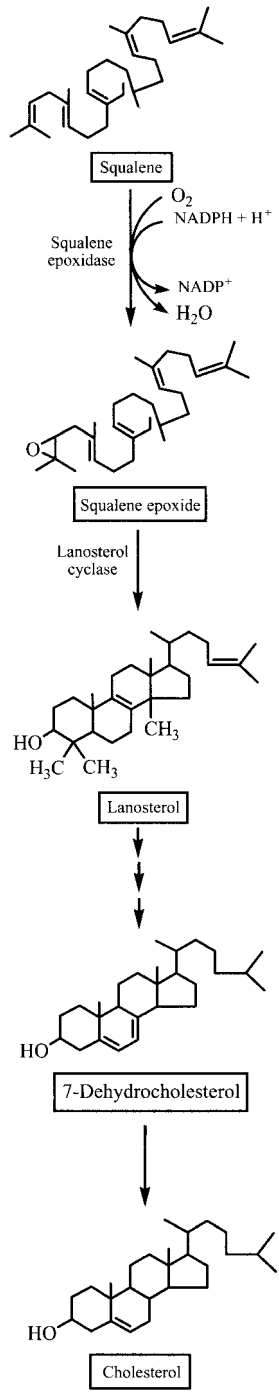


FIGURE 17-7 Synthesis of cholesterol from squalene.

17.3.6 Esterification of Cholesterol

Two key enzymes can esterify cholesterol: One is an intracellular enzyme, acyl-CoA: cholesterol acyltransferase (ACAT), which catalyzes the reaction

$$\text{fatty acyl-CoA} + \text{cholesterol} \rightarrow \text{cholesteryl ester} + \text{CoASH}$$

The other is an extracellular enzyme called lecithin:cholesterol acyltransferase (LCAT), which esterifies cholesterol molecules associated with the lipoprotein HDL. In this reaction, the source of the fatty acid is the phospholipid phosphatidylcholine:

$$\text{phosphatidylcholine} + \text{cholesterol} \rightarrow \text{cholesteryl ester} + \text{2-lysophosphatidylcholine}$$

17.3.7 Hydrolysis of Cholesteryl Esters

There are two distinct intracellular cholesteryl hydrolases. One is a lysosomal enzyme that hydrolyzes cholesteryl esters internalized through receptor-mediated endocytosis via the LDL receptor (see below), releasing free cholesterol for use by the cell. The other is a cytosolic enzyme that acts on cholesteryl esters in lipid droplets present in steroidogenic cells and which releases free cholesterol for synthesis of steroid hormones.

17.4 TRANSPORT OF CHOLESTEROL

17.4.1 Lipoproteins Transport Cholesterol in the Plasma

Cholesterol has very low solubility in water; at 30°C, the limit of solubility is approximately 0.01 mg per 100 mL. The total fasting cholesterol concentration in plasma of healthy people is usually 150 to 200 mg per 100 mL, which is about twice the normal plasma glucose concentration. Such a high concentration of cholesterol in plasma is possible due to cholesterol-rich plasma lipoproteins that solubilize and disperse cholesterol (see Table 12-1). Only about 30% of the total plasma cholesterol is free (unesterified); the rest is esterified with a long-chain fatty acid, usually linoleic acid, which increases the hydrophobicity of cholesterol. Along with proteins and phospholipids, free cholesterol is a key component of the surface coat of the plasma lipoproteins, whereas cholesteryl esters and TAGs are located within the core of the lipoproteins (see Fig. 12-3).

17.4.1.1 Chylomicrons. Chylomicrons are the main vehicle for transporting dietary-derived lipids, including cholesterol and cholesteryl esters in the circulation (Fig. 17-8). Like other lipoproteins, cholesterol is part of the surface coat of chylomicrons. Dietary cholesterol is also esterified within the intestinal mucosal cells and incorporated into the core of the developing chylomicrons. As the chylomicrons

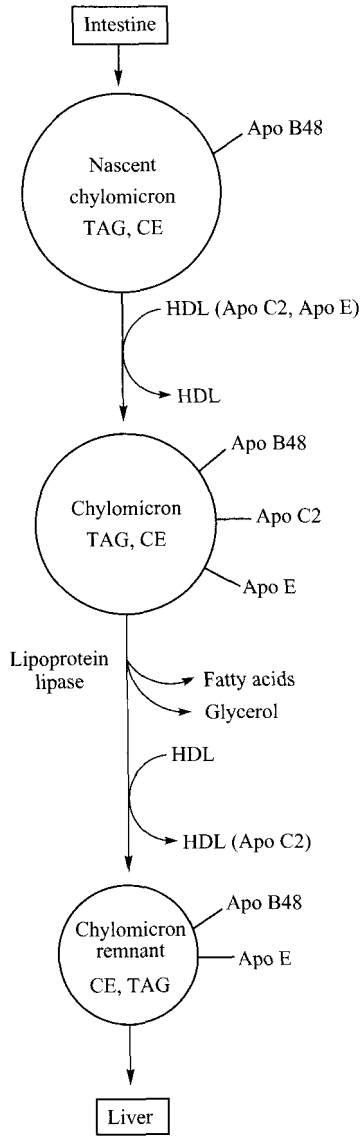


FIGURE 17-8 Simplified pathway for the transport of exogenous cholesterol in the blood. C, cholesterol; CE, cholesteryl ester, TAG, triacylglycerol.

circulate in the plasma, they lose TAG through hydrolysis by lipoprotein lipase (LpL), and shrink to become smaller particles called *chylomicron remnants*. Apoprotein E (apo E) on the surface of the chylomicron remnants facilitates binding of the remnants to the LDL receptor-related protein (LRP) on hepatocytes. The chylomicron remnants then enter the liver cells by receptor-mediated endocytosis. This process delivers

exogenous cholesterol and other lipophilic molecules (e.g., fat-soluble vitamins) to the liver.

17.4.1.2 VLDL. Very low-density lipoproteins (VLDL) are synthesized and secreted by the liver. Although the main function of VLDL is to export endogenous TAG that are made in the liver, VLDL is also involved in the transport of both free cholesterol and cholesteryl esters between tissues. Very low-density lipoproteins are the main vehicle for exporting both dietary-derived and endogenously synthesized cholesterol from hepatocytes into the plasma. Like chylomicrons, VLDL changes its composition and size as it circulates; it loses TAG through hydrolysis by LpL and by acquiring additional cholesteryl esters from HDL. As their triacylglycerol component undergoes hydrolysis, VLDL particles become remnants of various sizes (sometimes called IDL, or intermediate-density lipoproteins). The VLDL remnants contain apo E and enter hepatocytes by receptor-mediated endocytosis. Approximately two-thirds of the VLDL remnants are removed from the circulation by the liver; the remaining VLDL is converted in the circulation to LDL as a result of the actions of LpL and hepatic lipase (Fig. 17-9).

17.4.1.3 LDL. In humans, low-density lipoprotein (LDL) is the major lipoprotein that transports cholesterol in blood. Unlike chylomicrons and VLDL that are rich in TAG, the core of LDL contains primarily cholesteryl esters. The surface of each LDL particle contains one molecule of apo B100. Since its concentration in plasma is positively correlated with cardiovascular disease (stroke, myocardial infarction, blood clots), LDL-cholesterol is popularly termed the "bad" cholesterol.

LDL functions primarily to deliver cholesterol and cholesteryl esters to peripheral tissues such as the adrenal glands, testes, and ovaries. Since LDL acquires cholesteryl esters from HDL, it also contributes to *reverse cholesterol transport*, whereby cholesteryl esters are transported from peripheral tissues to the liver for excretion as cholesterol or as bile salts. The half-life of plasma LDL is about 3 days.

Both hepatocytes and peripheral cells express LDL receptors which recognize apo B100 and internalize LDL via receptor-mediated endocytosis (Fig. 17-9). The receptors are then recycled back to the cell surface, and the LDL is transported to lysosomes, where hydrolysis of cholesteryl esters generates free cholesterol.

17.4.1.4 HDL. The main role of high-density lipoproteins (HDL) is reverse cholesterol transport whereby HDL extracts cholesterol from peripheral tissues and transports that cholesterol to the liver for excretion (Fig. 17-10). Circulating HDL can also donate cholesteryl esters to other lipoproteins such as VLDL and IDL. HDL also plays a central role in lipoprotein metabolism by donating proteins such as apo C2 and apo E to chylomicrons and VLDL. Since the concentration of HDL is inversely correlated with cardiovascular disease, HDL-cholesterol is described as the "good" cholesterol. The plasma concentration of HDL can be increased by physical activity and by therapeutic agents such as statins, niacin, and cholestyramine.

The primary apoprotein in HDL is apoprotein A (apo A). The major form of apo A is apo A1, which is synthesized by both the liver and the intestine. Apo A1

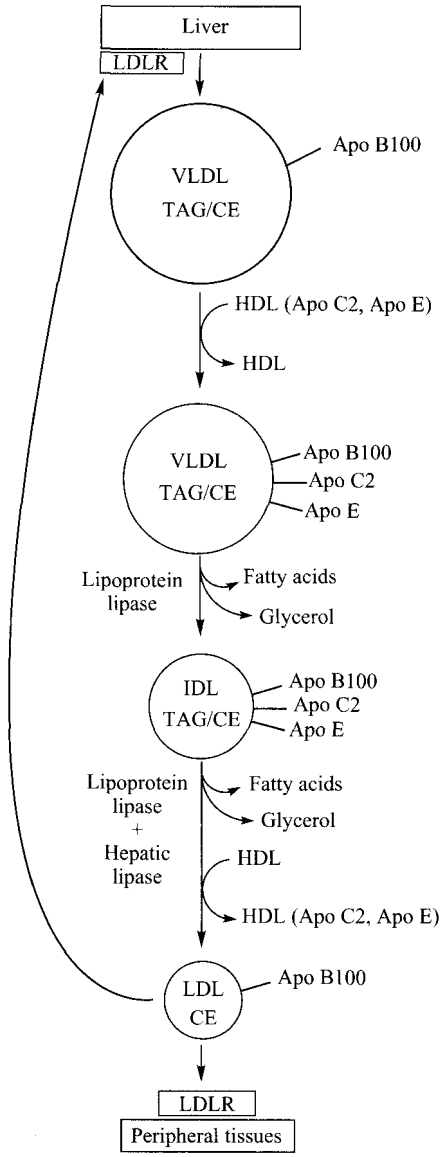


FIGURE 17-9 Simplified pathway for the transport of endogenous cholesterol. C, cholesterol; CE, cholesteryl ester; IDL, intermediate-density lipoprotein.

has a relatively long half-life (approximately 5 days), is recycled many times over, and acquires most of its lipid components in the circulation. There are three major structural forms of apo A1 circulating in the plasma: (1) amorphous or lipid-free HDL (apoA1), which does contain some phospholipid; (2) nascent or discoidal, lipid-poor HDL; and (3) mature, spherical HDL (HDL2, HDL3), which is rich in cholesteryl

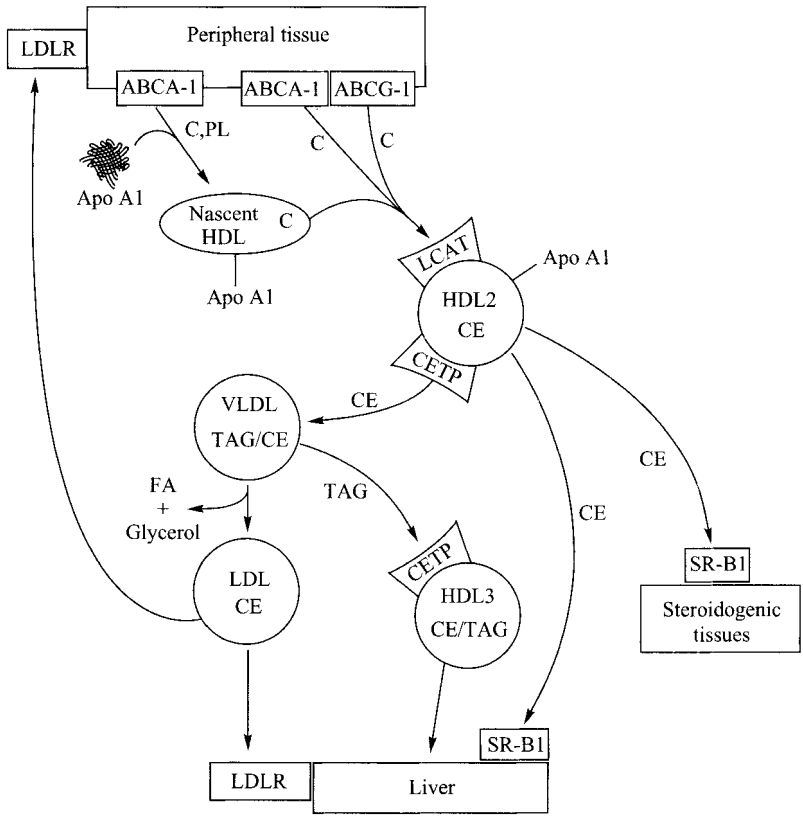


FIGURE 17-10 Simplified pathway for the formation of HDL and its role in the reverse transport of cholesterol. ABCA-1 and ABCG-1 are members of the ABC superfamily of ATP-binding casette transporters. Although not shown in the figure, HDL3 can also be converted back to HDL2 by the action of hepatic lipase. CETP, cholesteryl ester transfer protein; C, cholesterol; CE, cholesteryl ester; FA, fatty acid; G, glycerol; LDLR, LDL receptor; SR-B1, scavenger receptor B1.

esters. Lipid-free apo A1 is secreted by the liver and acquires free cholesterol and phospholipids in the plasma, thereby becoming discoidal or nascent HDL. As nascent HDL acquires additional free cholesterol, the cholesterol is esterified by the action of lecithin : cholesterol acyltransferase (LCAT) to generate cholesteryl esters, which account for the bulk of the core of the mature HDL, which is designated HDL2.

There are two mechanisms by which circulating HDL gives up some of its cholesteryl esters. One is the exchange of cholesteryl esters in HDL for TAG in VLDL, or, to a lesser extent, in LDL. The exchange process is mediated by cholesteryl ester transfer protein (CETP). Cholesteryl esters may also be removed by selective uptake by the liver, which is mediated by a scavenger receptor class BI (SR-BI) and occurs without the intracellular uptake of HDL proteins. The resulting HDL3 particles are

smaller and have a higher ratio of TAG to cholesteryl esters than HDL2 has, and can be cleared by the liver or the kidney. In the process of HDL3 formation from HDL2 some of the excess apo A1 and phospholipid is released from the surface of the particles, thus regenerating lipid-poor HDL. The lipid-poor HDL can then acquire and esterify additional cholesterol, as described above. Although not shown in Figure 17-10, HDL3 can also be converted back to HDL2 by the action of hepatic lipase, which hydrolyzes TAG, releasing free fatty acids and glycerol.

17.4.2 Lipoproteins Are Remodeled While They Circulate

Plasma lipoproteins are dynamic aggregates whose compositions change as they move through the blood. One example of lipoprotein remodeling is the maturation of HDL in the circulation. Another is the gradual hydrolysis—by lipoprotein lipase—of TAG in circulating chylomicrons and VLDL (see Chapter 12). Many other proteins contribute to the remodeling of plasma lipoproteins, as described below.

17.4.2.1 ATP-Binding Cassette Transporters. The plasma membrane of peripheral cells contains two large proteins, ABCA-1 and ABCG-1, which mediate the release of cholesterol from those cells (Fig. 17-10). ABCA-1 and ABCG-1 are members of the ABC superfamily of ATP-binding cassette transporters that are responsible for the translocation across membranes of certain drugs, peptides, and lipids, including cholesterol. ABCA-1 converts lipid-free apo A1 into nascent HDL. When apo A1 docks with ABCA-1 on the plasma membrane, several molecules of phospholipid and free cholesterol are transferred to apoA1 in an energy (ATP)-dependent process (Fig. 17-10). Both ABCA-1 and ABCG-1 can transfer additional free cholesterol to the nascent HDL.

17.4.2.2 Lecithin : Cholesterol Acyltransferase (LCAT). Lecithin : cholesterol acyltransferase is a soluble enzyme that is secreted by the liver. In the circulation, LCAT binds to the surface of HDL particles, where it is activated by apo A1. As HDL acquires free cholesterol from the plasma membrane of a cell in the periphery, LCAT transfers the fatty acid from the 2-position of phosphatidylcholine (lecithin) to free cholesterol to form cholesteryl ester:

$$\text{phosphatidylcholine} + \text{cholesterol} \rightarrow \text{cholesteryl ester} + \text{lysophosphatidylcholine}$$

LCAT prefers to use linoleic acid (18:2*n*-6) in this *trans*-esterification reaction. By contrast, acyl-CoA:cholesterol acyltransferase, the enzyme that generates intracellular cholesteryl esters, preferentially uses oleoyl-CoA.

17.4.2.3 Cholesterol Ester Transfer Protein (CETP). The transfer of cholesteryl esters between HDL and other lipoproteins is facilitated by a plasma protein called CETP, which is synthesized in and secreted by hepatocytes and adipocytes. CETP occurs in the circulation bound to the various subspecies of HDL. The function

of CETP is to catalyze the transfer of cholesteryl esters from HDL to VLDL and LDL in exchange for the transfer of TAG to HDL (Fig. 17-10).

17.4.2.4 Phospholipid Transfer Protein (PLTP). PLTP catalyzes the transfer of lipids, particularly phosphatidylcholine, between lipoproteins. As triacylglycerol hydrolysis occurs and both chylomicrons and VLDL particles shrink in size, PLTP serves to remove excess phospholipid from the surface of these lipoproteins and transfer it to HDL, providing substrate for the LCAT reaction.

17.4.3 Plasma Lipoproteins Exchange Proteins in the Circulation

When VLDL is secreted from the liver, the major lipid component is triacylglycerol and the major protein constituents are apo B100 and apo A1. Apo A1 then dissociates from the VLDL, and as it acquires cholesterol and phospholipids, it becomes HDL. Circulating VLDL acquires apo C2 and apo E from HDL. As the VLDL-associated triacylglycerol is hydrolyzed and VLDL becomes IDL and eventually LDL, VLDL particles also donate apo C2 and apo E back to HDL, so that the mature LDL particle contains only apo B100. HDL thus serves as a reservoir for apo C2 and apo E in the circulation (Figs. 17-8 and 17-9).

17.4.4 Lipoprotein Receptors

Receptor-mediated endocytosis of lipoproteins provides a mechanism both for their clearance from the circulation and for the delivery of key lipid components to target cells. Targeting of lipoproteins to sites of metabolism and removal is mediated primarily by the apoproteins on their surfaces.

17.4.4.1 LDL Receptor. The LDL receptor (LDLR) is a transmembrane glycoprotein with an apo B-100–binding domain. LDL receptors are expressed on liver cells and extrahepatic tissues and they recognize apo B100 but not the smaller apo B48 molecule present on chylomicrons and chylomicron remnants. Once the LDL receptor is occupied by LDL, the LDL : LDLR complex clusters in coated pits, which are then internalized by receptor-mediated endocytosis. Intracellularly, as the clathrin-coated vesicles lose their clathrin the LDL receptors are recycled back to the plasma membrane. The LDL-containing endosomes then fuse with lysosomes to form endolysosomes whose internal milieu is relatively acidic (approximately pH 5). Within the endolysosomes, the cholesteryl esters are hydrolyzed by "acid lipase" to free cholesterol and fatty acids, while the apo B-100 is hydrolyzed to amino acids.

17.4.4.2 LDL Receptor-Related Protein (LRP). LRP is expressed on the surface of hepatocytes but not peripheral cells. Its function is to bind and clear chylomicron remnants.

17.4.4.3 Scavenger Receptors A (SR-A's). Scavenger receptors A are a family of molecules that are expressed on tissue macrophages, Kupffer cells, and various

extrahepatic endothelial cells. SR-A's take up oxidized LDL particles, which having been oxidized by free radicals are no longer recognized by the LDL receptor. Unlike LDL receptors, the scavenger receptors are not down-regulated by intracellular cholesterol. The persistence of significant amounts of oxidized LDL in the circulation can lead to excessive accumulation of oxidized LDL in the macrophages, transforming the latter into "foam cells," which eventually form atherosclerotic plaques.

17.4.4.4 Scavenger Receptors B1 (SR-B1's). A different scavenger receptor, SR-B1, mediates uptake of HDL cholesteryl esters by the liver. In contrast to the lipoprotein receptors described above, SR-B1 is not internalized by receptor-mediated endocytosis. Instead, SR-B1 permits hepatocytes selectively to remove and internalize HDL-associated cholesteryl esters. The adrenal glands, ovaries, and other steroidogenic tissues use the same SR-B1 receptor-dependent mechanism to extract cholesteryl esters from circulating HDL particles to provide cholesterol substrate for steroid hormone synthesis (Fig. 17-10).

17.4.5 Transport of Cholesterol in the Brain

As noted earlier, cholesterol does not cross the blood–brain barrier and must therefore be synthesized within the central nervous system (CNS), primarily by Schwann cells and oligodendrocytes. The CNS also has a separate lipoprotein transport system by which cholesterol is exchanged among various cells via HDL-like lipoproteins. The most abundant apolipoprotein in the CNS is apo E, which is synthesized by glial cells. The primarly mechanism for export of excess cholesterol from the brain is as the oxysterol, 24*S*-hydroxycholesterol (Chapter 18), rather than as cholesterol itself.

17.5 REGULATION OF CHOLESTEROL METABOLISM

17.5.1 Regulation of HMG-CoA Reductase

HMG-CoA reductase is the rate-limiting step of cholesterol synthesis, and its activity is under strict metabolic control. The simultaneous regulation of HMG-CoA reductase synthesis and turnover can alter steady-state levels of the enzyme 200-fold. The central role of HMG-CoA reductase in cholesterol homeostasis is evidenced by the effectiveness of a family of drugs called *statins* that are used to lower plasma cholesterol levels. Statins (e.g., lovastatin, pravastatin, fluvastatin, cerivastatin, atorvastatin) inhibit HMG-CoA reductase activity, particularly in liver, and decrease a person's total plasma cholesterol concentration by as much as 50%.

17.5.1.1 Transcriptional Regulation. A regulatory protein called *sterol regulatory element binding protein* (SREBP) plays a central role in regulating the expression of HMG-CoA reductase levels. Scap, the SREBP-escort protein, transports SREBP into the nucleus when SREBP binds to the sterol regulatory element (SRE) and up-regulates HMG-CoA expression. Several other proteins are involved in this

regulatory process, including COPII and Insig. Cholesterol and oxysterols (e.g., 25-hydroxycholesterol) bind to COPII and Insig, respectively, and block the proteolytic activation and transport of SREBP, thereby preventing the up-regulation of HMG-CoA reductase.

The synthesis of mRNAs that encode at least three other enzymes in the cholesterol biosynthetic pathway—HMG-CoA synthase, farnesyl pyrophosphate synthase, and squalene synthase—is regulated in parallel with HMG-CoA reductase. Transcription of the LDL receptor is also regulated by the intracellular cholesterol concentration. Each of these three genes has a similar SRE in its promoter sequence that is recognized by SREBP.

There are multiple isoforms of SREBP. One of these, SREBP-2, selectively activates transcription of cholesterol biosynthetic genes and the LDL receptor gene. By contrast, SREBP-1 also activates transcription of acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase 2, and glycerol 3-phosphate acyltransferase. SREBP-1 thus controls not only cholesterol synthesis but also the synthesis of fatty acids, TAG, and phospholipids.

17.5.1.2 Proteolysis of HMG-CoA Reductase. In sterol-depleted cells, HMG-CoA reductase is slowly degraded with a half-life greater than 12 hours. Insig, one of the proteins involved in the regulation of transcription of HMG-CoA, modulates proteolytic degradation of the enzyme. Binding of sterols, particularly lanosterol, and oxysterols to Insig accelerates the degradation of existing HMG-CoA reductase.

17.5.1.3 Enzyme Phosphorylation. HMG-CoA reductase is inhibited when it is phosphorylated by AMP-activated kinase. This kinase, which also acts to phosphorylate acetyl-CoA carboxylase, plays an important role in inhibiting multiple biosynthetic pathways when cellular energy stores are low.

17.5.2 Regulatory Role of the LDL Receptor

LDL is the major transporter of plasma cholesterol and the LDLR is the major mechanism by which cholesterol is removed from the blood. As noted above, as the intracellular concentration of free cholesterol increases, LDLR transcription is suppressed. As a result, receptor-mediated endocytosis of LDL is reduced and the plasma LDL concentration increases. There are two pharmacological mechanisms for increasing the expression of LDLRs on hepatocytes and thus decreasing circulating LDL cholesterol: statins and bile salt sequestrants.

17.5.2.1 Statins Act to Up-regulate the LDL Receptor. As described above, statin drugs decrease cholesterol synthesis by inhibiting HMG-CoA activity. This, in turn, results in up-regulation of the LDLR, particularly in hepatocytes.

17.5.2.2 Bile Acid Sequestrants Up-regulate the LDL Receptor. On a mass basis, the major metabolic products of cholesterol metabolism are the bile salts. As described in Chapter 2, bile salts are normally reabsorbed in the distal ileum.

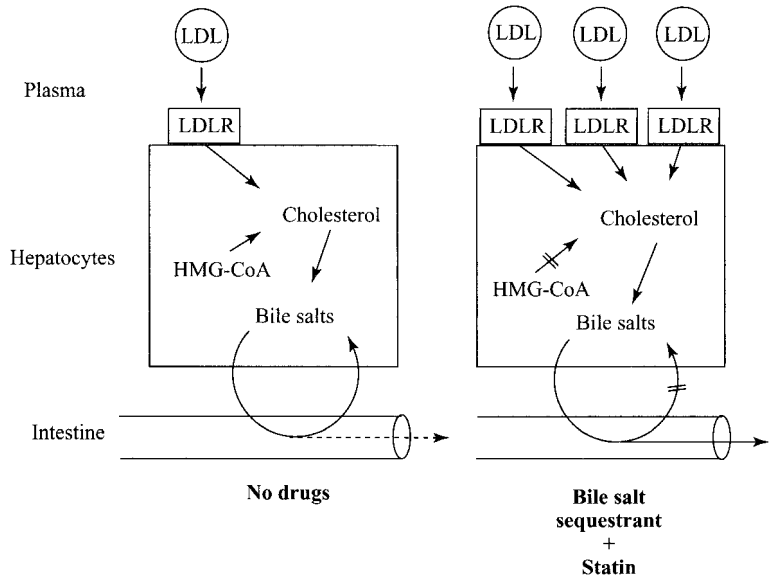


FIGURE 17-11 Synergistic effects of bile salt sequestrants and statins to up-regulate hepatic LDL-receptor expression and decrease plasma LDL-cholesterol.

Mucilaginous or soluble dietary fiber, such as that found in fruits and oat bran, binds bile salts and decrease their absorption. This, in turn, increases synthesis of bile salts in the liver, consuming cholesterol in the process and decreasing the concentration of cholesterol in hepatocytes. Bile-acid sequestrants such as cholestyramine and colestipol are anionic, insoluble polymers that bind bile acids strongly inside the resin matrix; the bile acid–sequestrant complex is excreted in the feces. Depletion of the body's bile acid pool results in up-regulation of hepatic 7α-hydroxylase, which increases the conversion of cholesterol to bile acids. The depletion of hepatic cholesterol increases the expression of LDL-receptor protein and lowers the plasma LDL-cholesterol concentration. However, since the decreased intrahepatic concentration of cholesterol also stimulates synthesis of HMG-CoA reductase, statins may be prescribed in conjunction with bile-acid sequestrants. Such a combination therapy usually results in a greater up-regulation of LDLR expression and a greater decrease in the plasma LDL-cholesterol level than is obtained from use of either a statin or a bile salt sequestrant alone (Fig. 17-11).

17.5.3 Regulation of Cholesterol Absorption

As noted earlier, much of the cholesterol that enters the small intestine is absorbed. By contrast, plant sterols (e.g., sitosterol) and related stanols (which are derivatives of sitosterol) are not well absorbed, and are selectively secreted from the enterocytes back into the intestinal lumen. Importantly, plant sterols and stanols interfere with the absorption of dietary cholesterol. For this reason, increased intake of vegetables

and therapeutic doses of plant sterols and stanols have been used to lower the plasma cholesterol level. Cholesterol absorption can also be reduced by Ezetimibe (Zetia), which inhibits the transporter (NPC1L1) that moves cholesterol from the intestinal lumen into enterocytes.

17.6 ABNORMALITIES IN CHOLESTEROL METABOLISM

17.6.1 Hypercholesterolemia

Hypercholesterolemia refers to plasma levels of cholesterol that exceed the normal range. The risk of coronary heart disease (CHD) is correlated with both the total cholesterol and LDL-cholesterol (LDL-C) levels, while a high fasting HDL-C level is a negative risk factor for CHD. Since the risk of developing CHD is related mainly to the LDL-component, treatment is aimed at decreasing the level of LDL-C. The current desirable fasting plasma LDL-C level is <100 mg/dL, 100 to 129 is considered nearly optimal, 130 to 159 is borderline high, 160 to 189 is high, and ≥ 190 is very high.

Treatment of persons with moderate hypercholesterolemia usually begins with dietary and other behavioral changes (e.g., exercise). Diets low in total fat, saturated fat, and cholesterol and relatively high in oleic acid (as in olive oil) tend to lower both the total cholesterol level and the LDL-C level. If additional reductions in cholesterol are called for, various medications are available. We have already discussed the use of sequestrants (e.g., cholestyramine, colestipol), which promote fecal excretion of bile acids; statins, which reduce endogenous cholesterol synthesis; and Ezetimibe, which blocks cholesterol absorption from the intestine. Other medications, such as niacin and gemfibrozil, can also be used to decrease plasma TAG levels and increase HDL-C levels.

17.6.2 Atherogenic Dyslipidemia

The dyslipidemic or atherogenic profile is a combination of three abnormalities in plasma lipoprotein levels: high VLDL triacylglycerol ($\geq$150 mg/dL), low HDL-C ($<$40 mg/dL for men and $<$50 mg/dL for women), and the presence of relatively small, dense LDL particles. Independent of the concentration of either total plasma cholesterol or LDL-C, the dyslipidemic profile is a major risk factor for coronary artery disease. The dyslipidemic profile is commonly associated with insulin resistance, type II diabetes, central obesity, and hypertension, which are included in a constellation of findings that have been termed the *metabolic syndrome*. Although the etiology of the metabolic syndrome is not fully understood, the condition is often responsive to a combination of weight loss and exercise, as well as to a myriad of pharmacological agents.

17.6.3 Familial Hypercholesterolemia

A deficiency of LDLR is the most common monogenetic cause of FH. In the United States, the incidence of heterozygous FH is approximately 1 in 500 persons and

that of homozygous FH is 1 in 1 million persons. FH heterozygotes usually exhibit an elevated fasting plasma LDL-C concentration (250 to 500 mg/dL; optimum, <100 mg/dL) and a normal triglyceride level. They have an increased risk of CHD, with onset in the fourth or fifth decade. Patients with heterozygous FH are generally responsive to treatment with HMG-CoA reductase inhibitors, which up-regulate the expression of the functional LDLR gene. In some patients, the addition of a bile acid sequestrant or nicotinic acid is required to achieve the desired LDL-C level.

Persons who are homozygous for FH usually have plasma LDL-C levels in the range 500 to 1200 mg/dL, even in early childhood. These patients invariably have cutaneous deposits of cholesterol called *xanthomas* on the hands, wrists, elbows, and/or knees. Coronary heart disease usually manifests within the first two decades of life. The disease is more severe in LDLR-negative patients (<2% normal LDLR activity) than in those whose cultured skin fibroblasts exhibit 2 to 20% residual LDLR activity in vitro. Due to the lack of functional receptors, patients with homozygous FH are largely unresponsive to drug therapies that up-regulate LDLR expression. Currently, the preferred treatment is LDL apheresis, which selectively removes the apoB-containing lipoproteins (VLDL, IDL, LDL) from the plasma.

17.6.4 Familial Dysbetalipoproteinemia

Familial dysbetalipoproteinemia or apo E defect is characterized by increased plasma concentrations of both cholesterol and triglycerides as a consequence of impaired clearance of chylomicron remnants and VLDL remnants by the liver. Normally, two major lipoprotein bands are visualized after electrophoresis of a fasting plasma sample from a normal person: an alpha band composed of HDL and a beta band composed of LDL; VLDL forms a small pre-beta band which runs ahead of the LDL band. Plasma from persons with familial dysbetalipoproteinemia, however, exhibits one broad band that stretches across both the beta and pre-beta positions and contains VLDL remnants of various sizes. Patients with this disease usually present in adulthood with premature coronary and/or peripheral vascular disease, xanthomas in the creases of the palms, and on the elbows and knees, as well as other locations.

There are three major allelic isoforms of apo E in the human population, designated E2, E3, and E4. Most patients with familial dysbetalipoproteinemia are homozygous for the E2 isoform (designated apo E2/E2). The E2 isoform binds less readily than other isoforms to hepatic lipoprotein receptors and delays clearance of both chylomicron remnants and VLDL from the plasma. Interestingly, although 80 to 90% of persons with familial dysbetalipoproteinemia are homozygous apo E2/E2, only 1 to 4% of apo E2/E2 homozygotes manifest this disorder, indicating that additional genetic and/or environmental factors are involved. Hypothyroidism, diabetes mellitus, obesity, and estrogen deficiency are among the factors that precipitate manifestation of familial dysbetalipoproteinemia in persons with the apo E2/E2 phenotype. Patients with this disorder usually respond well to low-cholesterol and low-fat diets, weight reduction, and drug therapy.

17.6.5 Alzheimer's Disease

There is evidence that abnormal cholesterol metabolism may be a factor in Alzheimer's disease (AD). Apo E, which is synthesized by astrocytes and microglia in the brain, is thought to play a central role in regulating cholesterol homeostasis in the central nervous system. Apo E is also associated with the β-amyloid deposits that are a neuropathologic hallmark of AD. Although the mechanisms by which apo E influences the onset and progression of AD are obscure, people who carry one E4 allele have about a twofold higher lifetime risk of developing AD than do those with other apo E genotypes, and the onset of disease occurs earlier.

17.6.6 Tangier Disease

Tangier disease is a rare disease that results from a genetic defect in reverse cholesterol transport in which there is a virtual absence of plasma HDL-C and very low levels of apo A1. Patients may present with enlarged yellow-orange tonsils loaded with cholesteryl esters and other lipophilic compounds, including retinyl esters and carotenoids. Cholesteryl esters also accumulate in reticuloendothelial cells of the thymus, spleen, liver, bone marrow, and intestinal mucosa. The disease is caused by a loss-of-function mutation in the ABC1 transporter, so that it cannot facilitate the transfer of free cholesterol from cells to apo A1 to form discoidal, nascent HDL; instead, the lipid-free apo A1 is cleared rapidly from the circulation by the kidneys. In the absence of ABC1 activity, cholesteryl esters accumulate in peripheral cells, particularly macrophages and cells of the reticuloendothelial system. The fasting plasma concentration of VLDL triacylglycerol is elevated, apparently from the loss of HDL as a reservoir for apo C2, thereby resulting in a deficiency of apo C2 that is required to activate lipoprotein lipase. Despite their lack of HDL, patients with Tangier disease do not have a markedly increased risk for premature CAD, apparently because of their low (40% of normal) LDL-C levels. The low LDL-C levels in the plasma of these patients may reflect both impaired generation of LDL from VLDL and up-regulation of hepatic LDL receptors in response to the lack of HDL-mediated return of cholesterol to the liver.

17.6.7 Familial HDL Deficiency

Familiar HDL deficiency or primary hypoalphalipoproteinemia is caused either by a mutation in the apo A1 gene or by heterozygosity for ABC1 transporter deficiency (i.e., the carrier state for Tangier disease) and is characterized by low levels of circulating HDL-C. This disorder occurs in 1% of the general population and is a common genetic cause of increased triglyceride levels in plasma and CHD. By contrast, although both complete and partial deficiencies of LCAT also result in markedly reduced HDL-C and circulating apo A1 levels, these conditions rarely lead to premature atherosclerotic disease.

17.6.8 Niemann–Pick C Disease

Niemann–Pick type C is an autosomal recessive disease that causes progressive neurological degeneration and the lysosomal accumulation of cholesterol and other lipids (e.g., gangliosides and bis-monoacylglycerol) in the central nervous system and reticulendothelial cells. The two forms of the disease are the result of mutations in two genes, *NPC1* and *NPC2*, which are required for the egress of cholesterol from late endosomes and lysosomes. In the absence of functional *NPC1* and *NPC2*, cholesterol delivered to the lysosomes via receptor-mediated endocytosis of LDL cannot be transported to the endoplasmic reticulum, thereby causing cholesterol to accumulate in lysosomes and eventually depleting the plasma membrane of cholesterol. As cells such as hepatocytes respond to the low concentration of membrane cholesterol by up-regulating expression of LDL receptors, increased internalization of LDL only exacerbates the problem. The severe neurological problems of patients with Niemann–Pick C disease may also be due, in part, to other, as yet not fully characterized functions of *NPC1* in neurons.

17.6.9 Smith–Lemli–Opitz Syndrome

Smith–Lemli–Opitz syndrome (SLOS) is the most common genetic defect of cholesterol biosynthesis, occurring in 1 in 30,000 births. Patients with SLOS are born with a variable spectrum of morphogenic anomalies, including microcephaly, dysmorphic craniofacial features, polydactyly, and congenital heart defects. SLOS was the first multiple malformation syndrome to be attributed to a deficiency of a single enzyme, in this case 3β-hydroxysterol Δ^7-reductase, which reduces the double bond of 7-dehydrocholesterol (7-DHC) in the last step of cholesterol synthesis. As a result, these patients have markedly increased amounts of 7-DHC and decreased levels of cholesterol in plasma and cultured skin fibroblasts. The multiple congenital malformations in SLOS are attributed to a lack of cholesterol for posttranslational modification of the Hedgehog proteins, which have been implicated as signaling molecules in embryonic patterning processes. Alternatively, the accumulation of 7-dehydocholesterol may alter formation of lipid rafts within the plasma membrane, and thus impair interaction of Hedgehog proteins with their membrane-bound receptors.

CHAPTER 18

STEROIDS AND BILE ACIDS

18.1 FUNCTIONS OF OXYGENATED DERIVATIVES OF CHOLESTEROL

Oxygenated derivatives of cholesterol play many roles in the body. Cholesterol is the precursor of two important classes of molecules, bile acids and steroid hormones. In addition, 7-dehydrocholesterol, the immediate precursor of cholesterol, can be converted to cholecalciferol (vitamin D_3), which ultimately produces 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$, calcitriol], the active hormone that regulates calcium metabolism.

18.1.1 Bile Acids

Cholesterol is a 27-carbon lipid containing a fused four-ring structure and a hydrocarbon chain (Fig. 18-1). Except for the one hydroxyl group at C3, cholesterol is completely nonpolar. By contrast, bile acids contain 24-carbon atoms and are more polar than cholesterol: The steroid ring of bile acids contains one or more additional hydroxyl groups and the shorter hydrocarbon side chain terminates in a carboxyl group (Fig. 18-2). In addition, the stereochemistry of the steroid nucleus is modified, resulting in a planar structure in which all of the hydroxyl groups are situated on the same side of the plane of the molecule (see Fig. 3-6).

The so-called "bile salts" are actually bile acids that contain an amino acid which is conjugated in amide linkage to the side chain of the carboxyl group of the bile acid.

Medical Biochemistry: Human Metabolism in Health and Disease By Miriam D. Rosenthal and Robert H. Glew

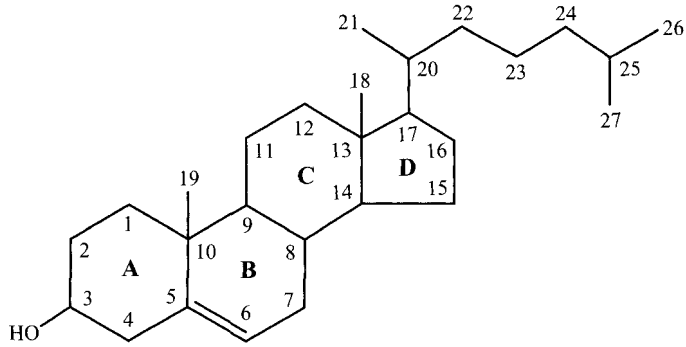


FIGURE 18-1 Numbering the carbon atoms and rings of cholesterol.

The two amino acids used most commonly by the liver to conjugate human bile acids are glycine, which is predominant in adults, and the sulfur amino acid taurine, which is predominant in infants (see Fig. 3-5). Conjugated bile acids are more ionized at the slightly acidic pH of the intestinal lumen than their nonconjugated counterparts and are therefore better emulsifying agents.

Bile salts play a major role in the digestion and absorption of triacylglycerols and cholesteryl esters. Bile salts emulsify dietary lipids in the gastrointestinal tract and stabilize the resulting mixed micelles. Along with phosphatidylcholine, bile salts solubilize the cholesterol and bile pigments present in bile, preventing formation of precipitates (stones) of cholesterol or bilirubin in the gallbladder and bile ducts. In addition, formation of bile salts represents the only significant metabolic mechanism for eliminating excess cholesterol from the body.

18.1.2 Steroid Hormones

Cholesterol serves as the precursor for the synthesis of all five classes of steroid hormones (Fig. 18-3). Three of these classes are 21-carbon structures: glucocorticoids, mineralocorticoids, and progestogens. The *glucocorticoids* (e.g., hydrocortisone,

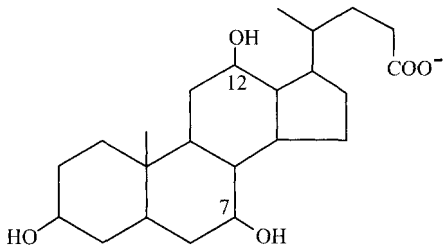


FIGURE 18-2 Cholic acid is the major bile acid produced in adults. Chenodeoxycholic acid (not shown) lacks the hydroxyl group at C12.

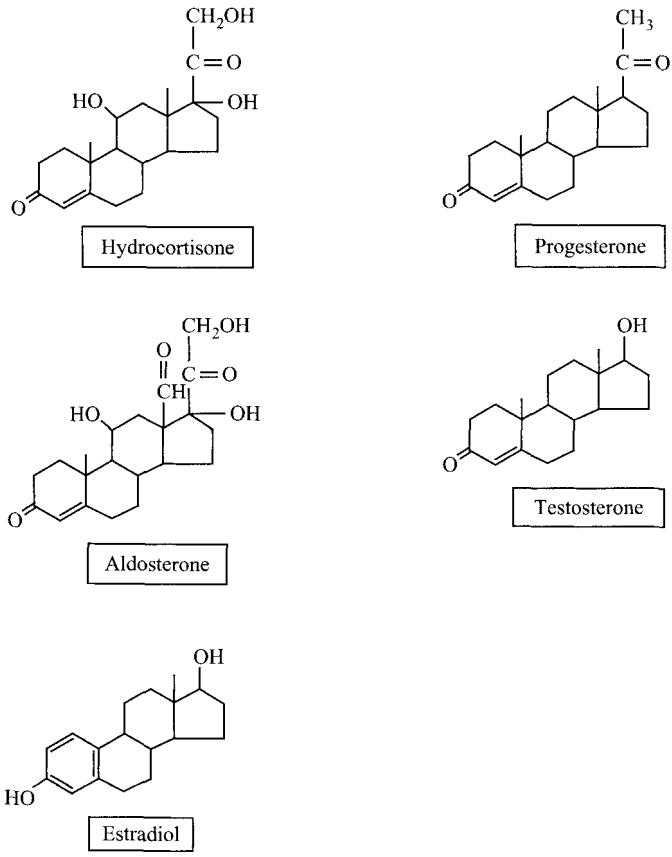


FIGURE 18-3 Representative members of each of the five classes of steroid hormones.

a.k.a. cortisol) mediate a person's response to physiological stress and are involved in coordinating carbohydrate, fat, and protein metabolism. *Mineralocorticoids* (e.g., aldosterone) regulate the body's electrolyte and water balance via their action on the renal tubules. Progesterone, a *progestogen*, regulates menses and breast development and is required for implantation of the fertilized ovum and maintenance of pregnancy. Testosterone and other androgens contain 19 carbons and are responsible for masculine characteristics, whereas estrogens, such as estradiol, have 18 carbons and are the female steroid sex hormones. As can be seen from the structures of the representative steroid hormones shown in Figure 18-3, all of these steroids have been modified from the original 27-carbon cholesterol molecule by shortening or elimination of the hydrocarbon side chain and by the addition of polar hydroxyl or keto substituents on the steroid ring structure. The basic steroid structure has been further modified in estrogens by the elimination of a methyl group and desaturation to generate an aromatic A ring.

Because of their lipid nature, steroid hormones pass readily through the plasma membrane of all target cells. Once inside its target cell, the steroid hormone binds to its specific receptor protein. The steroid receptors act in the nucleus, where the hormone-receptor complex binds to specific DNA sequences, resulting in increased transcription of those particular genes and ultimately an increase in the level of the protein encoded by those genes.

18.1.3 1,25-Dihydroxycholecalciferol (Calcitriol)

1,25-Dihydroxycholecalciferol [1,25-$(OH)_2D_3$] is a 27-carbon molecule that shares many similarities with the steroid hormones described above. It is actually derived from 7-dehydrocholesterol, the immediate precursor of cholesterol, rather than from cholesterol and differs from the classic steroid hormones in that the B ring of cholesterol has been opened (Fig. 18-4). 1,25-$(OH)_2D_3$ plays an important role in Ca^{2+} homeostasis: It increases the plasma calcium concentration both by promoting intestinal absorption of dietary calcium and by stimulating Ca^{2+} release from bone when dietary calcium is insufficient. However, the major function of 1,25-$(OH)_2D_3$ in bone is to promote mineralization. 1,25-Dihydroxycholecalciferol also has important effects on differentiation in many cell types other than bone and may be protective against colorectal cancers and other cancers.

18.2 LOCALIZATION OF THE SYNTHESIS OF OXYGENATED DERIVATIVES OF CHOLESTEROL

18.2.1 Bile Acids

The synthesis of the primary bile acids, cholic acid and chenodeoxycholic acid, and their conjugation with taurine or glycine to form bile salts occurs exclusively in the liver. As described in Chapter 3, the bile salts are secreted from the liver into the biliary tract and enter the duodenum, where they facilitate digestion and absorption of lipids. The conjugated bile acids are reabsorbed in the distal ilium and transported through the blood back to the liver. Some of the secondary bile acids, formed when bacterial enzymes in the intestine reduce (dehydroxylate) primary bile acids, are also reabsorbed and recycled.

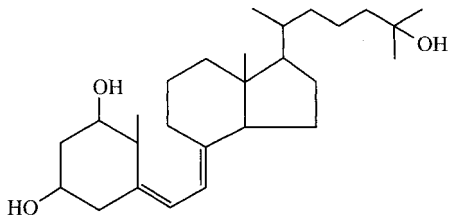


FIGURE 18-4 Structure of 1,25-dihydrocycholecalciferol [1,25-$(OH)_2D_3$].

18.2.2 Steroid Hormones

Estrogen is synthesized in the ovarian follicle, whereas the corpus luteum which develops following ovulation produces progesterone and some estrogen. The major site of androgen synthesis in men is the testis. In women, ovarian cells synthesize androgens as well as estrogens; indeed, androstenedione synthesis in the theca cells of the ovary is essential to the production of estradiol by the follicle. The adrenal cortex synthesizes glucocorticoids, mineralocorticoids, and the androgens androstenedione and dehydroepiandrosterone (DHEA).

18.2.3 1,25-Dihydroxycholecalciferol

The precursor for [1,25-$(OH)_2$-D_3] synthesis is cholecalciferol, which is either formed nonenzymatically in the skin from 7-dehydrocholesterol (vitamin D_3) or obtained in the diet. Conversion of vitamin D_3 to the active hormone 1,25-$(OH)_2$-D_3, requires two successive hydroxylation reactions in the liver and kidney, respectively.

18.3 PATHWAYS OF CHOLESTEROL METABOLISM

18.3.1 Monooxygenases

Many of the enzymes that catalyze oxygenation of cholesterol and cholesterol derivatives are monooxygenases or mixed-function oxidases. Mixed-function oxidases catalyze reactions in which one atom of molecular oxygen oxidizes an organic substrate, while the other atom of molecular oxygen oxidizes NADPH or NADH:

$$R{-}H + NADPH + H^+ + O_2 \rightarrow R{-}OH + NADP^+ + H_2O$$

The monooxygenases that modify cholesterol are members of the cytochrome P450 (CYP) superfamily of enzymes and usually utilize NADPH as a cofactor or second substrate. These monooxygenases are all membrane-bound and localized to either the endoplasmic reticulum or the inner mitochondrial membrane. The cytochrome P450 enzymes usually have two names, one identifying them as a member of the cytochrome P450 family (e.g., CYP7A1) and the other identifying the cholesterol carbon atom that is modified in the reaction (e.g., 7α-hydroxylase).

18.3.2 Synthesis of Bile Acids

The classical or neutral pathway of bile acid synthesis involves modification of the cholesterol ring structure before the hydrocarbon side chain is cleaved (Fig. 18-5). However, there is also an alternative or acidic pathway of bile acid synthesis in which the side-chain modification reactions occur first, followed by modification of the sterol nucleus. In adult humans, 75 to 95% of bile salts are synthesized via the classical pathway. The acidic pathway is more prevalent in the fetus and neonate.

The intracellular trafficking of intermediates in bile acid synthesis is complex, with reactions in the pathway occurring in mitochondria, peroxisomes, the endoplasmic reticulum, and cytosol.

18.3.2.1 Classical Pathway of Bile Acid Synthesis. The initial—and regulated—step of bile acid synthesis is hydroxylation of C7 on the B ring of cholesterol by 7α-hydroxylase (CYP7A1) (Fig. 18-5):

$$\text{cholesterol} + \text{NADPH} + \text{H}^+ + \text{O}_2 \rightarrow 7\alpha\text{-hydroxycholesterol} + \text{NADP}^+ + \text{H}_2\text{O}$$

This initial 7α-hydroxylation step is followed by a sequence of 14 reactions that:

- Epimerize the 3β group to form a 3α-hydroxyl group
- Hydrogenate the C5–C6 double bond to form a saturated bond

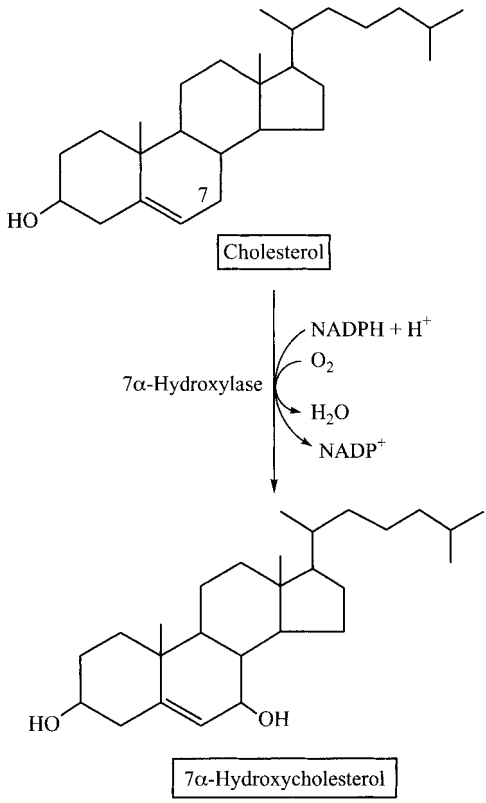


FIGURE 18-5 7α-Hydroxylase catalyzes the initial step in the classic pathway of bile acid synthesis.

- Introduce a hydroxyl group on the C12 carbon
- Cleave three carbons from the hydrocarbon side chain
- Oxidize the terminal carbon of the side chain to generate choloyl-CoA

Choloyl-CoA is then conjugated with either glycine or taurine to form glycocholate or taurocholate, respectively.

Synthesis of chenodeoxycholoyl-CoA follows the same pathway as that for choloyl-CoA but omits the hydroxylation of C12. The activity of the enzyme sterol 12α-hydroxylase thereby controls the resulting ratio of cholic acid to chenodeoxycholic acid and thus the overall detergent potency of the bile acid pool.

18.3.2.2 Alternate Pathway of Bile Acid Synthesis. The first step in the alternate pathway for bile acid synthesis is catalyzed by several different sterol hydroxylases which generate oxysterols by hydroxylating cholesterol at carbon 24, 25, or 27 (Fig. 18-6). The oxysterols are then 7α-hydroxylated by oxysterol hydroxylases CYP7B1 or CYP39A1 rather than by CYP7A1. Subsequent steps in the alternate pathway for bile acid synthesis utilize the same reactions as those of the classical pathway. The major product of the classical pathway is cholic acid whereas the alternate pathway generates relatively more chenodeoxycholic acid than cholic acid.

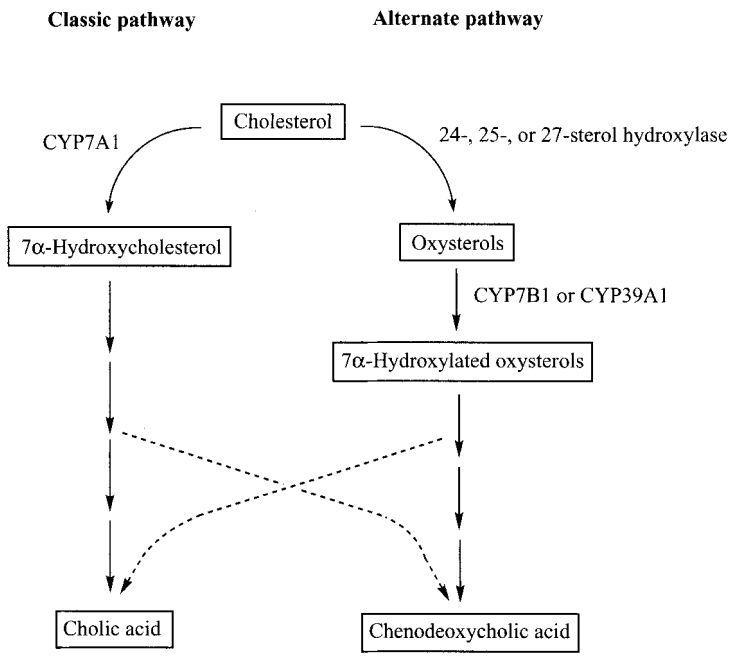


FIGURE 18-6 Classic and alternate pathways for bile acid synthesis. The dashed lines indicate minor pathways.

Although bile acid synthesis occurs only in the liver, the initial steps of the acidic pathway (generation and hydroxylation of oxysterols) can occur in a variety of extrahepatic tissues. For example, the brain, which cannot export cholesterol, expresses 24-sterol hydroxylase and exports the resulting oxysterol into the blood. The 27-hydroxylation of cholesterol by peripheral cells may also contribute to reverse transport of cholesterol by converting cholesterol into a more water-soluble product.

18.3.3 Synthesis of Steroid Hormones

The cholesterol utilized by the adrenal cortex and the gonads for steroid hormone synthesis is derived principally from LDL-cholesterol in blood. LDL binds to LDL receptors on steroidogenic tissues and is internalized by receptor-mediated endocytosis. The LDL-cholesteryl esters are then hydrolyzed by lysosomal cholesteryl esterase.

$$\text{cholesteryl ester} + H_2O \rightarrow \text{cholesterol} + \text{fatty acid}$$

The resulting cholesterol is then reesterified by acyl-CoA cholesterol acyl transferase (ACAT) and stored as cholesteryl esters in lipid droplets in the cytosol. Initiation of steroid hormone synthesis thus requires a second hydrolysis of cholesteryl esters by a cytosolic cholesteryl esterase. Cholesterol can also be synthesized de novo within the adrenal cortex and gonads.

18.3.3.1 Synthesis of Pregnenolone. The 21-carbon steroid pregnenolone is the common precursor for all of the steroid hormones. It is synthesized from cholesterol by the cytochrome P450 cholesterol side-chain cleavage enzyme (CYP11A, cholesterol desmolase) (Fig. 18-7). The removal of six carbons from the side chain as isocaproate and the introduction of a keto group on C20 is accomplished by three successive monooxygenase reactions, which result in hydroxylation of C20 and C22, followed by cleavage of the C20–C22 carbon–carbon bond. Three molecules of O_2 and three molecules of NADPH are consumed in the process of converting cholesterol to pregnenolone.

The side-chain cleavage enzyme complex is located on the inner mitochondrial membrane. Synthesis of pregnenolone therefore begins with the transport of cholesterol from the cytosol into the mitochondrion. In most steroidogenic cells except the placenta, the transport of cholesterol into the mitochondrion is mediated by a carrier called *steroidogenic acute regulatory protein* (StAR); the placenta lacks StAR but expresses another protein that has similar functional properties. Pregnenolone is transported back into the cytosol, where the subsequent steps of steroid synthesis occur.

18.3.3.2 Synthesis of Progesterone. Pregnenolone is converted to progesterone by 3β-hydroxysteroid dehydrogenase-isomerase (3β-HSD) (Fig. 18-8).

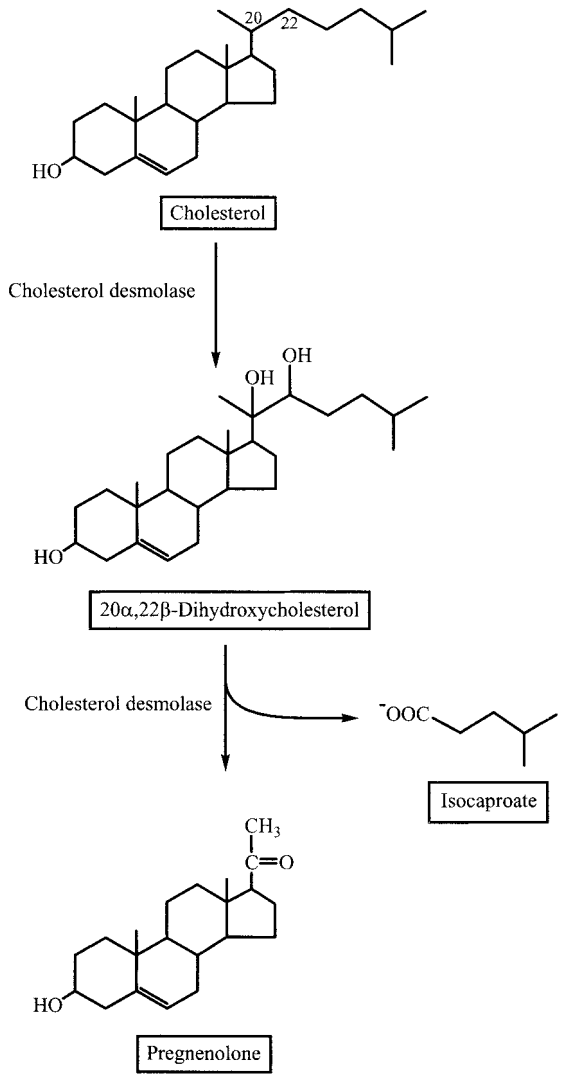


FIGURE 18-7 Synthesis of pregnenolone from cholesterol.

18.3.3.3 Synthesis of Hydrocortisone (Cortisol). Pregnenolone is converted by 17α-hydroxylase to 17-hydroxypregnenolone, which is converted by 3β-HSD to 17-hydroxyprogesterone. 17-Hydroxyprogesterone is then hydroxylated sequentially at C21 and then at C11 to form hydrocortisone (Fig. 18-9). Cortisone administered therapeutically is inactive, and is activated in the liver by 11β-dihydroxysteroid dehydrogenase, which generates hydrocortisone by reduction of the keto group on C11 of cortisone.

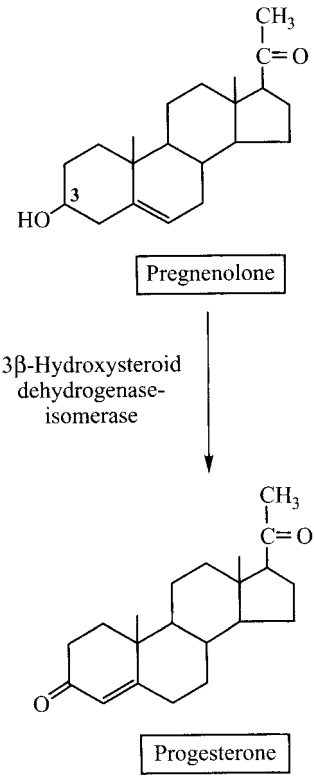


FIGURE 18-8 Conversion of pregnenolone to progesterone.

18.3.3.4 Synthesis of Aldosterone. Aldosterone synthesis is initiated by 21-hydroxylation of progesterone to form 21-hydroxyprogesterone, also known as deoxycorticosterone (Fig. 18-10). The subsequent steps in aldosterone synthesis involve two hydroxylation reactions at C11 and C18, respectively, followed by oxidation of the C18 hydroxyl group to a keto group. These three steps are all catalyzed by the same enzyme, CYP11B2.

18.3.3.5 Synthesis of Androgens. The pathway for the synthesis of testosterone in the testis is shown in Figure 18-11. Pregnenolone is first hydroxylated by CYP17 to 17-hydroxpregnenolone. CYP17 also has 17,20-lyase activity, thus allowing for the synthesis of dehydroepiandrosterone (DHEA). Synthesis of testosterone from DHEA involves successive hydroxylations by 3β-hydroxysteroid dehydrogenase (generating androstenedione) and then 17β-hydroxysteroid dehydrogenase. Both DHEA and androstenedione have weak androgenic activity and are synthesized in the adrenals as well as the gonads.

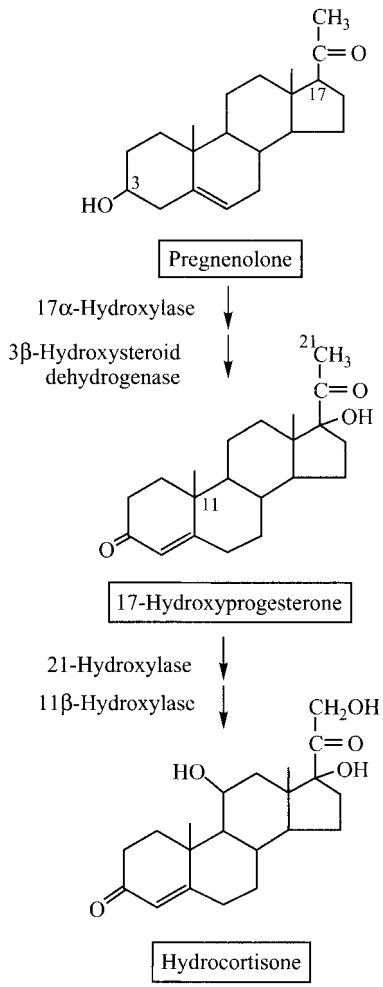


FIGURE 18-9 Synthesis of hydrocortisone from pregnenolone.

18.3.3.6 Synthesis of Estrogens. Aromatase (CYP19A1), the key enzyme complex that synthesizes estrogens from androgens, catalyzes a series of reactions that start with two successive hydroxylations of C19 of testosterone (Fig. 18-12). Subsequent cleavage of C19 as formate generates an 18-carbon steroid with an aromatic A ring. In women, aromatase is present in both the ovaries and peripheral tissues and acts on both testosterone and androstenedione, producing estradiol and estrone, respectively. Aromatase inhibitors have become an important treatment in women with breast cancer.

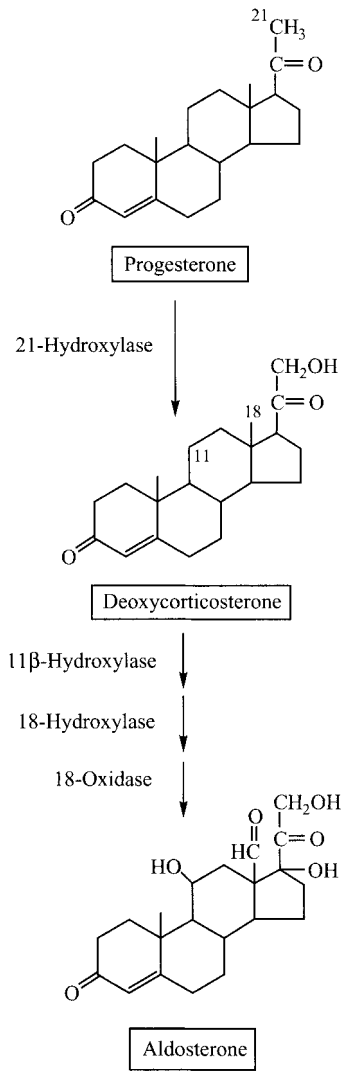


FIGURE 18-10 Synthesis of aldosterone from progesterone.

18.3.4 Synthesis of 1,25-Dihydroxycholecalciferol

Cholecalciferol (vitamin D_3) can either be obtained from the diet or formed in the skin by the nonenzymatic action of ultraviolet (UV) light on 7-dehydrocholesterol (Fig. 18-13). Ergocalciferol (vitamin D_2) is formed by the action of UV light on a structurally similar plant sterol (ergosterol). Vitamin D_3 is preferred for vitamin D supplementation.

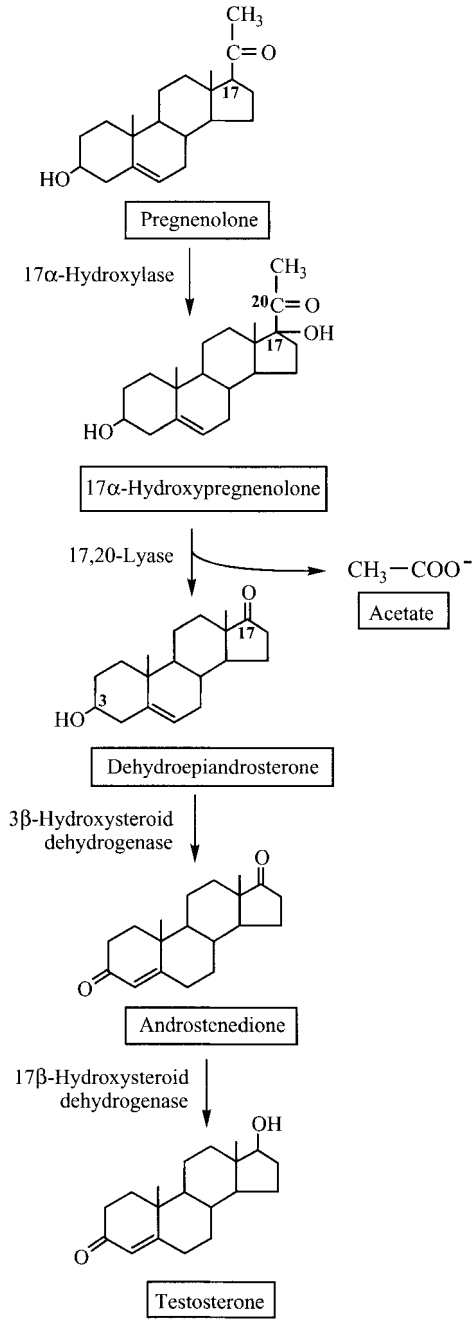


FIGURE 18-11 Synthesis of androgens.

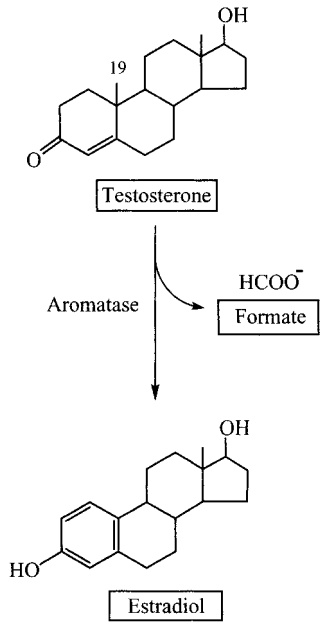


FIGURE 18-12 Conversion of testosterone to estradiol.

Conversion of cholecalciferol to its active hormonal form is a multiorgan process. First, cholecalciferol is hydroxylated in the liver by 25-hydroxylase to form 25-hydroxycholecalciferol (Fig. 18-13). A second hydroxylation step, catalyzed by 1α-hydroxylase in the kidney, generates the active hormone 1,25-dihydroxycholecalciferol. 1α-Hydroxylase is also expressed in placenta and placenta. Kidney, bone, cartilage, and intestine contain a 24-hydroxylase that converts 25-dihydroxycholecalciferol to the inactive 24,25-dihydroxycholecalciferol, thus preventing formation of excess active 1,25-$(OH)_2D_3$.

18.4 REGULATION OF THE SYNTHESIS OF METABOLITES OF CHOLESTEROL

18.4.1 Regulation of Bile Acid Synthesis

On a mass basis, bile acids are the main products of cholesterol metabolism. Under normal conditions, only 5 to 10% of the bile salts that enter the gut are actually excreted in the feces; their replacement requires de novo synthesis of approximately 400 mg/day of bile salts. Certain dietary fibers and pharmaceutical *bile acid sequestrants* such as cholestyramine decrease the reabsorption of bile acids and, as a result, increase hepatic synthesis of bile acids from cholesterol. Cholestyramine is therefore used to reduce the plasma concentration of cholesterol (see Chapter 17).

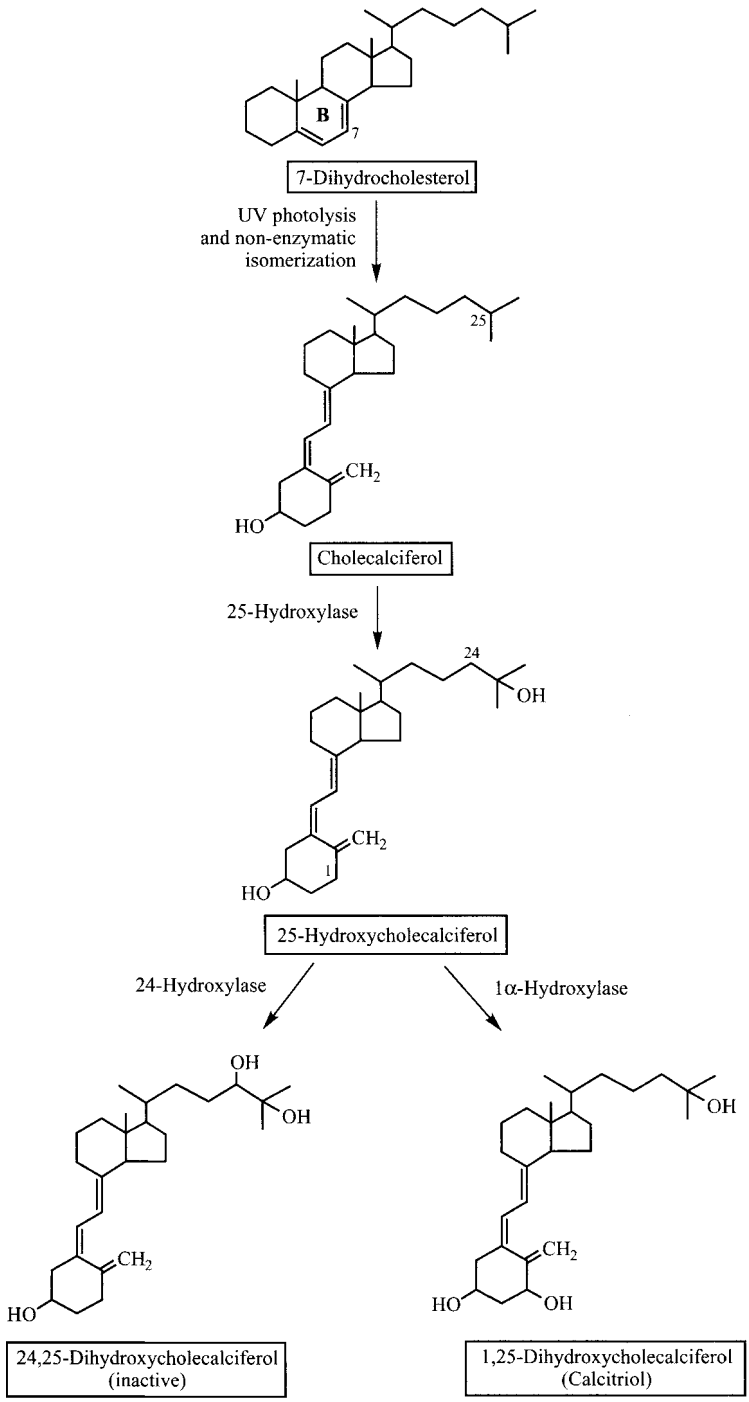


FIGURE 18-13 Synthesis of both 1,25-dihydroxycholecalciferol and the inactive molecule 24,25-dihydroxycholecalciferol from 7-dehydrocholesterol (vitamin D_3).

The regulated step in the classic pathway of bile salt synthesis is the initial hydroxylation of cholesterol by 7α-hydroxylase, an enzyme with a relatively short half-life. Synthesis of new 7α-hydroxylase protein is inhibited at the transcriptional level by primary bile acids. Cholestyramine and related resins that bind bile salts and decrease the efficiency of their reuptake by the ileum up-regulate 7α-hydroxylase and thus increase the metabolism of hepatic cholesterol to bile salts.

18.4.2 Regulation of Steroid Synthesis

Synthesis of steroid hormones by steroidogenic glands is stimulated by corresponding trophic hormones synthesized by the pituitary. For example, adrenocorticotrophic hormone (ACTH) stimulates the synthesis of steroids in the adrenal cortex, while the gonadotrophins LH (luteinizing hormone) and FSH (follicle-stimulating hormone) regulate steroid hormone synthesis in the gonads.

Binding of the trophic hormones to their corresponding plasma membrane receptors initiates a signal transduction cascade that results in increased concentrations of cAMP, which stimulates protein kinase A (PKA) (Fig. 18-14). PKA, in turn, phosphorylates and activates cholesteryl ester hydrolase, which makes cholesterol available

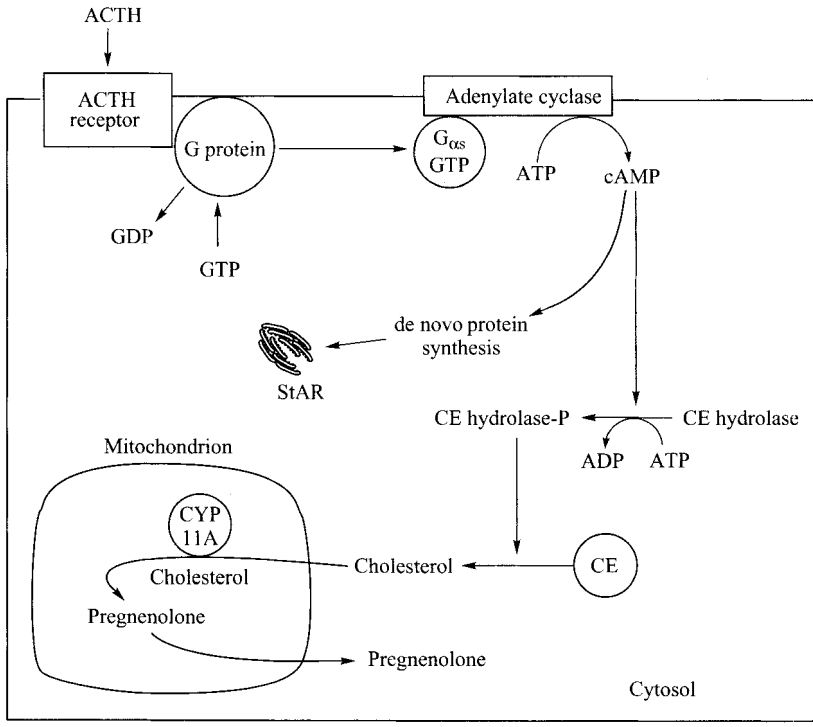


FIGURE 18-14 ACTH stimulates hydrolysis of cholesteryl esters and transport of free cholesterol into the mitochondrion. CE, cholesteryl ester; CYP 11A, cholesterol side-chain cleavage enzyme; PKA, protein kinase A; StAR, steroidogenic acute regulatory protein.

for steroid hormone synthesis. The action of PKA also induces de novo synthesis of StAR and thus stimulates transport of cholesterol to the inner mitochondrial membrane where the cholesterol P450 side-chain cleavage enzyme is located.

What determines which steroid hormones are produced by a particular cell? The common initial step in steroid hormone synthesis is the one that generates pregnenolone. The metabolic fate of pregnenolone is dependent primarily on the particular enzymes present within that cell. For example, cells of the zona fasciculata in the adrenal cortex contain 17α-hydroxylase, which converts pregnenolone to 17α-hydroxypregnenolone, which is subsequently metabolized to hydrocortisone. By contrast, cells of the zona glomerulosa in the adrenal cortex convert pregnenolone to aldosterone. Since several of the enzymes of steroid hormone pathways contribute to the generation of multiple hormones in the same gland, impairment of an enzyme activity resulting in decreased synthesis of some steroids may increase the availability of precursor molecules for other steroidogenic pathways.

18.4.3 Regulation of 1,25-Dihydroxycholecalciferol Synthesis

Formation of 1,25-$(OH)_2D_3$ is regulated primarily by the level of 1α-hydroxylase. Synthesis of 1α-hydroxylase in the proximal renal tubule is stimulated by parathyroid hormone and is inhibited by its product, 1,25-$(OH)_2D_3$, and by a high concentration of extracellular calcium.

18.5 ABNORMAL FUNCTION

18.5.1 Genetic Defects in Bile Acid Synthesis

The pathways that convert cholesterol to cholate and deoxycholate are affected by several inborn errors of metabolism, all of which result in progressive liver disease typically manifested in the neonatal period. Affected persons have cholestasis (blockage of bile flow) accompanied by hyperbilirubinemia, steatorrhea, and deficiencies of the fat-soluble vitamins. The disorders are diagnosed by the finding of unusual bile acids (e.g., 7α-hydroxy-3-oxo-4-cholenoic acid) in the urine. Treatment with oral chenodeoxycholic acid is usually effective in restoring the normal secretion of bile, improving the digestion and absorption of dietary lipids, and inhibiting the production of abnormal bile acids.

18.5.2 Congenital Adrenal Hyperplasia

The most common cause of congenital adrenal hyperplasia (CAH) is a genetic deficiency of the activity of the steroidogenic enzyme 21-hydroxylase (CYP21). This enzyme defect results in decreased synthesis of hydrocortisone. The resulting increase in pituitary ACTH secretion in turn produces adrenal hyperplasia and increases androgen production. Affected persons exhibit hydrocortisone insufficiency (hypoglycemia, hypotension) and are dependent on exogenous hydrocortisone. In severe cases, aldosterone deficiency also occurs, resulting in dehydration, hyponatremia due to salt wasting, and hyperkalemia.

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency is also the most common cause of genital ambiguity in newborn females. Lack of hydrocortisone results in increased ACTH secretion, which overstimulates 17-hydroxyprogesterone synthesis, resulting in increased synthesis of DHEA and androstenedione. Exposure of the female fetus to androgens in utero results in prenatal virilization (masculinization of the external genitalia). In affected newborn males, there are no abnormalities of the external genitalia. However, if a male infant with CAH is not treated promptly, excess androgen synthesis results in precocious development of secondary sex characteristics and increased stature. Hydrocortisone replacement therapy is effective in decreasing ACTH levels and suppressing excess androgen production. Hydrocortisone also restores normal glucocorticoid function; however, the more severely affected patients also require mineralocorticoid replacement therapy. In at-risk pregnancies, early maternal treatment with dexamethasone may prevent or significantly reduce virilization of the genitalia of female neonates.

18.5.3 Congenital Lipoid Adrenal Hyperplasia

A rare type of adrenal hyperplasia results from a genetic deficiency of StAR, the steroidogenic acute regulatory protein. Loss of the ability to transport cholesterol into mitochondria results in a loss of all steroidogenesis (defective glucocorticoid, mineralocorticoid, and androgen production) and accumulation of cholesteryl esters in the cytosol of the adrenal cortex. The lack of hydrocortisone production leads to increased ACTH stimulation, resulting in unusually large adrenal glands filled with cholesteryl esters. The condition is lethal unless appropriate mineralocorticoid- and glucocorticoid-replacement therapy is provided. Steroid biosynthesis is impaired in the gonads as well. Affected newborn males will show incomplete masculinization of the external genitalia. Affected females usually do not manifest symptoms of ovarian failure until puberty, which is the expected time of estrogen synthesis.

18.5.4 Rickets and Osteomalacia

Vitamin D deficiency results in rickets in children and osteomalacia in adults. Both diseases reflect impaired mineralization of newly synthesized organic bone matrix. In adults, the undermineralization occurs after linear growth has stopped and involves bone only. However, in children whose bones are still growing, decreased mineralization of osteoid occurs in growing cartilage in the growth plate as well as in bone, and bony deformities, including the characteristic bowed long bones of the legs, may occur.

Vitamin D deficiency usually results from a combination of inadequate exposure to sunlight and decreased dietary intake of vitamin D. Breast-fed infants who do not receive vitamin D supplements are at particular risk because human milk usually contains insufficient vitamin D. Also at high risk for lack of endogenously synthesized vitamin D are dark-skinned people living at latitudes where there is less sunlight, or persons who do not expose their skin to sunlight. Dietary vitamin D is present mainly in foods of animal origin, especially liver, eggs, and vitamin D–fortified milk and

dairy products. A decreased serum level of 25-hydroxycholecalciferol is diagnostic of vitamin D deficiency. Severe kidney or liver disease reduces conversion of vitamin D to 25-hydroxycholecalciferol and/or the hormonally active 1,25-$(OH)_2D_3$, resulting in rickets and osteomalacia even in the presence of adequate sunlight and/or dietary intakes of vitamin D.

In many parts of the world, rickets is caused by a dietary deficiency of calcium rather than vitamin D. Rickets may also result from an inherited deficit in renal 1α-hydroxylase or from genetic disorders that cause hypophosphatemia due to inhibition of phosphate reabsorption in the renal tubule.

CHAPTER 19

NITROGEN HOMEOSTASIS

19.1 FUNCTIONS OF NITROGEN METABOLISM

Each day, all humans turn over 1 to 2% of their total body protein, most of which is muscle protein. About three-fourths of the amino acids liberated during this process are reutilized for the synthesis of new proteins; the remaining 20 to 25% is catabolized. To maintain health, dietary protein must replace the degraded amino acids and provide amino acids that are precursors for the synthesis of proteins and other critical nitrogen-containing compounds, such as heme and nucleic acids.

There is no distinct storage form for amino acids in the body as there is for glucose in the form of glycogen; all body proteins serve one or another important function. The turnover of some proteins, particularly those in muscle, is increased under conditions of fasting and starvation when amino acids are needed to provide substrates for gluconeogenesis.

In general, catabolism of amino acids released during protein turnover and dietary amino acids in excess of those needed for protein synthesis involves (1) removal of the α-amino group and (2) oxidation of the carbon skeletons to CO_2 and H_2O, generating ATP in the process.

19.1.1 Urea

Humans excrete excess dietary nitrogen primarily as urea in the urine (Fig. 19-1). At plasma concentrations greater than 50 μM, ammonia ($NH_3 + NH_4^+$) is toxic to the

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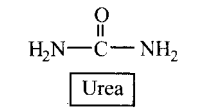
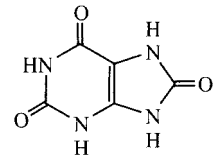
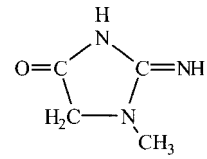


Urea



Urate



Creatinine

FIGURE 19-1 Major nitrogenous compounds excreted in the urine.

central nervous system. Urea, one of the simplest of organic compounds, is formed from CO_2, ammonium ion, and the α-amino group of aspartate by means of the urea cycle. Synthesis of urea provides a mechanism for detoxifying ammonia.

19.1.2 Urinary Excretion of Other Nitrogenous Compounds

Urine contains nitrogenous compounds other than urea, including uric acid, creatinine, and ammonia, the excretion of which serves other, distinct functions or represents breakdown products of certain metabolites (Fig. 19-1). For example, creatinine is a breakdown product of muscle creatine phosphate and, as such, provides the clinician with a convenient measure of muscle mass. For this reason, it is sometimes useful to quantify the urinary content of other metabolites by normalizing their values to urinary creatinine. Uric acid is the end product of purine catabolism. The kidney also excretes some nitrogen directly in the form of ammonium ions, which serve to buffer acidic, anionic waste products such as β-hydroxybutyrate, acetoacetate, and sulfate. Excretion of ammonium ions is thus increased during ketoacidosis and other metabolic conditions where excess organic acids are produced.

19.1.3 Other Losses of Nitrogen from the Body

Although the major route of nitrogen excretion is urea, there is also a loss of approximately 1.6 g of nitrogen per day (equivalent to 10 g of protein) in the feces.

Fecal nitrogen arises both from sloughing of cells into the gut and from incompletely absorbed dietary protein. In addition, there are minor losses of nitrogen-containing substances from the skin (both as sweat and shed skin cells) and from hair loss, nasal secretions, and menstrual fluid.

19.1.4 Nitrogen Balance

To maintain nitrogen homeostasis, the quantity of nitrogen excreted must be balanced by dietary nitrogen intake. A healthy adult who consumes 100 g of protein (16 g of nitrogen) per day will excrete 16 g of nitrogen per day, of which approximately 15 g will be in the form of urea. If the same person were to increase his or her protein intake further, the renal excretion of urea would be increased to eliminate the excess nitrogen.

19.2 LOCALIZATION OF NITROGEN METABOLISM

The synthesis of urea occurs in the liver, which is the only tissue in humans that contains the enzyme arginase. The liver is also a major site of aminotransferase activity. The resulting urea is released into the blood and excreted by the kidney. Under conditions of vigorous exercise, some urea is also eliminated from the body in sweat.

The major source of amino acid nitrogen that is excreted is from turnover of muscle proteins. A large fraction of the excess nitrogen from amino acid catabolism in muscle is released into the blood as alanine and glutamine. Most of the alanine secreted by muscle is utilized by the liver, with the carbon skeleton serving as a precursor for gluconeogenesis, while the amino acid nitrogen group is incorporated into urea. The glutamine secreted by muscle is used mainly by the kidney. The ammonium ions generated during the catabolism of glutamine in the kidney serve to buffer organic acids and other anions (e.g., sulfate) in the urine, while the carbon skeleton (α-ketoglutarate) becomes a substrate for renal gluconeogenesis.

19.3 PHYSIOLOGICAL STATES IN WHICH THE NITROGEN DETOXIFICATION AND EXCRETION PATHWAYS ARE ESPECIALLY ACTIVE

Since the synthesis of urea increases when there is a need to eliminate excess nitrogen from the body, urea synthesis is higher when a person is consuming a high-protein diet as opposed to a diet low in protein. By contrast, the excretion of other nitrogenous compounds (e.g., creatinine, uric acid, ammonium ion) is relatively unaffected by the amount of nitrogen in the diet.

Urea synthesis is also increased in the fasted state. This may seem paradoxical: If urea synthesis decreases as the protein intake decreases, one might expect urea synthesis to be lowest when the protein intake is zero. However, as discussed in Chapter 9, carbon skeletons of a number of amino acids play an important role

as substrates for gluconeogenesis. A person consuming carbohydrate without any dietary protein will carry out minimal urea synthesis and excrete only about 2 g of nitrogen per day as urea. By contrast, the same person who is fasted and therefore actively breaking down muscle proteins to support gluconeogenesis may excrete as much as 10 to 12 g of nitrogen per day as urea.

19.4 REACTIONS OF UREA SYNTHESIS

19.4.1 Overall Pathway

Each urea molecule contains two nitrogen atoms and one carbon atom (Fig. 19-1). The two nitrogen atoms of urea originate directly from ammonium ion and aspartate, although both nitrogens are ultimately derived from the amino groups of all 20 common amino acids and the non-α-amino group nitrogens of asparagine, glutamine, and histamine. The single carbon atom of urea is derived from bicarbonate. The urea molecule is assembled on ornithine, which is an α-amino acid but not one used for protein synthesis. The assembly of urea on ornithine generates the amino acid arginine; subsequent hydrolysis of arginine by arginase releases urea and regenerates ornithine:

$$\text{arginine} \rightarrow \text{ornithine} + \text{urea}$$

Indeed, excess dietary arginine is itself an immediate precursor of urea. Since arginase is expressed only in liver, that organ is the sole site of urea synthesis.

19.4.2 Intracellular Localization of the Urea Cycle

Synthesis of urea involves both mitochondrial and cytosolic enzymes (Fig. 19-2). The first two steps of the urea cycle that generate the intermediate citrulline occur in the mitochondrion. Citrulline is then transported out of the mitochondrion into the cytosol, where it is converted to arginosuccinate and then to arginine. After arginase releases a molecule of urea from arginine, ornithine, the other product, is transported back into the mitochondrion for another round of urea synthesis.

19.4.3 Aminotransferase Reactions Generate Glutamate

Aminotransferases catalyzes reversible transfer of an α-amino group from an amino acid to an acceptor α-ketoacid, producing a new amino acid and an α-ketoacid derived from the donor amino acid (Fig. 19-3). The most common acceptor utilized by aminotransferases is α-ketoglutarate. Thus, the process of transamination results ultimately in the collection of amino acid nitrogen in glutamate.

Pyridoxal phosphate (PLP), the active form of vitamin B_6, is a cofactor in transamination reactions (Fig. 19-4). The aldehyde group of PLP can accept the α-amino

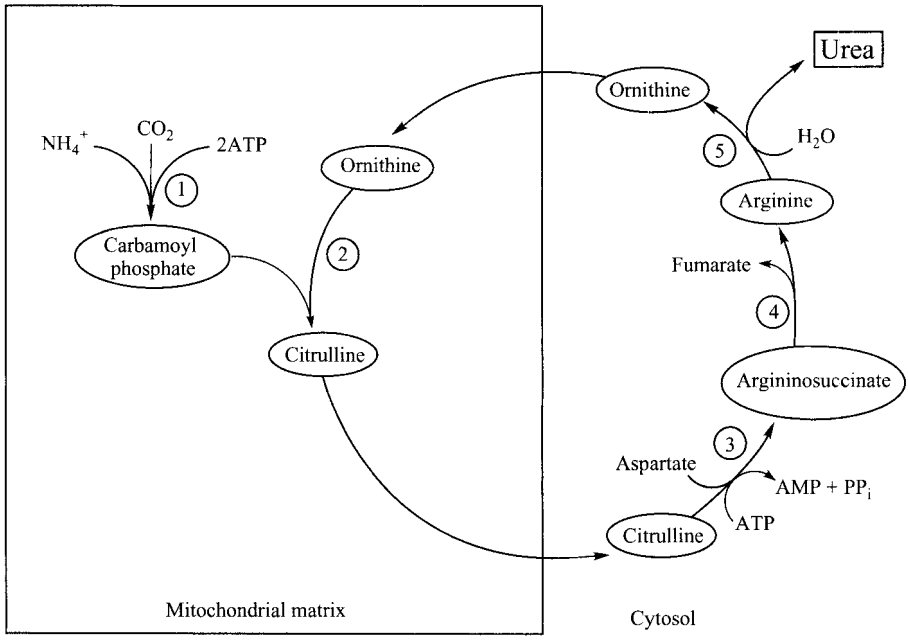


FIGURE 19-2 Localization of the reactions of the urea cycle: ①, carbamoyl phosphate synthase I; ②, ornithine transcarbamoylase; ③, arginosuccinate synthase; ④ arginosuccinate lyase; ⑤ arginase.

group from an amino acid, generating pyridoxamine phosphate, which in turn donates that amino group to an α-ketoacid, regenerating PLP. The activated intermediate in this process is a Schiff base. PLP-containing enzymes also catalyze many other reactions involving amino acids, including the decarboxylation reactions involved in the synthesis of the neurotransmitters dopamine and serotonin.

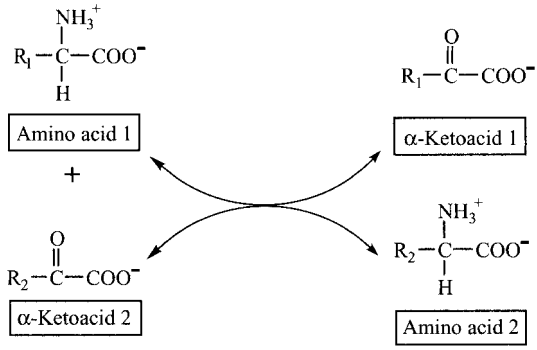


FIGURE 19-3 Transamination of amino acids.

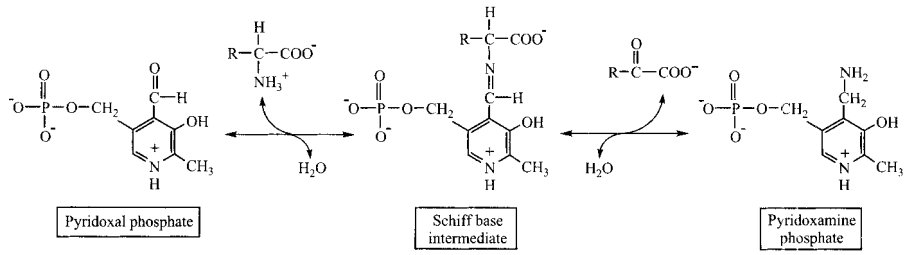


FIGURE 19-4 Role of pyridoxal phosphate in aminotransferase reactions.

19.4.3.1 Liver Is the Major Site of Aminotransferase Activity. The two principal liver transaminases are alanine aminotransferase (ALT), which catalyzes the reaction

$$\text{alanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{pyruvate} + \text{glutamate}$$

and aspartate aminotransferase (AST), which catalyzes the reaction

$$\text{aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{glutamate}$$

Most of the NH_4^+ generated when muscle proteins are broken down during a fast is exported in the form of alanine. In the liver, ALT catalyzes the transamination of alanine, generating pyruvate which can be utilized for gluconeogenesis, and glutamate which provides nitrogen atoms for urea synthesis. Some of the glutamate nitrogen is released as ammonium ions by the enzyme glutamate dehydrogenase (see below). Concurrently, AST utilizes some of the glutamate to generate aspartate by transfer of the amino group from glutamate to oxaloacetate. The NH_4^+ from the glutamate dehydrogenase reaction and the aspartate from the AST reaction provide the two nitrogens for urea synthesis.

19.4.3.2 Increased Plasma Levels of ALT and AST Are Indicative of Liver Damage. Aminotransferases are intracellular enzymes that have both cytosolic and mitochondrial isoforms. When there is liver damage, as occurs with cirrhosis or viral hepatitis, these aminotransferase are released from the hepatocytes. Increased plasma levels of ALT and AST are thus markers of liver damage. In the older clinical literature, these enzymes are sometimes referred to as SGPT (serum glutamate:pyruvate transaminase) and SGOT (serum glutamate:oxaloacetate transaminase), respectively.

19.4.4 Generation of Ammonium Ions from Amino Acids

Two steps are required to generate ammonium ions from most of the 20 common amino acids. The first is an aminotransferase reaction in which the α-amino group of

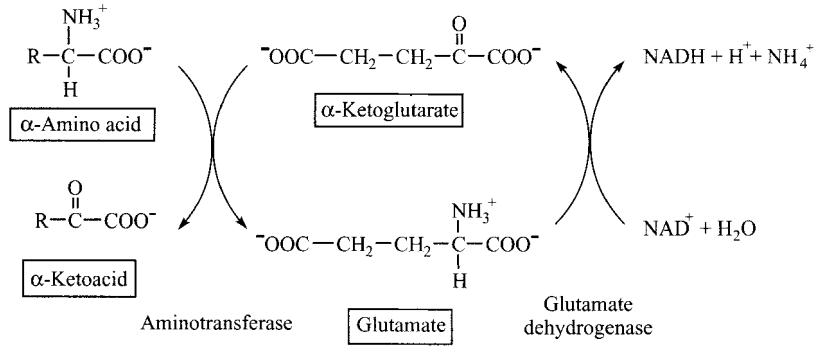


FIGURE 19-5 Central role of glutamate in the release of amino acid nitrogen as ammonium ions.

an amino acid is transferred to α-ketoglutarate, which converts the latter into glutamate. The second step is the NAD^+-dependent oxidative deamination of glutamate, which releases a free ammonium ion, regenerating α-ketoglutarate in the process (Fig. 19-5). Alternate catabolic pathways exist for those amino acids (e.g., serine, histidine, proline) that do not undergo transamination. Catabolism of amino acids and other nitrogenous molecules by bacteria in the gut is also a significant source of NH_4^+ in the body.

19.4.4.1 Glutamate Dehydrogenase. Glutamate dehydrogenase catalyzes an oxidative deamination reaction in which the amino group of glutamate is released as NH_4^+. This reaction occurs in the mitochondrial matrix and is readily reversible:

$$\text{glutamate} + NAD^+ \rightleftharpoons \alpha\text{-ketoglutarate} + NH_4^+ + NADH + H^+$$

The sequential activities of an aminotransferase that generates glutamate and glutamate dehydrogenase, which generates NH_4^+, thus provide a pathway for the generation of ammonium ions from the α-amino groups of most of the amino acids (Fig. 19-5).

19.4.4.2 Deamination of Other Amino Acids. Although all 20 common amino acids, except threonine, lysine, and proline, can be transaminated, many of the amino acids can also be catabolized by pathways that do not involve transamination. One such example is the dehydratase-catalyzed reactions that remove water from both serine and threonine and generate intermediates that contain unstable imino groups (Fig. 19-6). Spontaneous addition of water to these intermediates results in release of NH_4^+ and the generation of pyruvate and α-ketobutyrate, respectively.

Histidine is deaminated in one step by histidine ammonia lyase or histidase:

$$\text{histidine} \rightarrow \text{urocanate} + NH_4^+$$

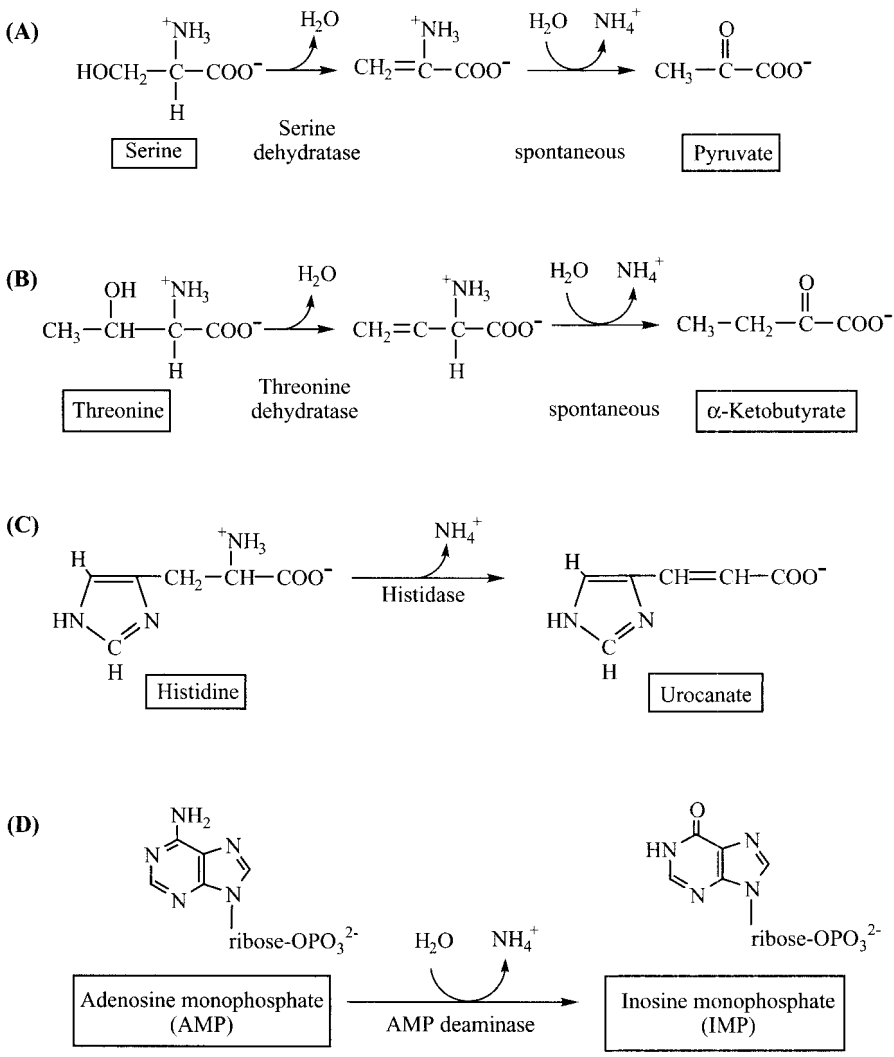


FIGURE 19-6 Alternate pathways for generation of ammonium ions: (A) serine dehydratase; (B) threonine dehydratase; (C) histidase; (D) AMP deaminase.

Subsequent metabolism of urocanate generates glutamate and NH_4^+ and results in the donation of one carbon from urocanate to the tetrahydrofolate pool in the form of N^5,N^{10}-methylenetetrahydrofolate.

The nitrogen group of proline forms part of its five-membered ring structure. Catabolism of proline involves oxidation and opening of the ring to generate glutamate semialdehyde, which is then converted to glutamate (see Chapter 20).

19.4.4.3 Purine Nucleotide cycle. Ammonium ion is also generated through the deamination of AMP to IMP (inosine monophosphate) by adenosine monophosphate deaminase (Fig. 19-6):

$$\text{AMP} + \text{H}_2\text{O} \rightarrow \text{IMP} + \text{NH}_4^+$$

This reaction is especially active in exercising muscle which generates AMP. When ATP levels are low, muscle can generate additional ATP directly from ADP by means of the myokinase (adenylate kinase) reaction:

$$2\text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$$

Removal of the resulting AMP is necessary if the reaction is to continue to the right. The pathway by which AMP is deaminated to IMP and IMP is subsequently utilized for resynthesis of AMP is referred to as the *purine nucleotide cycle* and is discussed further in Chapter 23.

19.4.4.4 Deamination of Glutamine and Asparagine. The amide nitrogens of glutamine and asparagine are removed hydrolytically by glutaminase:

$$\text{glutamine} + \text{H}_2\text{O} \rightarrow \text{glutamate} + \text{NH}_4^+$$

and asparaginase:

$$\text{asparagine} + \text{H}_2\text{O} \rightarrow \text{aspartate} + \text{NH}_4^+$$

Glutaminase is particularly important in the kidney, where NH_4^+ is used to buffer organic acids (e.g., ketones) and sulfate ions that are excreted in the urine.

19.4.5 The Urea Cycle

The reactions of the urea cycle are illustrated in Figure 19-7.

19.4.5.1 Synthesis of Carbamoyl Phosphate. The initial step in the detoxification of NH_4^+ is the synthesis of the nitrogenous organic compound carbamoyl phosphate:

$$\text{HCO}_3^- + \text{NH}_4^+ + 2\text{ATP} \rightarrow \text{carbamoyl phosphate} + 2\text{ADP} + \text{P}_i$$

The enzyme that catalyzes this reaction is carbamoyl phosphate synthetase I (CPS I), and it is localized to mitochondria. Carbamoyl phosphate synthetase II (CPS II) is a cytosolic enzyme that generates carbamoyl phosphate for pyrimidine synthesis.

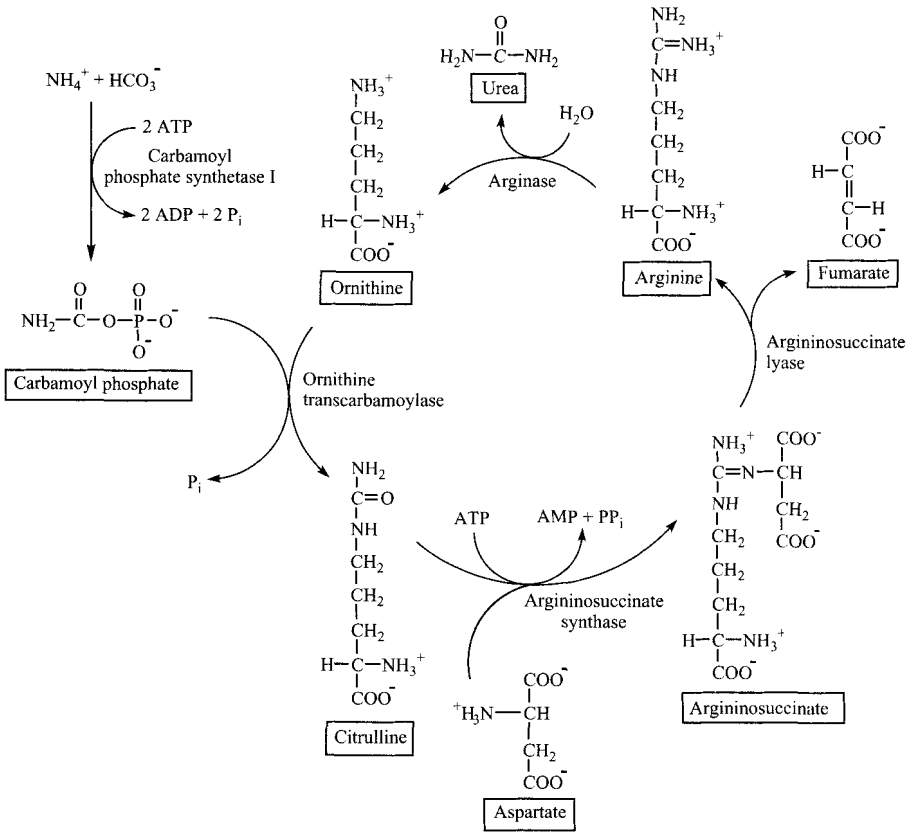


FIGURE 19-7 The urea cycle.

19.4.5.2 Ornithine Transcarbamoylase. Ornithine transcarbamoylase (OTC) catalyzes the transfer of the carbamyl group from carbamoyl phosphate to the amino group in the side chain of the amino acid ornithine, generating citrulline:

$$\text{ornithine} + \text{carbamoyl phosphate} \rightarrow \text{citrulline} + P_i$$

Although both ornithine and citrulline are α-amino acids, they are not utilized for protein synthesis. Citrulline is transported out of the mitochondrion in exchange for ornithine.

19.4.5.3 Argininosuccinate Synthase and Argininosuccinate Lyase. The effect of the next two steps in the urea cycle is to add an amino group to citrulline to generate arginine. This amino group is provided by the amino acid aspartate in the

reaction catalyzed by argininosuccinate synthase:

$$\text{citrulline} + \text{aspartate} + \text{ATP} \rightarrow \text{argininosuccinate} + \text{AMP} + \text{PP}_i$$

Subsequently, argininosuccinate is cleaved, retaining the amino group from aspartate and generating fumarate:

$$\text{argininosuccinate} \rightarrow \text{arginine} + \text{fumarate}$$

Fumarate is hydrated by cytosolic fumarase to form malate, which is oxidized to oxaloacetate, and then transaminated to regenerate aspartate.

19.4.5.4 Arginase. The urea cycle is completed by the hydrolysis of arginine, which generates urea plus ornithine:

$$\text{arginine} + \text{H}_2\text{O} \rightarrow \text{urea} + \text{ornithine}$$

Urea is secreted from hepatocytes and transported to the kidney for excretion in the urine. Ornithine generated by arginase is transported back into mitochondria to continue the cyclic process of urea synthesis.

19.4.5.5 De Novo Synthesis of Ornithine and Arginine. Some of the ornithine utilized in the urea cycle is derived through the action of arginase on dietary arginine. Ornithine can also be synthesized from glutamate by way of a pathway that involves reduction of glutamate by NADPH to produce glutamate semialdehyde, which contains one aldehyde and one carboxyl group (Fig. 19-8). Ornithine transaminase then acts on glutamate semialdehyde to generate ornithine:

$$\text{glutamate semialdehyde} + \text{glutamate} \rightleftharpoons \text{ornithine} + \alpha\text{-ketoglutarate}$$

Synthesis of Arginine from Ornithine Is Not Limited to the Liver. As described above, the enzymes of the urea cycle convert ornithine into arginine. Some of the

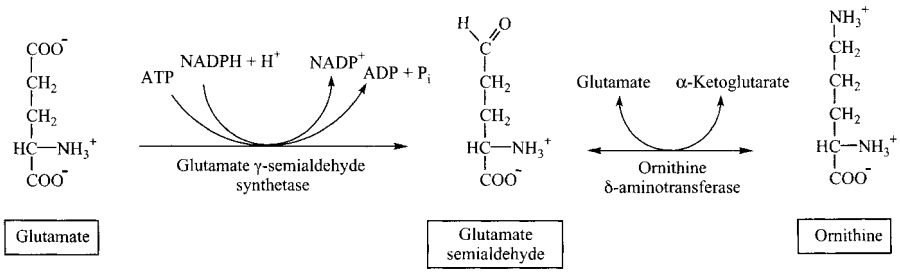


FIGURE 19-8 Synthesis of ornithine.

enzymes of the urea cycle are present in tissues other than the liver. For example, the intestinal mucosa can convert ornithine to citrulline, and the kidney can convert the resultant citrulline to arginine. However, since the kidney and intestine lack arginase, they cannot synthesize urea.

Arginine Is the Precursor of Nitric Oxide. Nitric oxide (NO) is an oxygen-containing free radical which functions as a neurotransmitter and vasodilatory autocoid or local hormone. At high concentrations, NO combines with molecular oxygen or superoxide to form other reactive oxygen species which can contribute to chronic inflammation and to neurodegenerative diseases. NO is synthesized from arginine in a reaction catalyzed by NO synthase:

$$\text{arginine} + O_2 + \text{NADPH} + H^+ \rightarrow \text{NO} + \text{citrulline} + \text{NADP}^+$$

There are three isozymes of NO synthase. The neural (nNOS) and endothelial (eNOS) forms are regulated by the intracellular calcium concentration and produce NO for its neurotransmitter and autocoid roles. By contrast, the isozyme induced in activated macrophages (iNOS) produces NO that contributes to the bacteriocidal response. The other product of the NO synthase reaction is citrulline, which is converted back to arginine through the sequential actions of argininosuccinate synthase and argininosuccinate lyase. Both argininosuccinate synthase and argininosuccinate lyase are induced under conditions that increase expression of iNOS and allow for regeneration of arginine in the absence of the full urea cycle.

19.5 REGULATION OF UREA SYNTHESIS

19.5.1 Allosteric Regulation of Carbamoyl Phosphate Synthetase I by *N*-Acetylglutamate

Carbamoyl phosphate synthetase I catalyzes the regulated step of urea synthesis. This enzyme is activated by *N*-acetylglutamate. Like fructose 2,6-bisphosphate which regulates glycolysis and gluconeogenesis, *N*-acetylglutamate is not an intermediate in the metabolic pathway that it regulates, but instead, is synthesized for the sole purpose of providing allosteric modulation of a key regulatory enzyme.

N-Acetylglutamate synthase catalyzes the following reaction:

$$\text{glutamate} + \text{acetyl-CoA} \rightarrow N\text{-acetylglutamate} + \text{CoASH}$$

N-Acetylglutamate synthase is allosterically activated by arginine (Fig. 19-9). Thus, *N*-acetylglutamate synthesis occurs only when the supply of both glutamate and ornithine is adequate.

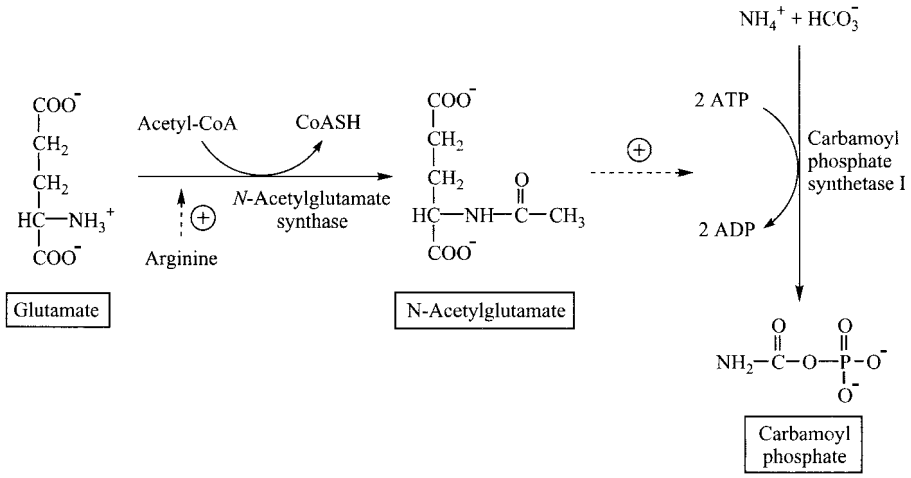


FIGURE 19-9 Regulation of carbamoyl phosphate synthetase I. The dashed arrows indicate the roles of allosteric regulators.

19.5.2 Induction of Enzymes

The levels of the urea cycle enzymes in the liver increase 10- to 20-fold in response to increased concentrations of amino acids and ammonia. Induction of urea cycle enzymes thus occurs under conditions of starvation and stress (e.g., sepsis, burns, trauma) when there is accelerated breakdown of body proteins to provide substrates for gluconeogenesis and the synthesis of acute-phase proteins, whose plasma concentrations increase during inflammatory states. The enzymes of the urea cycle are also induced by high-protein diets, which provide excess amino acids to the liver.

19.6 ABNORMAL FUNCTION OF THE PATHWAYS OF NITROGEN METABOLISM

19.6.1 Hyperammonemia

The finding of elevated blood levels of ammonia is evidence that the conversion of ammonia to urea is impaired in some way. Hyperammonemia in adults is usually the consequence of impaired liver function, secondary to liver disease (e.g., cirrhosis), organ transplantation, or chemotherapy. Transient hyperammonemia is often seen in premature neonates with immature liver function and/or inadequate hepatic blood flow. Impaired urea synthesis may also be the result of a genetic defect in one of the enzymes of the urea cycle. Regardless of its etiology, hyperammonemia is usually accompanied by increased plasma levels of glutamine, the amino acid that the brain uses as a vehicle to export excess ammonium ions.

Ammonia is toxic to the central nervous system, where it can cause both acute encephalopathy and long-term irreversible brain damage; however, the pathophysiologic mechanisms are not fully understood. One possible cause is the increased synthesis of the neurotransmitters glutamine and γ-aminobutyric acid (GABA) and subsequent derangements of neurotransmission. Another possible mechanism for ammonia toxicity in the brain involves the depletion of TCA-cycle intermediates by diversion of α-ketoglutarate to glutamate and glutamine synthesis, which would compromise the ability of the neural cells to generate ATP.

Treatment for hyperammonemia involves hemodialysis or peritoneal dialysis to remove the excess ammonia. In acute cases, oral sodium benzoate and sodium phenylbutyrate are sometimes administered to provide alternate pathways for nitrogen excretion as hippurate and phenylacetylglutamine, respectively (Fig. 19-10). Protein intake should also be severely restricted in patients with hyperammonemia. At the same time, it is important to provide adequate intake of carbohydrates to minimize further catabolism of endogenous protein.

19.6.2 Ornithine Transcarbamoylase Deficiency

Many inborn errors of urea-cycle metabolism have been described in the literature. All share a common set of biochemical symptoms, including hyperammonemia, respiratory alkalosis, and low blood urea nitrogen (BUN). Neonatal patients present with lethargy, irritability, and hypotonia. Prompt treatment is critical because prolonged hyperammonemia can result in irreversible brain damage, coma, and even death.

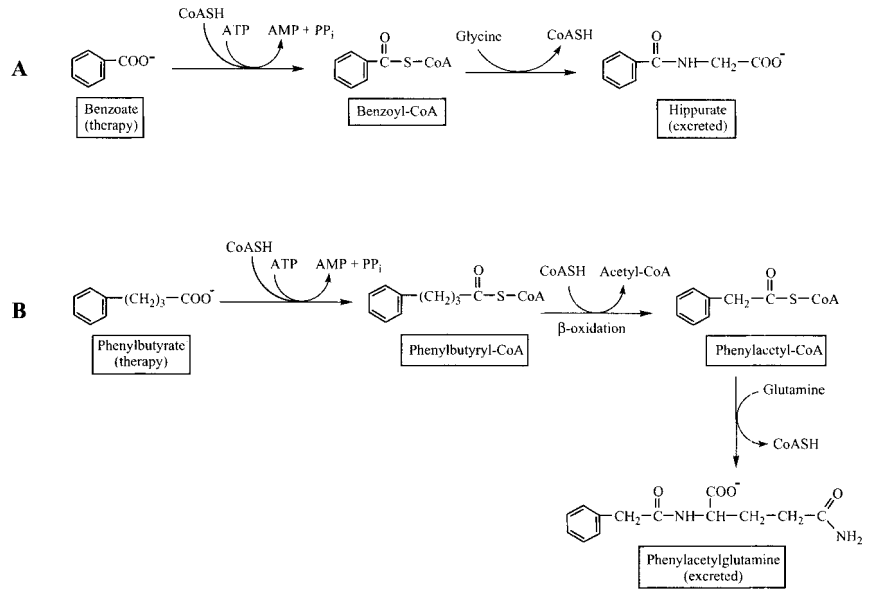


FIGURE 19-10 Alternate mechanisms of nitrogen excretion initiated by pharmacological therapy.

Older children may show a variety of neurological symptoms, including psychomotor retardation and recurrent cerebellar ataxia, with or without hyperammonemia. The differential diagnosis of inborn errors of the urea cycle is usually made from quantitative analysis of plasma amino acids, which identifies increased levels of urea-cycle intermediates upstream of the specific enzyme blockage.

The most common inborn error of the urea cycle is a deficiency of ornithine transcarbamoylase, an X-linked disorder with wide genotypic and phenotypic variability in hemizygous males. Heterozygous females are also affected; however, the severity is dependent on the pattern of X-chromosome inactivation. People with ornithine transcarbamoylase deficiency characteristically exhibit decreased plasma levels of citrulline and arginine. They also have elevated levels of orotic acid in their urine, which is a useful diagnostic indicator to rule out deficiency of carbamoyl phosphate synthetase or *N*-acetylglutamate synthetase. The increased synthesis of orotic acid is the result of the accumulation of intracellular carbamoyl phosphate, which bypasses the regulated step catalyzed by carbamoyl phosphate synthetase II and provides excess substrate for pyrimidine synthesis.

19.6.3 Metabolic Acidosis

The ketone bodies, β-hydroxybutyrate and acetoacetate, that accumulate during both starvation and poorly regulated type I diabetes mellitus, are organic acids. In addition, many inborn errors of metabolism (i.e., medium-chain acyl-CoA dehydrogenase deficiency) and pharmacological therapies (e.g., aspirin) increase renal acid excretion. Excretion of organic acids by the kidney is accompanied by increased excretion of ammonium ions which buffer the urine, resulting in an increase in the ratio of nitrogen excreted as ammonia relative to that excreted as urea.

19.6.4 Elevated BUN

Urea is synthesized in the liver and excreted primarily by the kidneys. Elevated BUN is an indication of a posthepatic failure in nitrogen excretion, the most common causes of which are impaired renal function and poor renal perfusion secondary to congestive heart failure or hypovolemic shock. Severe dehydration, with accompanied decreases in urinary output, may also result in an elevated BUN value.

19.6.5 Hypercatabolic States

Trauma, burns, and sepsis are characterized by increased fuel utilization and a negative nitrogen balance in which excretion of nitrogen—primarily as urea—exceeds dietary intake. These metabolic changes are mediated primarily by hydrocortisone, with contributions by inflammatory cytokines. In these hypermetabolic conditions there is an increased rate of protein degradation, primarily in skeletal muscle, which provides amino acids to support the biosynthetic needs associated with the immune response and wound healing. At the same time, there is increased synthesis and excretion of urea.

CHAPTER 20

AMINO ACIDS

20.1 FUNCTIONS OF AMINO ACID METABOLISM

Since 20 common amino acids, some with cyclic and branched structures, are utilized for protein synthesis, the synthesis and catabolism of amino acid carbon skeletons can be a complex and daunting subject with a myriad of details. Nonetheless, there are a number of common themes that are of major importance in understanding the overall metabolism of the body.

20.1.1 Synthesis of Amino Acids

Some of the 20 common amino acids can be synthesized in the body. The aminotransferase reactions that remove the amino group from most of these amino acids are readily reversible and can therefore be utilized to synthesize amino acids. For example, aspartate aminotransferase can be used to synthesize aspartate from the TCA-cycle intermediate oxaloacetate:

$$\text{oxaloacetate} + \text{glutamate} \rightleftharpoons \text{aspartate} + \alpha\text{-ketoglutarate}$$

Since the glutamate dehydrogenase reaction, too, is reversible, it can be used to incorporate NH_4^+ into α-ketoglutarate, generating glutamate. Glutamate, in turn, can donate its amino group for the synthesis of other amino acids:

$$\alpha\text{-ketoglutarate} + NH_4^+ + NADH^+ + H^+ \rightleftharpoons \text{glutamate} + NAD^+$$

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TABLE 20-1 Essential and Nonessential Amino Acids in Humans

Essential	Nonessential
Histidine	Alanine
Isoleucine	Arginine[a]
Leucine	Asparagine
Lysine	Aspartate
Methionine	Cysteine[b]
Phenylalanine	Glutamate
Threonine	Glutamine
Tryptophan	Glycine
Valine	Proline
	Serine
	Tyrosine[b]

[a]Essential in infants but not in adults.
[b]Conditionally essential. Synthesis of cysteine and tyrosine is dependent on adequate dietary intake of methionine and phenylalanine, respectively.

Thus, humans can synthesize a particular amino acid if they can synthesize its corresponding α-ketoacid carbon skeleton.

20.1.1.1 Essential Amino Acids. Certain amino acids, however, cannot be synthesized in the body; these are the essential amino acids and they must be obtained from the diet. The amino acids that are essential in adults are listed in Table 20-1. Two other amino acids, tyrosine and cysteine, can be synthesized from the essential amino acids phenylalanine and methionine, respectively. In addition, although arginine is not an essential amino acid in adults, its rate of synthesis in neonates is not adequate to meet their requirements for optimal growth. Exogenous arginine also becomes essential in cases of sepsis, when there is both a decrease in endogenous synthesis of arginine and an increased requirement of arginine for the synthesis of protein and nitric oxide.

20.1.1.2 Protein Quality. Protein synthesis requires that all 20 of the common amino acids be present, including the essential ones. Dietary proteins of both animal and vegetable origin usually provide all of the essential amino acids, but not necessarily in the proportions required to meet the body's needs for the synthesis of proteins and specialized nitrogen-containing molecules such as neurotransmitters (e.g., dopamine) and polyamines (e.g., spermine).

Nutritionists evaluate the quality of a particular dietary protein by comparing its amino acid composition to that of a reference protein which has the optimum proportions of all the essential amino acids. In general, animal proteins such as beef, fish, and egg white approximate those standards. Although vegetable proteins are sometimes referred to as "incomplete" proteins, they too usually contain all the essential amino acids, but not in the ideal proportions. For example, proteins in cereals such as wheat and rice are relatively deficient in lysine. The amino acid score of rice

protein is approximately 50, indicating that only half of the constituent amino acids can be utilized for protein synthesis before the supply of lysine is exhausted; the other 50% of the dietary amino acids must then be catabolized. By contrast, whereas the proteins of legumes such as peas and beans actually contain a higher level of lysine than the reference protein, they are relatively deficient in the sulfur-containing amino acids (methionine plus cysteine). However, when these two types of proteins are mixed as they would be in a meal of rice and beans, they complement each other to provide high-quality dietary protein. Improvement of the quality of vegetable proteins can also be achieved by including a small quantity of animal protein in the meal.

20.1.2 Glucogenic and Ketogenic Amino Acids

One function of amino acid catabolism is to provide their carbon skeletons as substrates for gluconeogenesis. Alanine and glutamine are major gluconeogenic substrates. In addition, the carbon skeletons of 11 of the other common amino acids are readily utilized for the synthesis of glucose. For example, catabolism of cysteine and serine yields pyruvate. The carbon skeletons of other glucogenic amino acids enter the TCA cycle; these include aspartate and asparagine, which are metabolized to oxaloacetate, and arginine and histidine, which are catabolized to α-ketoglutarate.

Two amino acids, lysine and leucine, cannot be utilized for glucose synthesis. Instead, the pathways that catabolize their carbon skeletons generate acetyl-CoA and acetoacetate. Since hepatocytes use acetyl-CoA to synthesize ketone bodies under fasting conditions, lysine and leucine are classified as ketogenic amino acids.

Five of the 20 common amino acids are both glucogenic and ketogenic, in that some of their carbon atoms can be utilized to produce glucose whereas the other carbon atoms generate ketogenic acetyl-CoA. These mixed glucogenic/ketogenic amino acids include tryptophan, isoleucine, threonine, phenylalanine, and tyrosine.

20.1.3 Amino Acids as Precursors of Neurotransmitters and Other Specialized Nitrogen-Containing Compounds

Amino acids provide nitrogen for the synthesis of purines, pyrimidines, nitrogen-containing phospholipids (e.g., phosphatidylcholine), and amino sugars (e.g., glucosamine). Tyrosine is the precursor of the catecholamine neurotransmitters (dopamine, norepinephrine, and epinephrine), the thyroid hormones (thyroxine and triiodothyronine), and the melanin pigments. Tryptophan is the precursor of serotonin, melatonin, and niacin. Other important amino acid–derived products include γ-aminobutyric acid, histamine, nitric oxide, and polyamines.

20.2 LOCALIZATION OF AMINO ACID METABOLISM

20.2.1 Synthesis of Nonessential Amino Acids

Although the liver is the major site of synthesis of nonessential amino acids, other tissues can synthesize and degrade amino acids. For example, the synthesis of arginine

for protein synthesis occurs by a two-step interorgan process: Glutamate is converted to ornithine and then to citrulline in the intestinal mucosa. The kidney then utilizes citrulline to synthesize arginine.

20.2.2 Synthesis of Conditionally Essential Amino Acids

The synthesis of tyrosine from the essential amino acid phenylalanine occurs exclusively in the liver. The transsulfuration pathway by which the sulfur atom of methionine is utilized to synthesize cysteine occurs primarily in liver, kidney, and intestinal mucosa.

20.2.3 Synthesis of Specialized Amino Acid–Related Products

Much of the synthesis of specialized metabolites of amino acids is localized to a few cell types. This is particularly true of neurotransmitters such as dopamine, norepinephrine, and serotonin, each of which is produced by specific groups of neurons. The adrenal medulla converts tyrosine to epinephrine, and the thyroid glands synthesize the thyroid hormones thyroxine and 3,5,3′-triiodothyronine from tyrosine.

20.2.4 Catabolism of Amino Acids

The liver is the major site for the catabolism of aromatic amino acid carbon skeletons (phenylalanine, tyrosine, and tryptophan) as well as for the synthesis of urea from amino acid nitrogen. By contrast, the initial transamination of the branched-chain amino acids leucine, isoleucine, and valine, and the subsequent oxidation of their carbon skeletons, occur primarily in skeletal muscle. As pointed out above, the carbon skeletons of alanine and glutamine are major substrates for gluconeogenesis in liver and kidney, respectively. Glutamine is a significant fuel for rapidly dividing cells such as lymphocytes, macrophage, and enterocytes, as well as for the kidney.

20.3 PHYSIOLOGICAL CONDITIONS DURING WHICH THE AMINO ACID CATABOLIC PATHWAYS ARE ESPECIALLY ACTIVE

There are two very different physiological conditions under which there is a high rate of catabolism of amino acids: following intake of a protein-rich diet and during fasting. In the fed state, there is active protein synthesis by muscle, liver, and other tissues; excess amino acids are oxidized for energy. In the fasting state, there is increased catabolism of muscle proteins. Most of the carbon skeletons of the branched-chain amino acids are utilized as fuel. The amino groups of the branched-chain amino acids are exported from muscle as alanine and glutamine, which are transported to the liver and kidney to provide substrates for gluconeogenesis.

20.4 PATHWAYS OF AMINO ACID METABOLISM

Human cells use a multiplicity of metabolic pathways to catabolize the 20 common amino acids and to synthesize the carbon skeletons of the nonessential amino acids. Rather than catalog all of these pathways, specific examples are cited to illustrate the types of reactions involved in amino acid metabolism.

20.4.1 Aminotransferase Reactions

Aminotransferases transfer the α-amino group from a donor amino acid to an acceptor α-ketoacid (see Fig. 19-3). This process initiates amino acid catabolism by transferring the α-amino group of various amino acids to α-ketoglutarate, thereby generating glutamate. Glutamate, in turn, serves as a nitrogen donor for urea synthesis. Transfer of the α-amino group of glutamate to various α-ketoacids can also be utilized to synthesize other nonessential amino acids (e.g., conversion of pyruvate to alanine or oxaloacetate to aspartate).

20.4.2 Synthesis of Glutamine

Glutamine is synthesized by the ATP-driven glutamine synthetase–catalyzed incorporation of amino acid–derived NH_4^+ into glutamate (Fig. 20-1):

$$\text{glutamate} + NH_4^+ + \text{ATP} \rightarrow \text{glutamine} + \text{ADP} + P_i$$

This reaction is irreversible. The opposite reaction, the hydrolytic removal of the amide group of glutamine, is catalyzed by glutaminase:

$$\text{glutamine} + H_2O \rightarrow \text{glutamate} + NH_4^+$$

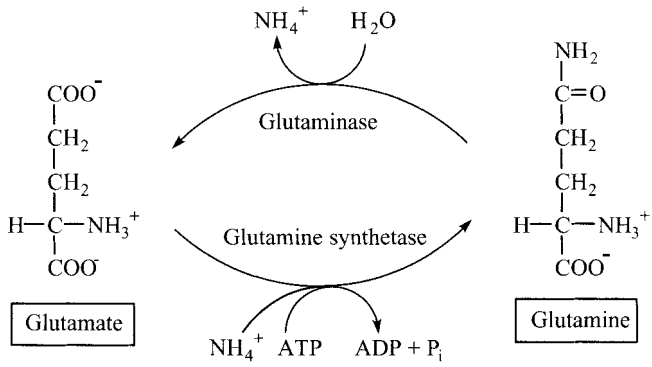

FIGURE 20-1 Synthesis and catabolism of glutamine.

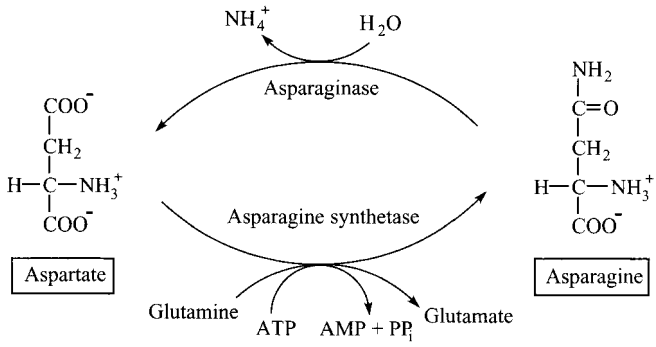

FIGURE 20-2 Synthesis and catabolism of asparagine.

20.4.3 Synthesis of Aspartate And Asparagine

An example of the process of endogenous amino acid synthesis is the synthesis of aspartate and its subsequent conversion to asparagine (Fig. 20-2). The precursors for this pathway are glucose, which is used to generate pyruvate, and amino acids, which donate the α-amino groups needed for glutamate synthesis. Pyruvate is first converted to oxaloacetate by pyruvate carboxylase:

$$\text{pyruvate} + CO_2 + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP}$$

Transamination of oxaloacetate by aspartate aminotransferase (AST) in turn generates aspartate:

$$\text{oxaloacetate} + \text{glutamate} \rightleftharpoons \text{aspartate} + \alpha\text{-ketoglutarate}$$

Finally, asparagine synthetase uses the amide nitrogen of glutamine to provide the amide nitrogen of asparagine:

$$\text{aspartate} + \text{glutamine} + \text{ATP} \rightarrow \text{asparagine} + \text{glutamate} + \text{AMP} + PP_i$$

20.4.4 Synthesis of Arginine and Proline

Arginine is synthesized from ornithine, which is generated from glutamate semialdehyde (see Fig. 9.8). Synthesis of glutamate semialdehyde by reduction of the γ-carboxyl group of glutamate to an aldehyde also serves as the initial step in the pathway of proline synthesis (Fig. 20-3):

$$\text{glutamate} + \text{ATP} + \text{NADPH} + H^+ \rightarrow \text{glutamate semialdehyde} + NADP^+ + \text{ADP} + P_i$$

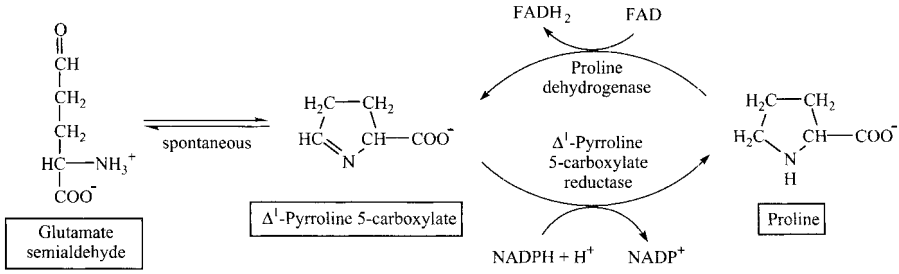

FIGURE 20-3 Synthesis and catabolism of proline.

The next step in the synthesis of proline is the spontaneous reversible cyclization of glutamate semialdehyde:

$$\text{glutamate semialdehyde} \rightleftharpoons \Delta^1\text{-pyrroline 5-carboxylate}$$

Δ^1-pyrroline 5-carboxylate is then reduced further to generate proline:

$$\Delta^1\text{-pyrroline 5-carboxylate} + \text{NADPH} + \text{H}^+ \rightarrow \text{proline} + \text{NADP}^+$$

Catabolism of proline involves essentially this same pathway, operating in reverse except that different enzymes catalyze the two oxidation steps. The enzyme that oxidizes proline to Δ^1-pyrroline 5-carboxylate uses FAD as a cofactor; the one that oxidizes glutamate semialdehyde to glutamate is NAD^+-dependent:

$$\text{proline} + \text{FAD} \rightarrow \Delta^1\text{-pyrroline 5-carboxylate} + \text{FADH}_2$$

$$\Delta^1\text{-pyrroline 5-carboxylate} \rightleftharpoons \text{glutamate semialdehyde}$$

$$\text{glutamate semialdehyde} + \text{NAD}^+ \rightarrow \text{glutamate} + \text{NADH} + \text{H}^+$$

20.4.5 Oxidation of Branched-Chain Amino Acids

The pathways by which the carbon skeletons of the three branched-chain amino acids—leucine, isoleucine, and valine—are oxidized nicely illustrate the general principles of amino acid catabolism and the ultimate metabolic fate of ketogenic and glucogenic amino acids (Fig. 20-4).

The first step in the catabolism of the branched-chain amino acids is the transfer of the α-amino group of each to α-ketoglutarate, generating the corresponding branched-chain α-ketoacid. Thus, for leucine,

$$\text{leucine} + \alpha\text{-ketoglutarate} \rightleftharpoons \alpha\text{-ketoisocaproate} + \text{glutamate}$$

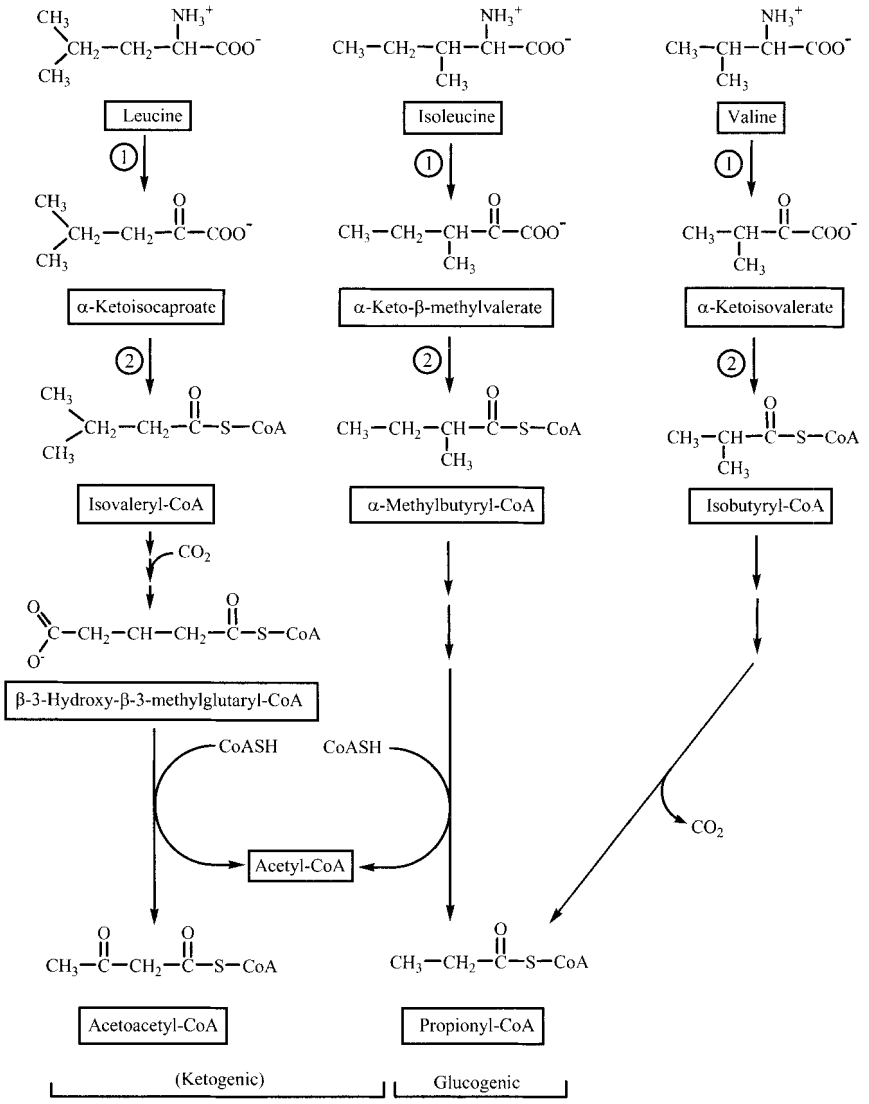

FIGURE 20-4 Catabolism of branched-chain amino acids. The enzymes common to catabolism of all three amino acids are ① branched-chain amino acid transaminase and ② α-ketoacid dehydrogenase.

The next step in the catabolism of the three branched-chain amino acids is an oxidative decarboxylation reaction catalyzed by the branched-chain α-ketoacid dehydrogenase complex. For example, α-ketoisocaproate is oxidized and decarboxylated to form isovaleryl-CoA:

$$\alpha\text{-ketoisocaproate} + NAD^+ + CoASH \rightarrow \text{isovaleryl-CoA} + NADH + H^+ + CO_2$$

This enzyme complex is similar to the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes in that it contains three catalytic components: branched-chain α-ketoacid decarboxylase, dihydrolipoamide branched-chain transacylase, and dihydrolipoamide dehydrogenase. Like the other α-ketoacid dehydrogenases, this enzyme complex catalyzes an irreversible reaction; it thus represents the committed step in the catabolism of branched-chain amino acids. Branched-chain α-ketoacid decarboxylase is similar to the other α-ketoacid dehydrogenases in that the E1 enzyme component requires thiamine pyrophosphate as a cofactor.

The subsequent steps in the oxidation of the three products of the branched-chain α-ketoacid decarboxylase reaction—isovaleryl-CoA (from leucine), α-methylbutyryl-CoA (from isoleucine), and isobutyryl-CoA (from valine)—are outlined in Figure 20-4. Although the specific details about the respective intermediates are beyond the scope of this book, the nature of the end products of the three branched-chain amino acids is illustrative of the ultimate metabolic fates of their amino acid carbon skeletons.

20.4.5.1 Leucine Is Ketogenic. Further catabolism of isovaleryl-CoA in mitochondria generates β-hydroxy-β-methylglutaryl-CoA (HMG-CoA). Mitochondrial HMG-CoA is an intermediate in the synthesis of ketone bodies; specifically, it is hydrolyzed by HMG-CoA lyase to yield acetoacetate:

$$\beta\text{-hydroxy-}\beta\text{-methylglutaryl-CoA} \rightarrow \text{acetoacetate} + \text{acetyl-CoA}$$

In peripheral tissues such as muscle, acetoacetate can be activated to acetoacetyl-CoA and then cleaved with the addition of a second molecule of CoASH to produce two molecules of acetyl-CoA. Since the carbon atoms of acetyl-CoA cannot be utilized for glucose synthesis, leucine is classified as a ketogenic rather than a glucogenic or mixed-type (glucogenic and ketogenic) amino acid.

20.4.5.2 Valine Is Glucogenic. The successive transamination and decarboxylation of valine generates isobutyryl-CoA. Subsequent oxidation of isobutyryl-CoA results in release of a second molecule of CO_2 and the generation of propionyl-CoA. As discussed in Chapter 10, the metabolism of propionyl-CoA involves carboxylation to methylmalonyl-CoA, which, in turn, is converted to succinyl-CoA. Since succinyl-CoA is a TCA-cycle intermediate that can be further metabolized to oxaloacetate, succinyl-CoA can be used to synthesize glucose. Valine is therefore a glucogenic amino acid.

20.4.5.3 Isoleucine Is Both Ketogenic and Glucogenic. Amino transfer and decarboxylation of isoleucine generate α-methylbutyryl-CoA, which is then oxidized to α-methylacetoacetyl-CoA and finally cleaved to produce propionyl-CoA plus acetyl-CoA:

$$\alpha\text{-methylacetoacetyl-CoA} + \text{CoA} \rightarrow \text{propionyl-CoA} + \text{acetyl-CoA}$$

As in the case of valine catabolism, the propionyl-CoA moiety can be used to synthesize glucose. By contrast, the acetyl-CoA moiety cannot be used for gluconeogenesis. The metabolic fate of isoleucine is thus mixed, being part glucogenic and part ketogenic.

20.4.6 Products of Phenylalanine Metabolism

20.4.6.1 Synthesis of Tyrosine from Phenylalanine. Phenylalanine, an essential amino acid, is the immediate precursor of tyrosine (Fig. 20-5). The reaction is catalyzed by phenylalanine hydroxylase with tetrahydrobiopterin (BH_4) as cofactor:

$$\text{phenylalanine} + O_2 + BH_4 \rightarrow \text{tyrosine} + BH_2 + H_2O$$

This pathway provides tyrosine for both protein synthesis and the synthesis of catecholamines and thyroid hormones. It is also the major pathway for the catabolism of excess phenylalanine.

The liver also contains a phenylalanine-specific aminotransferase:

$$\text{phenylalanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{phenylpyruvate} + \text{glutamate}$$

Phenylalanine aminotransferase requires a higher concentration of phenylalanine for activity than does phenylalanine hydroxylase and is therefore unable to prevent accumulation of deleterious levels of phenylalanine in people who are phenylalanine hydroxylase–deficient. Furthermore, the aromatic metabolites of phenylalanine represent a metabolic dead-end.

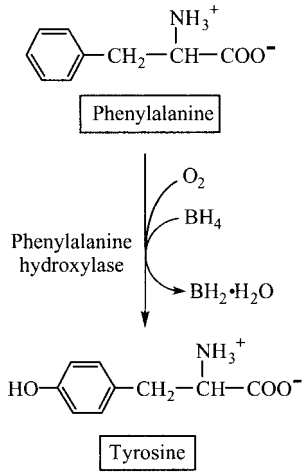

FIGURE 20-5 Synthesis of tyrosine from phenylalanine.

Tetrahydrobiopterin (BH_4) is a Cofactor for the Hydroxylation of Aromatic Amino Acids. Unlike so many of the other cofactors in intermediary metabolism, tetrahydrobiopterin, the cofactor for phenylalanine hydroxylase, is not a vitamin. Instead, BH_4 is synthesized from GTP (Fig. 20-6).

Recycling of Tetrahydrobiopterin. Phenylalanine hydroxylase is a mixed-function oxidase, which simultaneously oxidizes phenylalanine and removes two hydrogen atoms from tetrahydrobiopterin. The resulting pterin 4α-carbinolamine is then recycled to tetrahydrobiopterin, with NADPH serving as the reductant (Fig. 20-6):

$$\text{pterin 4}\alpha\text{-carbinolamine} \rightarrow \text{dihydrobiopterin} + H_2O$$

$$\text{dihydrobiopterin} + \text{NADPH} + H^+ \rightarrow \text{tetrahydrobiopterin} + \text{NADP}^+$$

20.4.6.2 Synthesis of Catecholamines. Dopamine and norepinephrine are neurotransmitters synthesized in the brain. Dopamine, norepinephrine, and epinephrine, collectively called *catecholamines*, have two hydroxyl groups on the aromatic or phenolic ring (catechol is *o*-dihydroxybenzene). The catecholamines are synthesized through a common pathway that starts with tyrosine (Fig. 20-7). The complete pathway, which generates epinephrine, occurs primarily in the adrenal gland.

Synthesis of DOPA (3.4-Dihydroxyphenylalanine). The first step in the synthesis of the catecholamines is the hydroxylation of tyrosine by tyrosine hydroxylase, which like phenylalanine hydroxylase is a mixed-function oxidase that also reduces BH_4:

$$\text{tyrosine} + O_2 + BH_4 \rightarrow \text{dihydroxyphenylalanine} + BH_2 + H_2O$$

Synthesis of Dopamine. Dopamine, the prominent neurotransmitter in the substantia nigra and several other parts of the brain, is synthesized by decarboxylation of DOPA. DOPA decarboxylase, like many other amino acid decarboxylases, utilizes pyridoxal phosphate (PLP) as a cofactor:

$$\text{dihydroxyphenylalanine} \rightarrow \text{dopamine} + CO_2$$

Norepinephrine. Norepinephrine is synthesized by oxidizing dopamine. Unlike the reactions catalyzed by phenylalanine hydroxylase and tyrosine hydroxylase, dopamine β-hydroxylase oxidizes the side chain rather than the phenyl ring and utilizes ascorbic acid rather than tetrahydrobiopterin as the cofactor and reducing agent:

$$\text{dopamine} + O_2 + \text{ascorbate} \rightarrow \text{norepinephrine} + \text{dehydroascorbate} + H_2O$$

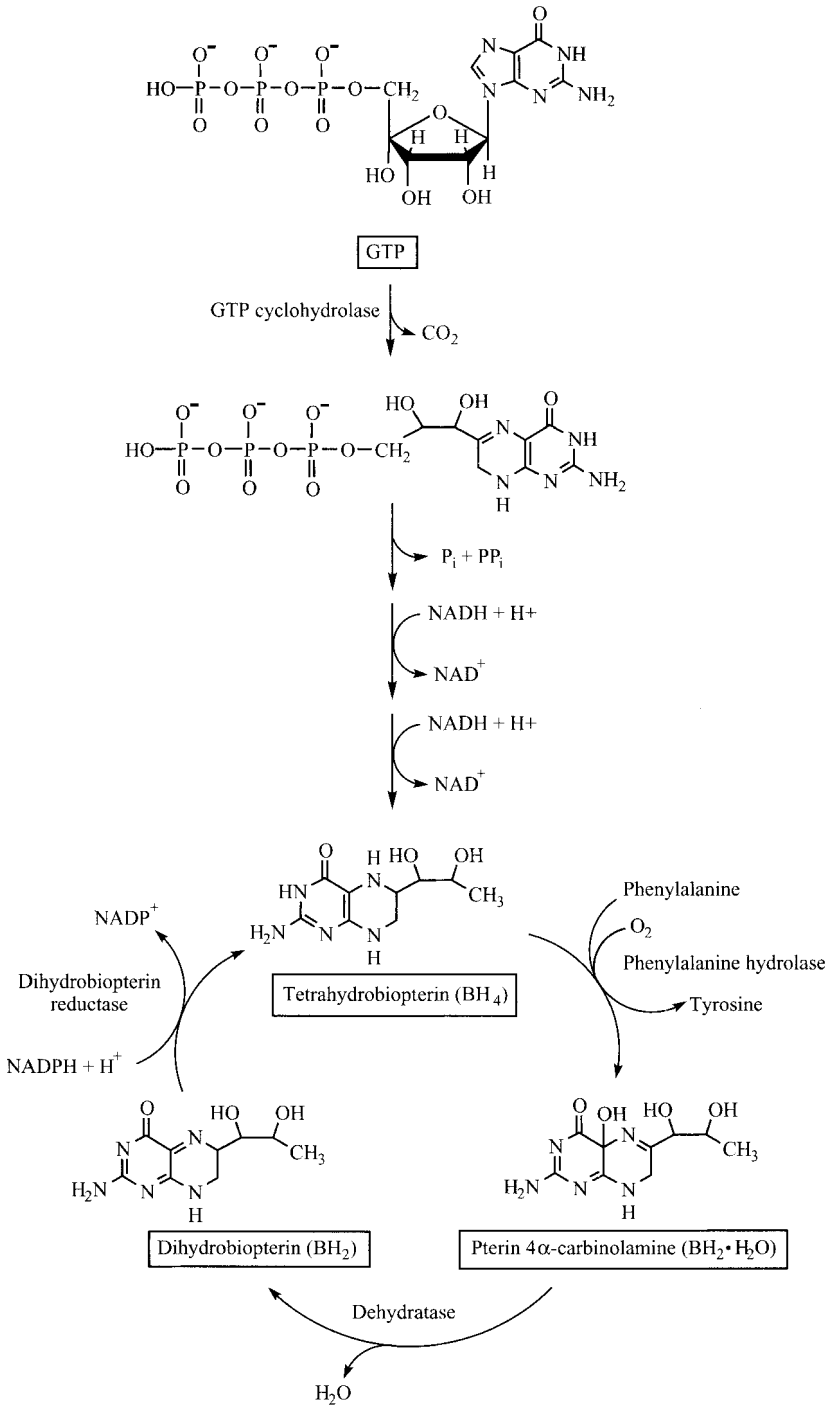

FIGURE 20-6 Synthesis and recycling of the cofactor tetrahydrobiopterin.

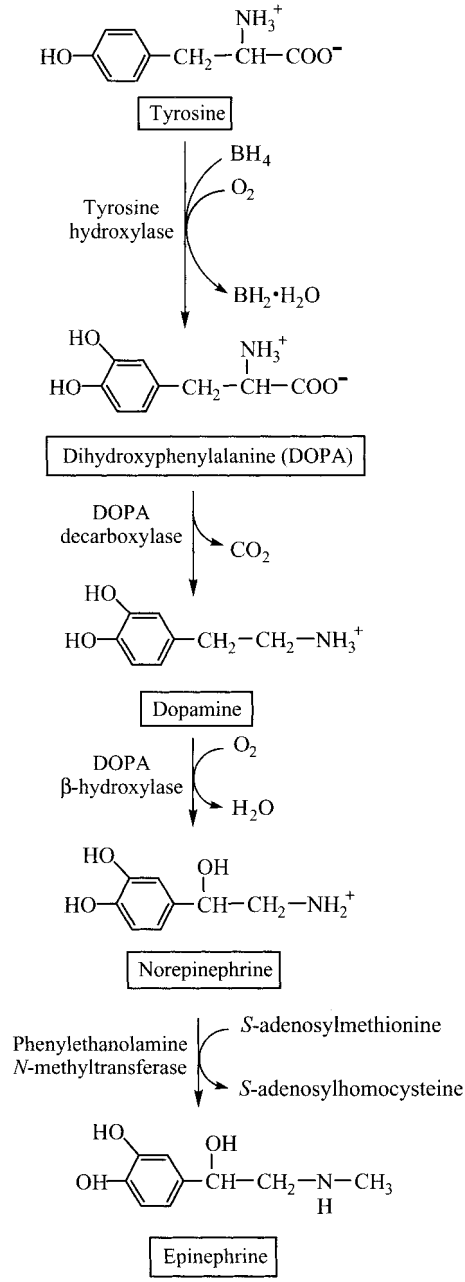

FIGURE 20-7 Synthesis of catecholamines.