Lipids II: Phospholipids, Glycosphingolipids, and Cholesterol

Phospholipids and glycosphingolipids are amphipathic lipid constituents of membranes (Chapter 10). They play an essential role in the synthesis of plasma lipoproteins (Chapter 20) and eicosanoids (Chapter 18). They function in transduction of messages from cell surface receptors to second messengers that control cellular processes (Chapter 30) and as surfactants. Cholesterol is mainly of animal origin and is an essential constituent of biomembranes (Chapter 10). In plasma, cholesterol is associated with lipoproteins (Chapter 20). Cholesterol is a precursor of bile acids formed in the liver; of steroid hormones secreted by adrenals, gonads, and placenta; and 7-dehydrocholesterol of vitamin D formed in the skin. In tissues, cholesterol exists primarily in the unesterified form (e.g., brain and erythrocytes), although appreciable quantities are esterified with fatty acids in liver, skin, adrenal cortex, and plasma lipoproteins.

19.1 Phospholipids

Phospholipids can be glycerolipids or sphingolipids. Examples of glycerolipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and phosphatidyglycerol (Figure 19-1). To distinguish between the two primary alcohol-carbon atoms of asymmetrically substituted glycerol derivatives, the glycerol carbon atoms are numbered 1 through 3 from top to bottom and the C₂ hydroxyl group is written to the left. This system is known as the stereochemical numbering (sn) convention. Thus, the structural formula for sn-1, 2-diacylglycerol is

Phosphatidylcholines

Phosphatidylcholines, or lecithins, are zwitterionic over a wide pH range because of the presence of a quaternary ammonium group and a phosphate moiety. Phosphatidylcholines are the most abundant phospholipids in animal tissues and typically contain palmitic, stearic, oleic, linoleic, or arachidonic acid, usually with saturated fatty acids in the sn-1 position and unsaturated fatty acids at sn-2.

The de novo pathways for phospholipid synthesis use cytidine triphosphate (CTP) for activation of intermediate species (analogous to the role of UTP in glycogen biosynthesis; Chapter 15). The principal pathway of
Lipids I: Phospholipids, Glycosphingolipids, and Cholesterol

**FIGURE 19-1**
Structure of some glycerophospholipids.

Phosphatidylcholine biosynthesis uses cytidine diphosphate (CDP) choline (Figure 19-2). Many reactions of phospholipid synthesis occur in the endoplasmic reticulum. Choline is first phosphorylated by ATP to phosphocholine, which reacts with CTP to form CDP-choline, from which phosphocholine is transferred to sn-1,2-diacylglycerol. The rate-limiting step in this pathway appears to be that catalyzed by CTP:phosphocholine cytidylyltransferase, which is activated by fatty acids.

Phosphatidylcholine can also be synthesized by the methylation pathway that converts phosphatidylethanolamine to phosphatidylcholine, principally in the liver. The methyl donor is S-adenosylmethionine (Chapter 17). Phosphatidylethanolamine-N-methyltransferase transfers three methyl groups in sequence to produce phosphatidylcholine. The fatty acid components of phosphatidylcholine can then be altered by deacylation-reacylation reactions.

Phosphatidylcholine is degraded by phospholipases that cleave preferentially at specific bonds (Chapter 18). Choline released is phosphorylated by choline kinase and reutilized in phosphatidylcholine synthesis. However, in liver mitochondria, choline is also oxidized to betaine (N-trimethylglycine):

Betaine functions as a methyl donor (e.g., in methionine biosynthesis from homocysteine; Chapter 17), and it can also be converted to glycine.

**Other Glycerophospholipids**

*Phosphatidylethanolamines*, or cephalins (so-called because they were first obtained from brain tissue), can be synthesized by reactions analogous to those of *de novo* synthesis of phosphatidylcholine. Ethanolamine is first phosphorylated by ATP and ethanolamine kinase to phosphoethanolamine, which then reacts with CTP to form CDP-ethanolamine. CTP:phosphoethanolamine cytidylyltransferase is not located on the endoplasmic reticulum, nor do fatty acids activate it as they do the analogous enzyme of phosphatidylcholine synthesis. Finally, 1,2-diacylglycerol phosphoethanolamine transferase catalyses the reaction of diacylglycerol with CDP-ethanolamine to form phosphatidylethanolamine.
FIGURE 19-2
Synthesis of phosphatidylcholine. The rate-limiting reaction is that catalyzed by cytidylyltransferase (reaction 2) which appears to be active only when attached to the endoplasmic reticulum, although it is also found free in the cytosol. Cytidylyltransferase is inactivated by a cAMP-dependent protein kinase and activated by a phosphatase. Translocation to the endoplasmic reticulum can be stimulated by substrates such as fatty acyl Coenzyme A (CoA). Choline deficiency can result in deposition of triacylglycerol in liver and reduced phospholipid synthesis. Enzymes: (1) choline kinase; (2) CTP:phosphocholine cytidylyltransferase; (3) glycerol kinase; (4) acyl-CoA:glycerol-3-phosphate acyltransferase; (5) acyl-CoA: acyl glycerol-3-phosphate acyltransferase; (6) phosphatidic acid phosphatase; (7) CDP-choline: diacylglycerol phosphocholine transferase.
Phosphatidylethanolamines can also be synthesized by decarboxylation of phosphatidylserine and in mammals principally through action of the Ca\(^{2+}\)-mediated base exchange enzyme (Figure 19-3). Phosphatidylserine production in liver occurs at the cytosolic face of the endoplasmic reticulum. In brain tissue, this phospholipid accounts for up to 15% of the total phospholipid content.

Phosphatidylinositols and phosphatidylglycerols are synthesized via CDP-diacylglycerol (Figure 19-4). Phosphatidylinositols are enriched with arachidonic acids by deacylation and reacylation. Two other phosphoinositides are synthesized and degraded in the phosphoinositol cycle (Chapter 30) by the enzymes on the plasma membrane, endoplasmic reticulum, and cytosol of brain and liver cells. These anionic phospholipids contain myo-inositol, which can be derived from food or produced via cyclization of glucose-6-phosphate. Phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate are derived from phosphatidylinositol by ATP-dependent phosphorylation of the 4- and 5-positions of the inositol ring. Dephosphorylation reactions are carried out by phosphomonoesterases. The exact proportion of the three forms in cell membranes is unknown, although the

**FIGURE 19-3**
Biosynthesis of phosphatidylethanolamine from phosphatidylserine. The base-exchange enzyme on the cytosolic face of the endoplasmic reticulum can interconvert these phospholipids in the presence of Ca\(^{2+}\) and the alternate head group, serine or ethanolamine. The decarboxylase is localized in the inner membrane of mitochondria and catalyzes the nonequilibrium conversion of phosphatidylserine to phosphatidylethanolamine.

**FIGURE 19-4**
Biosynthesis of phosphatidylinositols and phosphatidylglycerols. Enzymes: (1) glycerol kinase; (2) acyltransferases; (3) phosphatidate cytidylyltransferase; (4) CDP-diacylglycerol: inositol phosphatidate transferase; (5) CDP-diacylglycerol: sn-glycerol-3-phosphate phosphatidate transferase; (6) phosphatase; (7) phosphatidyglycerol: CDP-diacylglycerol phosphatidate transferase.
phosphatidylinositol content greatly exceeds that of the other two. The activity of a number of hormones, growth factors, and neurotransmitters depends upon hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol triphosphate and diacylglycerol, which serve to initiate parallel metabolic cascades that can mobilize intracellular calcium stores, activate protein kinase C and release arachidonic acid. Each of these events in turn can regulate a number of cellular processes (Chapter 30).

The transfer of phosphatidic acid from CDP-diacylglycerol to phosphatidylglycerol yields diphosphatidylglycerol, or cardiolipin (Figure 19-4), which is found in highest concentration in the inner membranes of mitochondria of cardiac muscle. Cardiolipin isolated from beef heart is used as an antigen in serological flocculation and precipitation tests for syphilis, a sexually transmitted disease caused by Treponema pallidum. Anticardiolipin antibodies are formed in response to lipoidal material released from damaged host cells early in the infection and that present on the cell surface of the treponeme. This test is nonspecific. In a specific test for syphilis, antigen derived from T. pallidum itself is used to detect the presence of antibodies specific to it.

In the synthesis of phosphatidic acid, the starting material may be glycerol, or dihydroxyacetone phosphate, a product of aldolase action on fructose-1,6-bisphosphate (Chapter 13). Analogous reactions are used in the synthesis of plasmalogen, which, like phosphoglycerides, have the common glycerol backbone. However, at the C₁ position, an α,β-unsaturated fatty ether is present rather than a fatty acid ester:

The α,β-unsaturated fatty ether is an aldehydogenic group because its hydrolysis releases an α,β-unsaturated primary alcohol that readily tautomerizes to an aldehyde. Choline, ethanolamine, and serine plasmalogens are found in cardiac and skeletal muscle, brain, and liver. The biosynthesis of phosphatidylethanolamine is shown in Figure 19-5.

**FIGURE 19-5**
Biosynthesis of ethanolamine plasmalogen. Enzymes: (1) acyltransferase; (2) synthase; (3) oxidoreductase; (4) acyltransferase; (5) phosphatase; (6) transferase; (7) Δ¹-alkyl desaturase.
Synthesis of phosphatidylcholine can occur by base-exchange reaction, by methylation of phosphatidylethanolamine, or by the coupled action of phospholipase and CDP-choline:cholinephosphotransferase on phosphatidylethanolamine. Although the function of most ether-containing phosphoglycerides is unknown, a "platelet-activating factor" (PAF) that promotes platelet aggregation has the following structure:

$$\text{H}_2\text{COR}$$

$$\text{H}_2\text{C}=\text{C}-\text{O}-\text{CH}_{\text{II}}$$

$$\text{H}_2\text{C}=\text{O}-\text{P}-\text{O}-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)$$

1-Alkyl-2-acetyl-sn-glyceryl-3-phosphocholine

PAF released from IgE-sensitized basophilic leukocytes (and probably other mononuclear cells, such as mast cells) in response to antigen stimulation causes aggregation of platelets and release of their granular constituents (e.g., serotonin). This action of PAF is potent and rivals that of thromboxane A2 (Chapter 18). PAF also is a potent anti-hypertensive agent when given intravenously to hypertensive rats. It is inactivated by deacetylation by a specific acetylhydrolase.

Phosphosphingolipids

The sphingomyelins are structurally similar to phosphatidylcholine but contain N-acylsphingosine (ceramide) instead of sn-1,2-diacylglycerol (Figure 19-6). They occur in high concentration in myelin and in the brain and are a nearly ubiquitous constituent of membranes.

Sphingolipid biosynthesis is catalyzed by membrane-bound enzymes of the endoplasmic reticulum. Sphingosine, an acylaminoalcohol, is synthesized from palmitoyl-CoA and serine in a reaction that requires pyridoxal phosphate, NADPH, and Mn$^{2+}$ (Figure 19-7). The exact pathway of ceramide synthesis is not known. The acyl group may be added to the 2-amino group of sphinganine, either as a fatty acid (the reverse of hydrolysis of ceramide) or from an acyl-CoA by an acyltransferase. In such a pathway, the double bond in the aliphatic chain would be inserted after the acylation step. Direct transfer of an acyl group to sphingosine is an alternative pathway of ceramide synthesis. The acyl groups of sphingolipids may be those of long-chain fatty acids synthesized by the fatty acid synthase complex (e.g., palmitic or stearic), very-long-chain fatty acids synthesized by the microsomal chain elongation system (e.g., behenic, lignoceric), monoenoic fatty acids (e.g., oleic, nervonic), or $\alpha$-hydroxy very-long-chain fatty acids. The presence of transferases specific for different chain lengths determines the acyl composition of sphingolipids in a given tissue.

Sphingomyelin is probably synthesized by an exchange reaction in which the phosphorylcholine moiety of phosphatidylcholine is transferred to ceramide:

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

Synthesis of glycosphingolipids and sulfoglycosphingolipids involves the addition of sugar and sulfate residues to ceramide from UDP-sugar derivatives or the activated sulfate donor 3'-phosphoadenosine-5'-phosphosulfate (Chapter 17), and appropriate transferases. These pathways are discussed in Chapter 16. Catabolism of sphingolipids is by specific lysosomal hydrolases. Several inherited disorders associated with the deficiencies of these enzymes are discussed below.

19.2 Phospholipids and Glycosphingolipids in Clinical Medicine

Pulmonary Surfactant Metabolism and Respiratory Distress Syndrome

Pulmonary surfactant is a complex of lipids and proteins with unique surface active properties that is synthesized exclusively in alveolar type II cells. The composition of surfactant is 90% lipids and 5–10% surfactant-specific proteins. The lipid component is made up of dipalmitoylphosphatidylcholine (also called lecithin, 70–80%) and another major phospholipid, phosphatidylglycerol (PG, 10%). The remainder of the phospholipids of surfactant are phosphatidylinositol (PI), phosphatidylethanolamine (PE), and phosphatidylserine (PS). Immature surfactant contains higher amounts of PI compared to PG. Thus, a low ratio of PG to PI indicates lung immaturity. Cholesterol, a neutral lipid, is also a constituent of the lipid component of surfactant.
FIGURE 19-7
Biosynthesis of sphingosine and ceramide.

After synthesis in the various compartments of endoplasmic reticulum of alveolar type II cells, surfactant components are assembled in the cytosol into lamellar bodies. In the process of formation of lamellar bodies, the transfer of phospholipids between membranes is facilitated by phospholipid transfer proteins, which are nonenzymatic proteins found in all eukaryotic cells and which play an important role in lipid metabolism. There are three well-characterized phospholipid transfer proteins:

1. PC-specific transfer protein.
2. PI- and PC-specific transfer protein.
3. Phospholipids and cholesterol-nonspecific lipid transfer protein (also known as sterol carrier protein).

All three of these proteins are present in the lung. The lamellar bodies are secreted into alveolar lumen where they are transformed into an extracellular form of surfactant that has a quadratic lattice structure called tubular myelin. The three-dimensional tubulin-myelin structures spread in a monolayer at the air-liquid interface. This spreading decreases the surface tension, prevents alveolar collapse at the end of expiration, and confers mechanical stability to the alveoli. The surfactant system is in a continuous state of flux, and surfactant is recycled by uptake.
and endocytosis in alveolar type II cells. Thus, the type II cells are involved in both the synthesis and the recycling of surfactant.

The phospholipids are mainly synthesized starting from glycerol 3-phosphate which is derived from glucose. The CDP-choline pathway is utilized in the synthesis of phosphatidylcholine or lecithin (Figure 19-2). The protein component of surfactant is lung-specific and consists of four proteins designated SP-A, SP-B, SP-C, and SP-D. These surfactant proteins perform important functions that lead to a reduction in alveolar surface tension during respiration. These include structural transformation of lamellar body to tubular myelin (SP-A and SP-B in the presence of Ca$^{2+}$), enhancement of surface-tension lowering properties and promotion of adsorption of surfactant phospholipids at the air-liquid interface (SP-B and SP-C), reuptake by endocytosis of surfactant by type II cells, and the activation of alveolar macrophages to facilitate surfactant clearance. Both SP-A and SP-D possess antimicrobial properties. SP-A is chemotactic for macrophages and promotes bacterial phagocytosis.

The most abundant surfactant protein is a water-soluble asialoglycoprotein that is a multimer consisting of six triple helical structures. The primary structure of SP-A is highly conserved among several species. It has two domains: the N terminus is collagen-like with Gly-X-Y repeats (where Y is frequently a prolyl residue), and the C terminus has lectin-like properties. SP-B and SP-C are highly hydrophobic proteins. SP-D is a glycoprotein and has a structure similar to SP-A. The importance of one surfactant protein is demonstrated in neonates who are born with an inherited deficiency of SP-B. Infants with a SP-B deficiency require ventilatory support and extracorporeal membrane oxygenation. However, almost all die during the first year of life due to progressive respiratory failure. Lung transplantation is the only therapy by which the SP-B-deficient infants can be saved from death.

Surfactant biosynthesis is developmentally regulated. The capacity for the fetal lung to synthesize surfactant occurs relatively late in gestation. Although the type II cells are identifiable at 20–22 weeks of gestation, the secretion of surfactant into the amniotic fluid occurs during 30–32 weeks of gestation. Thus, for the infant a consequence of prematurity is respiratory distress syndrome (RDS), which is a leading cause of neonatal morbidity and mortality in developed countries. The synthesis of surfactants is regulated by factors that include glucocorticoids, thyroid hormones, prolactin, estrogens, androgens, catecholamine (functioning through $\beta$-adrenergic receptors and cAMP), growth factors, and cytokines. Glucocorticoids stimulate lung maturation, thus glucocorticoid therapy in women in preterm labor prior to 34 weeks of gestation can significantly decrease the incidence of RDS in the premature neonates.

Thyroid hormones also accelerate fetal lung maturation. Fetal thyroid hormone levels may be increased by antenatal administration of thyrotropin-releasing hormone (TRH), a tripeptide that crosses the placental barrier, stimulates fetal pituitary production of thyroid stimulating hormone (TSH), and which, in turn, increases fetal thyroid hormone production (Chapter 33). This indirect method of enhancement of fetal thyroid hormone production is utilized because thyroid hormones do not readily cross the placental barrier. Insulin delays surfactant synthesis and so fetal hyperinsulinemia in diabetic mothers may increase the incidence of RDS even in the full-term infant. Androgen synthesized in the fetal testis is the probable cause of a slower onset of surfactant production in male fetuses. Prophylactic, or after onset of RDS, administration of synthetic or natural pulmonary surfactants intratracheally to preterm infants improves oxygenation and decreases pulmonary morbidity.

In adults, a severe form of lung injury can develop in association with sepsis, pneumonia, and injury to the lungs due to trauma or surgery. This catastrophic disorder is known as acute respiratory distress syndrome (ARDS) and has a mortality rate of more than 40%. In ARDS, one of the major problems is a massive influx of activated neutrophils which damage both vascular endothelium and alveolar epithelium and result in massive pulmonary edema and impairment of surfactant function. Neutrophil proteinases (e.g., elastase) break down surfactant proteins. A potential therapeutic strategy in ARDS involves administration of both surfactant and antiproteinases (e.g., recombinant $\alpha_{1}$-antitrypsin).

Biochemical Determinants of Fetal Lung Maturity

The need for surfactant production does not become essential until birth because no air-liquid interface exists in the alveoli in utero, and fetal oxygen needs are met by maternal circulation. The pulmonary system, including surfactant production, is among the last of the fetal organ systems to attain functional maturity. Since preterm birth is associated with significant neonatal morbidity and mortality in inadequate oxygen supply to an immature pulmonary lung system, the assessment of antenatal fetal lung maturity is necessary to develop a therapeutic strategy in the management of a preterm infant. The biochemical determinants are measured primarily in the amniotic fluid obtained by amniocentesis.

In a normal pregnancy, the lung is adequately developed by about the 36th or 37th week. Biochemical changes occurring during this period of gestation can be used to
evaluate fetal lung maturity when early delivery is planned. One such measurement is that of lecithin to sphingomyelin (L/S) ratio in amniotic fluid. In a normal pregnancy, the L/S ratio is less than 1 before the 31st week, rises to about 2 by the 34th week, to about 4 at the 36th week, and to about 8 at term (39 weeks). The change is due to an increase in lecithin synthesis rather than a decrease in synthesis of sphingomyelin. These values vary in normal gestations and in abnormal pregnancies (due to maternal, fetal, or placental disorders), the ratio may be elevated or reduced without regard to gestational age. A low L/S ratio is not inevitably associated with RDS. While an L/S ratio greater than 2 is associated with the absence of serious RDS, one lower than 2 is not uniformly predictive of the development of RDS.

Pulmonary surfactant in amniotic fluid can also be measured by its ability to generate foam stable in the presence of ethanol. This foam stability test (FST), or shake test, correlates well with the L/S ratio and with fetal lung maturity. In some instances, in the presence of an L/S ratio of less than 2, the FST has indicated lung maturity (without subsequent respiratory distress). This discrepancy may be due to the presence of surfactants other than lecithin that stabilize the neonatal alveoli, namely, phosphatidylglycerol (PG) and phosphatidylinositol (PI). These acidic phospholipids are synthesized in stepwise fashion during the last trimester of normal pregnancy.

The test for PG employs thin-layer chromatographic separation or a slide agglutination test using an antisera specific for PG. The advantage of PG measurement is that its value is not altered by the blood, meconium, vaginal secretions, or other contaminants, whereas the L/S is altered by the same contaminants. However, a disadvantage of PG determination in the assessment of fetal lung maturity is its late appearance (after 35 weeks of gestation) during pregnancy. Other amniotic fluid tests of lung maturity include measurement of lamellar bodies, either by measuring optical absorbance at 650 nm or by actual counting by procedures using standard hematological counters. An optical density of 0.15 or greater and a lamellar body count of 30,000–50,000/μL indicate pulmonary maturity. A fluorescent polarization technique which consists of competitive binding of a fluorescent probe to albumin and surfactant is employed in the assessment of fetal lung maturity. The net polarization for the albumin bound probe yields a high value, whereas a surfactant-bound probe yields a low value. A value of 55 mg of surfactant or greater per gram of albumin indicates maturity. In unanticipated premature births, the risk of RDS can be assessed by measurement of surfactant in gastric aspirates from the newborn, since the newborn swallows amniotic fluid in utero.

A number of factors (such as hypoxia and acidosis) depress phospholipid synthesis, and administration of glucocorticoids to mothers accelerates the rate of fetal lung maturation. The fetal lung undergoes an abrupt transition from a PO2 of about 20 mm Hg to a PO2 of 100 mm Hg. This change from a hypoxic to a relatively hyperoxic condition may lead to increased production of potentially cytotoxic O2 metabolites such as superoxide radical (O2·−), hydrogen peroxide (H2O2), hydroxyl radical (OH·), singlet oxygen (1O2), and peroxide radical (ROO·). The antioxidant enzyme system consists of superoxide dismutase, glutathione peroxidase, and catalase (see Chapter 14). In addition to these enzymes, other potential antioxidants are vitamin E, ascorbate, β-carotene, and thiol compounds (e.g., glutathione, cysteine). Infants born immaturely are particularly susceptible to deficiency of both surfactant and antioxidant defense. Administration of surfactant and the antioxidant enzymes using liposome technology has potential application in the management of RDS. Administration of surfactant to the lungs of very premature infants through an endotracheal tube has reduced morbidity and mortality from RDS.

Catabolism and Storage Disorders of Sphingolipids

There are four groups of glycosphingolipids: cerebrosides, sulfatides, globosides, and gangliosides. Cerebrosides contain a single sugar residue linked to ceramide, which is an N-acylsphingosine. Sulfatides contain a sulfate group attached to sugar residue. Globosides contain two or more sugar residues and an N-acetylgalactosamine group linked to ceramide. Gangliosides (G) contain oligosaccharide chains that contain sialic acid residues. They are classified based upon the number of sialic acid (N-acetylneuraminic acid, NANA) residues they contain and the sequence of sugar residues. Gm, Gp, GT and GQ contain gangliosides with one, two, three, and four sialic acid residues, respectively. The number associated with M, D, T, and Q signifies the sequence of sugar residues:

3. Represents Gal–Glc–ceramide. Thus, the structure of ganglioside Gm1, is:

\[
\text{Gal} \rightarrow \text{NacGal} \rightarrow \text{Gal} \rightarrow \text{Glc} \rightarrow \text{Ceramide}
\]

\[
\text{NANA} \quad \text{(sialic acid)}
\]

Sphingolipids are in a continuous state of turnover. They are catabolized by lysosomal enzymes by stepwise removal of sugar residues beginning at the nonreducing end.
<table>
<thead>
<tr>
<th>Disorders</th>
<th>Major Lips Accumulated</th>
<th>Other Compounds Affected</th>
<th>Enzyme or Activator Protein Lacking*</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_{M2}$ gangliosidosis, type II</td>
<td>Globoside and $G_{M2}$</td>
<td>Hexosaminidases A and B</td>
<td>Same clinical picture as Tay-Sachs disease but progresses more rapidly; no racial predilection; hepatosplenomegaly, cardiomyopathy.</td>
<td></td>
</tr>
<tr>
<td>(Tay-Sachs variant; Sandhoff's disease)</td>
<td>ganglioside</td>
<td>($\circledast$ and $\circledR$) $G_{M2}$-activator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fabry's disease</td>
<td>Gal-(4→1α)-Lac-Cer</td>
<td>Gal-(4→1α)-Gal-Cer accumulates.</td>
<td>$\alpha$-Galactosidase ($\circledast$)</td>
<td>X-linked recessive; hemizygous males have a characteristic skin lesion usually lacking in heterozygous females; pain in the extremities; death usually in the fourth decade results from renal failure or cerebral or cardiovascular disease.</td>
</tr>
<tr>
<td>(glycosphingolipid lipidosis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceramide lactoside lipidosis</td>
<td>Gal-(4→1β)-Glc-Cer</td>
<td>Ceramide lactoside $\beta$-galactosidase ($\circledast$)</td>
<td>Liver and spleen enlargement; slowly progressive brain damage neurological impairment.</td>
<td></td>
</tr>
<tr>
<td>Gaucher's disease</td>
<td>Glc-Cer</td>
<td>$G_{M3}$ ganglioside accumulates most frequently; other compounds occasionally.</td>
<td>Hepatosplenomegaly; frequently fatal; no known treatment; occurrence of Gaucher's cells (reticuloendothelial cells that contain accumulations of erythrocyte-derived glucocerebroside).</td>
<td></td>
</tr>
<tr>
<td>(glucosyl ceramide lipidosis; three types; see text)</td>
<td></td>
<td>$\beta$-Glucocerebrosidase (glucosylceramidase; $\circledast$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$G_{M1}$ gangliosidosis (two types; see text)</td>
<td>$G_{M1}$- and desialo-$G_{M1}$-gangliosides</td>
<td>Keratan sulfate-related polysaccharide accumulates.</td>
<td>Mental and motor deterioration; accumulation of mucopolysaccharides is as significant as accumulation of gangliosides; invariably fatal; autosomal recessive; blindness, cherry red macula (30%); hepatosplenomegaly; vacuolated lymphocytes; startle response to sound, dysostosis multiplex.</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 19-1**

*Characteristics of Glycosphingolipid Storage Disorders*
<table>
<thead>
<tr>
<th>Disorder</th>
<th>Compound/Enzyme</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>G(_M2) gangliosidosis, type I (Tay-Sachs disease; see text)</td>
<td>(G_{M2}^-) and desialo-(G_{M2}^-)-gangliosides</td>
<td>Other desialo hexosyl ceramides accumulate; other compounds occasionally. Hexosaminidase A ((\Box)) Red spot in retina; mental retardation; severe psychomotor retardation; seizures; blindness; startle response to sound; invariably fatal; autosomal recessive; panracial but especially prevalent among Northern European Jews.</td>
</tr>
<tr>
<td>Metachromatic leukodystrophy (MLD; sulfatide lipidoses; at least three types; see text)</td>
<td>3-sulfate-galactosylcerebroside</td>
<td>Cerebrosides other than sulfatides are decreased; ceramide dihexoside sulfate accumulates. Sulfatidases ((\Box)); arylsulfatases Activator protein sap-B Demyelination; progressive paralysis and dementia; death usually occurs within the first decade; autosomal recessive inheritance.</td>
</tr>
<tr>
<td>Krabbe’s disease (globoid cell leukodystrophy; galactosyl ceramide lipidosis)</td>
<td>Galactocerebroside</td>
<td>Sulfatides are also greatly decreased, probably as a secondary feature. Galactocerebroside-(\beta)-galactosidase ((\Box)) Mental retardation; demyelination; psychomotor retardation; failure to thrive; progressive spasticity; globoid cells in brain white matter; invariably fatal; autosomal recessive inheritance.</td>
</tr>
</tbody>
</table>

*The circled numbers refer to reactions in Figure 19-8. The abbreviations are the same as in that figure.*
of the molecule. Each sugar residue removed involves a specific exoglycosidase. Sulfatases are required for the removal of sulfate groups from sulfolipids. The degradation of sphingolipids, in addition to their requirement for specific hydrolases, is also dependent on nonenzymatic glycoproteins, known as sphingolipid activator proteins (SAPs). SAP-stimulated degradation of sphingolipids is thought to involve the binding of the activator protein with the sphingolipids so that the water-soluble hydrolases can access the specific sites of hydrolysis. Genes for SAPs are located in chromosomes 5 and 10. The SAP gene that resides in chromosome 5 codes for the activator of hexoseaminidase A, which hydrolyzes GM₂. The gene on chromosome 10 codes for a precursor which, after synthesis in the endoplasmic reticulum, is exported to the cell surface followed by its importation into the lysosomal compartment. In the lysosomes, the precursor protein is processed to yield four mature activator proteins: sap-A, sap-B, sap-C, and sap-D. The activator function of these proteins are as follows: sap-A stimulates glucosylceramidase and galactosylceramidase in the presence of detergents; sap-B is a nonspecific activator that stimulates hydrolysis of about 20 glycolipids as well as hydrolysis of sulfatide by arylsulfatidase A; sap-C is essential for the action of glucosylceramidase; and function of sap-D is unknown.

The importance of SAPs is exemplified in disorders where these activator proteins are not made as a result of mutations. A defect in the synthesis of the enzyme or its activator protein can both result in the same phenotype. Examples are hexoseaminidase A deficiency or its activator protein (Ganglioside GM₂ activator) resulting in Tay-Sachs disease; arylsulfatase A deficiency or its activator protein sap-B resulting in juvenile metachromatic leukodystrophy; and glucosylceramidase deficiency or its activator protein sap-C resulting in Gaucher’s disease. All of these disorders are accompanied by pronounced accumulation of the respective precursor lipids in the reticuloendothelial system. Sphingomyelin is hydrolyzed to ceramide and phosphorylcholine by sphingomyelinase:

Sphingomyelin + H₂O → phosphorylcholine + ceramide

Ceramide is hydrolyzed to sphingosine and fatty acid by ceramidase:

Ceramide + H₂O → sphingosine + fatty acid

A nonlysosomal ceramidase in some tissues functions optimally at neutral or alkaline pH and participates in the synthesis and breakdown of ceramide. Deficiency of lyosomal (acid) ceramidase in Farber’s disease (lipogranulomatosis) causes accumulation of ceramide. The disease is inherited as an autosomal recessive trait and is characterized by granulomatous lesions in the skin, joints, and larynx and moderate nervous system dysfunction; it may also involve heart, lungs, and lymph nodes. It is usually fatal during the first few years of life.

Sphingosine is catabolized to trans-2-hexadecanal and phosphoethanolamine by way of sphingosine phosphate and its cleavage by a lyase. Catabolism of glycosphingolipids involves removal of successive glycosyl residues from their nonreducing end until ceramide is released.

Abnormalities usually involve specific exoglycosidases and their activator proteins (discussed earlier) that hydrolyze the glycosidic bonds, except in metachromatic leukodystrophy, in which there is deficiency of a sulfatidase.

Catabolic pathways for the glycosphingolipids are given in Figure 19-8 and associated disorders are summarized in Table 19-1. Some comments are warranted:
1. Accumulation of a specific lipid in these disorders is frequently accompanied by deposition of one or more polysaccharides structurally related to the lipid.

2. Treatment is generally palliative or nonexistent. Enzyme replacement therapy has proved useful in some of these disorders. Because the exogenous enzymes are unable to cross the blood-brain barrier, their efficacy in the glycosphingolipidoses that have neurological involvement is doubtful. Attempts to modify the enzymes to overcome this difficulty offer some hope.

3. Considerable progress has been made in the identification of carriers and in prenatal diagnosis of homozygotes. Thus, laboratory assays of enzyme activity in leukocytes or cultured skin cells using chromogenic or fluorogenic synthetic substrates have dramatically reduced the incidence of Tay-Sachs disease.

Gaucher’s disease is the most common lysosomal storage disorder and also the most common inherited disease among Ashkenazi Jews, with a carrier frequency of about 1 in 14. Four mutations in the gene encoding β-glucocerebrosidase account for at least 90% of the symptomatic patients. Gaucher’s disease has three forms in which genetic effects appear to be due to errors in the same or related genetic loci. Type I, chronic nonneuropathic (adult), is the most common variety. It comprises a heterogeneous group of patients characterized by the presence of hematological abnormalities (anemia, thrombocytopenia) and erosion of the cortices of long bones. Type II, acute neuropathic, usually appears before 6 months of age and is fatal by 2 years. Mental damage is a primary characteristic, and the disease progresses rapidly from its onset. Type III, subacute neuropathic (juvenile), comprises a heterogeneous group in which death occurs between infancy and about 30 years of age. The cerebral abnormalities usually appear at least 2 years postnatally.

All three types share common features: hepatosplenomegaly, Gaucher cells in the bone marrow (accumulation of glucocerebroside in reticuloendothelial cells in liver, spleen, and bone marrow), and autosomal recessive inheritance. Some studies have shown a correlation between the residual β-glucocerebrosidase activity and clinical severity, but the molecular basis for the genetic heterogeneity is not known. The Gaucher cells obtained from bone marrow aspirates exhibit a characteristic appearance of the cytoplasm owing to rod-shaped striated inclusion bodies composed primarily of glucocerebroside. Patients with Gaucher’s disease have elevated levels of acid phosphatase activity in serum and spleen, increased iron stores, increased angiotensin converting enzyme activity, and a relative deficiency of clotting factor IX (Chapter 36).

Enzyme replacement therapy with purified macrophage-targeted human β-glucocerebrosidase in type I Gaucher’s disease causes breakdown of stored glucocerebrosides. This results in a reduction in the size of the liver and spleen, in improvement in hematological abnormalities (anemia and thrombocytopenia), increased bone mineralization, and decreased bone pain. Two sources of human β-glucocerebrosidase are available; one of them is derived from human placenta (aglucerase) and the other is synthesized by recombinant DNA technology (imiglucerase). Both enzymes are modified in their oligosaccharide side chains to expose terminal mannose residues. Macrophages, through their mannose receptors, internalize the modified enzyme.

Studies of cases of GM1-gangliosidosis have revealed two distinct types. In generalized gangliosidosis, GM1 and desialo-GM1-gangliosides accumulate in brain and viscera. The three β-galactosidase activities isolated from normal human liver all are absent. The disease begins at or near birth, progresses rapidly, and ends fatally, usually by 2 years of age. In juvenile GM1-gangliosidosis, psychomotor abnormalities usually begin at about 1 year, and death ensues at 3–10 years. Two liver β-galactosidase activities are absent, possibly accounting for the lack of lipid accumulation in this organ. This enzymatic finding supports the genetic separation into two forms.

GM2-gangliosidosis is of two types: Tay-Sachs disease, due to β-hexosaminidase A (Hex-A) deficiency, and Sandhoff’s disease, due to deficiency of β-hexosaminidase A and B (Hex-A, Hex-B). The relationship between these diseases is based on the subunit composition of the two affected enzymes. Hex-A, a heteropolymer, consists of two α-chains (coded for on chromosome 15), a β-chain (coded for on chromosome 5), and an activator protein. Hex-B is a tetramer of β-chains. Mutations at the α-locus give rise to Tay-Sachs disease. A variant form can arise from mutation at the activator protein locus; however, it shows normal in vitro Hex-A activity with chromogenic substrates. Mutations at the β-locus yield Sandhoff’s disease and affect Hex-A and Hex-B, both of which contain the β-subunit.

Treatment of sphingolipidoses is primarily symptomatic and supportive. For example, in patients with anemia due to Gaucher’s disease, thrombocytopenia associated with hypersplenism is relieved by splenectomy. Infusion of appropriate purified human placental tissue enzymes in patients with Gaucher’s disease and Fabry’s disease reduced the accumulated glycolipids in the circulation and liver. Recent advances in the cloning and amplification of human DNA segments in bacterial plasmids...
and subsequent isolation of the gene product has yielded enough enzyme required for treatment (Chapter 23). Use of the recipient’s erythrocytes in which the enzyme is entrapped is under investigation to minimize immunological complications. Exposing erythrocytes to hypotonic conditions in the presence of the enzyme causes formation of pores in the membrane that allow rapid exchange of the enzyme with the cellular contents. Restoration to isotonicity reseals the membrane and entraps some of the enzyme. Other enzyme carriers are liposomes, concentric lipid bilayers prepared from cholesterol, lecithin, and phosphatidic acid. The ideal treatment for these disorders would be addition, or replacement, of genetic material coding for the missing gene product. Replacement therapy with a polyethylene glycol-modified form of the missing enzyme, which has an extended half-life and reduced immunogenicity, may provide a promising approach to treatment (see Adenosine deaminase deficiency, Chapter 27).

**Alterations in Cell Surface Glycosphingolipids**

Changes in cell surface glycosphingolipids occur during fetal development and are reflected in several properties of the cell, namely, receptor specificity, antigenic specificity, adhesion, and possibly cell growth regulation. Glycosphingolipid metabolism is affected during oncogenic transformation in cultured cells and may be responsible for some properties of tumor cells (e.g., lack of contact inhibition of growth).

### 19.3 Cholesterol

Cholesterol (3-hydroxy-5,6-cholestene) is a steroid and contains the carbon skeleton of cyclopentanoperhydrophenanthrene, which consists of three six-membered rings and a five-membered ring. It is also a monohydroxylalcohol and contains a double bond between C5 and C6:

![Cholesterol structure](image)

Cholestanol (dihydrocholesterol) has the following conformation:

![Cholestanol structure](image)

In this structure, all of the ring fusions (between A and B, B and C, and C and D) are trans, the hydrogen atoms or methyl groups attached to the bridgehead carbons project to opposite sides of the rings, and the rings are in the more stable chair conformation. In cholesterol, the double bond between C5 and C6 distorts the conformation of the rings A and B and leads to the conformation shown below:

![Cholesterol conformation](image)

The angular methyl groups at C18 and C19, the 3-hydroxyl group, and the side chain at C17 all project toward the same side of the ring system. These substituents are indicated by solid lines and designated as β. A substituent group situated below the plane of the ring is in the α-orientation and is indicated with a dotted line. In general, the angular methyl groups are β-oriented, but the 3-hydroxyl group may be present in either the α- and β-orientation. In cholesterol, the 3-OH is in the β-orientation.

In some naturally occurring compounds (e.g., β-coprostanol), the junction between rings A and B is cis. This compound occurs in large quantities in feces, where it is produced from cholesterol by action of the intestinal flora.

Adults normally synthesize approximately 1 g of cholesterol and consume about 0.3 g/day. Dietary cholesterol is primarily derived from foods of animal origin such as
eggs and meat. Plants, yeasts, and fungi contain sterols that are structurally similar to cholesterol—sitosterols and ergosterols—but are poorly absorbed by the human intestinal tract. A rare inherited autosomal sterol storage disorder (sitosterolemia) is due to defects in the ATP-binding cassette-family of transporters that mediate cholesterol efflux. Treatment consists of diets low in plant sterol content and with cholestyramine to enhance sterol excretion (Chapter 20). In intestinal mucosal cells, most of the absorbed cholesterol is esterified with fatty acids and incorporated into chylomicrons that enter the blood through the lymph. After chylomicrons unload most of their triacylglycerol content at the peripheral tissues, chylomicron remnants are rapidly taken up by the liver (Chapter 20). The routing of nearly all of the cholesterol derived from dietary sources to the liver facilitates the balance of the steroid content in the organism, since the liver is the principal site of cholesterol production. Although the intestinal tract, adrenal cortex, testes, skin, and other tissues can also synthesize cholesterol, their contribution is a minor one.

Cholesterol biosynthesis proceeds via the isoprenoids in a multistep pathway. The end product, cholesterol, and the intermediates of the pathway participate in diverse cellular functions. The isoprenoid units give rise to dolichol, CoQ, heme A, isopentenyl-tRNA, farnesylated proteins, and vitamin D (in the presence of sunlight and 7-dehydrocholesterol). Dolichol is used in the synthesis of glycoproteins, CoQ in the mitochondrial electron transport chain, farnesylation and geranylgeranylation by posttranslational lipid modification that is required for membrane association and function of proteins such as p21 ras and G-protein subunits.

Cholesterol has several functions including involvement in membrane structure, by modulation of membrane fluidity and permeability, serving as a precursor for steroid hormone and bile acid synthesis, in the covalent modification of proteins, and formation of the central nervous system in embryonic development. The latter role of cholesterol was discovered through mutations and pharmacological agents that block cholesterol biosynthesis that occurs in six steps:

1. Conversion of acetyl-CoA to 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA);
2. Conversion of HMG-CoA to mevalonate, the rate-limiting step in cholesterol biosynthesis;
3. Conversion of mevalonate to isoprenyl pyrophosphates with loss of CO2;
4. Conversion of isoprenyl pyrophosphates to squalene;
5. Conversion of squalene to lanosterol; and
6. Conversion of lanosterol to cholesterol.

The biosynthetic reactions involve a series of condensation processes and are distributed between cytosol and microsomes. All of the carbons of cholesterol are derived from acetyl-CoA, 15 from the "methyl" and 12 from the "carboxyl" carbon atoms. Acetyl-CoA is derived from mitochondrial oxidation of metabolic fuels (e.g., fatty acids) and transported to cytosol as citrate (Chapter 18) or by activation of acetate (e.g., derived from ethanol oxidation) by cytosolic acetyl-CoA synthase (Chapter 18). All of the reducing equivalents are provided by NADPH.

### Conversion of Acetyl-CoA to HMG-CoA

In the cytosol, three molecules of acetyl-CoA are condensed to HMG-CoA through successive action of thiolase and HMG-CoA synthase, respectively (Figure 19-9). HMG-CoA synthase is under transcriptional regulation by the sterol end products.

HMG-CoA is also synthesized in mitochondria by the same sequence of reactions but yields the ketone bodies acetoacetate, D(-)-β-hydroxybutyrate, and acetone (Figure 19-10). Mitochondrial HMG-CoA also arises from oxidation of leucine (Chapter 17), which is ketogenic. Although HMG-CoA derived from leucine is not utilized in mevalonate synthesis, the carbon of leucine can be incorporated into cholesterol by way of acetyl-CoA. Thus, two distinct pools of HMG-CoA exist: one

![Figure 19-9](image-url)
CHAPTER 19 Lipids I: Phospholipids, Glycosphingolipids, and Cholesterol

FIGURE 19-10
Mitochondrial and cytosolic biosynthesis and utilization of HMG-CoA in the liver. The molecules indicated by an asterisk are the ketone bodies. Acetoacetate and $\beta$-hydroxybutyrate (after conversion to acetoacetate) are metabolized in extrahepatic tissues. Acetone is excreted in the lungs. Note the cytosolic multifunctional isoprenoid pathway for cholesterol biosynthesis. The double arrow indicates a multistep pathway.

mitochondrial and concerned with formation of ketone bodies, the other extramitochondrial and involved with synthesis of isoprenoid units.

Conversion of HMG-CoA to Mevalonate

This two-step reduction reaction is the rate-limiting step in cholesterogenesis. Cytosolic HMG-CoA is reduced by NADPH to mevalonate by HMG-CoA reductase through the production of an enzyme-bound aldehyde intermediate:

$\text{O} \quad \text{CH}_3 \quad \text{O}$
$\text{O}--\text{C}--\text{CH}_2--\text{C}--\text{CH}_2--\text{SCoA} + 2\text{NADPH} + 2\text{H}^+$
$\text{OH}$
$\text{HMG-CoA}$

HMG-CoA reductase is an integral protein of the endoplasmic reticulum and the primary site of regulation of synthesis of cholesterol and nonsterol isoprenoid derivatives. Its activity has a well-defined diurnal rhythm in rats and mice, coinciding with that of the enzyme’s synthesis and of the mRNA concentration. Activity is highest at about the middle of the dark period and lowest at about the middle of the light period. Its mechanism may be related to food consumption. Rats are nocturnal animals and consume food in the dark; the increased bile production and excretion depletes liver cholesterol and may stimulate the increased synthesis of HMG-CoA reductase as a compensatory mechanism.

HMG-CoA reductase is regulated via synthesis-degradation and phosphorylation-dephosphorylation. Phosphorylation decreases activity, whereas dephosphorylation increases it (Figure 19-11). The reductase kinase phosphorylation is not cAMP-dependent. However, cAMP dependence arises by way of activation of a protein kinase, which phosphorylates a protein inhibitor of phosphatase. The two phosphatases are identical. Thus, increase in cAMP concentration inhibits phosphatase activity, resulting in marked decrease in HMG-CoA reductase activity. Elevation of the plasma glucagon level (e.g., during fasting) activates cAMP production and reduces cholesterol production. Activity is also inhibited by oxygenated sterols (e.g., 27-hydroxycholesterol) but
not in enucleated cells, indicating involvement of the nucleus. The oxygenated sterols are synthesized in mitochondria and may repress the HMG-CoA reductase gene or activate genes for enzymes that degrade the reductase. A rare familial sterol storage disease, cerebrotendinous xanthomatosis, is characterized by accumulation of cholesterol (and its reduced product cholestanol) in every tissue, especially in brain, tendons, and aorta, causing progressive neurological dysfunction, tendon xanthomas, premature atherosclerosis, and myocardial infarction.

Patients also develop cholesterol gallstones from a defect in bile acid synthesis. The defect is in the mitochondrial C_{27}-steroid 27-hydroxylase. In these patients, the reduced formation of normal bile acids, particularly chenodeoxycholic acid, leads to the up-regulation of the rate limiting enzyme 7α-hydroxylase of the bile acid synthetic pathway (discussed later). This leads to accumulation of 7α-hydroxylated bile acid intermediates that are not normally utilized.

The inhibition of cholesterol synthesis by oxygenated sterols involves the following steps. After synthesis in the mitochondria, oxygenated sterols in the cytoplasm inhibit the activation of sterol regulatory element binding proteins (SREBPs), eventually leading to suppression of cholesterol biosynthesis. In cholesterol depleted states, activation of SREBPs requires participation of the SREBP-cleavage activating protein and two proteases. The mature SREBPs are translocated to the nucleus. In the nucleus, SREBPs function as transcription factors and activate, along with other factors, several genes by interacting at promoter sites consisting of a 10-base pair cis element known as sterol regulatory element-1 (SRE-1). Examples of activated genes include HMG-CoA synthase, HMG-CoA reductase, and low-density lipoprotein (LDL) receptors. The latter internalizes LDL to provide cholesterol to cells.

HMG-CoA reductase is inhibited competitively by structural analogues. These compounds are commonly known as “statins” and are used pharmacologically in cholesterol reduction which can reduce the risk for coronary artery disease and stroke (Chapter 20). Statins inhibit HMG-CoA reductase at a much lower concentration (1 μM) compared to the $K_m$ for HMG-CoA (10 μM). The structures of clinically effective statins are shown in Figure 19-12. Lovastatin, simvastatin, and pravastatin are derivatives of naturally occurring fungal products and fluvastatin, atorvastatin and cerivastatin are entirely synthetic compounds. Lovastatin and simvastatin are inactive lactones that are activated by the liver; others are active hydroxy-acids. Naturally occurring statins are found in a dietary supplement known as cholestin, which is obtained from rice fermented in red yeast. In China red yeast rice has been used as a coloring and flavoring agent. Cholestin’s safety and effectiveness as hypocholesterolemic agent awaits long-term clinical studies.
FIGURE 19-12
Structures of HMG-CoA reductase inhibitors (statins).
Despite inhibition of HMG-CoA reductase by statins, cells compensate by increasing enzyme expression several fold. However, the total body cholesterol is reduced by 20–40% due to increased expression of LDL-receptors after statin administration; this enhances LDL (the major cholesterol carrying lipoprotein) clearance from serum with a net reduction of serum cholesterol (Chapter 20). Individuals who lack functional LDL-receptors (homozygous familial hypercholesterolemia, Chapter 20) do not benefit from statin therapy. However, statin therapy is useful in the treatment of heterozygous familial hypercholesterolemia. Since HMG-CoA reductase plays a pivotal role in the synthesis of many products vital for cellular metabolism, inhibitors of the enzyme may have toxic effects. Monitoring of liver and muscle function may be necessary to detect any toxicity of statin drug therapy. A decreased risk of bone fractures with statin therapy has been observed in subjects age 50 years or older, who are being treated for hypercholesterolemia. The mechanism of action of statins in bone metabolism may involve inhibition of prenylation of signaling proteins of osteoclast cell membrane (Chapter 37).

Conversion of Mevalonate to Isoprenyl Pyrophosphate

Isoprenyl pyrophosphates are synthesized by successive phosphorylation of mevalonate with ATP to yield the 5-monophosphate, 5-pyrophosphate, and 5-pyrophospho-3-monophospho derivatives. This last compound is very unstable and loses the 3-phosphate and the carboxyl group to yield isopentenyl pyrophosphate (IPPP), which is isomerized to 3,3-dimethylallyl pyrophosphate (DMAPP). These reactions, catalyzed by cytosolic enzymes, are shown in Figure 19-13.

Patients with severe forms of inherited mevalonate kinase deficiencies exhibit mevalonic aciduria, failure to thrive, developmental delay, anemia, hepatosplenomegaly, gastroenteropathy, and dysmorphic features during neonatal development. These clinical manifestations underscore the importance of the formation of isoprenyl...
pyrophosphates not only for sterol synthesis but also for the nonsterol isoprene compounds dolichol, CoQ, heme A, isopentenyl-tRNA and farnesylated proteins.

**Condensation of Isoprenyl Pyrophosphate to Form Squalene**

IPPP, a nucleophile (by virtue of its terminal vinyl group), and DMAPP, an electrophile, undergo condensation with elimination of pyrophosphate to yield geranyl pyrophosphate (an electrophile), which condenses with a molecule of IPPP to yield a farnesyl pyrophosphate and pyrophosphate. These reactions are probably catalyzed by the same cytosolic enzyme complex. Two molecules of farnesyl pyrophosphate and then condense head-to-head to form squalene by action of microsomal squalene synthase (Figure 19-14).

The farnesyl pyrophosphate generated in this pathway is also used in the farnesylation of proteins. The farnesyl group is attached to a protein via the thioether linkage involving a cysteine residue found in the C terminus. Several proteins that are modified by farnesyl groups have been identified, e.g., growth-regulating ras proteins (Chapter 26) and nuclear envelope proteins. Proteins attached to a geranyl-geranyl group (a 20-C isoprene unit) have also been identified. The modification of proteins by these lipid moieties increases their hydrophobicity and may be required for these proteins to interact with other hydrophobic proteins and for proper anchoring in the cell membrane. The importance of farnesylation of proteins is exemplified by blockage of cell growth when mevalonate synthesis is inhibited.

**Conversion of Squalene to Lanosterol**

This step comprises cyclization of squalene to lanosterol (the first sterol to be formed) and conversion of lanosterol to cholesterol. The cyclization begins with conversion of squalene to squalene-2,3-epoxide by a microsomal mixed-function oxidase that requires O2, NADPH, and FAD (Figure 19-15). Cyclization of squalene-2,3-epoxide to lanosterol occurs by a series of concerted 1,2-methyl group

**FIGURE 19-14**

Synthesis of squalene from isomeric pentenyl pyrophosphates.

**FIGURE 19-15**

Cyclization of squalene to lanosterol. Supernatant protein factor (SPF), a cytosolic protein, promotes both stages of the cyclization.
and hydride shifts along the squalene chain. In both stages, the reactants are bound to supernatant protein factor (SPF), a cytosolic carrier that promotes conversion of squalene to lanosterol.

Conversion of Lanosterol to Cholesterol

Transformation of lanosterol to cholesterol (Figure 19-16) is a complex, multistep process catalyzed by enzymes of the endoplasmic reticulum (microsomes). A cytosolic sterol carrier protein is also required and presumably functions as a carrier of steroid intermediates from one catalytic site to the next but may also affect activity of the enzymes. The reactions consist of removal of the three methyl groups attached to C4 and C14, migration of the double bond from the 8,9- to the 5,6-position, and saturation of the double bond in the side chain. Conversion of lanosterol to cholesterol occurs principally via 7-dehydrocholesterol and to a minor extent via desmosterol.

The importance of cholesterol biosynthesis in embryonic development and formation of the central nervous system is reflected in patients with disorders in the pathway for the conversion of lanosterol to cholesterol. Three enzyme deficiencies have been identified (Figure 19-16):

1. 3β-Hydroxysteroid-Δ24-reductase (also known as sterol-Δ24-reductase);
2. 3β-Hydroxysteroid-Δ8,Δ7-isomerase (commonly known as sterol-Δ8-isomerase);
3. 3β-Hydroxysteroid-Δ7-reductase (also known as 7-dehydrocholesterol reductase).

Sterol-Δ8-isomerase deficiency, known as Conradi-Hünermann syndrome (CDPX2), is an X-linked dominant disorder. Clinical manifestations of this disorder include skeletal abnormalities, chondrodysplasia punctata, craniofacial anomalies, cataracts, and skin abnormalities. The 7-dehydrocholesterol reductase deficiency, known as Smith–Lemli–Opitz syndrome (SLO) is an autosomal recessive disorder occurring in about 1 in 20,000 births. Clinical manifestations of affected individuals include craniofacial abnormalities, microcephaly, congenital heart disease, malformation of the limbs, psychomotor retardation, cerebral maldevelopment, and urogenital anomalies. Measurement of 7-dehydrocholesterol in amniotic fluid during second trimester or in neonatal blood specimen has been useful in the identification of the disorder.

The sterol-Δ24-reductase deficiency causes a developmental phenotype similar to SLO syndrome and is associated with accumulation of desmosterol. The inability of de novo fetal synthesis of cholesterol combined with its inadequate transport from the mother to the fetus appears to be involved in the multiple abnormalities of morphology. SLO infants treated with rich sources of dietary cholesterol after birth have shown fewer growth abnormalities. However, it is not known whether long-term dietary cholesterol supplement can improve cognitive development, particularly since cholesterol is not transported across the blood-brain barrier.

An appreciation of the relationship between cellular cholesterol metabolism and a family of signaling molecules that participate in embryonic development is emerging. These signaling molecules are known as hedgehog proteins which were initially identified in Drosophila. The vertebrate counterparts of hedgehog proteins, participate in embryonic processing, including the neural tube and its derivatives, the axial skeleton, and the appendages. The hedgehog protein is a self-splicing protein that undergoes an autocatalytic proteolytic processing giving rise to an N-terminal and a C-terminal product. In Drosophila, hedgehog protein cleavage occurs between Gly-257 and Cys-258. Cholesterol is covalently attached to the carboxy terminal end of the N-terminal cleavage product. Both the autocatalytic proteolysis and intramolecular cholesterol transferase activities are located in the C-terminal portion of the hedgehog protein. The covalent modification of the N-terminal segment of the hedgehog protein is necessary for proper localization on the cell membrane at target sites to initiate downstream events (e.g., transcription of target genes). Thus, perturbations of cholesterol biosynthesis due to mutations or pharmacological agents can lead to defects in embryonic development.

Utilization of Cholesterol

Cholesterol is utilized in formation of membranes (Chapter 10), steroid hormones (Chapters 30, 32, and 34), and bile acids. 7-Dehydrocholesterol is required for production of vitamin D (Chapter 37). Under steady-state conditions, the cholesterol content of the body is maintained relatively constant by balancing synthesis and dietary intake with utilization. The major consumer of cholesterol is formation of bile acids, of which about 0.8–1 g/day are produced in the liver and lost in the feces. However, secretion of bile acids by the liver is many times greater (15–20 g/day) than the rate of synthesis because of their enterohepatic circulation (Chapter 12). Cholesterol is also secreted into bile, and some is lost in feces as cholesterol and as coprostanol, a bacterial reduction product (about 0.4–0.5 g/day). Conversion of cholesterol to steroid hormones and of 7-dehydrocholesterol to vitamin D and elimination of their inactive metabolites, are of minor significance in the disposition of cholesterol, amounting to approximately 50 mg/day. A small amount of cholesterol...
FIGURE 19-16
A partial pathway for the conversion of lanosterol to cholesterol. The complete process consists of 19 steps. The C24 = C25 double bond can be reduced by 3β-hydroxysteroid-Δ^{24}-reductase at several steps along the pathway (indicated by 1), and deficiency of this enzyme leads to accumulation of desmosterol. Deficiency of enzyme 2 and enzyme 3 results in CDPX2 and SLO syndromes, respectively (see text).
(about 80 mg/day) is also lost through shedding of the outer layers of the skin.

**19.4 Bile Acids**

Bile acids are 24-carbon steroid compounds. Primary bile acids (cholic and chenodeoxycholic) are synthesized in the liver from cholesterol (Figure 19-17). In human bile, about 45% is chenodeoxycholic acid, 31% cholic acid and 24% deoxycholic acid (a secondary bile acid formed in the intestine). Early studies in rodents showed the preferred substrate to be newly synthesized cholesterol. However, whole-body turnover studies in humans using radioactive markers indicate that approximately two thirds of bile acid is derived from HDL cholesterol delivered to the liver. Formation is initiated by 7α-hydroxylation, the committed and rate-limiting step catalyzed by microsomal
7α-hydroxylase; the reaction requires NADPH, O₂, cytochrome P-450, and NADPH: cytochrome P-450 reductase. Reactions that follow are: oxidation of the 3β-hydroxyl group to a 3-keto group, isomerization of the Δ⁵ double bond to the Δ⁴-position, conversion of the 3-keto group to a 3α-hydroxy group, reduction of the Δ⁴ double bond, 12α-hydroxylation in the case of cholic acid synthesis, and oxidation of the side chain. 12α-Hydroxylase, like 7α-hydroxylase, is associated with microsomes and requires NADPH, molecular oxygen, and cytochrome P-450. Unlike 7α-hydroxylase, its activity does not exhibit diurnal variation. Its activity determines the amount of cholic acid synthesized. Side chain oxidation starts with 27-hydroxylation and is followed by oxidative steps similar to those of β-oxidation of fatty acids (Chapter 18). The 27-hydroxylation catalyzed by a mixed-function hydroxylase probably occurs in mitochondria and requires NADPH, O₂, and cytochrome P-450. Bile acid deficiency in cerebrotendinous xanthomatosis (see above) is due to a deficiency of 27-hydroxylase. Since the substrates for bile acid formation are water insoluble, they require sterol carrier proteins for synthesis and metabolism.

Bile acids are conjugated with glycine or taurine (Figure 19-18) before being secreted into bile, where the ratio of glycine- to taurine-conjugated acids is about 3:1. Sulfate esters of bile acids are also formed to a small extent. At the alkaline pH of bile and in the presence of alkaline cations (Na⁺, K⁺), the acids and their conjugates are present as salts (ionized forms), although the terms bile acids and bile salts are used interchangeably.

Regulation of Bile Acid Synthesis

Regulation of bile acid formation from cholesterol occurs at the 7α-hydroxylation step and is mediated by the concentration of bile acids in the enterohepatic circulation. 7α-Hydroxylase is modulated by phosphorylation-dephosphorylation cascade similar to that of HMG-CoA reductase (Figure 19-11) except that the phosphorylated form of 7α-hydroxylase is more active.

As noted earlier, the major rate-limiting step of cholesterol biosynthesis is reduced synthesis of HMG-CoA. 7α-hydroxycholesterol, the first intermediate of bile acid formation, inhibits HMG-CoA reductase. The activities of 7α-hydroxycholesterol and HMG-CoA reductase undergo parallel changes under the influence of bile acid levels. In the rat, they show similar patterns of diurnal variation, with highest activities during the dark period. Bile acids and intermediates do not appear to function as allosteric modifiers. In the intestines, bile acids may regulate cholesterol biosynthesis, in addition to their role in cholesterol absorption. In humans, the presence of excess cholesterol does not increase bile acid production proportionally, although it suppresses endogenous cholesterol synthesis and increases excretion of fecal neutral steroids.

Hepatic bile acid synthesis amounts to about 0.8–1 g/day. When loss of bile occurs owing to drainage
SECTION 19.4  Bile Acids

through a biliary fistula, to administration of bile acid-complexing resins (e.g., cholestyramine), or to ileal exclusion, activity of 7α-hydroxylase increases several fold, with consequent increase in bile acid formation. The latter two methods are used to reduce cholesterol levels in hypercholesterolemic patients (Chapter 20).

Disposition of Bile Acids in the Intestines and Their Enterohepatic Circulation

Bile is stored and concentrated in the gallbladder, a saccular, elongated, pear-shaped organ attached to the hepatic duct. Bile contains bile acids, bile pigments (i.e., bilirubin glucuronides; see Chapter 29), cholesterol, and lecithin. The pH of gallbladder bile is 6.9–7.7. Cholesterol is solubilized in bile by the formation of micelles with bile acids and lecithin. Cholesterol gallstones can form as a result of excessive secretion of cholesterol or of insufficient amounts of bile acids and lecithin relative to cholesterol in bile. Inadequate amounts of bile acids result from decreased hepatic synthesis, decreased uptake from the portal blood by hepatocytes, or increased loss from the gastrointestinal tract.

With ingestion of food, cholecystokinin (Chapter 12) is released into the blood and causes contraction of the gallbladder, whose contents are rapidly emptied into the duodenum by way of the common bile duct. In the duodenal wall, the bile duct fuses with the pancreatic duct at the ampulla of Vater. Bile functions include absorption of lipids and the lipid-soluble vitamins A, D, E, and K by the emulsifying action of bile salts (Chapter 12), neutralization of acid chyme, and excretion of toxic metabolites (e.g., bile pigments, some drugs and toxins) in the feces.

The secondary bile acids, deoxycholic and lithocholic acids, are derived by 7-dehydroxylation from the deconjugated primary bile acids, cholic and chenodeoxycholic acids, respectively (Figure 19-19), through action of bacterial enzymes primarily in the large intestine. The major portion (> 90%) of bile acids in the intestines is reabsorbed by an active transport system into the portal circulation at the distal ileum and transported bound to albumin. They are taken up by the liver, promptly reconjugated with taurine and glycine, and resecreted into bile. Both ileal absorption and hepatic uptake of bile acids may be mediated by Na⁺-dependent (carrier) transport mechanisms. This cyclic transport of bile acids from intestine to liver and back to the intestine is known as the enterohepatic circulation (Figure 19-20). During a single passage of portal blood through the liver, about 90% of the bile acids are extracted. The bile acid pool size in the enterohepatic circulation is 2–4 g and circulates about twice during digestion.
FIGURE 19-20
Formation, enterohepatic circulation, and disposition of the bile acids. CDCA = Chenodeoxycholic acid.

of each meal. The amount of bile acids lost in feces is about 0.8–1 g/day and consists mostly of secondary bile acids (particularly lithocholic acid, the least soluble of the bile acids). The loss is made up by synthesis of an equal amount in the liver.

Bile Acid Metabolism and Clinical Medicine
In liver disorders, serum levels of bile acids are elevated, and their measurement is a sensitive indicator of liver disease. Bile acids are not normally found in urine owing to efficient uptake by the liver and excretion into the intestines. In hepatocellular disease and obstructive jaundice, however, their urinary excretion increases.

Lithocholic acid is toxic and can cause hemolysis and fever. Effects associated with hyperbile acidemia include pruritus, steatorrhea, hemolytic anemia, and further liver injury.

The main cause of cholesterol (presence or formation of gallstones) is precipitation of cholesterol in bile. Elevated biliary concentrations of bile pigments (bilirubin glucuronides) can also lead to formation of concretions known as pigment stones (Chapter 29). Since biliary cholesterol is solubilized by bile acids and lecithin, an excess of cholesterol along with decreased amounts of bile acids and lecithin, cause bile to become supersaturated with cholesterol with the risk of forming cholesterol stones. The limits of solubility of cholesterol in the presence of bile salts and lecithin has been established by using a ternary phase diagram. If the mole ratio of bile salts and phospholipids to cholesterol is less than 10:1, the bile is considered to be lithogenic (stone forming), but this ratio is not absolute. The pattern of food intake in Western nations, which consists of excess fat, cholesterol, and an interval of 12–14 hours between the evening meal and breakfast, leads to a fasting gallbladder bile that is saturated or supersaturated with cholesterol. In individuals who have gallstones, the problems associated with production of lithogenic bile appear to reside in the liver and not in the gallbladder. When the activities of HMG-CoA reductase and 7α-hydroxylase were measured in the liver of patients who had gallstones and compared with those
of controls, cholesterogenesis was found to be increased in the patients with gallstones, and bile acid synthesis was reduced. Under these conditions, the ratio of cholesterol to bile acids secreted by the liver is increased. The gallbladder provides an environment where concentration of the lithogenic bile triggers formation of gallstones. A genetic predisposition to formation of cholesterol gallstones is common in certain groups (e.g., 70% of Native American women older than 30 years have cholesterol gallstones). In general, the incidence of gallstones in women is three times higher than in men, and this proportion increases with age. Obesity and possibly multiparity are also associated with formation of gallstones. Any disorder of the ileum (e.g., Crohn’s disease) or its resection can result in gallstones owing to impaired absorption of bile acids and depletion of the bile acid pool. Agents that increase biliary secretion of cholesterol (e.g., estrogen, oral contraceptives, and clofibrate) and those which prevent bile acid reabsorption in the intestines (e.g., cholestyramine) are also predisposing factors.

Cholelithiasis is frequently treated by surgical removal of the gallbladder (cholecystectomy). Oral treatment with ursodeoxycholic (ursodiol) and chenodeoxycholic (chenodiol) acids has effectively solubilized gallstones in a number of patients. These bile acids apparently reduce HMG-CoA reductase activity, thereby lowering cholesterol levels while enriching the bile acid pool. The increased bile acid to cholesterol ratio in the bile apparently aids in solubilizing the stones already present. However, the effect of chenodiol is transient, and therapy may have to be long-term. In addition, the treatment appears to be promising only in patients who have radiolucent gallstones and functioning gallbladders; and because it raises serum transaminase levels, the significance of which is not clear, the measurement of other liver function tests may be necessary. However, ursodiol has few side effects and is effective at a lower dosage. Ursodiol is not a human metabolite and differs from chenodiol only in the orientation of a hydroxyl group at C7 (Figure 19-17). Chenodiol is in the α-orientation, whereas ursodiol is in the β-orientation. Two other nonsurgical treatments undergoing clinical evaluation are extracorporeal shock-wave lithotripsy to fragment gallstones and direct infusion into the gallbladder with the solvent methyl tert-butyl ether to dissolve cholesterol stones. Oral treatment with ursodiol in conjunction with extracorporeal shock-wave lithotripsy is more effective than lithotripsy alone.

**Supplemental Readings and References**


