Chapter 9—
Lipid Metabolism I:
Utilization and Storage of Energy in Lipid Form

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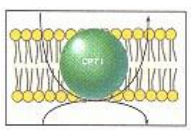


9.1— Overview

As the human body builds and renews its structures, obtains and stores energy, and performs its various functions, there are many circumstances in which it is essential to use molecules or parts of molecules that do not associate with water. This property of being **nonpolar** and **hydrophobic** is the defining characteristic of substances classed as **lipids.** Most of these are molecules that contain or are derived from **fatty acids.** In the early stages of biochemical research, lipids were not investigated as intensively as other body constituents, largely because techniques for studying aqueous systems were easier to develop. This benign neglect led to assumptions that lipids were relatively inert and their metabolism was of lesser importance than that of carbohydrates, for instance.

As the methodology for analyzing lipid metabolism developed, it became evident that fatty acids and their derivatives had at least two major roles in the body. On the one hand, oxidation of fatty acids was shown to be a major means of metabolic energy production. It also became clear that their storage in the form of **triacylglycerols** was more efficient and quantitatively more important than storage of carbohydrates as glycogen. On the other hand, as details of the chemistry of biological structures were defined, hydrophobic structures were found to be largely composed of fatty acids and their derivatives. Thus the major separation of cells and subcellular structures into separate aqueous compartments is accomplished with membranes whose hydrophobic characteristics are largely supplied by the fatty acid moieties of complex lipids. These latter compounds contain constituents other than fatty acids and glycerol. They frequently have significant covalently bound hydrophilic moieties, notably carbohydrates in the glycolipids and organic phosphate esters in phospholipids.

Lipids have several other quantitatively less important roles, which are nonetheless of great functional significance. These include the use of surface active properties of some complex lipids for specific functions, such as maintenance of lung alveolar integrity and solubilization of nonpolar substances in body fluids. In addition, several classes of lipids, for example, steroid hormones and prostaglandins, have highly potent and specific physiological roles in control of metabolic processes. The interrelationships of some processes involved in lipid metabolism are outlined in Figure 9.1. The metabolism of fatty acids and triacylglycerols is so crucial to proper functioning of the human body that imbalances and deficiencies in these processes can have serious pathological consequences. Disease states related to fatty acid and triacylglycerol metabolism include obesity, diabetes, ketoacidosis, and abnormalities in transport of lipids in blood. In addition, some unique deficiencies have been found, such as Refsum's disease and familial hypercholesterolemia, which have helped to elucidate some pathways in lipid metabolism.

This chapter is concerned primarily with the structure and metabolism of fatty acids and of their major storage form, triacylglycerols. After a discussion of the structures of the more important fatty acids found in humans, how they

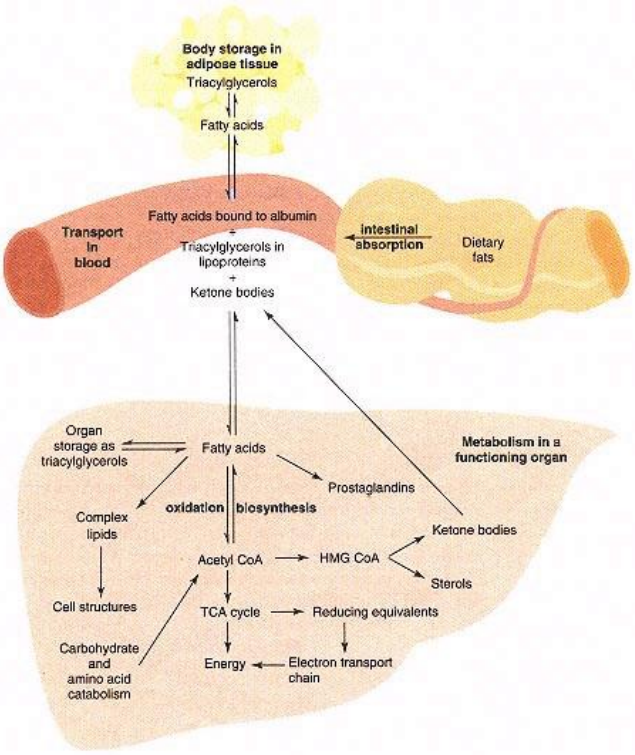


Figure 9.1
Metabolic interrelationships of fatty acids in the human.

are supplied from the diet or by biosynthesis is described. The mechanism for storage as triacylglycerols and how fatty acids are mobilized and transported throughout the body to sites where they are needed are discussed. The central process of energy production from fatty acids is then examined, and finally the mechanisms by which the ketone bodies are synthesized and used are presented.

The Appendix includes the nomenclature and chemistry of lipids and in Chapter 26 there is a discussion of digestion and absorption of lipids.

9.2— Chemical Nature of Fatty Acids and Acylglycerols

Fatty Acids Are Alkyl Chains Terminating in a Carboxyl Group

Fatty acids consist of an **alkyl chain** with a terminal carboxyl group, the basic formula of completely saturated species being $CH_3–(CH_2)_n–COOH$. The important fatty acids for humans have relatively simple structures, although in some organisms they may be quite complex, containing cyclopropane rings or extensive branching. Unsaturation occurs commonly in human fatty acids, with up to six double bonds per chain, these being almost always of the cis configuration. If there is more than one double bond per molecule, they are always separated by a **methylene** ($–CH_2–$) **group.** The most common fatty acids

in biological systems have an even number of carbon atoms, although some organisms do synthesize those with an odd number of carbon atoms. Humans can use the latter for energy but incorporate them into complex lipids to a minimal degree.

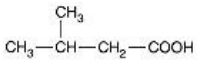


Figure 9.2
Isovaleric acid.

A few fatty acids with an α-OH group are produced and used structurally by humans. However, more oxidized forms are normally produced only as metabolic intermediates during energy production or for specific physiological activity in the case of prostaglandins and thromboxanes. Some animals, including humans, also produce relatively simple **branched-chain acids,** branching being limited to methyl groups along the chain at one or more positions. These are apparently produced to contribute specific physical properties to some secretions and structures. For instance, large amounts of branched-chain fatty acids, particularly isovaleric acid (Figure 9.2), occur in lipids of echo-locating structures in marine mammals. Elucidation of the role of these compounds in sound focusing should be fascinating.

Most fatty acids in humans have C16, C18, or C20 atoms, but there are several with longer chains that occur principally in lipids of the nervous system. These include nervonic acid and a C22 acid with six double bonds (Figure 9.3).

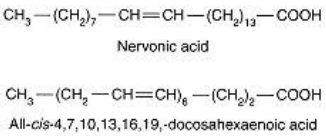


Figure 9.3
Long-chain fatty acids.

Nomenclature of Fatty Acids

The most abundant fatty acids have common names that have been accepted for use in the official nomenclature. Examples are given in Table 9.1 with official systematic names. The approved abbreviations consist of the number of carbon atoms followed, after a colon, by the number of double bonds. Carbon atoms are numbered with the carboxyl carbon as number 1, and double bond locations are designated by the number of the carbon atom on the carboxyl side of it. These designations of double bonds are in parentheses after the rest of the symbol.

Most Fatty Acids in Humans Occur As Triacylglycerols

Fatty acids occur primarily as esters of glycerol, as shown in Figure 9.4, when they are stored for future use. Compounds with one **(monoacylglycerols)** or two **(diacylglycerols)** acids esterified are present only in relatively minor amounts and occur largely as metabolic intermediates in biosynthesis and degradation of glycerol-containing lipids. Most fatty acids in humans exist as **triacylglycerols,** in which all three hydroxyl groups on glycerol are esterified with a fatty acid. These compounds have been called "neutral fats or triglycerides." There are other types of "neutral fats" in the body, and the terms "triglyceride," "monoglyceride," and "diglyceride" are chemically incorrect and should not be used.

The distribution of different fatty acids in the three positions of the glycerol moiety of triacylglycerols in the body at any given time is influenced by many

TABLE 9.1 Fatty Acids of Importance to Humans

Numerical Symbol	*Structure*	*Trivial Name*	*Systematic Name*
16: 0	CH_3–$(CH_2)_{14}$–COOH	Palmitic	Hexadecanoic
16: 1(9)	CH_3–$(CH_2)_5$–CH=CH–$(CH_2)_7$–COOH	Palmitoleic	*cis*-9-Hexadecenoic
18: 0	CH_3–$(CH_2)_{16}$–COOH	Stearic	Octadecanoic
18: 1(9)	CH_3–$(CH_2)_7$–CH=CH–$(CH_2)_7$–COOH	Oleic	*cis*-9-Octadecenoic
18: 2(9,12)	CH_3–$(CH_2)_3$–$(CH_2$–CH=CH$)_2$–$(CH_2)_7$–COOH	Linoleic	*cis,cis*-9,12-Octadecadienoic
18: 3(9,12,15)	CH_3–$(CH_2$–CH=CH$)_3$–$(CH_2)_7$–COOH	Linolenic	*cis,cis,cis*-9,12,15-Octadecatrienoic
20: 4(5,8,11,14)	CH_3–$(CH_2)_3$–$(CH_2$–CH=CH$)_4$–$(CH_2)_3$–COOH	Arachidonic	*cis,cis,cis,cis*-5,8,11,14-Eicosatetraenoic

factors, including diet and anatomical location of the triacylglycerol. Compounds with the same fatty acid in all three positions of glycerol are rare; the usual case is for a complex mixture.

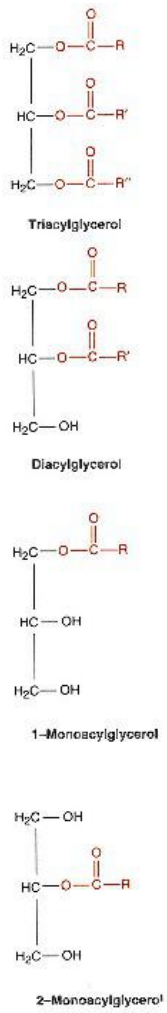


Figure 9.4
Acylglycerols.

The Hydrophobic Nature of Lipids Is Important for Their Biological Function

One significant property of fatty acids and triacylglycerols is their lack of affinity for water. Long hydrocarbon chains have negligible possibility for hydrogen bonding. Acids, whether unesterified or in a complex lipid, have a much greater tendency to associate with each other or other hydrophobic structures, such as sterols and hydrophobic side chains of amino acids, than they do with water or polar organic compounds. This hydrophobic character is essential for construction of complex biological structures such as membranes.

The **hydrophobic nature** of triacylglycerols and their highly reduced state make them efficient compounds in comparison to glycogen for storing energy. Three points deserve emphasis. First, on a weight basis pure triacylglycerols yield near two and one-half times the amount of ATP on complete oxidation than does pure glycogen. Second, triacylglycerols can be stored without associated water, whereas glycogen is very hydrophilic and binds about twice its weight of water when stored in tissues. Thus the equivalent amount of metabolically recoverable energy stored as hydrated glycogen would weigh about four times as much as if it were stored as triacylglycerols. Third, the average 70-kg person stores about 350 g of carbohydrate as liver and muscle glycogen. This represents about 1400 kcal of available energy, barely enough to sustain bodily functions for 24 hours of fasting. By contrast, a normal complement of fat stores will provide sufficient energy to allow several weeks of survival during total food deprivation.

In humans most of the fatty acids are either saturated or contain only one double bond. Although they are readily catabolized by appropriate enzymes and cofactors, they are fairly inert chemically. The highly unsaturated fatty acids in tissues are much more susceptible to oxidation.

9.3— Sources of Fatty Acids

Both diet and biosynthesis supply the fatty acids needed by the human body for energy and for construction of hydrophobic parts of biomolecules. Excess amounts of protein and carbohydrate in the diet are readily converted to fatty acids and stored as triacylglycerols.

Most Fatty Acids Are Supplied in the Diet

Various animal and vegetable lipids are ingested, hydrolyzed at least partially by digestive enzymes, and absorbed through the intestinal mucosa to be distributed through the body, first in the lymphatic system and then in the bloodstream. These processes are discussed in Chapter 25. To a large extent dietary supply governs the composition of fatty acids in body lipids. Metabolic processes in various tissues modify both dietary and *de novo* synthesized fatty acids to produce nearly all the required structures. With one exception, the actual composition of fatty acids supplied in the diet is relatively unimportant. This exception involves the need for appropriate proportions of relatively highly unsaturated fatty acids because many higher mammals, including humans, are unable to synthesize fatty acids with double bonds near the methyl end of the molecule. Certain **polyunsaturated acids** with double bonds within the last seven linkages toward the methyl end are essential for specific functions. Although all

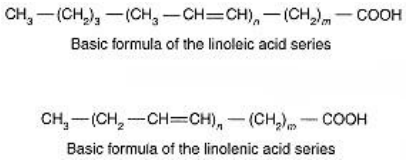


Figure 9.5
Linoleic and linolenic acid series.

the reasons for this need are not yet explained, one is that some of these acids are precursors of prostaglandins, very active oxidation products (see p. 431).

In humans a dietary precursor is essential for two series of fatty acids. These are the linoleic series and the linolenic series (Figure 9.5).

Palmitate Is Synthesized from Acetyl CoA

The second major source of fatty acids for humans is their biosynthesis from small-molecule intermediates derived from metabolic breakdown of sugars, some amino acids, and other fatty acids. In a majority of instances the saturated, straight-chain C16 acid, **palmitic acid,** is first synthesized, and all other fatty acids are made by modification of palmitic acid. Acetyl CoA is the direct source of all carbon atoms for this synthesis. Fatty acids are synthesized by sequential addition of two-carbon units to the activated carboxyl end of a growing chain. In mammalian systems the sequence of reactions is carried out by **fatty acid synthase.**

Fatty acid synthase is a fascinating enzyme complex that is still studied intensely. In bacteria it is a complex of several proteins, whereas in mammalian cells it is a single multifunctional protein. Either acetyl CoA or butyryl CoA is the priming unit for fatty acid synthesis, and the methyl end of these primers becomes the methyl end of palmitate. Addition of the rest of the two-carbon units requires activation of the methyl carbon of acetyl CoA by carboxylation to malonyl CoA. However, CO_2 added in this process is lost when condensation of malonyl CoA to the growing chain occurs, so carbon atoms in the palmitate chain originate only from acetyl CoA.

Formation of Malonyl CoA Is the Commitment Step of Fatty Acid Synthesis

The reaction that commits acetyl CoA to fatty acid synthesis is its carboxylation to **malonyl CoA** by the enzyme **acetyl-CoA carboxylase** (Figure 9.6). This reaction is similar in many ways to carboxylation of pyruvate, which starts the process of gluconeogenesis. The reaction requires ATP and HCO_3^- as the source of CO_2. As with pyruvate carboxylase, the first step is formation of activated CO_2 on the biotin moiety of acetyl-CoA carboxylase using energy from ATP. This is then transferred to acetyl CoA.

Acetyl-CoA carboxylase, a key control point in the overall synthesis of fatty acids, can be isolated in a protomeric state that is inactive. The protomers aggregate to form enzymatically active polymers upon addition of citrate *in*

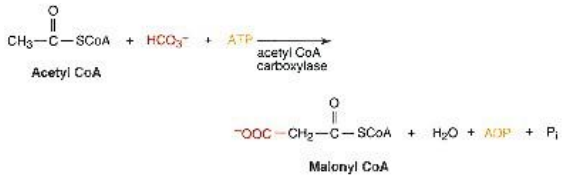


Figure 9.6
Acetyl-CoA carboxylase reaction.

vitro. Pamitoyl CoA *in vitro* inhibits the active enzyme. The action of these two effectors is very logical. Increased synthesis of fatty acids to store energy is desirable when citrate is in high concentration, and decreased synthesis is necessary if high levels of product accumulate. However, the degree to which these regulatory mechanisms actually operate *in vivo* is still unclear.

Acetyl-CoA carboxylase is also controlled by a cAMP-mediated phosphorylation–dephosphorylation mechanism in which the phosphorylated enzyme is less active than the dephosphorylated one. There is evidence suggesting that phosphorylation is promoted by glucagon (via cAMP) as well as by AMP (via an AMP-activated kinase) and that the active form is fostered by insulin. These effects of hormone-mediated phosphorylation are separate from the allosteric effects of citrate and palmitoyl CoA (see Table 9.2).

TABLE 9.2 Regulation of Fatty Acid Synthesis

Enzyme	***Regulatory Agent***		***Effect***
Palmitate Biosynthesis			
Acetyl-CoA carboxylase	Short term	Citrate	Allosteric activation
		C16–C18 acyl CoAs	Allosteric inhibition
		Insulin	Stimulation
		Glucagon	Inhibition
		cAMP-mediated phosphorylation	Inhibition
		Dephosphorylation	Stimulation
	Long term	High-carbohydrate diet	Stimulation by increased enzyme synthesis
		Fat-free diet	Stimulation by increased enzyme synthesis
		High-fat diet	Inhibition by decreased enzyme synthesis
		Fasting	Inhibition by decreased enzyme synthesis
		Glucagon	Inhibition by decreased enzyme synthesis
Fatty acid synthase	Phosphorylated sugars		Allosteric activation
	High-carbohydrate diet		Stimulation by increased enzyme synthesis
	Fat-free diet		Stimulation by increased enzyme synthesis
	High-fat diet		Inhibition by decreased enzyme synthesis
	Fasting		Inhibition by decreased enzyme synthesis
	Glucagon		Inhibition by decreased enzyme synthesis
Biosynthesis of Fatty Acids Other than Palmitate			
Fatty acid synthase	High ratio of methylmalonyl CoA/malonyl CoA		Increased synthesis of methylated fatty acids
	Thioesterase cofactor		Termination of synthesis with short-chain product
Stearyl CoA desaturase	Various hormones		Stimulation of unsaturated fatty acid synthesis by increased enzyme synthesis
	Dietary polyunsaturated fatty acids		Decreased activity

The rate of synthesis of acetyl-CoA carboxylase is also regulated. More enzyme is produced by animals on high-carbohydrate or fat-free diets, whereas on fasting or high-fat diets the rate of enzyme synthesis is decreased.

Reaction Sequence for Synthesis of Palmitic Acid

The first step catalyzed by fatty acid synthase in bacteria is transacylation of the primer molecule, either acetyl CoA or butyryl CoA, to a 4 -phosphopantetheine moiety on a protein constituent of the enzyme complex. This protein is ***a*cyl *c*arrier *p*rotein (ACP),** and its phosphopantetheine unit is identical with that in CoA. The mammalian enzyme also contains a phosphopantetheine. Six or seven two-carbon units are then added sequentially to the enzyme complex until the palmitate molecule is completed. After each addition of a two-carbon unit a series of reductive steps takes place. The reaction sequence starting with an acetyl CoA primer and leading to butyryl-ACP is as presented in Figure 9.7.

The next round of synthesis is initiated by transfer of the newly formed fatty acid chain from 4 -phosphopantetheine moiety of ACP to a functional –SH group of ***β*-ketoacyl-ACP synthase** (analogous to Reaction 3a). This liberates the –SH group of ACP for acceptance of a second malonyl unit from

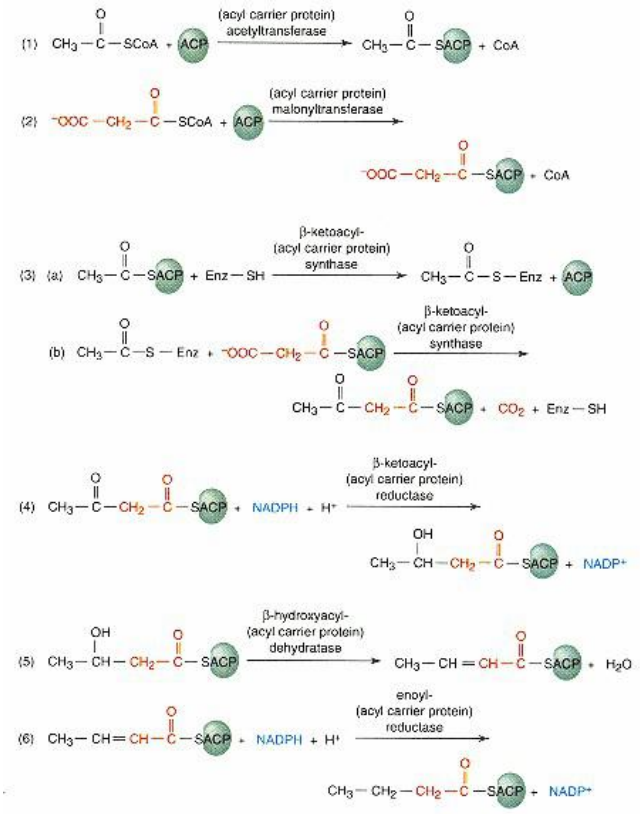


Figure 9.7
Reactions catalyzed by fatty acid synthase.

malonyl CoA (Reaction 2) and allows Reactions 3b to 6 to generate hexanoyl-ACP. The process is repeated five more times at which point palmitoyl-ACP is acted on by a **thioesterase** with production of free palmitic acid (Figure 9.8). Note that at this stage the sulfhydryl groups of ACP and β-ketoacyl-ACP synthase are both free so that another cycle of fatty acid synthesis can begin.

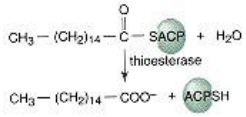


Figure 9.8
Release of palmitic acid from fatty acid synthase.

Mammalian Fatty Acid Synthase Is a Multifunctional Polypeptide

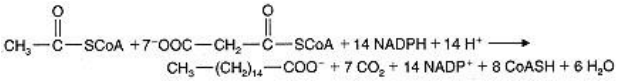
The reaction sequence given above is the basic pattern for fatty acid biosynthesis in living systems. The details of the reaction mechanisms are still unclear and may vary between species. The enzyme complex termed fatty acid synthase catalyzes all these reactions, but its structure and properties vary considerably. The individual enzymes in *Escherichia coli* are dissociable. By contrast, **mammalian synthase** is composed of two possibly identical subunits, each of which is a multienzyme polypeptide containing all of the necessary catalytic activities in a linear array. Even between mammalian species and tissues there are variations.

It appears that the growing fatty acid chain is continually bound to the multifunctional protein and is sequentially transferred between the 4 -phospho-pantetheine group of ACP, a domain on the protein, and the sulfhydryl group of a cysteine residue on β-ketoacyl-ACP synthase during the condensation reaction (Reaction 3, Figure 9.7) (see also Figure 9.9). An intermediate acylation to a serine residue probably takes place when acyl CoA units add to enzyme-bound ACP in the transacylase reactions.

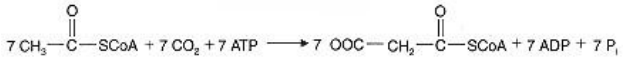
Regulation of palmitate biosynthesis probably occurs primarily by controlling the rate of synthesis and degradation of the enzyme. The agents and conditions that do this are given in Table 9.2. They are logical in terms of balancing an efficient utilization of the various biological energy substrates.

Stoichiometry of Fatty Acid Biosynthesis

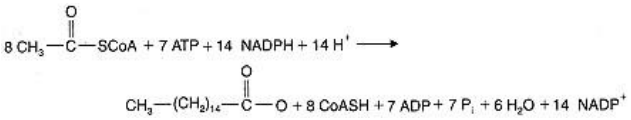
If acetyl CoA is the primer for palmitate biosynthesis, the overall reaction is



To calculate the energy needed for the overall conversion of acetyl CoA to palmitate, we must add the ATP used in formation of malonyl CoA:



Then the stoichiometry for conversion of acetyl CoA to palmitate is



Acetyl CoA Must Be Transported from Mitochondria to the Cytosol for Palmitate Synthesis

Fatty acid synthase and acetyl-CoA carboxylase are found primarily in the cytosol where biosynthesis of palmitate occurs. Mammalian tissues must use special processes to ensure an adequate supply of acetyl CoA and NADPH for this synthesis in the cytosol. The major source of acetyl CoA is the pyruvate dehydro-

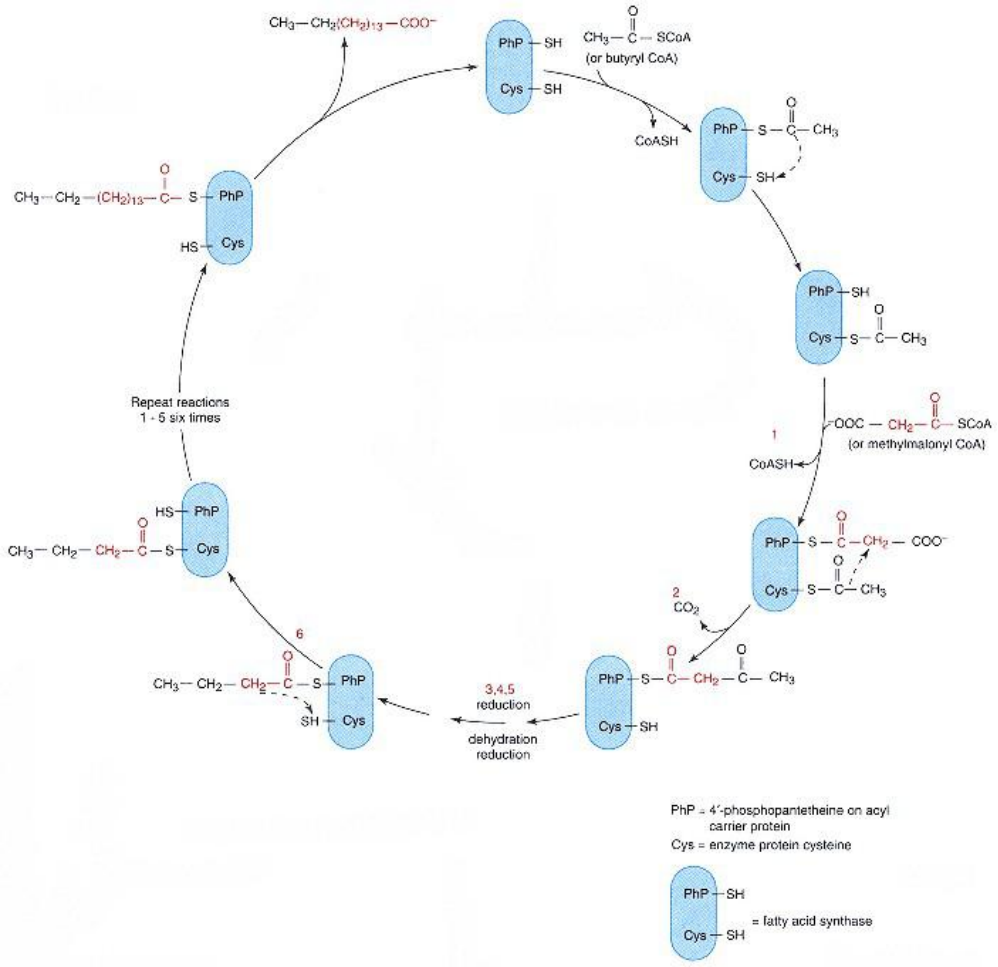


Figure 9.9
Proposed mechanism of elongation reactions taking place on mammalian fatty acid synthase.

genase reaction in the matrix of mitochondria. Since the mitochondrial inner membrane is not readily permeable to acetyl CoA, a process involving citrate moves the C2 unit to the cytosol for palmitate biosynthesis. This mechanism (Figure 9.10) takes advantage of the facts that citrate exchanges freely from mitochondria to cytosol (see p. 243) and that an enzyme exists in cytosol to convert citrate to acetyl CoA and oxaloacetate. When there is an excess of citrate from the TCA cycle, this intermediate will pass into the cytosol and supply acetyl CoA for fatty acid biosynthesis. The cleavage reaction, which is

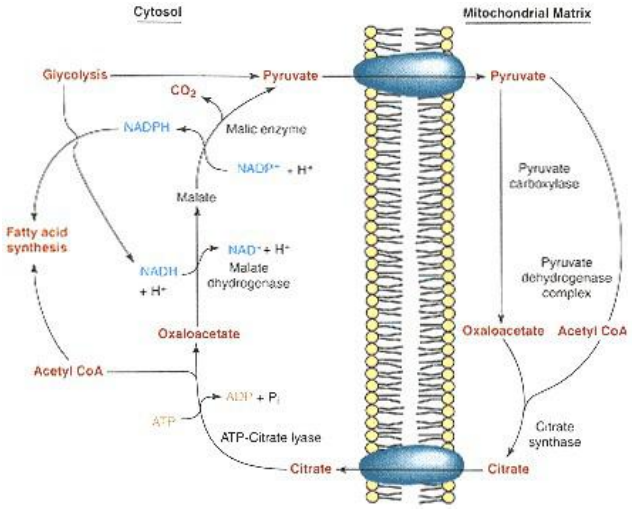


Figure 9.10
Mechanism for transfer of acetyl CoA from mitochondria to cytosol for fatty acid biosynthesis.

energy requiring, is catalyzed by **ATP-citrate lyase:**

$$\text{Citrate} + \text{ATP} + \text{CoA} \longrightarrow \text{acetyl CoA} + \text{ADP} + P_i + \text{oxaloacetate}$$

This mechanism has other advantages because CO_2 and NADPH for synthesis of palmitate can be produced from excess cytosolic oxaloacetate. As shown in Figure 9.10, NADH reduces oxaloacetate to malate via **malate dehydrogenase,** and malate is then decarboxylated by **NADP-linked malic enzyme** (malate: $NADP^+$ oxidoreductase-decarboxylating) to produce NADPH, pyruvate, and CO_2. Thus NADPH is produced from NADH generated in glycolysis. The cycle is completed by return of pyruvate to the mitochondrion where it can be carboxylated to regenerate oxaloacetate, as described in the process of gluconeogenesis (see p. 299).

In summary, 1 NADH is converted to NADPH for each acetyl CoA transferred from mitochondria to cytosol, each transfer requiring 1 ATP. The transfer of the 8 acetyl CoA used for each molecule of palmitate supplies 8 NADPH. Since palmitate biosynthesis requires 14 NADPH mol^{-1}, the other 6 NADPH must be supplied by the cytosolic pentose phosphate pathway. This stoichiometry is, of course, hypothetical. The *in vivo* relationships are complicated because transport of citrate and other di- and tricarboxylic acids across the inner mitochondrial membrane occurs by one-for-one exchanges. The actual flow rates are probably controlled by a composite of the concentration gradients of several of these exchange systems.

Palmitate Is the Precursor of Other Fatty Acids

Humans can synthesize all of the fatty acids they need from palmitate except the essential, polyunsaturated fatty acids (see p. 365). These syntheses involve a variety of enzyme systems in a number of locations. Palmitate produced by fatty acid synthase is modified by three processes: elongation, desaturation, and hydroxylation.

Elongation Reactions

In mammals **elongation of fatty acids** occurs in either the endoplasmic reticulum or mitochondria; the processes are slightly different in these two loci. In the endoplasmic reticulum the sequence of reactions is similar to that occurring in the cytosolic fatty acid synthase with malonyl CoA as the source of two-carbon units and NADPH providing the reducing power. The preferred substrate for elongation is palmitoyl CoA. In contrast to palmitate synthesis, intermediates in subsequent reactions are CoA esters rather than attached to a protein, suggesting that the process is carried out by separate enzymes rather than a complex like fatty acid synthase. In most tissues this elongation system in the endoplasmic reticulum converts palmitate to stearate almost exclusively. Brain, however, contains one or more additional elongation systems, which synthesize longer chain acids (up to C24) needed for brain lipids. These other systems also use malonyl CoA as substrate.

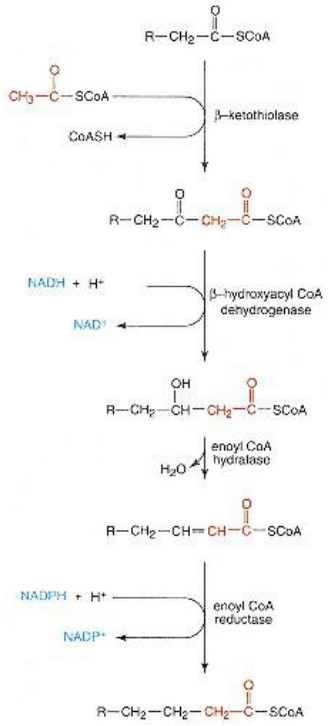


Figure 9.11
Mitochondrial elongation of fatty acids.

The elongation system in mitochondria differs in that acetyl CoA is the source of the added two-carbon units and both NADH and NADPH serve as reducing agents (Figure 9.11). This system operates by reversal of the pathway of fatty acid β-oxidation (see Section 9.6) with the exception that NADPH-linked enoyl-CoA reductase (last step of elongation) replaces FAD-linked acyl-CoA dehydrogenase (first step in β-oxidation). The process has little activity with acyl CoA substrates of C16 atoms or longer, suggesting that it serves primarily in elongation of shorter chain species.

Formation of Monoenoic Acids by Stearoyl CoA Desaturase

In higher animals **desaturation of fatty acids** occurs in the endoplasmic reticulum, and the oxidizing system used to introduce cis double bonds is significantly different from the main fatty acid oxidation process in mitochondria. The systems in endoplasmic reticulum have sometimes been termed "**mixed function oxidases**" because the enzymes simultaneously oxidize two substrates. In fatty acid desaturation one of these substrates is NADPH and the other is the fatty acid. Electrons from NADPH are transferred through a specific flavoprotein reductase and a cytochrome to "active" oxygen so that it will then oxidize the fatty acid. Although the complete mechanism has not been determined, this latter step may involve a hydroxylation. The three components of the system are the **desaturase enzyme, cytochrome b_5,** and **NADPH-cytochrome b_5 reductase.** The overall reaction is

$$R{-}CH_2{-}CH_2{-}(CH_2)_7{-}COOH + NADPH + H^+ + O_2 \longrightarrow R{-}CH{=}CH{-}(CH_2)_7{-}COOH + NADP^+ + 2\,H_2O$$

The enzyme specificity is such that the R group must contain at least six carbon atoms.

The regulatory mechanisms that govern the conversion of palmitate to unsaturated fatty acids are largely unexplored. An important consideration is the control of the proportions of unsaturated fatty acids for proper maintenance of the physical state of stored triacylglycerols and membrane phospholipids. A committed step in the formation of unsaturated fatty acids from palmitate or stearate is introduction of the first double bond between C-9 and C-10 atoms by **stearoyl CoA desaturase** to produce palmitoleic or oleic acid, respectively. The activity of this enzyme and its synthesis are controlled by both dietary and hormonal mechanisms. Increasing the amounts of polyunsaturated fatty acids in the diet of experimental animals decreases the activity of stearoyl CoA desaturase in liver, and insulin, triiodothyronine, and hydrocortisone cause its induction.

Formation and Modification of Polyunsaturated Fatty Acids

A variety of **polyunsaturated fatty acids** are synthesized by humans through a combination of elongation and desaturation reactions. Once the initial double

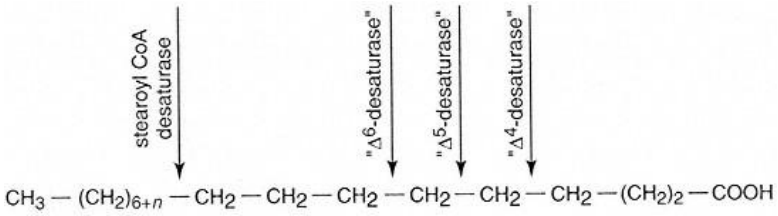
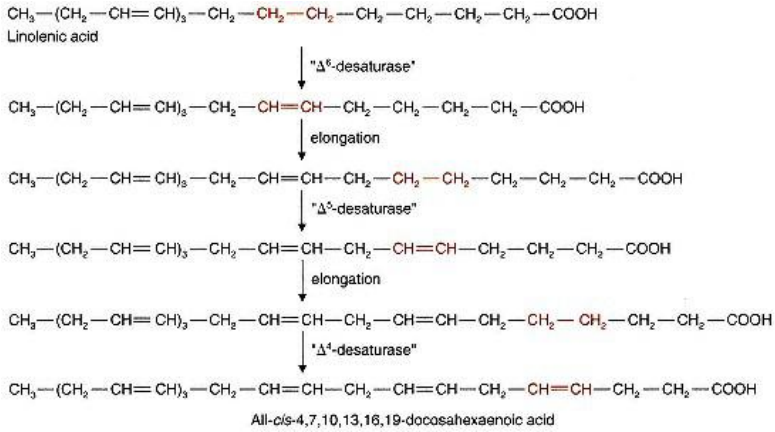


Figure 9.12
Positions in the fatty acid chain where desaturation can occur in the human.
There must always be at least six single bonds in the chain toward the methyl end of the molecule just beyond the bond being desaturated.

bond has been placed between carbons 9 and 10 by stearoyl CoA desaturase, additional double bonds can be introduced just beyond C-4, C-5, or C-6 atoms. Desaturation at C-8 probably occurs also in some tissues. The positions of these desaturations are shown in Figure 9.12. The relative specificities of the various enzymes are still to be determined completely, but it seems likely that elongation and desaturation can occur in either order. Conversion of linolenic acid to all *cis*-4, 7, 10, 13, 16, 19-docosahexaenoic acid in brain is a specific example of such a sequence.



Polyunsaturated fatty acids, particularly arachidonic acid, are precursors of the highly active prostaglandins and thromboxanes. Different classes of prostaglandins are formed depending on the precursor fatty acid and the sequence of oxidations that convert the acids to active compounds. A detailed discussion of these substances and their formation is found in Chapter 10. Polyunsaturated fatty acids in living systems have a significant potential for auto-oxidation, a process that may have important physiological and/or pathological consequences. Auto-oxidation reactions cause rancidity in fats and curing of linseed oil in paints.

Formation of Hydroxy Fatty Acids in Nerve Tissue

There are apparently two different processes that produce **α-hydroxy fatty acids** in higher animals. One occurs in the mitochondria of many tissues and acts on relatively short-chain fatty acids (see Section 9.6). The other has been demonstrated only in tissues of the nervous system where it produces long-chain fatty acids with a hydroxyl group on C-2. These are needed for the formation of some myelin lipids. The specific case of α-hydroxylation of lignoceric acid to cerebronic acid has been studied. These enzymes preferentially use C22 and C24 fatty acids and show characteristics of the "mixed function oxidase" systems, requiring molecular oxygen and NADH or NADPH. This

synthesis may be closely coordinated with biosynthesis of sphingolipids that contain hydroxylated fatty acids.

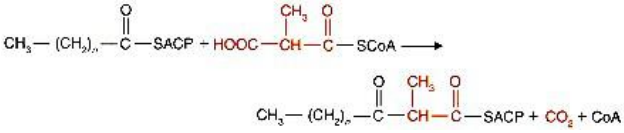
Fatty Acid Synthase Can Produce Fatty Acids Other than Palmitate

The schemes outlined, which synthesize and modify palmitate, account for the great bulk of fatty acid biosynthesis in the human body, particularly that involved in energy storage. There are, however, many special instances where smaller amounts of different fatty acids are needed for specific structural or functional purposes. These acids are produced by modification of the process carried out by fatty acid synthase. Two examples are production of fatty acids shorter than palmitate in mammary glands and synthesis of branched-chain fatty acids in certain secretory glands.

Milk produced by many animals contains varying amounts of fatty acids with shorter chain lengths than palmitate. The amounts produced by **mammary gland** apparently vary with species and especially with the physiological state of the animal. This is probably true of humans, although most investigations have been carried out with rats, rabbits, and various ruminants. The same fatty acid synthase that produces palmitate synthesizes shorter chain acids when the linkage of the growing chain with acyl carrier protein is split before the full C16 chain is completed. This hydrolysis is caused by soluble **thioesterases** whose activity is under hormonal control.

There are relatively few branched-chain fatty acids in higher animals. Until recently, their metabolism has been studied mostly in primitive species such as mycobacteria, where they are present in greater variety and amount. Simple branched-chain fatty acids are synthesized by tissues of higher animals for specific purposes, such as the production of waxes in sebaceous glands and avian preen glands and the elaboration of structures in echo-locating systems of porpoises.

The majority of branched-chain fatty acids in higher animals are synthesized by fatty acid synthase and are methylated derivatives of saturated, straight-chain acids. When **methylmalonyl CoA** is used as a substrate instead of malonyl CoA, a methyl side chain is inserted in the fatty acid, and the reaction is as follows:



Regular reduction steps then follow. Apparently these reactions occur in many tissues normally at a rate several orders of magnitude lower than the utilization of malonyl CoA to produce palmitate. The proportion of branched-chain fatty acids synthesized is largely governed by the relative availability of the two precursors. An increase in branching can occur by decreasing the ratio of malonyl CoA to methylmalonyl CoA. A malonyl-CoA decarboxylase capable of causing this decrease occurs in many tissues. It has also been suggested that increased levels of methylmalonyl CoA in pathological situations, such as vitamin B_{12} deficiency, can lead to excessive production of branched-chain fatty acids.

Fatty Acyl CoAs May Be Reduced to Fatty Alcohols

As discussed in Chapter 10, many phospholipids contain fatty acid chains in ether linkage rather than ester linkage. The biosynthetic precursors of these

ether-linked chains are **fatty alcohols** (Figure 9.13) rather than fatty acids. These alcohols are formed in higher animals by a two-step, NADPH-linked reduction of fatty acyl CoAs in the endoplasmic reticulum. In organs that produce relatively large amounts of ether-containing lipids, the concurrent production of fatty acids and fatty alcohols is probably closely coordinated.

$$CH_3—(CH_2)_n—CH_2OH$$

Figure 9.13
Fatty alcohol.

9.4— Storage of Fatty Acids As Triacylglycerols

Most tissues in the body can convert fatty acids to triacylglycerols (Figure 9.14) by a common sequence of reactions, but liver and adipose tissue carry out this process to the greatest extent. Adipose tissue is a specialized connective tissue designed for synthesis, storage, and hydrolysis of triacylglycerols. This is the main system for long-term energy storage in humans. We are concerned here with white adipose tissue as opposed to brown adipose tissue, which occurs in much lesser amounts and has other specialized functions. Triacylglycerols are stored as liquid droplets in the cytoplasm, but this is not "dead storage" since they turn over with an average half-life of only a few days. Thus, in a homeostatic situation, there is continuous synthesis and breakdown of triacylglycerols in adipose tissue. Some storage also occurs in skeletal and cardiac muscle, but only for local consumption.

Triacylglycerol synthesis in liver is used primarily for production of blood lipoproteins, although the products can serve as energy sources for other liver

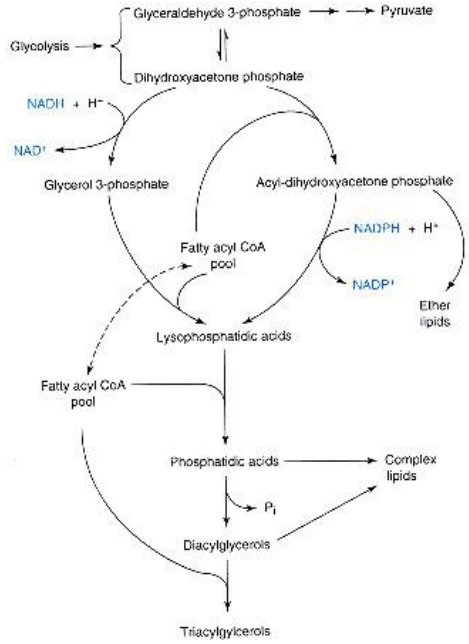
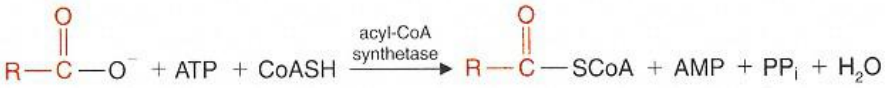


Figure 9.14
Alternative pathways for biosynthesis of triacylglycerols from dihydroxyacetone phosphate.

functions. Required fatty acids may come from the diet, from adipose tissue via blood transport, or from *de novo* biosynthesis. Acetyl CoA for biosynthesis is derived principally from glucose catabolism.

Triacylglycerols Are Synthesized from Fatty Acyl CoAs and Glycerol 3-Phosphate in Most Tissues

Triacylglycerols are synthesized in most tissues from activated fatty acids and a phosphorylated three-carbon product of glucose catabolism (see Figure 9.14), which can be either **glycerol 3-phosphate** or **dihydroxyacetone phosphate.** Glycerol 3-phosphate is formed either by reduction of dihydroxyacetone phosphate produced in glycolysis or by phosphorylation of glycerol. White adipose tissue contains little or no glycerol kinase, so it derives glycerol phosphate from glycolytic intermediates. Fatty acids are activated by conversion to their CoA esters in the following reaction:



This two-step reaction has an acyl adenylate as intermediate and is driven by hydrolysis of pyrophosphate to P_i.

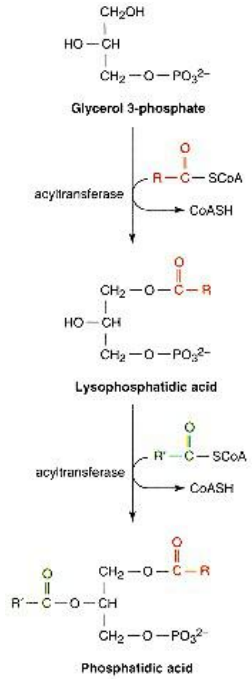


Figure 9.15
Synthesis of phosphatidic acid from glycerol 3-phosphate.

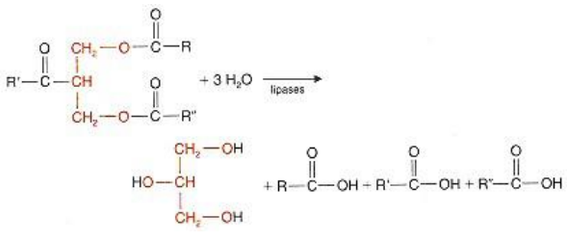
Synthesis of triacylglycerols from phosphorylated three-carbon fragments involves formation of **phosphatidic acid,** which is a key intermediate in synthesis of other lipids as well (see Chapter 10). This may be formed by two sequential acylations of glycerol 3-phosphate, as shown in Figure 9.15. Alternatively, dihydroxyacetone phosphate may be acylated directly at C-1 followed by reduction at C-2. The resultant lysophosphatidic acid can then be further esterified, as illustrated in Figure 9.16. If phosphatidic acid from either of these routes is used for synthesis of triacylglycerol, the phosphate group is next hydrolyzed by **phosphatidate phosphatase** to yield diacylglycerol, which is then acylated to triacylglycerol (Figure 9.17).

There is at least one tissue, **intestinal mucosa,** in which the synthesis of triacylglycerols does not require formation of phosphatidic acid as described above. A major product of intestinal digestion of lipids is 2-monoacylglycerols, which are absorbed as such into mucosa cells. An enzyme in these cells catalyzes acylation of these monoacylglycerols with acyl CoA to form 1,2-diacylglycerols, which then can be further acylated as shown above.

The specificity of the acylation reactions in all these steps is still quite controversial. Analysis of **fatty acid patterns** in triacylglycerols from various human tissues shows that the distribution of different acids on the three positions of glycerol is neither random nor absolutely specific. The patterns in different tissues show some characteristic tendencies. Palmitic acid tends to be concentrated in position 1 and oleic acid in positions 2 and 3 of human adipose tissue triacylglycerols. Two main factors that determine localization of a particular fatty acid to a given position on glycerol are the specificity of acyltransferase involved and relative availability of different fatty acids in the fatty acyl CoA pool. Other factors are probably involved but their relative importance has not been determined.

Mobilization of Triacylglycerols Requires Hydrolysis

The first step in recovering stored fatty acids for energy production is hydrolysis of triacylglycerols. A variety of lipases catalyze this reaction, the sequence of hydrolysis from the three positions on glycerol depending on the specificities of the particular lipases involved.



Lipases in adipose tissue are, of course, key enzymes for release of the major energy stores. The lipase that removes the first fatty acid is a controlled enzyme, which is sensitive to a variety of circulating hormones. This control of triacylglycerol hydrolysis must be balanced with the process of triacylglycerol synthesis to assure adequate energy stores and avoid obesity (see Clin. Corr. 9.1 and 9.2). Fatty acids and glycerol produced by adipose tissue lipases are released to circulating blood, where fatty acids are bound by serum albumin and transported to tissues for use. Glycerol returns to liver, where it is converted to dihydroxyacetone phosphate and enters glycolytic or gluconeogenic pathways.

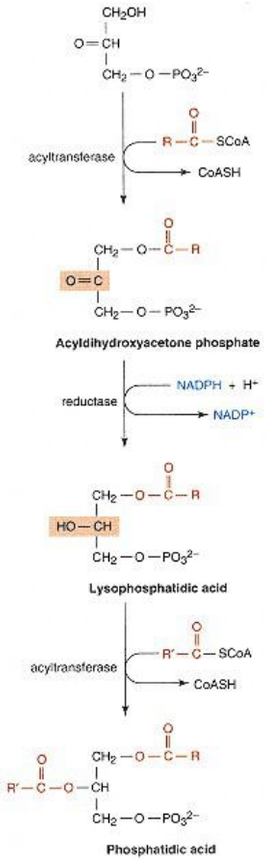


Figure 9.16
Synthesis of phosphatidic acid from dihydroxyacetone phosphate.

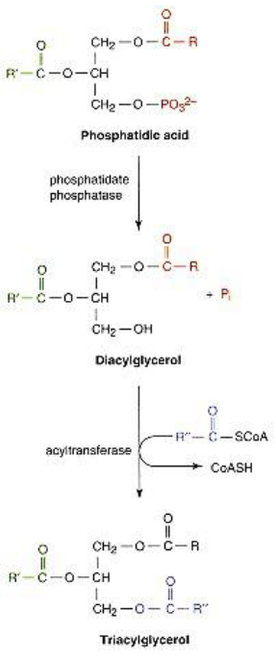


Figure 9.17
Synthesis of triacylglycerol from phosphatidic acid.

CLINICAL CORRELATION 9.1

Obesity

The terms obesity and overweight refer to excess in body weight relative to height. Their definitions are arbitrary and are based on actuarial estimates of ideal body weight (IBW), that is, body weight associated with the lowest morbidity and mortality. Relative weight is body weight relative to IBW: overweight is defined as relative weight up to 20% above normal and obesity is relative weight over 20% above IBW. The body mass index (BMI) is well correlated with measures of body fat and is defined as weight (kg) divided by height^2 (m_2). Overweight is defined as a BMI of 25–30 kg per m_2 and obesity as a BMI > 30 kg per m_2. Skinfold thickness also is a measure of body fat stores.

The cause of most cases of obesity is not known. Endocrine diseases such as hypothyroidism or Cushing's disease (overproduction of corticosteroids) are rare causes. Genetic factors interact with environmental factors: 80% of children of two obese parents will be obese, while only 14% of children of normal weight parents are obese. The major mechanism of weight gain is consumption of calories in excess of daily energy requirements, but the normal processes controlling food intake are not very well understood. Rarely, tumors of the hypothalamus result in pathological overeating (hyperphagia). However, a specific defect in most cases of human obesity has not been demonstrated.

The treatment of obesity revolves about dietary restriction, increased physical activity, and behavior modification. The real problem is to modify the patients' eating patterns long term, and even in those who lose weight, regain of the weight is very common. Currently, no pharmacological agents are effective in promoting long-term weight control. Surgery to limit the size of the gastric reservoir can be considered for patients over 100% above IBW. Medical complications of obesity include a two- to threefold increase in hypertension, gallstones, and diabetes, and fivefold increase in risk of endometrial carcinoma. Obese patients have decreased plasma antithrombin III levels, which predisposes them to venous thrombosis (see Clin. Corr. 8.8).

Bray, G. A. Complications of obesity. *Ann. Intern. Med.* 103:1052, 1985; and Bray, G. A. The syndromes of obesity: an endocrine approach. In: L. J. DeGroot (Ed.), *Endocrinology*, Vol. 3, 3rd ed. Philadelphia: Saunders, 1995, p. 2624.

9.5— Methods of Interorgan Transport of Fatty Acids and Their Primary Products

Lipid-Based Energy Is Transported in Blood in Different Forms

The energy available in fatty acids needs to be distributed throughout the body from the site of fatty acid absorption, biosynthesis, or storage to functioning tissues that consume them. This transport is closely integrated with that of other lipids, especially cholesterol, and is intimately involved in pathological processes leading to atherosclerosis. Various mechanisms are being intensively studied, but many important questions are still unanswered.

In humans, three types of substances are used as vehicles to transport lipid-based energy: (1) chylomicrons and other plasma lipoproteins in which

CLINICAL CORRELATION 9.2

Leptin and Obesity

In 1994 the *OB* gene of mice, its protein product, and their human homologues were identified. The human gene encodes a polypeptide of 166 amino acids that is expressed in adipose tissue in proportion to the severity of the obesity. The secreted protein, called leptin, contains 146 amino acids, can be measured by immunoassay, and is highly homologous to the murine protein.

Mice of the ob/ob strain that inherit a nonsense mutation in the leptin gene, leading to a truncated protein of 104 amino acids that is not secreted, are obese, diabetic, and exhibit reduced activity, metabolism, and body temperature. Injection of recombinant leptin into mice homozygous for this mutation lowered their food intake, body weight, percentage of body fat, and serum glucose and insulin concentrations, and increased their metabolic rate, body temperature, and activity level.

There is no difference in the structure of leptin between lean and obese human subjects. This suggests that the problem in obese individuals might be decreased sensitivity to leptin. Interestingly, a leptin receptor present in the hypothalamus has been shown to be defective in the db/db mouse and the fa/fa Zucker rat. In both cases the phenotype is similar to that of the ob/ob mouse. Whether an analogous situation applies in human obesity remains to be established.

Considine, R. V., Sinha, M. K., Heiman, M. L., et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N. Engl. J. Med.* 334:292, 1996; and Lee, G. H., Proenca, R., Montez, J. M., et al. Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 379:632, 1996.

triacylglycerols are carried in protein-coated lipid droplets, both of which contain other lipids; (2) fatty acids bound to serum albumin; and (3) so-called ketone bodies, acetoacetate and β-hydroxybutyrate. These three vehicles are used in varying proportions to carry energy in the bloodstream via three routes. One is transport of dietary fatty acids as chylomicrons throughout the body from the intestine after absorption. Another is transport of lipid-based energy processed by or synthesized in liver and distributed either to adipose tissue for storage or to other tissues for use; this includes "ketone bodies" and plasma lipoproteins other than chylomicrons. Finally, there is transport of energy released from storage in adipose tissue to the rest of the body in the form of fatty acids that are bound to serum albumin.

The proportion of energy being transported in any one of the modes outlined above varies considerably with metabolic and physiological state. At any time, the largest amount of lipid in blood is in the form of triacylglycerols in various lipoproteins. Fatty acids bound to albumin, however, are utilized and replaced very rapidly so total energy transport for a given period by this mode may be very significant.

Plasma Lipoproteins Carry Triacylglycerols and Other Lipids

Plasma lipoproteins are synthesized in both intestine and liver and are a heterogeneous group of lipid–protein complexes composed of various types of lipids and apoproteins (see p. 56 for a detailed discussion of structure). The two most important vehicles for delivery of lipid-based energy are **chylomicrons** and **very low density lipoprotein (VLDL),** because they contain relatively large amounts of triacylglycerols. Chylomicrons are formed in the intestine and function in absorption and transport of dietary triacylglycerol, cholesterol, and fat-soluble vitamins. The exact precursor–product relationships between the other types of plasma lipoproteins have yet to be completely defined, as do the roles of various protein components. Liver synthesizes VLDL and fatty acids from triacylglycerols in VLDL are taken up by adipose tissue and other tissues. In the process VLDLs are converted to **low density lipoproteins (LDLs).** The role of **high density lipoprotein (HDL)** in transport of lipid-based energy is yet to be clarified. All of these lipoproteins are integrally involved in transport of other lipids, especially cholesterol. Lipid components can interchange to some extent between different classes of lipoprotein, and some apoproteins probably have functional roles in modifying enzyme activity during exchange of lipids between plasma lipoproteins and tissues. Other apoproteins serve as specific recognition sites for cell surface receptors. Such interaction constitutes the first step in receptor-mediated endocytosis of certain lipoproteins. Studies of rare genetic abnormalities have been helpful in explaining the roles of some of these apoproteins (see Clin. Corr. 9.3).

Fatty Acids Are Bound to Serum Albumin

Serum albumin acts as a carrier for a number of substances in blood, some of the most important being fatty acids. These acids are water insoluble in themselves, but when they are released into plasma during triacylglycerol hydrolysis they are quickly bound to albumin. This protein has a number of binding sites for fatty acid, some of them having very high affinity. At any one time the number of sites on albumin actually occupied with fatty acids is far from maximal, but the turnover of these fatty acids is high, so binding by this mechanism constitutes a major route of energy transfer.

Ketone Bodies Are a Lipid-Based Energy Supply

The third mode of transport of lipid-based energy-yielding molecules is in the form of small water-soluble molecules, **acetoacetate** and **β-hydroxybutyrate** (Figure 9.18), produced primarily by liver during oxidation of fatty acids. The reactions involved in their formation and utilization will be discussed later.

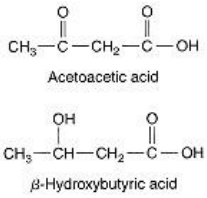


Figure 9.18
Structures of ketone bodies.

CLINICAL CORRELATION 9.3

Genetic Abnormalities in Lipid-Energy Transport

Diseases that affect the transport of lipid-based energy frequently result in abnormally high plasma triacylglycerols, cholesterol, or both. They are classified as hyperlipidemias. Some of them are genetically transmitted, and presumably they result from the alteration or lack of one or more proteins involved in the production or processing of plasma lipids. The nature and function of all of these proteins are yet to be determined, so the elucidation of exact causes of the pathology in most of these diseases is still in the early stages. However, in several cases a specific protein abnormality has been associated with altered lipid transport in the patient's plasma.

In the extremely rare disease, analbuminemia, there is an almost complete lack of serum albumin. In a rat strain with analbuminemia, a 7 base-pair deletion in an intron of the albumin gene results in the inability to process the nuclear mRNA for albumin. Despite the many functions of this protein, the symptoms of the disease are surprisingly mild. Lack of serum albumin effectively eliminates the transport of fatty acids unless they are esterified in acylglycerols or complex lipids. However, since patients with analbuminemia do have elevated plasma triacylglycerol levels, presumably the deficiency in lipid-based energy transport caused by the absence of albumin to carry fatty acids is filled by increased use of plasma lipoproteins to carry triacylglycerols.

A more serious genetic defect is the absence of lipoprotein lipase. The major problem here is the inability to process chylomicrons after a fatty meal. Pathological fat deposits occur in the skin (eruptive xanthomas) and the patients typically suffer from pancreatitis. If patients are put on a low-fat diet they respond reasonably well.

Another rare but more severe disease, abetalipoproteinemia, is caused by defective synthesis of apoprotein B, an essential component in the formation of chylomicrons and VLDL. Under these circumstances the major pathway for transporting lipid-based energy from the diet to the body is unavailable. Chylomicrons, VLDL, and LDL are absent from the plasma and fat absorption is deficient or nonexistent. There are other serious symptoms, including neuropathy and red cell deformities, whose etiology is less clear.

Havel, R. J., and Kane, J. P. Structure and metabolism of plasma lipoproteins. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, Vol. II, 7th ed. New York: McGraw-Hill, 1995, p. 1841; and Brunzell, J. D. Familial lipoprotein lipase deficiency and other causes of the chylomicronemia syndrome. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, Vol. II, 7th ed. New York: McGraw-Hill, 1995, p. 1913.

Under certain conditions, these substances can reach excessive concentrations in blood, leading to ketosis and acidosis. Spontaneous decarboxylation of acetoacetate to **acetone** also occurs, which is detectable as the smell of acetone in the breath when acetoacetate concentrations are high. This led early investigators to call the group of soluble products **"ketone bodies."** In fact, β-hydroxybutyrate and acetoacetate are continually produced by liver and, to a lesser extent, by kidney. Skeletal and cardiac muscle utilize them to produce ATP. Nervous tissue, which normally obtains almost all of its energy from glucose, is unable to take up and use fatty acids bound to albumin for energy production. However, it can use ketone bodies when glucose supplies are insufficient.

Lipases Must Hydrolyze Blood Triacylglycerols for Their Fatty Acids to Become Available to Tissues

Fatty acids bound to albumin and ketone bodies are readily taken up by various tissues for oxidation and production of ATP. The energy in fatty acids stored or circulated as triacylglycerols, however, is not directly available, but rather triacylglycerols must be enzymatically hydrolyzed to release the fatty acids and glycerol. Two types of lipases are involved: (1) **lipoprotein lipase,** which hydrolyzes triacylglycerols in plasma lipoproteins; and (2) **"hormone-sensitive triacylglycerol lipase,"** which initiates hydrolysis of triacylglycerols in adipose tissue and release of fatty acids and glycerol into plasma.

Lipoprotein lipase is located on the surface of endothelial cells of capillaries and possibly of adjoining tissue cells. It hydrolyzes fatty acids from the 1 and/ or 3 position of tri- and diacylglycerols present in VLDL or chylomicrons. One of the lipoprotein apoproteins (ApoC-II) must be present to activate the process. Fatty acids released are either bound to serum albumin or taken up by the tissue. Monoacylglycerol products may either pass into the cells or be further hydrolyzed by serum **monoacylglycerol hydrolase.**

A completely distinct type of lipase controls mobilization of fatty acids from triacylglycerols stored in adipose tissue. One of them is hormonally controlled

TABLE 9.3 Regulation of Triacylglycerol Metabolism

Enzyme	*Regulatory Agent*	*Effect*
	Triacylglycerol Mobilization	
"Hormone-sensitive" lipase	"Lipolytic hormones," e.g., epinephrine, glucagon, and ACTH	Stimulation by cAMP-mediated phosphorylation of relatively inactive enzyme
	Insulin	Inhibition
	Prostaglandins	Inhibition
Lipoprotein lipase	Apolipoprotein C-II	Activation
	Insulin	Activation
	Triacylglycerol Biosynthesis	
Phosphatidate phosphatase	Steroid hormones	Stimulation by increased enzyme synthesis

by a cAMP-mediated mechanism. There are a number of lipase activities in the tissue, but the enzyme attacking triacylglycerols initiates the process. Two other lipases then rapidly complete the hydrolysis of mono- and diacylglycerols, releasing fatty acids to plasma where they are bound to serum albumin. Triacylglycerol metabolism is tightly controlled by both hormones and required cofactors. Some of the key regulatory factors are presented in Table 9.3.

9.6— Utilization of Fatty Acids for Energy Production

Fatty acids that arrive at the surface of cells are taken up and used for energy production primarily in mitochondria in a process intimately integrated with energy generation from other sources. Energy-rich intermediates produced from fatty acids are the same as those obtained from sugars, that is, NADH and $FADH_2$. The final stages of the oxidation process are exactly the same as for carbohydrates, that is, the metabolism of acetyl CoA by the TCA cycle and production of ATP in the mitochondrial electron transport system.

The degree of utilization of fatty acids for energy production varies considerably from tissue to tissue and depends to a significant extent on the metabolic status of the body, whether it is fed or fasted, exercising or at rest, and so on. For instance, nervous tissue oxidizes fatty acids to a minimal degree, if at all, but cardiac and skeletal muscle depend heavily on fatty acids as a major energy source. During prolonged fasting most tissues can use fatty acids or ketone bodies for their energy requirements.

β-Oxidation of Straight-Chain Fatty Acids Is the Major Energy-Producing Process

For the most part, fatty acids are oxidized by a mechanism that is similar to, but not identical with, a reversal of the process of palmitate synthesis. That is, two-carbon fragments are removed sequentially from the carboxyl end of the acid after steps of **dehydrogenation, hydration,** and **oxidation** to form a β-keto acid, which is split by **thiolysis.** These processes take place while the acid is activated in a thioester linkage to the 4 -phosphopantetheine of CoA.

Fatty Acids Are Activated by Conversion to Fatty Acyl CoA

The first step in oxidation of a fatty acid is its activation to a fatty acyl CoA. This is the same reaction described for synthesis of triacylglycerols in Section 9.4 and occurs in the endoplasmic reticulum or the outer mitochondrial membrane.

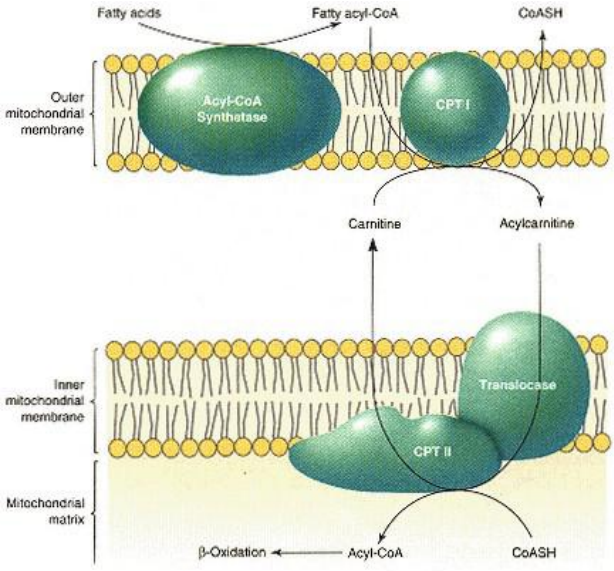
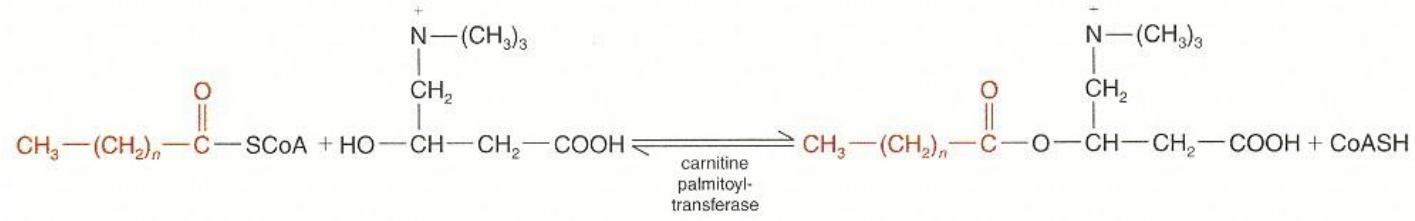


Figure 9.19
Mechanism for transfer of fatty acids from the cytosol through the inner mitochondrial membrane for oxidation.

Carnitine Carries Acyl Groups across the Mitochondrial Membrane

Whereas most fatty acyl CoAs are formed outside mitochondria, the oxidizing machinery is inside the inner membrane, which is impermeable to CoA and its derivatives. The cell overcomes this problem by using **carnitine (4-trimethylamino-3-hydroxybutyrate)** as the carrier of acyl groups across the membrane. The steps involved are outlined in Figure 9.19. Enzymes on both sides of the membrane transfer fatty acyl groups between CoA and carnitine.



On the outer mitochondrial membrane the acyl group is transferred to carnitine catalyzed by **carnitine palmitoyltransferase I (CPT I).** Acyl carnitine then exchanges across the inner mitochondrial membrane with free carnitine by a carnitine–acylcarnitine antiporter translocase. Finally, the fatty acyl group is transferred back to CoA by **carnitine palmitoyltransferase II (CPT II)** located on the matrix side of the inner membrane. This process functions primarily in mitochondrial transport of fatty acyl CoAs with chain lengths of C12–C18. Genetic abnormalities in the system lead to serious pathology (see Clin. Corr. 9.4). By contrast, entry of shorter chain fatty acids is independent of carnitine because they cross the inner mitochondrial membrane directly and become activated to their CoA derivatives in the matrix compartment.

β-Oxidation Is a Sequence of Four Reactions

The four reactions of β-oxidation are presented in Figure 9.20. Once the fatty acyl groups have been transferred back to CoA at the inner surface of the inner

mitochondrial membrane, they can be oxidized (see Reaction 1) by a group of **acyl-CoA dehydrogenases.** Dehydrogenases are present on the inner membrane and remove hydrogen atoms to form enoyl CoA with a trans double bond between C-2 and C-3 atoms. There are several different dehydrogenases with different specificities for chain length of the acyl CoA. All are flavoproteins (see Clin. Corr. 9.5). As in the TCA cycle, enzyme-bound $FADH_2$ transfers electrons through several other electron-transferring flavoproteins to ubiquinone in the electron transport system, yielding two ATPs for each double bond formed.

The second step in β-oxidation is hydration of the trans double bond to a **3-L-hydroxyacyl CoA.** This reaction is stereospecific, in that the L isomer is the product when the trans double bond is hydrated. The stereospecificity of the oxidative pathway is governed by the enzyme catalyzing the third reaction, which is specific for the L isomer as its substrate. The final step is the cleavage of the two-carbon fragment by a thiolase, which, like the preceding two enzymes, has relatively broad specificity with regard to chain length of the acyl group being oxidized. In the overall process then, an acetyl CoA is produced and the acyl CoA product is ready for the next round of oxidation starting with acyl-CoA dehydrogenase.

It has been impossible to show conclusively that any of the enzymes in the β-oxidation scheme are control points, although under rather rigid *in vitro* conditions some apparently have slower maximum rates of reaction than others. It is assumed that control is exerted by availability of substrates and cofactors and by the rate of processing of acetyl CoA by the TCA cycle. One way in which substrate availability is controlled is by regulation of the carnitine shuttle mechanism that transports fatty acids into mitochondria, a phenomenon of central importance in the regulation of hepatic ketone body production (see p. 387).

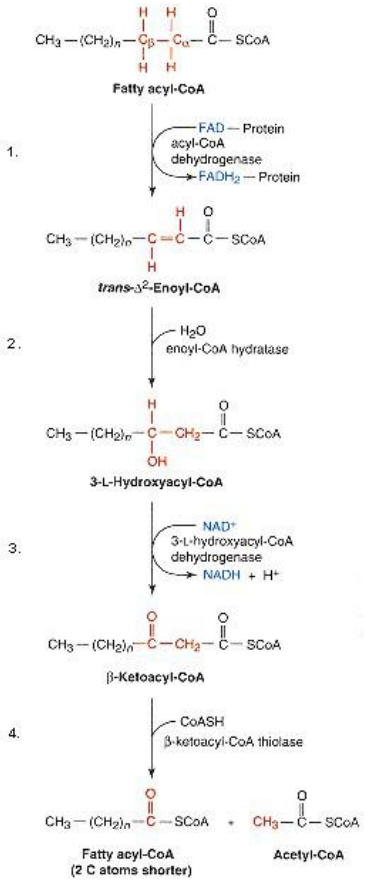


Figure 9.20
Pathway of fatty acid -oxidation.

Energy-Yield from β-Oxidation of Fatty Acids

Each set of oxidations resulting in production of a two-carbon fragment yields, in addition to acetyl CoA, one reduced flavoprotein and one NADH. In the oxidation of palmitoyl CoA, seven such cleavages take place, and in the last cleavage two acetyl CoA molecules are formed. Thus the products of β-oxidation of palmitate are eight acetyl CoAs, seven reduced flavoproteins, and seven NADH. Each of the reduced flavoproteins can yield two ATP and each NADH can yield three when oxidized by the electron transport chain, so the reduced nucleotides yield 35 ATP per palmitoyl CoA. As described in Chapter 6, oxidation of each acetyl CoA through the TCA cycle yields 12 ATP, so the eight two-carbon fragments from a palmitate molecule produce 96 ATP. However, 2 ATP equivalents (1 ATP going to 1 AMP) were used to activate palmitate to palmitoyl CoA. Therefore each palmitic acid entering the cell from the action of lipoprotein lipase or from its combination with serum albumin can yield 129 ATP mol^{-1} by complete oxidation. The significance of the role of fatty acids in supplying the energy needs in humans is discussed on page 536.

Comparison of the β-Oxidation Scheme with Palmitate Biosynthesis

In living metabolic systems the reactions in a catabolic pathway are sometimes quite similar to those in a reversal of the corresponding anabolic sequence, but there are usually mechanisms that provide for separate control of the two schemes. This is true in the case of fatty acid biosynthesis and β-oxidation. The critical differences between the two pathways are outlined in Table 9.4. They include separation by subcellular compartmentation (β-oxidation occurs in the mitochondria and palmitate biosynthesis in the cytosol) and use of different cofactors (NADPH in biosynthesis, FAD and NAD^+ in oxidation).

CLINICAL CORRELATION 9.4

Genetic Deficiencies in Carnitine Transport or Carnitine Palmitoyltransferase

A number of diseases result from genetic abnormalities in the transport of long-chain fatty acids across the inner mitochondrial membrane. They stem from deficiencies either in the level of carnitine or in the functioning of the carnitine palmitoyltransferase (CPT) enzyme system.

The clinical symptoms of carnitine deficiency range from mild, recurrent muscle cramping to severe weakness and death. Two categories of the disorder, primary and secondary, are now recognized. Primary carnitine deficiency is caused by a defect in the high-affinity plasma membrane carnitine transporter in tissues such as muscle, kidney, heart, and fibroblasts (but apparently not in liver where a different transporter is operative). It results in extremely low levels of carnitine in affected tissues and also in plasma (because of failure to the kidneys to reabsorb carnitine). The very low carnitine level in heart and skeletal muscle seriously compromises long-chain fatty acid oxidation. Dietary carnitine therapy, by raising the plasma concentration of carnitine and forcing its entry into tissues in a nonspecific manner, is frequently beneficial. Secondary carnitine deficiency is often associated with inherited defects in the β-oxidation pathway that give rise to the accumulation of acyl CoAs and, in turn, acylcarnitines. The latter compounds can be excreted in the urine (see Clin. Corr. 9.5), thus draining the body's carnitine pool; in addition, they are thought to impair the tissue uptake of free carnitine.

CPT deficiency also presents as distinct clinical entities. The most common deficiency results from mutations in the CPT II gene that give rise to a partial loss of enzyme activity. The patient generally experiences muscle weakness during prolonged exercise when muscle relies heavily on fatty acids as an energy source. Myoglobinuria, due to breakdown of muscle tissue, is a frequent accompaniment. The disorder is usually referred to as the "muscular" form of CPT II deficiency. Mutations causing more severe (90% or greater) loss of CPT II activity can have serious consequences in early infancy. These are usually precipitated by periods of fasting and include hypoketotic hypoglycemia, hyperammonemia, cardiac malfunction, and sometimes death. Similar morbidity and mortality are associated with mutations in the gene for liver CPT I. To date only a few patients with hepatic CPT I deficiency have been reported, the small number possibly indicating that the disease is frequently lethal and has gone undiagnosed. Muscle CPT I is now known to be a different isoform from its liver counterpart, but no defects at this locus have yet been reported.

The first patient with carnitine–acylcarnitine translocase deficiency was described as recently as 1992. Clinical features included intermittent hypoglycemic coma, hyperammonemia, muscle weakness, and cardiomyopathy. The condition proved fatal at age 3 years. Three additional cases with similar symptomatology have since been reported.

The hallmark of treatment for all inherited disorders of the carnitine transport/CPT system is avoidance of starvation and a diet low in long-chain fatty acids. Supplementary dietary medium-chain triacylglycerols, the fatty acids of which are oxidized by a carnitine-independent mechanism, have proved beneficial.

Stanley, C. A., Hale, D. E., Berry, G. T., Deleeno, S., Boxer, J., and Bonnefont, J.-P. A deficiency of carnitine–acylcarnitine translocase in the inner mitochondrial membrane. *N. Engl. J. Med.* 327:19, 1992; and Roe, C R., and Coates, P. M. Mitochondrial fatty acid oxidation disorders. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, Vol. II, 7th ed. New York: McGraw-Hill, 1995, p. 1501.

Some Fatty Acids Require Modification of β-Oxidation for Metabolism

The β-oxidation scheme accounts for the bulk of energy production from fatty acids in the human. These reactions, however, must be supplemented by other mechanisms so that ingested odd-chain and unsaturated fatty acids can be oxidized. In addition, reactions catalyze α- and ω-oxidation of fatty acids. **α-Oxidation** occurs at C-2 instead of C-3, as in the β-oxidation scheme. **ω-Oxidation** occurs at the methyl end of the fatty acid molecule. Partial oxidation of fatty acids with cyclopropane ring structures probably occurs in humans, but the mechanisms are not worked out.

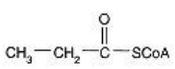


Figure 9.21
Propionyl CoA.

Propionyl CoA Is Produced by Oxidation of Odd-Chain Fatty Acids

Oxidation of fatty acids with an odd number of carbon atoms proceeds exactly as described above, but the final product is a molecule of propionyl CoA (Figure 9.21). For this compound to be further oxidized, it undergoes carboxylation to methylmalonyl CoA, molecular rearrangement, and conversion to succinyl CoA. These reactions are identical with those described on page 479 for the metabolism of **propionyl CoA** formed in the metabolic breakdown of some amino acids.

Oxidation of Unsaturated Fatty Acids Requires Additional Enzymes

Many unsaturated fatty acids in the diet are available for production of energy by humans. Structures encountered in these dietary acids may differ from those

CLINICAL CORRELATION 9.5

Genetic Deficiencies in the Acyl-CoA Dehydrogenases

The acyl-CoA dehydrogenase deficiencies represent a recently discovered group of inherited defects that impair the β-oxidation of fatty acids at different stages of the chain shortening process. The affected enzyme may be the long-chain acyl-CoA dehydrogenase (LCAD), the medium-chain acyl-CoA dehydrogenase (MCAD), or the short-chain acyl-CoA dehydrogenase (SCAD), whose substrate specificities are for acyl CoA chains of greater than C12, C6–C12, and C4–C6, respectively. The three conditions are inherited in autosomal recessive fashion and share many of the same clinical features. The best characterized is MCAD deficiency, which, though first recognized as late as 1982, is now thought to be one of the most common of all inborn errors of metabolism.

Medium-chain acyl-CoA dehydrogenase deficiency usually manifests itself within the first 2 years of life after a fasting period of 12 h or more. Typical symptoms include vomiting, lethargy, and frequently coma, accompanied by hypoketotic hypoglycemia and dicarboxylic aciduria. The absence of starvation ketosis is accounted for by the block in hepatic fatty acid oxidation, which also causes a slowdown of gluconeogenesis. This, coupled with impaired fatty acid oxidation in muscle, which promotes glucose utilization, leads to profound hypoglycemia. Accumulation of medium-chain acyl CoAs in tissues forces their metabolism through alternative pathways including ω-oxidation and transesterification to glycine or carnitine. Excessive urinary excretion of the reaction products (medium-chain dicarboxylic acids together with medium-chain esters of glycine and carnitine) provide diagnostic clues.

Most patients with this disorder do well simply by avoiding prolonged periods of starvation, which is consistent with the fact that the metabolic complications of MCAD deficiency are seen only when body tissues become heavily dependent on fatty acids as a source of energy (e.g., with carhohydrate deprivation). In retrospect, it now seems likely that many cases previously diagnosed loosely as "Reye-like syndrome" or "sudden infant death syndrome" were in fact due to MCAD deficiency.

Coates, P. M., and Tanaka, K. Molecular basis of mitochondrial fatty acid oxidation defects. *J. Lipid Res.* 33:1099, 1992.

required by the specificity of enzymes in β-oxidation pathway. Oxidation of linoleoyl CoA, outlined in Figure 9.22, illustrates two special reactions required for oxidation of unsaturated fatty acids.

One problem is that in β-oxidation of unsaturated fatty acids the sequential excision of C2 fragments can generate an acyl CoA intermediate with a double bond between C-3 and C-4 atoms instead of between C-2 and C-3 atoms as

TABLE 9.4 Comparison of Schemes for Biosynthesis and β-Oxidation of Palmitate

Parameter	*Biosynthesis*	*β-Oxidation*
Subcellular localization	Primarily cytosolic	Primarily mitochondrial
Phosphopantetheine-containing active carrier	Acyl carrier protein	Coenzyme A
Nature of small carbon fragment added or removed	C-1 and C-2 atoms of malonyl CoA after initial priming	Acetyl CoA
Nature of oxidation–reduction coenzyme	NADPH	FAD when saturated chain dehydrogenated, NAD^+ when hydroxy acid dehydrogenated
Stereochemical configuration of β-hydroxy intermediates	D-β-Hydroxy	L -Hydroxy
Energy equivalents yielded or utilized in interconversion of palmitate and acetyl CoA	7 ATP + 14 NADPH = 49 ATP equiv	7 $FADH_2$ + 7 NADH – 2 ATP – 33 ATP equiv

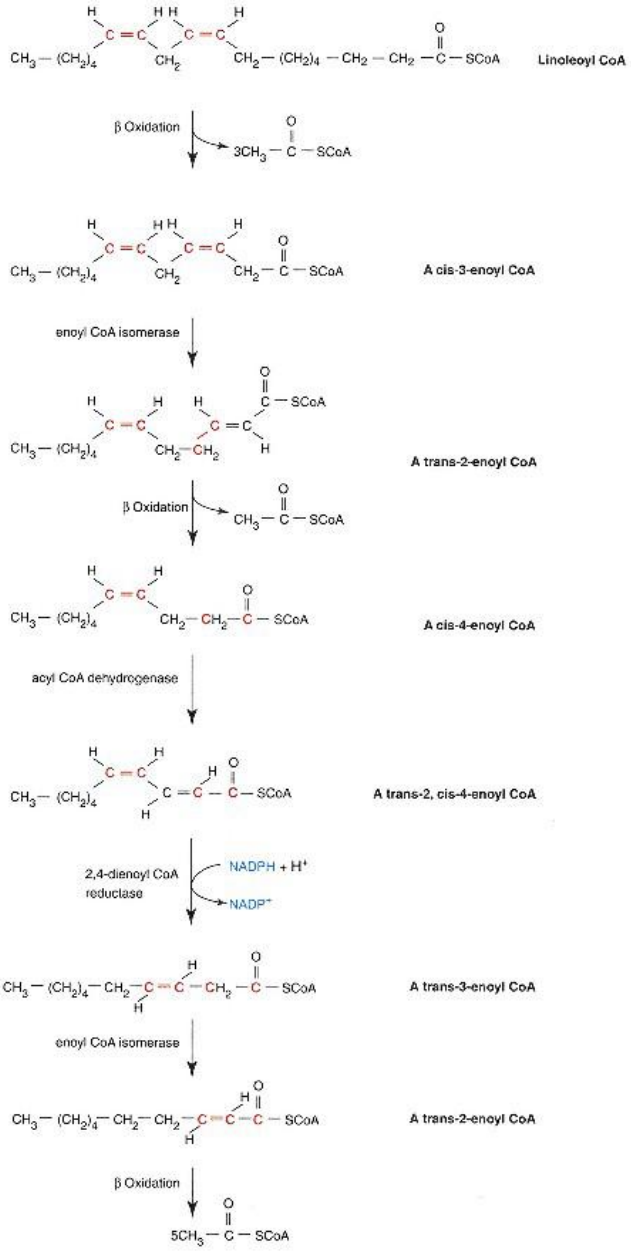


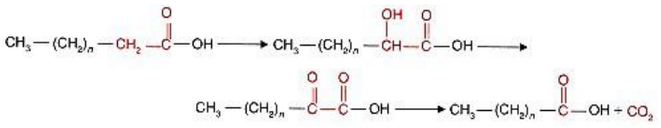
Figure 9.22
Oxidation of linoleoyl CoA.

required for the enoyl CoA hydratase reaction. If so, the cis bond between C-3 and C-4 atoms is isomerized into a trans bond between C-2 and C-3 atoms by an auxiliary enzyme, enoyl CoA isomerase. The regular process can then proceed.

A second problem occurs if the cis double bond of the acyl CoA intermediate resides between C-4 and C-5 atoms. In this case the action of acyl-CoA dehydrogenase gives rise to a *trans*-2, *cis*-4-enoyl CoA. This is acted on by 2,4-dienoyl CoA reductase that, using reducing equivalents from NADPH, produces a *trans*-3-enoyl CoA. This will serve as a substrate for enoyl CoA isomerase producing *trans*-2-enoyl CoA needed for the next round of β-oxidation.

Some Fatty Acids Undergo α-Oxidation

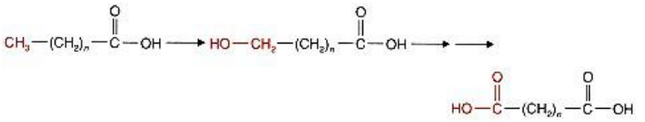
As noted earlier, there are several mechanisms for **hydroxylation of fatty acids**. The one discussed previously is for a hydroxylation of long-chain acids needed for synthesis of sphingolipids. In addition, there are systems in other tissues that hydroxylate the α carbon of shorter chain acids in order to start their oxidation. The sequence is as follows:



These hydroxylations probably occur in the endoplasmic reticulum and mitochondria and involve the "mixed function oxidase" type of mechanism discussed previously, because they require molecular oxygen, reduced nicotin-amide nucleotides and specific cytochromes. Such reactions are particularly important in oxidation of methylated fatty acids (see Clin. Corr. 9.6).

ω-Oxidation Gives Rise to a Dicarboxylic Acid

Another minor pathway for fatty acid oxidation also involves hydroxylation and occurs in the endoplasmic reticulum of many tissues. In this case hydroxylation takes place on the methyl carbon at the other end of the molecule from the carboxyl group or on the carbon next to the methyl end. It uses the "mixed function oxidase" type of reaction requiring cytochrome P450, O_2, and NADPH, as well as the necessary enzymes (see Chapter 23). Hydroxylated fatty acid can be further oxidized to a **dicarboxylic acid** via sequential action of cytosolic **alcohol** and **aldehyde dehydrogenases**. The process occurs primarily with medium-chain fatty acids. The overall reactions are



The dicarboxylic acid so formed can be activated at either end of the molecule to form a CoA ester, which in turn can undergo β-oxidation to produce shorter chain dicarboxylic acids such as adipic (C6) and succinic (C4) acids. This process appears to occur primarily in peroxisomes (see p. 19).

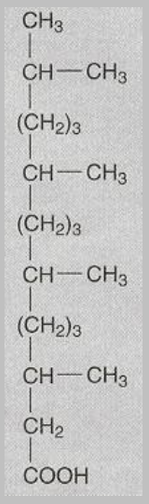
Ketone Bodies Are Formed from Acetyl CoA

The ketone bodies are water-soluble forms of lipid-based energy and consist mainly of acetoacetic acid and its reduction product β-hydroxybutyric acid. β-Hydroxybutyryl CoA and acetoacetyl CoA are intermediates near the end of the β-oxidation sequence, and it was initially presumed that enzymatic removal

CLINICAL CORRELATION 9.6

Refsum's Disease

Although the use of the α-oxidation scheme is a relatively minor one in terms of total energy production, it is significant in the metabolism of dietary fatty acids that are methylated. A principal example of these is phytanic acid,



Phytanic acid

a metabolic product of phytol, which occurs as a constituent of chlorophyll. Phytanic acid is a significant constituent of milk lipids and animal fats, and normally it is metabolized by an initial α-hydroxylation followed by dehydrogenation and decarboxylation. β-Oxidation cannot occur initially because of the presence of the 3-methyl group, but it can proceed after the decarboxylation. The whole reaction produces three molecules of propionyl CoA, three molecules of acetyl CoA, and one molecule of isobutyryl CoA.

In a rare genetic disease called Refsum's disease, the patients lack the α-hydroxylating enzyme and accumulate large quantities of phytanic acid in their tissues and sera. This leads to serious neurological problems such as retinitis pigmentosa, peripheral neuropathy, cerebellar ataxia, and nerve deafness. The restriction of dietary dairy products and meat products from ruminants results in lowering of plasma phytanic acid and regression of neurologic symptoms.

Steinberg. D. Refsum disease. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, Vol. II, 7th ed. New York: McGraw-Hill, 1995, p. 2351.

of CoA from these compounds was the main route for production of the free acids. However, β-oxidation proceeds completely to acetyl CoA production without accumulation of any intermediates, and acetoacetate and β-hydroxybutyrate are formed subsequently from acetyl CoA by a separate mechanism.

HMG CoA Is an Intermediate in the Synthesis of Acetoacetate from Acetyl CoA

The primary site for formation of ketone bodies is liver, with lesser activity occurring in kidney. The entire process takes place within the mitochondrial matrix and begins with condensation of two acetyl CoA molecules to form acetoacetyl CoA (Figure 9.23). The enzyme involved, ***β*-ketothiolase**, is probably an isozyme of that which catalyzes the reverse reaction as the last step of β-oxidation. Acetoacetyl CoA then condenses with another molecule of acetyl CoA to form ***β*-hydroxy-*β*-methylglutaryl coenzyme A (HMG CoA).** Cleavage of HMG CoA then yields acetoacetic acid and acetyl CoA.

Acetoacetate Forms Both D-β-Hydroxybutyrate and Acetone

In mitochondria a fraction of the acetoacetate is reduced to D-β-hydroxybutyrate depending on the intramitochondrial [NADH]/[NAD^+] ratio. Note that the product of this reaction is D-β-hydroxybutyrate, whereas β-hydroxybutyryl CoA formed during β-oxidation is of the L configuration. ***β*-Hydroxybutyrate dehydrogenase** is tightly associated with the inner mitochondrial membrane and, because of its high activity in liver, the concentrations of substrates and products

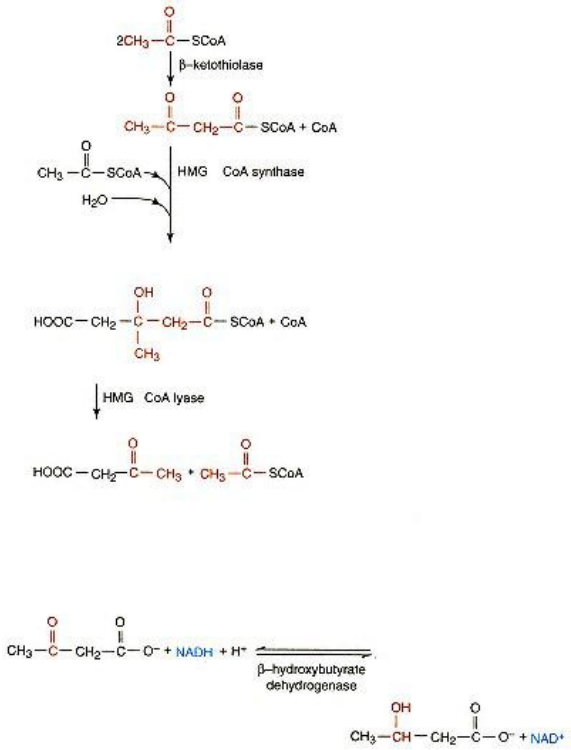
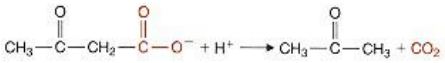


Figure 9.23
Pathway of acetoacetate formation.

of the reaction are maintained close to equilibrium. Thus the ratio of β-hydroxybutyrate to acetoacetate in blood leaving liver can be taken as a reflection of the mitochondrial [NADH]/[NAD^+ ratio.

Some acetoacetate continually undergoes slow, spontaneous nonenzymatic decarboxylation to acetone:



Under normal conditions acetone formation is negligible, but when pathological accumulations of acetoacetate occur, as, for example, in severe diabetic ketoacidosis (see Clin. Corr. 9.7), the amount of acetone in blood can be sufficient to cause it to be detectable in a patient's breath.

As seen from Figure 9.24, the pathway leading from acetyl CoA to HMG CoA also operates in the cytosolic space of liver cells (indeed, this applies to essentially all tissues of the body). However, in this compartment HMG CoA lyase is absent and the HMG CoA formed is used for cholesterol biosynthesis (see Chapter 10). What distinguishes liver from nonhepatic tissues is its high complement of intramitochondrial **HMG CoA synthase**, thus providing an enzymological basis for the primacy of this organ in ketone body production.

Utilization of Ketone Bodies by Nonhepatic Tissues Requires Formation of Acetoacetyl CoA

Acetoacetate and β-hydroxybutyrate produced by liver serve as excellent fuels for a variety of nonhepatic tissues, such as cardiac and skeletal muscle, particularly when glucose is in short supply (starvation) or inefficiently used (insulin deficiency). But since under these conditions the same tissues can readily use free fatty acids (whose blood concentration rises as insulin levels fall) as a source of energy, a nagging question for many years was why liver should produce ketone bodies in the first place. The answer emerged in the late 1960s with the recognition that during prolonged starvation in humans the ketone bodies replace glucose as the major fuel of respiration for the central nervous system, which has a low capacity for fatty acid oxidation. Also noteworthy is the fact that during the neonatal period of development, acetoacetate and β-hydroxybutyrate serve as important precursors for cerebral lipid synthesis.

Use of ketone bodies requires that acetoacetate first be reactivated to its CoA derivative. This is accomplished by a mitochondrial enzyme, acetoacetate:succinyl CoA CoA transferase, present in most nonhepatic tissues but absent from liver. Succinyl CoA serves as the source of the coenzyme. The reaction is depicted in Figure 9.25. Through the action of β-ketothiolase, acetoacetyl CoA is then converted into acetyl CoA, which in turn enters the TCA cycle with production of energy. Mitochondrial β-hydroxybutyrate dehydrogenase in non-hepatic tissues reconverts β-hydroxybutyrate into acetoacetate as the concentration of the latter is decreased.

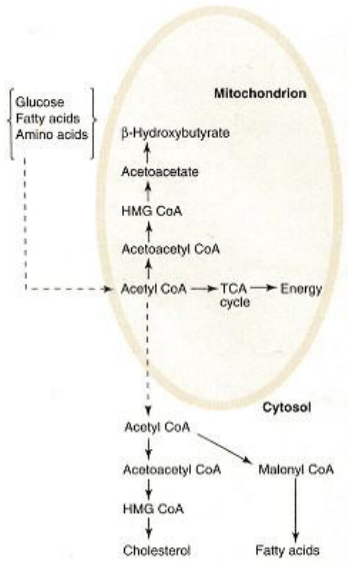


Figure 9.24
Interrelationships of ketone bodies with lipid, carbohydrate, and amino acid metabolism in liver.

Starvation and Certain Pathological Conditions Lead to Ketosis

Under normal feeding conditions, hepatic production of acetoacetate and β-hydroxybutyrate is minimal and the concentration of these compounds in the blood is very low ($\leq$0.2 mM). However, with food deprivation ketone body synthesis is greatly accelerated, and the circulating level of acetoacetate plus β-hydroxybutyrate may rise to the region of 3–5 mM. This is a normal response of the body to a shortage of carbohydrate and serves a number of crucial roles. In the early stages of fasting, use of ketone bodies by heart and skeletal muscle conserves glucose for support of the central nervous system. With more prolonged starvation, increased blood concentrations of acetoacetate and β-hydroxybutyrate ensure their efficient uptake by brain, thereby further sparing glucose consumption.

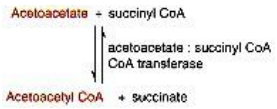


Figure 9.25
Initial step in utilization of acetoacetate by nonhepatic tissues.

CLINICAL CORRELATION 9.7

Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) is a common illness among patients with insulin-dependent diabetes mellitus. Although mortality rates have declined, they are still in the range of 6–10%. The condition is triggered by severe insulin deficiency coupled with glucagon excess and is frequently accompanied by concomitant elevation of other stress hormones, such as epinephrine, norepinephrine, cortisol, and growth hormone. The major metabolic derangements are marked hyperglycemia, excessive ketonemia, and ketonuria. Blood concentrations of acetoacetic acid plus β-hydroxybutyric acid as high as 20 mM are not uncommon. Because these are relatively strong acids (pK 3.5), the situation results in life-threatening metabolic acidosis.

The massive accumulation of ketone bodies in the blood in DKA stems from a greatly accelerated hepatic production rate such that the capacity of nonhepatic tissues to use them is exceeded. In biochemical terms the initiating events are identical with those operative in the development of starvation ketosis; that is, increased glucagon/insulin ratio elevation of liver cAMP decreased malonyl CoA deinhibition of CPT I activation of fatty acid oxidation and ketone production (see text for details). However, in contrast to physiological ketosis, where insulin secretion from the pancreatic β cells limits free fatty acid (FFA) availability to the liver, this restraining mechanism is absent in the diabetic individual. As a result, plasma FFA concentrations can reach levels as high as 3–4 mM, which drive hepatic ketone production at maximal rates.

Correction of DKA requires rapid treatment that will be dictated by the severity of the metabolic abnormalities and the associated tissue water and electrolyte imbalance. Insulin is essential. It lowers the plasma glucagon level, antagonizes the catabolic effects of glucagon on the liver, inhibits the flow of ketogenic and gluconeogenic substrates (FFA and amino acids) from the periphery, and stimulates glucose uptake in target tissues.

Foster, J. D., and McGarry, J. D. Metabolic derangements and treatment of diabetic ketoacidosis. *N. Engl. J. Med.* 309:159, 1983; and Foster, D. W., and McGarry, J. D. Acute complications of diabetes: ketoacidosis, hyperosmolar coma, lactic acidosis. In: L. J. DeGroot (Ed.), *Endocrinology*, Vol. 2, 3rd ed. Philadelphia: Saunders, 1995, p. 1506.

In contrast to the **physiological ketosis of starvation,** certain pathological conditions, most notably **diabetic ketoacidosis** (see Clin. Corr. 9.7), are characterized by excessive accumulation of ketone bodies in the blood (up to 20 mM). Hormonal and biochemical factors operative in the overall control of hepatic ketone body production are discussed in detail in Chapter 14.

Peroxisomal Oxidation of Fatty Acids Serves Many Functions

Although the bulk of cellular fatty acid oxidation occurs in mitochondria it has recently become clear that a significant fraction also takes place in **peroxisomes** of liver, kidney, and other tissues. Peroxisomes are a class of subcellular organelles with distinctive morphological and chemical characteristics. Their initial distinguishing property was a high content of the enzyme catalase and it has been suggested that peroxisomes may function in a protective role against oxygen toxicity. Several lines of evidence suggest that they are also involved in lipid catabolism. First, the analogous structures in plants, glyoxysomes, are capable of oxidizing fatty acids. Second, a number of drugs used clinically to decrease triacylglycerol levels in patients cause a marked increase in peroxisomes. Third, liver peroxisomes, isolated by differential centrifugation, oxidize fatty acids and contain most of the enzymes needed for the β-oxidation process.

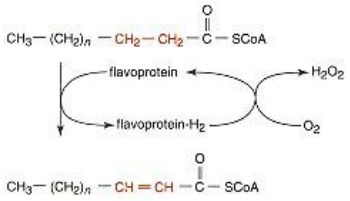


Figure 9.26
Initial step in peroxisomal fatty acid oxidation.

The mammalian **peroxisomal fatty acid oxidation** scheme is similar to that in plant glyoxysomes but differs from the mitochondrial β-oxidation system in three important respects. First, the initial dehydrogenation is accomplished by a cyanide-insensitive oxidase system, as shown in Figure 9.26. $\mathbf{H_2O_2}$ formed is eliminated by **catalase,** and the remaining steps are the same as in the mitochondrial system. Second, there is evidence that the peroxisomal and mitochondrial enzymes are slightly different and that the specificity in peroxisomes is for somewhat longer chain length. Third, although rat liver mitochondria will oxidize a molecule of palmitoyl CoA to eight molecules of acetyl CoA, the β-oxidation system in peroxisomes from the same organ will not proceed beyond the stage of octanoyl CoA (C8). The possibility is thus raised that one function

of peroxisomes is to shorten the chains of relatively long-chain fatty acids to a point at which β-oxidation can be completed in mitochondria.

Other peroxisomal reactions include chain shortening of dicarboxylic acids, as noted earlier, conversion of cholesterol into bile acids, and formation of ether lipids. Given these diverse metabolic roles it is not surprising that the congenital absence of functional peroxisomes, an inherited defect known as Zellweger syndrome, has such devastating effects (see Clin. Corr. 1.3).

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Questions

C. N. Angstadt and J. Baggott

1. Fatty acids occurring in humans most commonly:

   A. are highly branched structures.

   B. have double bonds present in trans configuration.

   C. contain an even number of carbon atoms.

   D. are limited to 16 or fewer carbon atoms.

   E. if polyunsaturated, have a conjugated double-bond system.

2. Triacylglycerols:

   A. would be expected to be good emulsifying agents.

   B. yield about the same amount of ATP on complete oxidation as would an equivalent weight of glycogen.

   C. are stored as hydrated molecules.

   D. in the average individual, represent sufficient energy to sustain life for several weeks.

   E. are generally negatively charged molecules at physiological pH.

3. In humans, fatty acids:

   A. can be synthesized from excess dietary carbohydrate or protein.

   B. are not required at all in the diet.

   C. containing double bonds cannot be synthesized.

   D. must be supplied entirely by the diet.

   E. other than palmitate, must be supplied by the diet.

4. All of the following statements about acetyl-CoA carboxylase are correct EXCEPT:

   A. it undergoes protomer–polymer interconversion during its physiological regulation.

   B. it requires biotin.

   C. it is inhibited by cAMP-mediated phosphorylation.

   D. it is activated by both palmitoyl CoA and citrate.

   E. its content in a cell responds to changes in fat content in the diet.

5. In the synthesis of palmitate:

   A. the addition of malonyl CoA to fatty acid synthase elongates the growing chain by three carbon atoms.

   B. a β-keto residue on the 4 -phosphopantetheine moiety is reduced to a saturated residue by NADPH.

   C. palmitoyl CoA is released from the synthase.

   D. transfer of the growing chain from ACP to another –SH occurs after the addition of the next malonyl CoA.

   E. the first compound to add to fatty acid synthase is malonyl CoA.

6. Citrate stimulates fatty acid synthesis by all of the following EXCEPT:

   A. allosterically activating acetyl-CoA carboxylase.

   B. providing a mechanism to transport acetyl CoA from the mitochondria to the cytosol.

   C. participating in a pathway that ultimately produces CO_2 and NADPH in the cytosol.

   D. participating in the production of ATP.

7. Fatty acyl CoAs shorter than 16 carbon atoms are the preferred substrates for:

A. fatty acid elongation in the brain.

B. carnitine transport into the mitochondria.

C. fatty acid elongation in mitochondria.

D. fatty acid elongation in the endoplasmic reticulum.

E. all of the above.

8. Fatty acid synthase:

A. synthesizes only palmitate.

B. yields an unsaturated fatty acid by skipping a reductive step.

C. produces hydroxy fatty acids in nerve tissue.

D. can stop synthesis with the release of a fatty alcohol instead of an acid.

E. can produce a branched-chain fatty acid if methylmalonyl CoA is used as a substrate.

9. In humans, desaturation of fatty acids:

A. occurs primarily in mitochondria.

B. is catalyzed by an enzyme system that uses NADPH and a cytochrome.

C. introduces double bonds primarily of trans configuration.

D. can occur only after palmitate has been elongated to stearic acid.

E. introduces the first double bond at the methyl end of the molecule.

10. All of the following events are usually involved in the synthesis of triacylglycerols in adipose tissue EXCEPT:

A. addition of a fatty acyl CoA to a diacylglycerol.

B. addition of a fatty acyl CoA to a lysophosphatide.

C. a reaction catalyzed by glycerol kinase.

D. hydrolysis of phosphatidic acid by a phosphatase.

E. reduction of dihydroxyacetone phosphate.

11. Plasma lipoproteins:

A. are the only carriers of lipid-based energy in the blood.

B. usually have a nonpolar core containing triacylglycerols and cholesterol esters.

C. are composed primarily of free (unesterified) fatty acids.

D. include chylomicrons generated in the liver.

E. include high density lipoproteins (HDL) as the major carrier of lipid-based energy.

12. Lipoprotein lipase:

A. is an intracellular enzyme.

B. is stimulated by cAMP-mediated phosphorylation.

C. functions to mobilize stored triacylglycerols from adipose tissue.

D. is stimulated by one of the apoproteins present in VLDL.

E. readily hydrolyzes three fatty acids from a triacylglycerol.

13. A deficiency of carnitine might be expected to interfere with:

A. β-oxidation.

B. ketone body formation from acetyl CoA.

C. palmitate synthesis.

D. mobilization of stored triacylglycerols from adipose tissue.

E. uptake of fatty acids into cells from the blood.

14. β-Oxidation of fatty acids:

A. generates ATP only if acetyl CoA is subsequently oxidized.

B. is controlled primarily by allosteric effectors.

C. uses only even-chain, saturated fatty acids as substrates.

D. uses $NADP^+$

E. occurs by a repeated sequence of four reactions.

15. Ketone bodies:

A. are formed by removal of CoA from the corresponding intermediate of β-oxidation.

B. are synthesized from cytoplasmic β-hydroxy-β-methylglutaryl coenzyme A (HMG CoA).

C. are excellent energy substrates for liver.

D. include both β-hydroxybutyrate and acetoacetate, the ratio reflecting the intramitochondrial [NADH]/[NAD^+] ratio in liver.

E. form when β-oxidation is interrupted.

16. The high glucagon/insulin ratio seen in starvation:

A. promotes mobilization of fatty acids from adipose stores.

B. stimulates β-oxidation by inhibiting the production of malonyl CoA.

C. leads to increased concentrations of ketone bodies in the blood.

D. all of the above.

E. none of the above.

17. Peroxisomal oxidation of fatty acids:

A. is identical to β-oxidation in the mitochondria.

B. involves a flavoprotein that produces H_2O_2.

C. has a preference for fatty acids shorter than 12 carbons.

D. does not use NAD^+.

E. is effective only for dicarboxylic acids.

Answers

1. C A: Some may have methyl branches but most are straight chain. B: Most naturally occurring double bonds are cis, an important factor in β-oxidation of unsaturated fatty acids. D: C18 and C20 fatty acids are very common. E: Double bonds are separated by $–CH_2–$ (p. 363).

2. D A, C, and E: Triacylglycerols are neutral, hydrophobic molecules with no hydrophilic portion and therefore are not emulsifying agents and are stored anhydrously. B: Their more reduced state, compared to carbohydrates, makes them more energy-rich (p. 365).

3. A It is important to realize that triacylglycerol is the ultimate storage form of excess dietary intake. B–E: We can synthesize most fatty acids, including those with double bonds, except for the essential fatty acids, linoleic and linolenic acids (p. 365).

4. D A: Acetyl-CoA carboxylase shifts between its protomeric (inactive) and polymeric (active) forms under the influence of a variety of regulatory factors. C: Since cAMP increases at times when energy is needed, it is consistent that a process that uses energy would be inhibited. E: Long-term control is

related to enzyme synthesis and responds appropriately to dietary changes (Table 9.2, p. 367).

5. B A: Splitting CO_2 from malonyl CoA is the driving force for the condensation reaction so the chain grows two carbon atoms at a time. C: Palmitate is released as the free acid; the conversion to the CoA ester is by a different enzyme (p. 369). D: It is important to realize that only ACP binds the incoming malonyl CoA so it must be freed before another addition can be made. E: Acetyl CoA adds first to form the foundation for the rest of the chain (p. 368).

6. D Citrate consumes ATP when acted upon by citrate cleavage enzyme (p. 371). A: Table 9.2. B: Acetyl CoA is generated primarily in mitochondria but does not cross the membrane readily. C: Oxaloacetate generated by citrate cleavage enzyme, when converted to malate, yields CO_2 and NADPH by the malic enzyme (Figure 9.10).

7. C C and D:The role of mitochondrial fatty acid elongation seems to be to elongate short-chain fatty acids; the cytoplasmic system is most active with palmitate. A: Brain elongates 18 carbon acids to meet its needs. B: Short- and medium-chain fatty acids are capable of entering mitochondria and their activation to CoA esters occurs there (p. 372).

8. E This is much slower than reaction with malonyl CoA, but it is significant. A: In certain tissues, for example, mammary glands, shorter chain products are formed. B–D: These products are all formed by other processes. Reactions proceeding on a multienzyme complex generally do not "stop" at intermediate steps (p. 374).

9. B A: Desaturation occurs in the endoplasmic reticulum. C: Naturally occurring fatty acids are cis. D: Elongation and unsaturation can occur in any order. E: If this were true we could make linoleic acid (p. 372).

10. C This does not occur to any significant extent in adipose tissue. A, B, and D: The sequential addition of fatty acyl CoAs to glycerol 3-phosphate forms lysophosphatidic acid, then phosphatidic acid whose phosphate is removed before the addition of the third fatty acyl residue. E: This is the formation of α-glycerol phosphate in adipose tissue (p. 375).

11. B All lipoproteins (Section 9.5) have this same general structure, a nonpolar core surrounded by a more polar shell. A and C: Fatty acids bound to serum albumin and ketone bodies are other sources. D: Chylomicrons carry dietary lipid from the intestine. E: HDL function is to carry cholesterol away from tissues (p. 379).

12. D A–C: These are characteristics of hormone-sensitive lipase. E: It generally requires more than one lipase to hydrolyze all of the fatty acids (p. 381, Table 9.3).

13. A Carnitine functions in transport of fatty acyl CoA esters formed in cytosol into the mitochondria (p. 382).

14. E A and D: It is important to realize that β-oxidation, itself, generates $FADH_2$ and NADH, which can be reoxidized to generate ATP. B: Carnitine transport to provide the substrate and reoxidation of reduced cofactors control β-oxidation. C: β-Oxidation is a general process requiring only minor modifications to oxidize nearly any fatty acid in the cell (p. 385, Table 9.4).

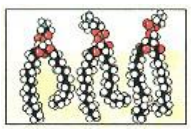
15. D A and E: β-Oxidation proceeds to completion; ketone bodies are formed by a separate process. B and C: Ketone bodies are formed, but not used, in liver mitochondria; cytosolic HMG CoA is a precursor of cholesterol (pp. 387–389).

16. D High glucagon/insulin ratio results in cAMP-mediated phosphorylations that activate hormone-sensitive lipase and inhibit acetyl-CoA carboxylase. Both of these, as well as other events, promote ketone body formation by greatly increasing acetyl CoA production in mitochondria, thereby assuring efficient uptake and utilization by brain (pp. 377, 381, 389).

17. B A and B: This is one of the differences between the peroxisomal and mitochondrial systems. C and E: Role seems to be to oxidize longer chain fatty acids to a point where mitochondrial oxidation can work. D: Except for the flavoprotein, the reactions are the same as the mitochondrial process (p. 390).

Chapter 10—
Lipid Metabolism II:
Pathways of Metabolism of Special Lipids

Robert H. Glew



Clinical Correlations

10.1— Overview

Lipid is a general term that describes substances that are relatively water insoluble and extractable by nonpolar solvents. Complex lipids of humans fall into one of two broad categories: nonpolar lipids, such as triacylglycerols and cholesterol esters, and polar lipids, which are amphipathic in that they contain both a hydrophobic domain and a hydrophilic region in the same molecule. This chapter discusses polar lipids including *phospholipids, sphingolipids*, and *eicosanoids*. The hydrophobic and hydrophilic regions are bridged by a glycerol moiety in glycerophospholipids and by sphingosine in sphingomyelin and glycosphingolipids. Triacylglycerol is confined largely to storage sites in adipose tissue, whereas polar lipids occur primarily in cellular membranes. Oils of soybean, oil palm, rapeseed, sunflower, cottonseed, and peanut account for 80% of worldwide plant oil production and consist mainly of palmitic, stearic, oleic, linoleic, and α-linolenic acids. Membranes generally contain 40% of their dry weight as lipid and 60% as protein.

Cell–cell recognition, phagocytosis, contact inhibition, and rejection of transplanted tissues and organs are all phenomena of medical significance that involve highly specific recognition sites on the surface of plasma membranes. Synthesis of the complex glycosphingolipids that play a role in these important biological events will be described. Glycolipids are worthy of study because ABO antigenic determinants of blood groups are primarily glycolipid in nature. In addition, various sphingolipids are the storage substances that accumulate in liver, spleen, kidney, or nervous tissue of persons suffering from certain genetic disorders called sphingolipidoses. In order to understand the basis of

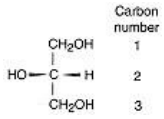


Figure 10.1
Stereospecific numbering of glycerol.

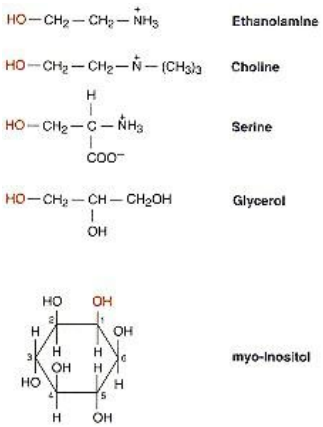


Figure 10.2
Structure of some common polar groups of phospholipids.

these enzyme-deficiency states, a knowledge of relevant chemical structures involved is required.

A very important lipid is *cholesterol*. This chapter describes the pathway of cholesterol biosynthesis and its regulation and shows how cholesterol functions as a precursor to bile salts and steroid hormones. Also described is the role of high-density lipoprotein (HDL) and lecithin:cholesterol acyltransferase (LCAT) in the management of plasma cholesterol.

Finally, the metabolism and function of two pharmacologically powerful classes of hormones derived from arachidonic acid, namely, prostaglandins and leukotrienes, will be discussed. See the Appendix, for a discussion of nomenclature and chemistry of lipids.

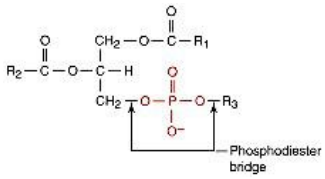
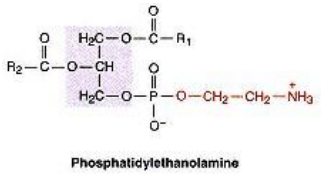
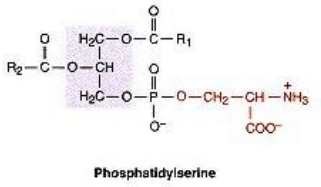


Figure 10.3
Generalized structure of a phospholipid where R_1 and R_2 represent the aliphatic chains of fatty acids, and R_3 represents a polar group.

10.2— Phospholipids

Two principal classes of **acylglycerolipids** are **triacylglycerols** and **glycerophospholipids**. They are referred to as glycerolipids because the core of these compounds is provided by the C3 polyol, glycerol. Two primary alcohol groups of glycerol are not stereochemically identical and in the case of phospholipids, it is usually the same hydroxyl group that is esterified to the phosphate residue. The stereospecific numbering system is the best way to designate different hydroxyl groups. In this system, when the structure of glycerol is drawn in the Fischer projection with the C-2 hydroxyl group projecting to the left of the page, the carbon atoms are numbered as shown in Figure 10.1. When the stereospecific numbering (*sn*) system is employed, the prefix *sn-* is used before the name of the compound. Glycerophospholipids usually contain an *sn*-glycerol 3-phosphate moiety. Although each contains the glycerol moiety as a fundamental structural element, neutral triacylglycerols and charged ionic phospholipids have very different physical properties and functions.





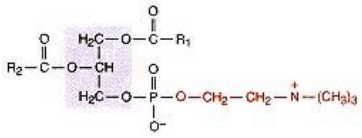


Figure 10.4
Structures of some common phospholipids.

Phospholipids Contain 1,2-Diacylglycerol and a Base Connected by a Phosphodiester Bridge

Phospholipids are polar, ionic lipids composed of 1,2-diacylglycerol and a phosphodiester bridge that links the glycerol backbone to some base, usually a nitrogenous one, such as choline, serine, or ethanolamine (Figures 10.2 and 10.3). The most abundant phospholipids in human tissues are **phosphatidylcholine** (also called lecithin), **phosphatidylethanolamine,** and **phosphatidylserine** (Figure 10.4). At physiologic pH, phosphatidylcholine and phosphatidylethanolamine have no net charge and exist as dipolar zwitterions, whereas phosphatidylserine has a net charge of –1, causing it to be an acidic phospholipid. Phosphatidylethanolamine (PE) is related to phosphatidylcholine in that trimethylation of PE produces lecithin. Most phospholipids contain more than one kind of fatty acid per molecule, so that a given class of phospholipids from any tissue actually represents a family of molecular species. Phosphatidylcholine (PC) contains mostly palmitic acid (16:0) or stearic acid (18:0) in the *sn*-1 position and primarily unsaturated C18 fatty acids oleic, linoleic, or α-linolenic in the *sn*-2 position. Phosphatidylethanolamine has the same saturated fatty acids as PC at the *sn*-1 position but contains more of the long-chain polyunsaturated fatty acids—namely, 18:2, 20:4, and 22:6—at the *sn*-2 position.

Phosphatidylinositol is an acidic phospholipid that occurs in mammalian membranes (Figure 10.5). Phosphatidylinositol is rather unusual because it often contains almost exclusively stearic acid in the *sn*-1 position and arachidonic acid (20:4) in the *sn*-2 position.

Another phospholipid comprised of a polyol polar head group is **phosphatidylglycerol** (Figure 10.5), which occurs in relatively large amounts in

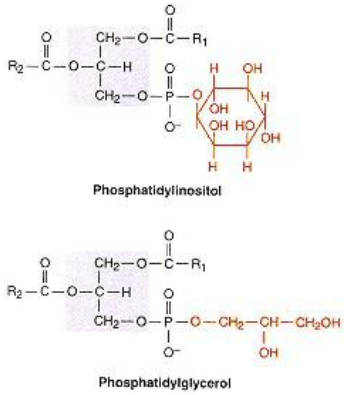


Figure 10.5
Structures of phosphatidylglycerol and phosphatidylinositol.

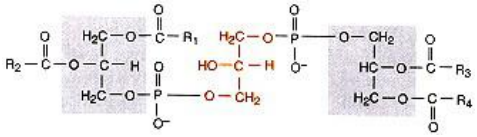


Figure 10.6
Structure of cardiolipin.

mitochondrial membranes and pulmonary surfactant and is a precursor of **cardiolipin**. Phosphatidylglycerol and phosphatidylinositol both carry a formal charge of –1 at neutral pH and are therefore acidic lipids. Cardiolipin, a very acidic (charge, –2) phospholipid, is composed of two molecules of phosphatidic acid linked together covalently through a molecule of glycerol (Figure 10.6). It occurs primarily in the inner membrane of mitochondria and in bacterial membranes.

Phospholipids mentioned so far contain only *O*-acyl residues attached to glycerol. *O*-(1-Alkenyl) substituents occur at C-1 of the *sn*-glycerol in phosphoglycerides in combination with an *O*-acyl residue esterified to the C-2 position; compounds in this class are known as **plasmalogens** (Figure 10.7). Relatively large amounts of ethanolamine plasmalogen (also called plasmenylethanolamine) occur in myelin with lesser amounts in heart muscle where choline plasmalogen is abundant.

An unusual phospholipid called "**platelet-activating factor**" (PAF) (Figure 10.8) is a major mediator of hypersensitivity, acute inflammatory reactions and anaphylactic shock. In hypersensitive individuals, cells of the polymorphonu-clear (PMN) leukocyte family (basophils, neutrophils, and eosinophils), macrophages, and monocytes are coated with IgE molecules that are specific for a particular antigen (e.g., ragweed pollen and bee venom). Subsequent reexposure to the antigen and formation of antigen–IgE complexes on the surface of the aforementioned inflammatory cells provoke synthesis and release of PAF. Platelet-activating factor contains an *O*-alkyl moiety at the *sn*-1 position and an acetyl residue instead of a long-chain fatty acid (e.g., stearic acid) in position 2 of the glycerol moiety. PAF is not stored; it is synthesized and released when PMNs are stimulated. Platelet aggregation, cardiovascular and pulmonary changes, edema, hypotension, and PMN cell chemotaxis are affected by PAF.

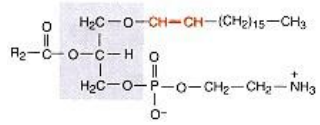


Figure 10.7
Structure of ethanolamine plasmalogen.

Phospholipids in Membranes Serve a Variety of Roles

Although present in body fluids such as plasma and bile, phospholipids are found in highest concentration in various cellular membranes where they serve as structural and functional components. Nearly one-half the mass of the erythrocyte membrane is comprised of various phospholipids (see Chapter 5). Phospholipids also activate certain enzymes. β-Hydroxybutyrate dehydrogenase, an enzyme imbedded in the inner membrane of mitochondria (see p. 388), has an absolute requirement for phosphatidylcholine; phosphatidylserine and phosphatidylethanolamine cannot substitute.

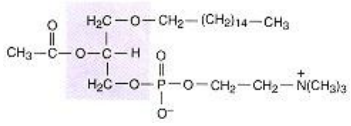


Figure 10.8
Structure of platelet activating factor (PAF).

Dipalmitoyllecithin Is Necessary for Normal Lung Function

Normal lung function depends on a constant supply of **dipalmitoyllecithin** in which the lecithin molecule contains palmitic acid (16:0) residues in both the *sn*-1 and *sn*-2 positions. More than 80% of the phospholipid in the extracellular liquid layer that lines alveoli of normal lungs is dipalmitoyllecithin. This particular phospholipid, called **surfactant,** is produced by type II epithelial cells and prevents atelectasis at the end of the expiration phase of breathing

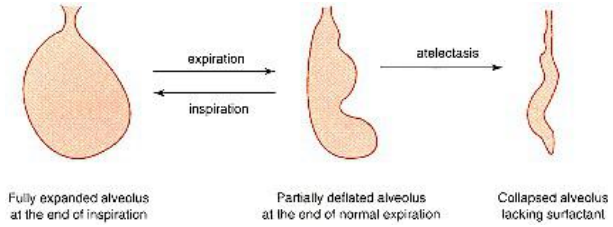


Figure 10.9
Role of surfactant in preventing atelectasis.

(Figure 10.9). This lipid decreases surface tension of the aqueous surface layer of the lung. Lecithin molecules that do not contain two residues of palmitic acid are not effective in lowering surface tension of the fluid layer lining alveoli. Surfactant also contains phosphatidylglycerol, phosphatidylinositol, and 18- and 36-kDa proteins (designated surfactant proteins), which contribute significantly to the surface tension lowering property of pulmonary surfactant.

During the third trimester—before the 28th week of gestation—fetal lung synthesizes primarily sphingomyelin. Normally, at this time, glycogen that has been stored in epithelial type II cells is converted to fatty acids and then to dipalmitoyllecithin. During lung maturation there is a good correlation between increase in lamellar inclusion bodies that represent the intracellular pulmonary surfactant (phosphatidylcholine) storage organelles, called lamellar bodies, and the simultaneous decrease in glycogen content of type II pneumocytes. At the 24th week of gestation the type II granular pneumocytes appear in the alveolar epithelium, and within a few days they produce their typical osmiophilic lamellar inclusion bodies. The number of type II cells increases until the 32nd week, at which time surface active agent appears in the lung and amniotic fluid. Surface tension decreases when inclusion bodies increase in the type II cells. In the few weeks before term one can perform screening tests on amniotic fluid to detect newborns that are at risk for respiratory distress syndrome (RDS) (see Clin. Corr. 10.1). These tests are useful in timing elective deliveries, in applying vigorous preventive therapy to the newborn infant, and to determine if the mother should be treated with a glucocorticoid drug to accelerate maturation of the fetal lung. Dexamethasone therapy has also been used in neonates with chronic lung disease (bronchopulmonary dysplasia); however, while such corticosteroid therapy may be effective in some cases in improving lung function, in others it causes periventricular abnormalities in the brain.

Respiratory failure due to an insufficiency in surfactant can also occur in adults whose type II cells or surfactant-producing pneumocytes have been destroyed as an adverse side effect of the use of immunosuppressive medications or chemotherapeutic drugs.

The **detergent properties** of phospholipids, especially phosphatidylcholine, play an important role in bile where they function to solubilize cholesterol. An impairment in phospholipid production and secretion into bile can result in formation of cholesterol stones and bile pigment gallstones. Phosphatidylinositol and phosphatidylcholine also serve as sources of arachidonic acid for synthesis of prostaglandins, thromboxanes, leukotrienes, and related compounds.

Inositides Play a Role in Signal Transduction

Inositol-containing phospholipids (inositides) play a central role in signal transduction systems; the most important is **phosphatidylinositol 4,5-bisphosphate** (PIP_2) (Figure 10.10). When certain ligands bind to their respective receptors on the plasma membrane of mammalian cells (see Chapter 19), PIP_2

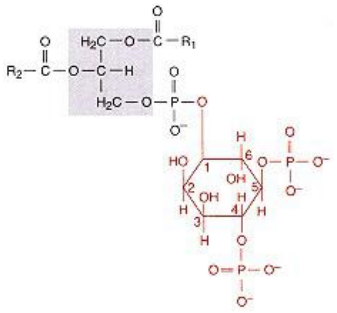


Figure 10.10
Structure of phosphatidylinositol 4,5-bisphosphate (PIP_2 or PtdIns $(4,5)P_2$).

CLINICAL CORRELATION 10.1

Respiratory Distress Syndrome

Respiratory distress syndrome (RDS) is a major cause of neonatal morbidity and mortality in many countries. It accounts for approximately 15–20% of all neonatal deaths in Western countries and somewhat less in developing countries. The disease affects only premature babies and its incidence varies directly with the degree of prematurity. Premature babies develop RDS because of immaturity of their lungs, resulting from a deficiency of pulmonary surfactant. The maturity of the fetal lung can be predicted antenatally by measuring the lecithin/sphingomyelin (L/S) ratio in the amniotic fluid. The mean L/S ratio in normal pregnancies increases gradually with gestation until about 31 or 32 weeks when the slope rises sharply. The ratio of 2.0 that is characteristic of the term infant at birth is achieved at the gestational age of about 34 weeks. For predicting pulmonary maturity, the critical L/S ratio, is 2.0 or greater. The risk of developing RDS when the L/S ratio is 1.5–1.9 is approximately 40%, and for a ratio less than 1.5 about 75%. Although the L/S ratio in amniotic fluid is still widely used to predict the risk of RDS, the results are unreliable if the amniotic fluid specimen has been contaminated by blood or meconium obtained during a complicated pregnancy.

In recent years determinations of saturated palmitoylphosphatidylcholine (SPC), phosphatidylglycerol, and phosphatidylinositol have been found to be additional predictors of the risk of RDS. Exogenous surfactant replacement therapy using surfactant from human and animal lungs is effective in the prevention and treatment of RDS.

Merritt, T. A., Hallman, M., Bloom, B.T., et al. Prophylactic treatment of very premature infants with human surfactant. *N. Engl. J. Med.* 315:785, 1986; and Simon, N. V., Williams, G. H., Fairbrother, P. F., Elser, R. C., and Perkins, R. P. Prediction of fetal lung maturity by amniotic fluid fluorescence polarization, L/S ratio, and phosphatidylglycerol. *Obstet. Gynecol.* 57:295, 1981.

localized to the inner leaflet of the membrane becomes a substrate for a receptor-dependent phosphoinositidase C (PIC), which hydrolyzes it into two intracellular signals (Figure 10.11): **inositol 1,4,5-trisphosphate** (IP_3), which triggers release of Ca^{2+} from special vesicles of the endoplasmic reticulum, and **1,2-diacylglycerol,** which stimulates activity of protein kinase C. Regulatory functions of these products of the PIC reaction are discussed in Chapter 19. Phosphatidic acid, a product of phospholipase D action on phospholipids, has been implicated as a second messenger.

The complex pathways of inositol phosphate metabolism serve three roles: (1) removal and inactivation of the potent intracellular signal IP_3; (2) conservation of inositol; and (3) synthesis of polyphosphates such as inositol pentakis-

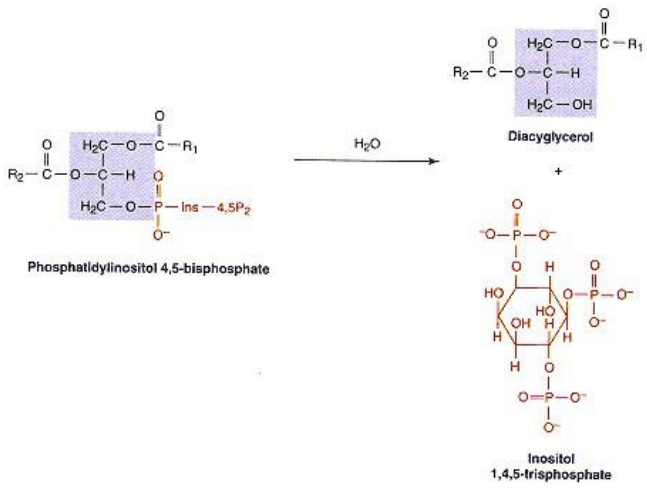


Figure 10.11
Generation of 1,2-diacylglycerol and inositol 1,4,5-trisphosphate by action of phospholipase C on phosphatidylinositol 4,5-bisphosphate.

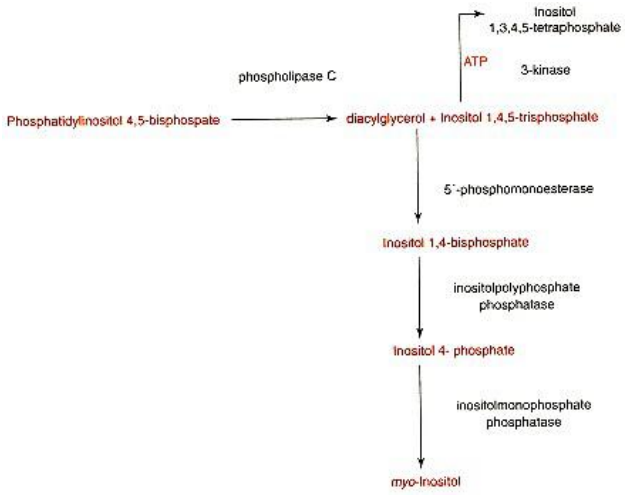


Figure 10.12
Pathways for the removal of intracellular inositol 1,4,5-trisphosphate.

phosphate ($InsP_5$) and inositol hexakisphosphate ($InsP_6$) whose functions have not been determined. Inositol 1,4,5-trisphosphate is metabolized by two enzymes: first a 5-phosphomonoesterase that converts IP_3 to inositol 1,4-bisphosphate and second a 3-kinase that forms inositol 1,3,4,5-tetraphosphate. A family of phosphatases in turn convert $Ins(1,4)P_2$ to *myo*-inositol (Figure 10.12). Inositol is eventually reincorporated into the phospholipid pool.

Phosphatidylinositol Serves to Anchor Glycoproteins to the Plasma Membrane

In addition to its role as a structural component of membranes and source of arachidonic acid for prostaglandin and leukotriene synthesis (see p. 431), phosphatidylinositol serves as an anchor to tether certain glycoproteins to the external surface of plasma membranes. In trypanosomal parasites (e.g., *Trypanosoma brucei,* which causes sleeping sickness), the external surface of the plasma membrane is coated with a protein called **variable surface glycoprotein** (VSG) linked to the membrane through a glycophospholipid anchor, specifically phosphatidylinositol (Figure 10.13). The salient structural features of the protein–lipid linkage region of the **glycosylphosphatidylinositol** (GPI) **anchor** are: (1) the diacylglyceride (DAG) moiety of phosphatidylinositol is integrated into the outer leaflet of the lipid bilayer of the plasma membrane; (2) the inositol residue is linked to DAG through a phosphodiester bond; (3) inositol is bonded to glucosamine, which contains a free, unacetylated amino group; (4) the presence of a mannose-rich glycan domain; and (5) a phosphoethanolamine residue linked to the carboxy terminus of the protein. Depending on the protein to which it is attached and the tissue or organism in which it is expressed, the GPI core may be decorated with additional carbohydrates and phosphatidylethanolamines that extend from the core mannoses; these include mannose, glucose, galactose, *N*-acetylgalactose, *N*-acetylneuraminic acid, and *N*-acetylgalactosamine. Some other proteins that are attached to the external surface of the plasma membrane include acetylcholine esterase, alkaline phosphatase, and 5 -nucleotidase.

The GPI anchor serves several functions. First, it confers on the protein to which it is attached unrestricted lateral mobility within the lipid bilayer, thereby allowing the protein to move about rapidly on the surface of the plasma membrane. Second, the presence of phospholipase C-type activity on the cell surface

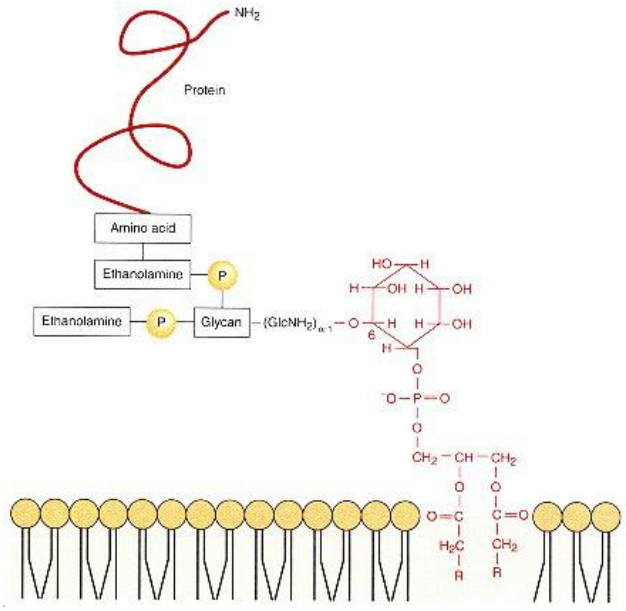


Figure 10.13
Structure of a typical phosphatidylinositol membrane protein anchor; $GlcNH_2$, glucosamine.

permits shedding of the phosphatidylinositol-anchored protein. As an example, this provides trypanosomes with a means for discarding surface antigens, thus changing their coat and escaping antibodies of the host's immune system. Third, the action of phospholipase C on the phosphatidylinositol anchor releases diacylglyceride, a second messenger that can activate protein kinase C (see p. 865). Biosynthesis of GPI anchors has been characterized extensively.

Other types of protein lipidation (co- or posttranslational modification of proteins by specific lipids) include *N*-myristoylation at the amino terminus of proteins, *S*-palmitoylation at internal cysteines, and *S*-prenylation by farnesyl or geranylgeranyl residues at cysteines at the carboxyl terminus of proteins.

Biosynthesis of Phospholipids

Phosphatidic Acid Is Synthesized from α-Glycerophosphate and Fatty Acyl CoA

1-α-Phosphatidic acid (commonly called phosphatidic acid) and 1,2-diacyl-*sn*-glycerol are common intermediates in the pathways of phospholipid and triacylglycerol biosynthesis (Figure 10.14) and both pathways share some of the same enzymes (see Chapter 9). Essentially all cells are capable of synthesizing phospholipids to some degree (except mature erythrocytes), whereas triacylglycerol biosynthesis occurs only in liver, adipose tissue, and intestine. In most tissues, the pathway for phosphatidic acid synthesis begins with α-glycerophosphate (*sn*-glycerol 3-phosphate). The most general source of α-glycerophosphate, particularly in adipose tissue, is from reduction of the glycolytic intermediate, dihydroxyacetone phosphate, in the reaction catalyzed by α-glycerophosphate dehydrogenase:

$$\text{Dihydroxyacetone phosphate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{α-glycerol 3-phosphate} + \text{NAD}^+$$

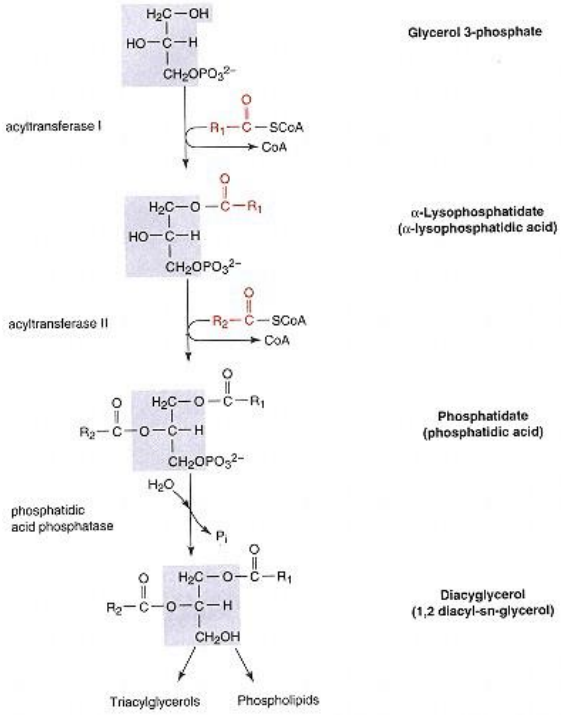


Figure 10.14
Phosphatidic acid biosynthesis from glycerol 3-phosphate and the role of phosphatidic acid phosphatase in synthesis of phospholipids and triacylglycerols.

A few specialized tissues, including liver and kidney, derive α-glycerophosphate by means of the glycerol kinase reaction:

$$\text{Glycerol} + \text{ATP} \overset{Mg^{2+}}{\rightleftharpoons} \alpha\text{-glycerol 3-phosphate} + \text{ADP}$$

The next two steps in phosphatidic acid biosynthesis involve stepwise transfer of long-chain fatty acyl groups from fatty acyl CoA. The first acyltransferase (I) is called **glycerol phosphate:acyltransferase** and attaches predominantly saturated fatty acids or oleic acid to the *sn*-1 to produce 1-acylglycerol phosphate or α-lysophosphatidic acid. The second enzyme (II), **1-acylglycerol phosphate:acyltransferase,** acylates the *sn*-2 position, usually with an unsaturated fatty acid (Figure 10.14). In both cases the donor of acyl groups is the CoA thioester derivative of the appropriate long-chain fatty acid.

The specificity of the two acyltransferases does not always match the fatty acid asymmetry found in the phospholipids of a particular cell. Remodeling reactions, discussed below, modify the fatty acid composition at C-1 and C-2 of the glycerol phosphate backbone.

Cytosolic phosphatidic acid phosphatase (also called phosphatidic acid phosphohydrolase) hydrolyzes phosphatidic acid (1,2-diacylglycerophosphate) that is generated on the endoplasmic reticulum, thereby yielding 1,2-diacyl-*sn*-glycerol that serves as the branch point in triacylglycerol and phospholipid synthesis (Figure 10.14). Phosphatidic acid can also be formed by a second pathway that begins with DHAP. This is usually an alternative supportive route used by some tissues to produce phosphatidic acid (see Chapter 9).

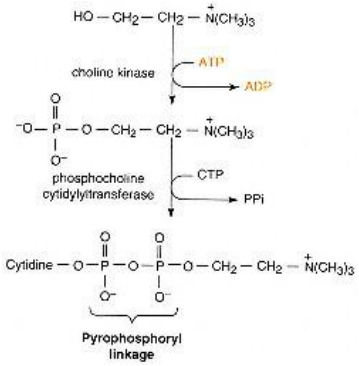


Figure 10.15
Biosynthesis of CDP-choline from choline.

Specific Phospholipids Are Synthesized by Addition of a Base to Diacylglycerol

The major pathway for biosynthesis of phosphatidylcholine (lecithin) involves sequential conversion of choline to phosphocholine, CDP-choline, and phosphatidylcholine. In this pathway, the phosphocholine polar head group is activated using CTP, according to the following reactions. Free choline, a dietary requirement for most mammals, is first phosphorylated by ATP by choline kinase (Figure 10.15). Phosphocholine is converted to CDP-choline at the expense of CTP in the reaction catalyzed by **phosphocholine cytidylyltransferase.** Note inorganic pyrophosphate (PP_i) is a product of this reaction. The high-energy pyrophosphoryl bond in CDP-choline is very unstable and reactive so that the phosphocholine moiety can be transferred readily to the nucleophilic center provided by the OH group at position 3 of 1,2-diacylglycerol by choline phosphotransferase (Figure 10.16). This is the principal pathway for the synthesis of dipalmitoyllecithin in lung.

The rate-limiting step for phosphatidylcholine biosynthesis is the cytidylyl-transferase reaction that forms CDP-choline (Figure 10.15). This enzyme is regulated by a novel mechanism involving exchange of enzyme between cytosol and endoplasmic reticulum. The cytosolic form of cytidylyltransferase is inactive and appears to function as a reservoir of enzyme; binding of the enzyme to the membrane results in activation. Translocation of cytidylyltransferase from the cytosol to the endoplasmic reticulum is regulated by cAMP and fatty acids. Reversible phosphorylation of the enzyme by a cAMP-dependent kinase causes it to be released from the membrane, rendering it inactive. Subsequent dephos-

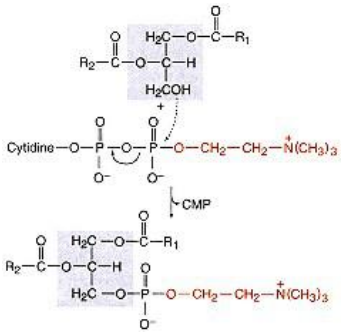


Figure 10.16
Choline phosphotransferase reaction.

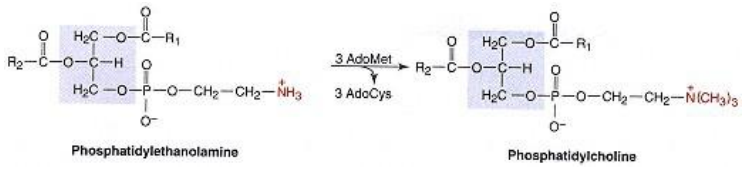


Figure 10.17
Biosynthesis of phosphatidylcholine from phosphatidylethanolamine and *S*-adenosylmethionine (AdoMet) and *S*-adenosylhomocysteine (AdoCys).

phorylation will cause cytidylyltransferase to rebind to the membrane and become active. Fatty acyl CoAs activate the enzyme by promoting its binding to the endoplasmic reticulum. In liver only, phosphatidylcholine is formed by repeated methylation of phosphatidylethanolamine. **Phosphatidylethanol-amine *N*-methyltransferase,** an enzyme of the endoplasmic reticulum, catalyzes transfer of methyl groups one at a time from ***S*-adenosylmethionine (AdoMet)** to phosphatidylethanolamine to produce phosphatidylcholine (Figure 10.17).

The primary pathway for phosphatidylethanolamine synthesis in liver and brain involves **ethanolamine phosphotransferase** of the endoplasmic reticulum that catalyzes the reaction shown in Figure 10.18. This enzyme is particularly abundant in liver. CDP-ethanolamine is formed by **ethanolamine kinase:**

$$\text{Ethanolamine} + \text{ATP} \overset{Mg^{2+}}{\rightleftharpoons} \text{phosphoethanolamine} + \text{ADP}$$

and **phosphoethanolamine cytidylyltransferase:**

$$\text{Phosphoethanolamine} + \text{CTP} \overset{Mg^{2+}}{\rightleftharpoons} \text{CDP-ethanolamine} + PP_i$$

Liver mitochondria also generate phosphatidylethanolamine by decarboxylation of phosphatidylserine; however, this is thought to represent only a minor pathway (Figure 10.19).

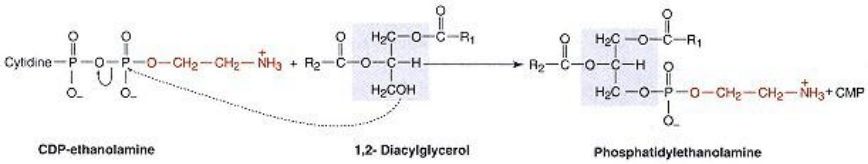


Figure 10.18
Biosynthesis of phosphatidylethanolamine from CDP-ethanolamine and diacylglycerol; the reaction is catalyzed by ethanolamine phosphotransferase.

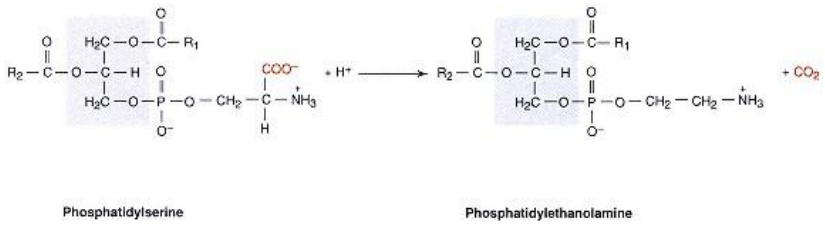


Figure 10.19
Formation of phosphatidylethanolamine by the decarboxylation of phosphatidylserine.

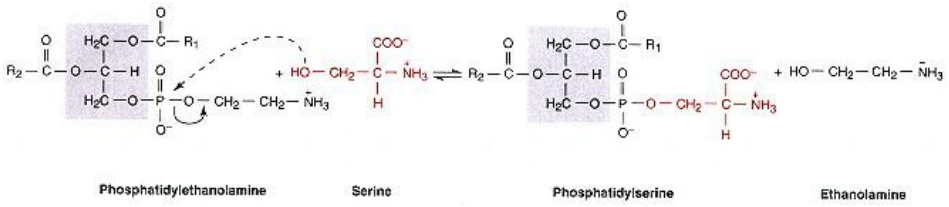


Figure 10.20
Biosynthesis of phosphatidylserine from serine and phosphatidylethanolamine by "base exchange."

The major source of phosphatidylserine in mammalian tissues is provided by the "base-exchange" reaction (Figure 10.20) in which the polar head group of phosphatidylethanolamine is exchanged for serine. Since there is no net change in the number or kinds of bonds, this reaction is reversible and has no requirement for ATP or any other high-energy compound. The reaction is initiated by attack on the phosphodiester bond of phosphatidylethanolamine by the hydroxyl group of serine.

Phosphatidylinositol is made via CDP-diacylglycerol and free *myo*-inositol (Figure 10.21) in a reaction catalyzed by **phosphatidylinositol synthase,** another enzyme of the endoplasmic reticulum.

The Asymmetric Distribution of Fatty Acids in Phospholipids Is Due to Remodeling Reactions

Two phospholipases, phospholipase A_1 and phospholipase A_2, occur in many tissues and play a role in the formation of specific phospholipid structures containing appropriate fatty acids in the *sn*-1 and *sn*-2 positions. Most fatty acyl CoA transferases and phospholipid synthesizing enzymes discussed above lack the specificity required to account for the asymmetric position or distribution of fatty acids found in many tissue phospholipids. The fatty acids found in the *sn*-1 and *sn*-2 positions of the various phospholipids are often not the same ones transferred to the glycerol backbone in the initial acyl transferase reactions of the phospholipid biosynthetic pathways. **Phospholipases A_1** and **A_2** catalyze reactions indicated in Figure 10.22 where X represents the polar head group of a phospholipid. The products of the action of phospholipases A_1 and A_2 are called lysophosphatides.

If it becomes necessary for a cell to remove some undesired fatty acid, such as stearic acid from the *sn*-2 position of phosphatidylcholine, and replace it by a more unsaturated one like arachidonic acid, then this can be accomplished by the action of phospholipase A_2 followed by a reacylation reaction. Insertion of arachidonic acid into the 2 position of *sn*-2-lysophosphatidylcholine can

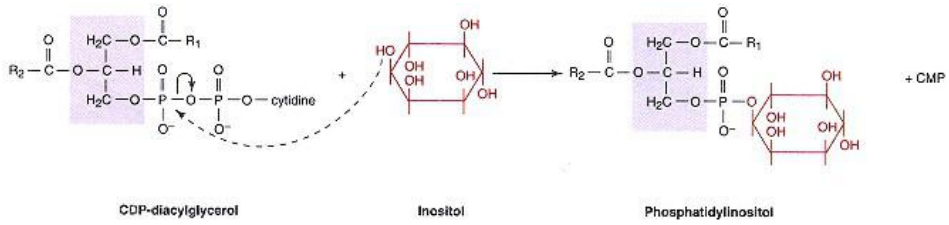


Figure 10.21
Biosynthesis of phosphatidylinositol.

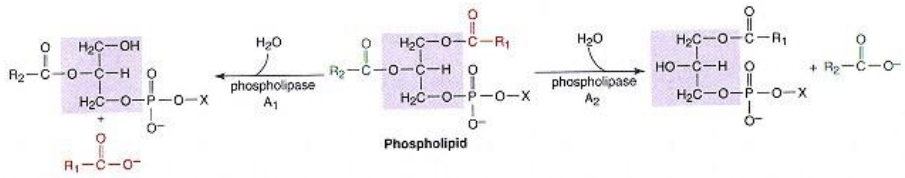


Figure 10.22
Reactions catalyzed by phospholipase A_1 and phospholipase A_2.

be accomplished either by direct acylation from arachidonoyl CoA involving **arachidonic acid-specific acyl CoA transacylase** (Figure 10.23) or from some other arachidonic acid-containing phospholipid by an exchange-type reaction (Figure 10.24) catalyzed by **lysolecithin: lecithin acyltransferase** (LLAT) (Figure 10.24). Since there is no change in either number or nature of the bonds involved in products and reactants, ATP is not required. Reacylation of lysophosphatidylcholine from acyl CoA is the major route for remodeling of phosphatidylcholine.

Lysophospholipids, particularly *sn*-1-lysophosphatidylcholine, can also serve as sources of fatty acid in remodeling reactions. Those involved in synthe-

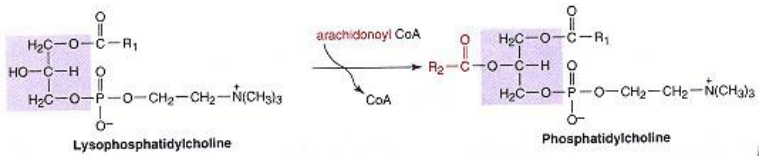


Figure 10.23
Synthesis of phosphatidylcholine by reacylation of lysophosphatidylcholine
where $R_2-C(=O)-O-$ represents arachidonic acid.
This reaction is catalyzed by acyl CoA:1-acylglycerol-3-phosphocholine *O*-acyltransferase

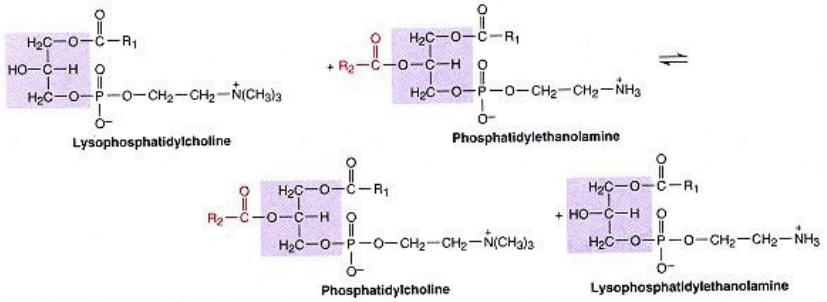


Figure 10.24

Formation of phosphatidylcholine by lysolecithin exchange, where $R_2-C(=O)-O-$ represents arachidonic acid.

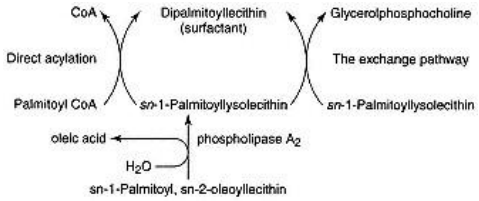


Figure 10.25
Two pathways for biosynthesis of
dipalmitoyllecithin from *sn*-1 palmitoyl-lysolecithin.

sis of dipalmitoyllecithin (surfactant) from 1-palmitoyl-2-oleoylphosphatidyl-choline are presented in Figure 10.25. Note that *sn*-1-palmitoyllysolecithin is the source of palmitic acid in the acyltransferase exchange reaction.

Plasmalogens Are Synthesized from Fatty Alcohols

Ether glycerolipids are synthesized from DHAP, long-chain fatty acids, and long-chain fatty alcohols; the reactions are summarized in Figure 10.26. Acyldihydroxyacetone phosphate is formed by **acyl CoA: dihydroxyacetone phosphate acyltransferase** (enzyme 1) acting on dihydroxyacetone phosphate and long-chain fatty acyl CoA. The ether bond is introduced by **dihydroxyacetone phosphate synthase** (Figure 10.26, enzyme 2), which exchanges the 1-*O*-acyl

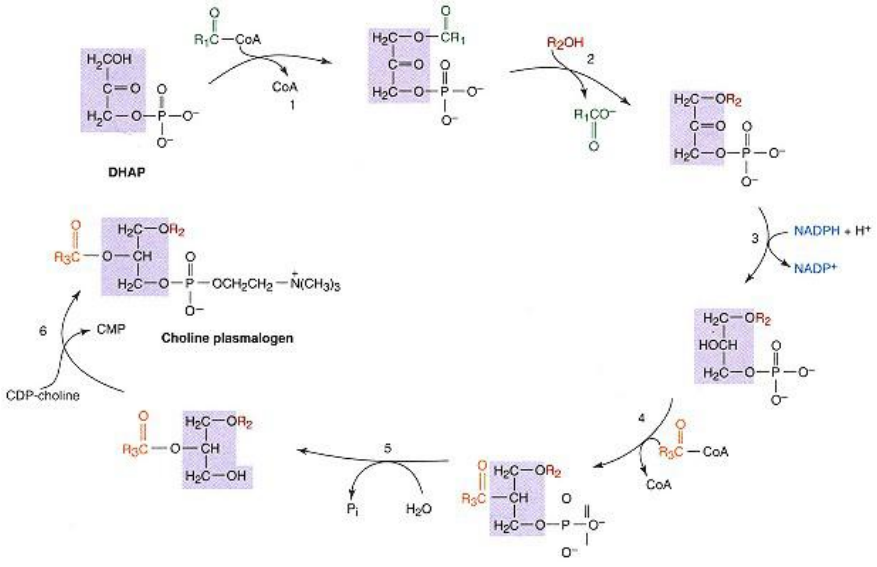


Figure 10.26
Pathway of choline plasmalogen biosynthesis from DHAP.
1, acyl CoA: dihydroxyacetone phosphate acyltransferase; 2, alkyldihydroxyacetone phosphate synthase; 3, NADPH: alkyldihydroxyacetone phosphate oxidoreductase; 4, acyl CoA:1-alkyl-2- lyso-*sn*-glycero-3-phosphate acyltransferase; 5, 1-alkyl-2-acyl-*sn*- glycerol-3-phosphate phosphohydrolase; 6, CDP-choline: 1-alkyl-2-acyl-*sn*-glycerol cholinephosphotransferase.

group of acyldihydroxyacetone phosphate with a long-chain fatty alcohol. The synthase occurs in peroxisomes. Plasmalogen synthesis is completed by transfer of a long-chain fatty acid from its respective CoA donor to the *sn*-2 position of 1-alkyl-2-lyso-*sn*-glycero-3-phosphate (Figure 10.26, Reaction 4). Patients with Zellweger's disease lack peroxisomes and cannot synthesize adequate amounts of plasmalogen.

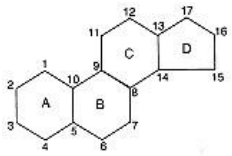


Figure 10.27
The cyclopentanophenanthrene ring.

10.3— Cholesterol

Cholesterol, an Alicyclic Compound, Is Widely Distributed in Free and Esterified Forms

Cholesterol is an alicyclic compound whose structure includes: (1) the perhy-drocyclopentanophenanthrene nucleus with its four fused rings; (2) a single hydroxyl group at C-3, (3) an unsaturated center between C-5 and C-6 atoms; (4) an eight-membered branched hydrocarbon chain attached to the D ring at position 17; and (5) a methyl group (designated C-19) attached at position 10 and another methyl group (designated C-18) attached at position 13 (see Figures 10.27 and 10.28).

In terms of physical properties, cholesterol is a lipid with very low solubility in water; at 25°C, the limit of solubility is approximately 0.2 mg/100 mL. The actual concentration of cholesterol in plasma of healthy people is usually 150–200 mg/100 mL; this value is almost twice the normal concentration of blood glucose. This high solubility of cholesterol in blood is due to plasma lipoproteins (mainly LDL and VLDL) that have the ability to bind and thereby solubilize large amounts of cholesterol (see p. 56). Actually, only about 30% of the total plasma cholesterol occurs free; approximately 70% of the cholesterol in plasma lipoproteins exists in the form of **cholesterol esters** where some long-chain fatty acid, usually linoleic acid, is attached by an ester bond to the OH group on C-3 of the A ring. The long-chain fatty acid residue enhances the hydrophobicity of cholesterol (Figure 10.29). Cholesterol is a ubiquitous and essential component of mammalian cell membranes.

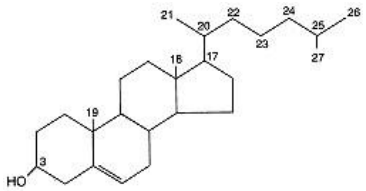


Figure 10.28
Structure of cholesterol (5-cholesten-3 -ol).

Cholesterol is also abundant in bile where the normal concentration is 390 mg/100 mL. Only 4% of cholesterol in bile is esterified to a long-chain fatty acid. Bile does not contain appreciable amounts of lipoproteins and solubilization of free cholesterol is achieved in part by the detergent property of phospholipids present in bile that are produced in liver (see p. 1078). A chronic disturbance in phospholipid metabolism in liver can result in deposition of cholesterol-rich gallstones. Bile salts, which are derivatives of cholesterol, also aid in keeping cholesterol in solution in bile. Cholesterol also appears to protect membranes of the gallbladder from potentially irritating or harmful effects of bile salts.

In the clinical laboratory, total cholesterol is estimated by the Liebermann–Burchard reaction. The proportions of free and esterified cholesterol can be determined by gas–liquid chromatography or reverse-phase high-pressure liquid chromatography (HPLC).

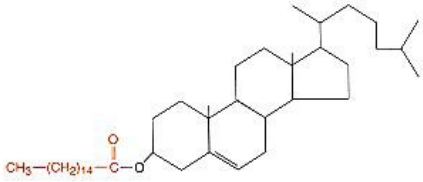


Figure 10.29
Structure of cholesterol (palmitoyl) ester.

Cholesterol Is a Membrane Component and Precursor of Bile Salts and Steroid Hormones

Cholesterol, derived from the diet or synthesized *de novo* in virtually all cells of humans, has a number of important roles. It is the major sterol in humans and a component of virtually all plasma and intracellular membranes. Cholesterol is especially abundant in myelinated structures of brain and central nervous system but is present in small amounts in the inner membrane of the mitochondrion (see p. 186). In contrast to the situation in plasma, most cholesterol in cellular membranes occurs in the free, unesterified form.

Cholesterol is the immediate precursor of **bile acids** synthesized in liver and that function to facilitate absorption of dietary triacylglycerols and fat-soluble vitamins (Chapter 26). It is important to realize that the ring structure of cholesterol cannot be metabolized to CO_2 and water in humans. Excretion of cholesterol is by way of the liver and gallbladder through the intestine in the form of bile acids.

Another physiological role of cholesterol is as the precursor of various **steroid hormones** (Chapter 21). Progesterone is the C_{21} keto steroid sex hormone secreted by the corpus luteum of the ovary and by placenta. The metabolically powerful corticosteroids of adrenal cortex are derived from cholesterol; these include deoxycorticosterone, corticosterone, cortisol, and cortisone. The mine ralocorticoid aldosterone is derived from cholesterol in the zona glomerulosa tissue of the cortex of the adrenal gland. Cholesterol is also the precursor of female steroid hormones (estrogens, e.g., estradiol) in the ovary and of male steroids (e.g., testosterone) in the testes. Although all steroid hormones are structurally related to and biochemically derived from cholesterol, they have widely different physiological properties that relate to spermatogenesis, pregnancy, lactation and parturition, mineral balance, and energy (amino acids, carbohydrate, and fat) metabolism.

The hydrocarbon skeleton of cholesterol is also found in plant sterols, for example, **ergosterol,** a precursor of vitamin D (Figure 10.30). Ergosterol is converted in skin by ultraviolet irradiation to vitamin D_2. Vitamin D_2 is involved in calcium and phosphorus metabolism (Chapter 28).

Cholesterol Is Synthesized from Acetyl CoA

Although *de novo* biosynthesis of cholesterol occurs in virtually all cells, this capacity is greatest in liver, intestine, adrenal cortex, and reproductive tissues, including ovaries, testes, and placenta. From an inspection of its structure it is apparent that cholesterol biosynthesis will require a source of carbon atoms and considerable reducing power to generate the numerous carbon–carbon and carbon–hydrogen bonds. All carbon atoms of cholesterol are derived from acetate. Reducing power in the form of NADPH is provided mainly by glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of the hexose monophosphate shunt (see p. 336). The pathway of cholesterol synthesis occurs in the cytosol and is driven in large part by hydrolysis of high-energy thioester bonds of acetyl CoA and phosphoanhydride bonds of ATP.

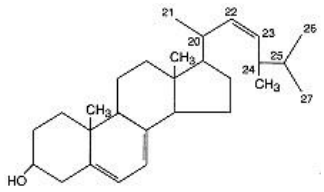
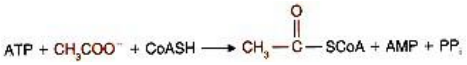


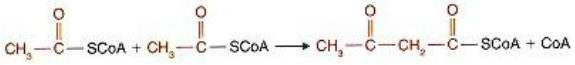
Figure 10.30
Structure of ergosterol.

Mevalonic Acid Is a Key Intermediate

The first compound unique to cholesterol biosynthesis is mevalonic acid derived from acetyl CoA. Acetyl CoA can be obtained from several sources: (1) the β-oxidation of fatty acids (Chapter 9); (2) the oxidation of ketogenic amino acids such as leucine and isoleucine (Chapter 11); and (3) the pyruvate dehydrogenase reaction. Free acetate can be activated to its thioester derivative at the expense of ATP by **acetokinase,** also referred to as **acetate thiokinase:**



The first two reactions in cholesterol biosynthesis are shared by the pathway that produces ketone bodies (see p. 387). Two molecules of acetyl CoA condense to form acetoacetyl CoA in a reaction catalyzed by acetoacetyl CoA thiolase (acetyl CoA:acetyl CoA acetyltransferase):



Formation of the carbon–carbon bond in acetoacetyl CoA in this reaction is favored energetically by cleavage of a thioester bond and generation of free coenzyme A.

The next step introduces a third molecule of acetyl CoA into the cholesterol pathway and forms the branched-chain compound **3-hydroxy-3-methylglutaryl CoA** (HMG CoA) (Figure 10.31). This condensation reaction is catalyzed by **HMG CoA synthase** (3-hydroxy-3-methylglutaryl CoA:acetoacetyl CoA lyase). Liver parenchymal cells contain two isoenzyme forms of HMG CoA synthase; one in the cytosol is involved in cholesterol synthesis, while the other has a mitochondrial location and functions in synthesis of ketone bodies (see p. 388). In the HMG CoA synthase reaction, an aldol condensation occurs between the methyl carbon of acetyl CoA and the β-carbonyl group of acetoacetyl CoA with the simultaneous hydrolysis of the thioester bond of acetyl CoA. The thioester bond in the original acetoacetyl CoA substrate molecule remains intact. HMG CoA can also be formed from oxidative degradation of the branched-chain amino acid leucine, through the intermediates 3-methylcrotonyl CoA and 3-methylglutaconyl CoA (Chapter 11).

The step that produces the unique compound mevalonic acid from HMG CoA is catalyzed by the important microsomal enzyme **HMG CoA reductase** (mevalonate:NAD^+ oxidoreductase) that has an absolute requirement for NADPH as the reductant (Figure 10.32). This reductive step (1) consumes two molecules of NADPH, (2) results in hydrolysis of the thioester bond of HMG CoA, and (3) generates a primary alcohol residue in mevalonate. This reduction reaction is irreversible and produces (*R*)-(+)mevalonate, which contains six carbon atoms. HMG CoA reductase catalyzes the rate-limiting reaction in the pathway of cholesterol biosynthesis. HMG CoA reductase is an intrinsic membrane protein of the endoplasmic reticulum whose carboxyl terminus extends into the cytosol and carries the enzyme's active site. Phosphorylation regulates

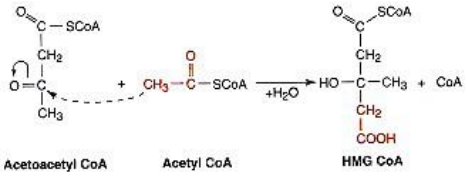


Figure 10.31
HMG CoA synthase reaction.

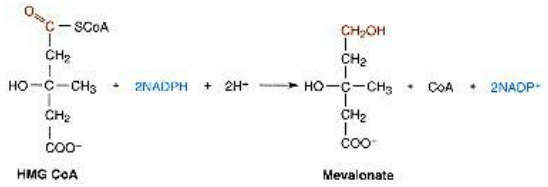


Figure 10.32
HMG CoA reductase reaction.

HMG CoA reductase activity of the cell by diminishing its catalytic activity (V_{max}) and enhancing the rate of its degradation by increasing its susceptibility to proteolytic attack. Increased amounts of intracellular cholesterol stimulate phosphorylation of HMG CoA reductase.

Mevalonic Acid Is a Precursor of Farnesyl Pyrophosphate

Reactions involved in conversion of mevalonate to **farnesyl pyrophosphate** are summarized in Figure 10.33. The stepwise transfer of the terminal γ-phosphate group from two molecules of ATP to mevalonate (A) to form 5-pyrophosphomevalonate (B) are catalyzed by mevalonate kinase (enzyme I) and phosphomevalonate kinase (enzyme II). The next step affects decarboxylation of 5-pyrophosphomevalonate and generates Δ^3-isopentenyl pyrophosphate (D); this reaction is catalyzed by pyrophosphomevalonate decarboxylase. In this ATP-dependent reaction in which ADP, P_i, and CO_2 are produced, it is thought that decarboxylation–dehydration proceeds by way of the triphosphate intermediate, 3-phosphomevalonate 5-pyrophosphate (C). Isopentenyl pyrophosphate is converted to its allylic isomer 3,3-dimethylallyl pyrophosphate (E) in a reversible reaction catalyzed by isopentenyl pyrophosphate isomerase. The condensation of 3,3-dimethylallyl pyrophosphate (E) and Δ^3-isopentenyl pyrophosphate (D) generates **geranyl pyrophosphate** (F).

The stepwise condensation of three C_5 isopentenyl units to form the C15 unit farnesyl pyrophosphate (G) is catalyzed by one enzyme, a cytoplasmic prenyl transferase called geranyltransferase.

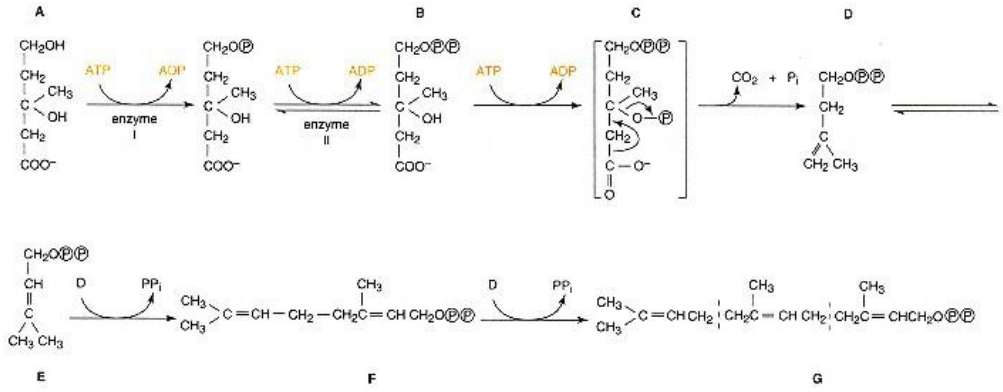


Figure 10.33
Formation of farnesyl pyrophosphate (F) from mevalonate (A).
Dotted lines divide molecules into isoprenoid-derived units. D is 3-isopentenyl pyrophosphate.

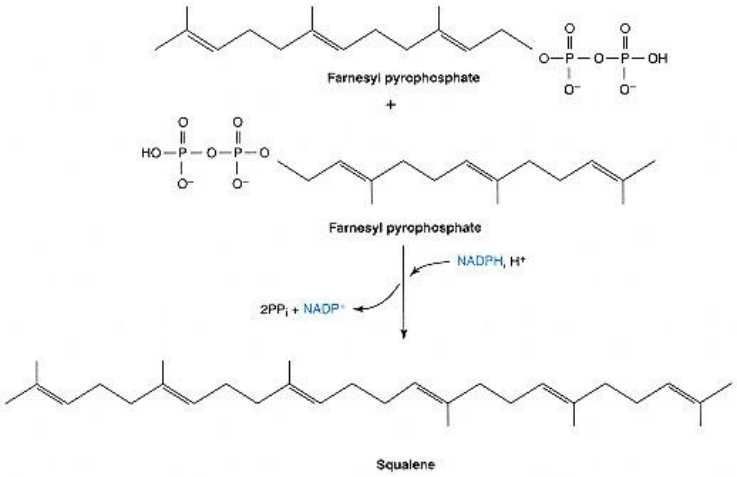


Figure 10.34
Formation of squalene from two molecules of farnesly pyrophosphate.

Cholesterol Is Formed from Farnesyl Pyrophosphate via Squalene

The last steps in cholesterol biosynthesis involve "head-to-head" fusion of two molecules of farnesyl pyrophosphate to form **squalene** and finally cyclization of squalene to yield cholesterol. The reaction that produces the C_{30} squalene molecule from two C_{15} farnesyl pyrophosphate moieties (Figure 10.34) and is unlike the previous carbon–carbon bond-forming reactions in the pathway (Figure 10.33). **Squalene synthase,** present in the endoplasmic reticulum, releases two pyrophosphate groups, with loss of a hydrogen atom from one molecule of farnesyl pyrophosphate and replacement by a hydrogen from NADPH. Several different intermediates probably occur between farnesly pyrophosphate and squalene. By rotation about carbon–carbon single bonds, the conformation of squalene indicated in Figure 10.35 can be obtained. Note the similarity of the overall shape of the compound to cholesterol and that squalene is devoid of oxygen atoms.

Cholesterol biosynthesis from squalene proceeds through the intermediate **lanosterol,** which contains the fused tetracyclic ring system and a C_8 side chain:

Squalene → squalene 2,3-epoxide → lanosterol

The many carbon–carbon bonds formed during cyclization of squalene are generated in a concerted fashion as indicated in Figure 10.36. The OH group

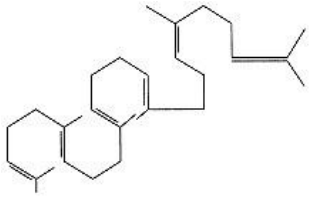


Figure 10.35
Structure of squalene, C_{30}.

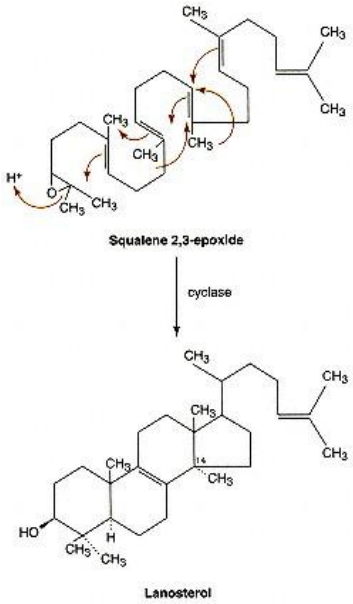


Figure 10.36
Conversion of squalene 2,3-epoxide to lanosterol.

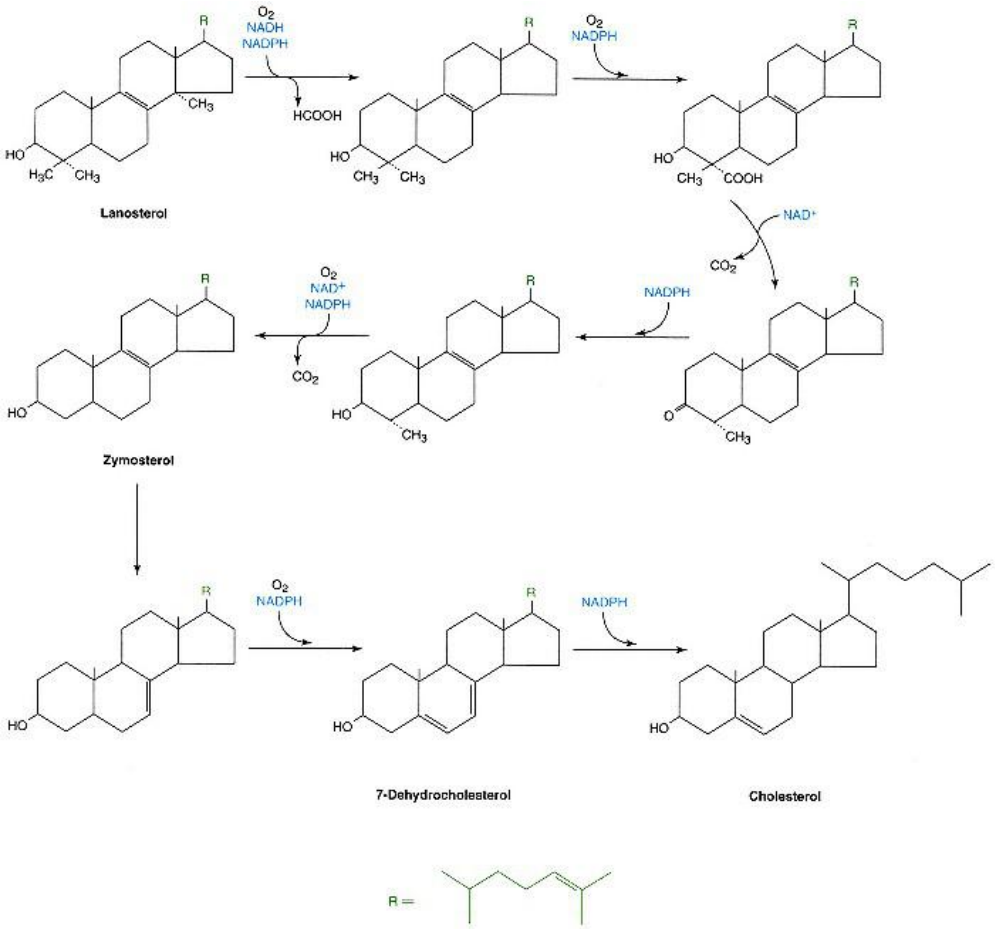


Figure 10.37
Conversion of lanosterol to cholesterol.

of lanosterol projects above the plane of the A ring; this is referred to as the β orientation. Groups that extend down below the ring in a trans relationship to the OH group are designated as α by a dotted line. During this reaction sequence an OH group is added to C-3, two methyl groups undergo shifts, and a proton is eliminated. The oxygen atom is derived from molecular oxygen. The reaction is catalyzed by an endoplasmic reticulum enzyme, **squalene oxidocyclase,** that is composed of at least two activities, squalene epoxidase or monooxygenase and a cyclase (lanosterol cyclase).

The cyclization process is initiated by epoxide formation at the expense of NADPH:

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

This reaction is catalyzed by the monooxygenase or epoxidase component. Hydroxylation at C-3 by way of the epoxide intermediate triggers the cyclization of squalene to form lanosterol (Figure 10.36). In the cyclization, two hydrogen atoms and two methyl groups migrate to neighboring positions.

Transformation of lanosterol to cholesterol involves many poorly understood steps and a number of different enzymes. These steps include: (1) removal of the methyl group at C-14; (2) removal of two methyl groups at C-4; (3) migration of the double bond from C-8 to C-5; and (4) reduction of the double bond between C-24 and C-25 in the side chain (see Figure 10.37).

Cholesterol Biosynthesis Is Carefully Regulated

The cholesterol pool of the body is derived from absorption of dietary cholesterol and biosynthesis primarily in liver and intestine. When the amount of dietary cholesterol is reduced, cholesterol synthesis is increased in liver and intestine to satisfy the needs of other tissues and organs. Cholesterol synthesized *de novo* is transported from liver and intestine to peripheral tissues in the form of lipoproteins. These are the only tissues that manufacture **apolipoprotein B,** the protein component of cholesterol transport proteins LDL and VLDL. Most apolipoprotein B is secreted into the circulation as VLDL, which is converted into LDL by removal of triacylglycerol and apolipoprotein C components, probably in plasma and liver. When the quantity of dietary cholesterol increases, cholesterol synthesis in liver and intestine is almost totally suppressed. Thus the rate of *de novo* cholesterol synthesis is inversely related to the amount of dietary cholesterol taken up by the body.

The primary site for control of cholesterol biosynthesis is HMG CoA reductase, which catalyzes the step that produces mevalonic acid. This is the committed step and the rate-limiting reaction in the pathway of cholesterol biosynthesis (Figure 10.38). Cholesterol effects feedback inhibition of its own synthesis by inhibiting the activity of preexisting HMG CoA reductase and also by promoting rapid inactivation of the enzyme by mechanisms that remain to be elucidated.

In a normal healthy adult on a low-cholesterol diet, about 1300 mg of cholesterol is returned to the liver each day for disposal. This cholesterol comes from cholesterol reabsorbed from the gut by means of the enterohepatic circulation and HDL, which carries cholesterol to the liver from peripheral tissues. Liver disposes of cholesterol by: (1) excretion in bile as free cholesterol and

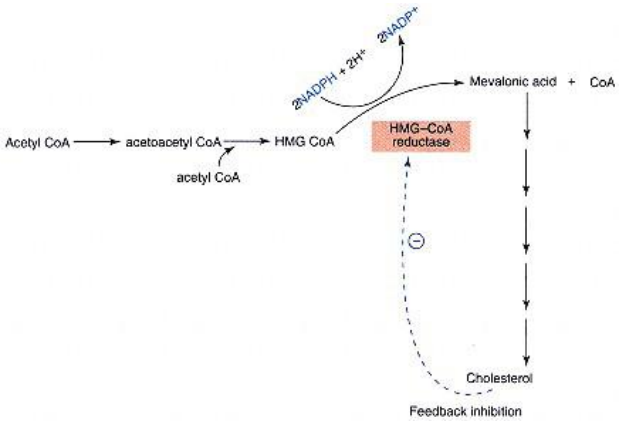


Figure 10.38
Summary of the pathway of cholesterol synthesis indicating feedback inhibition of HMG CoA reductase by cholesterol.

after conversion to bile salts; each day, about 250 mg of bile salts and 550 mg of cholesterol are lost from the enterohepatic circulation; (2) esterification and storage in liver as cholesterol esters; and (3) incorporation into lipoproteins (VLDL and LDL) and secretion into the circulation. On a low-cholesterol diet, liver will synthesize ~800 mg of cholesterol per day to replace bile salts and cholesterol lost from the enterohepatic circulation in the feces.

The mechanism of suppression of cholesterol biosynthesis by LDL-bound cholesterol involves specific **LDL receptors** that project from the surface of human cells. The first step of the regulatory mechanism involves the binding of the lipoprotein LDL to these LDL receptors, thereby extracting the LDL particles from the blood. The binding reaction is characterized by its saturability, high affinity, and high degree of specificity. The receptor recognizes only LDL and VLDL, the two plasma lipoproteins that contain apolipoprotein B-100. Once binding to receptor occurs at sites on the plasma membrane that contain pits coated with a protein called **clathrin,** the cholesterol-charged lipoprotein is endocytosed in the form of clathrin-coated vesicles. Intracellularly, the coated vesicle loses its clathrin and becomes an endosome (see p. 379). This process is termed **receptor-mediated endocytosis.** The next step involves the fusion of the endosome with a lysosome that contains numerous hydrolytic enzymes, including proteases and cholesterol esterase. The LDL receptor separates from LDL and returns to the cell surface. Inside the lysosome the cholesterol esters of LDL are hydrolyzed by cholesterol esterase to produce free cholesterol and a long-chain fatty acid. Free cholesterol then diffuses into the cytoplasm where, by some unknown mechanism, it inhibits the activity of HMG CoA reductase and suppresses the synthesis of HMG CoA reductase enzyme. There is evidence that cholesterol acts at the level of DNA and protein synthesis to decrease the rate of synthesis of HMG CoA reductase. At the same time, **fatty acyl CoA:cholesterol acyltransferase** (ACAT) in the endoplasmic reticulum is activated by cholesterol, promoting the formation of cholesterol esters, principally cholesterol oleate. Accumulation of intracellular cholesterol eventually inhibits the replenishment of LDL receptors on the cell surface, a phenomenon called **down regulation,** thereby blocking further uptake and accumulation of cholesterol.

The LDL receptor is a single-chain glycoprotein; numerous mutations in its gene are associated with familial hypercholesterolemia. The receptor spans the plasma membrane once with the carboxyl terminus on the cytoplasmic face and the amino terminus, which contains the LDL-binding site, extending into the extracellular space. **Apoprotein B-100** and **apoprotein E,** which is present in IDL (intermediate density lipoprotein) and some forms of HDL, are the two proteins through which particular lipoproteins bind to the LDL receptor.

CLINICAL CORRELATION 10.2

Treatment of Hypercholesterolemia

Many authorities recommend screening asymptomatic individuals by measuring plasma cholesterol. A level less than 200 mg% is considered desirable, and a level over 240 mg% requires lipoprotein analysis, especially determination of LDL cholesterol. Reduction of LDL cholesterol depends on dietary restriction of cholesterol to less than 300 mg day^{-1}, of calories to attain ideal body weight, and of total fat intake to less than 30% of total calories. Approximately two-thirds of the fat should be mono- or polyunsaturated. The second line of therapy is with drugs. Cholestyramine and colestipol are bile salt-binding drugs that promote excretion of bile salts in the stool. This in turn increases the rate of hepatic bile salt synthesis and of LDL uptake by the liver. Lovastatin is an inhibitor of HMG CoA reductase. Since this enzyme is limiting for cholesterol synthesis, lovastatin decreases endogenous synthesis of cholesterol and stimulates uptake and LDL via the LDL receptor. The combination of lovastatin and cholestyramine is sometimes used for severe hyperlipidemia.

Expert Panel. Evaluation and treatment of high blood cholesterol in adults. *Arch. Intern. Med.* 148:36, 1988.

The correlation between high levels of blood cholesterol, particularly LDL cholesterol, and heart attacks and strokes have led to the development of dietary and therapeutic approaches to lower blood cholesterol (see Clin. Corr. 10.2). Patients with **familial** (genetic) **hypercholesterolemia** suffer from accelerated atherosclerosis (see Clin. Corr. 10.3). In most cases, there is a lack of functional LDL receptors on the cell surface because the mutant alleles produce little or no LDL receptor protein; these patients are referred to as receptor-negative. In others the LDL receptor is synthesized and transported normally to the cell surface; an amino acid substitution or other alteration in the protein's primary structure, however, adversely affects the LDL-binding region of the receptor. As a result, there is little or no binding of LDL to the cell, cholesterol is not transferred into the cell, cholesterol synthesis is not inhibited, and the blood cholesterol level increases. Another LDL-deficient group of hypercholesterolemic patients is able to synthesize the LDL receptor but has a defect in the transport mechanism that delivers the glycoprotein to its proper location on the plasma membrane. And finally, there is another subclass of genetically determined hypercholesterolemics whose LDL receptors have a defect in the

cytoplasmic carboxyl terminus; they populate their cell surfaces with LDL receptors normally but are unable to internalize the LDL–LDL receptor complex due to an inability to cluster this complex in coated pits.

In specialized tissues such as the adrenal gland and ovary, the cholesterol derived from LDL serves as a precursor to the steroid hormones made by these organs, such as cortisol and estradiol, respectively. In liver, cholesterol extracted from LDL and HDL is converted into bile salts that function in intestinal fat digestion.

CLINICAL CORRELATION 10.3

Atherosclerosis

Atherosclerosis is the leading cause of death in Western industrialized countries. The risk of developing it is directly related to the plasma concentration of LDL cholesterol and inversely related to that of HDL cholesterol. This explains why the former is frequently called "bad" cholesterol and the latter "good" cholesterol, though chemically there is only one cholesterol. Atherosclerosis is a disorder of the arterial wall characterized by accumulation of cholesteryl esters in cells derived from the monocyte–macrophage line, smooth muscle cell proliferation, and fibrosis. The earliest abnormality is migration of blood monocytes to the subendothelium of the artery. Once there, they differentiate into macrophages. These cells accumulate cholesterol esters derived from plasma LDL. Why these cells do not regulate cellular cholesterol stores normally is not completely understood. Some of the LDL may be taken up via pathways distinct from the classical LDL receptor pathway. For instance, receptors that mediate uptake of acetylated LDL or LDL complexed with dextran sulfate have been described and these are not regulated by cellular cholesterol content. Distortion of the subendothelium leads to platelet aggregation on the endothelial surface and release of platelet-derived mitogens such as platelet-derived growth factor (PDGF). This is thought to stimulate smooth muscle cell growth. Death of the foam cells results in the accumulation of a cellular lipid that can stimulate fibrosis. The resulting atherosclerotic plaque narrows the blood vessel and serves as the site of thrombus formation, which precipitates myocardial infarction (heart attack).

Ross, R. The pathogenesis of atherosclerosis—an update. *N. Engl. J. Med.* 314:488, 1986.

Plasma Cholesterol Is in a Dynamic State

Plasma cholesterol is in a dynamic state, entering the blood complexed with lipoproteins that keep the lipid in solution and leaving the blood as tissues take up cholesterol from these lipoproteins. Plasma lipoproteins contain free cholesterol and cholesterol esterified to a long-chain fatty acid. From 70% to 75% of plasma cholesterol is esterified to long-chain fatty acids. It is the free, unesterified form of cholesterol that exchanges readily between different lipoproteins and the plasma membrane of cells.

The mechanism for entry of cholesterol into liver cells from the three types of plasma lipoprotein is quite different. While the metabolism of chylomicrons and LDL has been quite well defined, that of HDL is just beginning to be understood. Chylomicrons that have had their triacylglycerol content reduced by plasma lipoprotein lipase become chylomicron remnants, which are rich in dietary cholesterol (free and esterified) and in fat-soluble vitamins. They are taken up by receptor-mediated endocytosis into liver cells, as is LDL.

High-density lipoproteins and the enzyme **lecithin: cholesterol acyltransferase** (LCAT) play important roles in the elimination of cholesterol from the body. LCAT catalyzes the freely reversible reaction (Figure 10.39), which transfers the fatty acid in the *sn*-2 position of phosphatidylcholine to the 3-hydroxyl of cholesterol. LCAT is a plasma enzyme produced mainly by liver. The actual substrate for LCAT is cholesterol contained in HDL. The LCAT–HDL system functions to protect cells, especially their plasma membrane, from the damaging effects of excessive amounts of free cholesterol. Cholesterol ester generated in the LCAT reaction diffuses into the core of the HDL particle where it is then transported from the tissues and plasma to liver, the latter being the only organ capable of metabolizing and excreting cholesterol. Thus, by this mechanism, referred to as the reverse transport of cholesterol, LCAT acting on HDL provides a vehicle for transporting cholesterol from peripheral tissues to the liver.

Cholesterol Is Excreted Primarily As Bile Acids

The bile acids are the end products of cholesterol metabolism. Primary bile acids are synthesized in hepatocytes directly from cholesterol. The most abundant bile

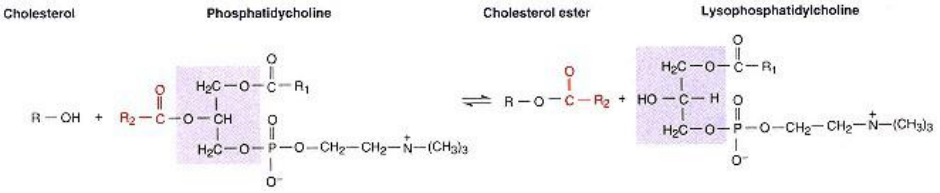


Figure 10.39
Lecithin:cholesterol acyltransferase (LCAT) reaction, where R—OH indicates cholesterol.

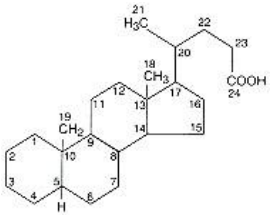


Figure 10.40
Structure of cholanic acid.

acids in humans are derivatives of cholanic acid (Figure 10.40), that is, **cholic acid** and **chenodeoxycholic acid** (Figure 10.41). The primary bile acids are composed of 24 carbon atoms, contain two or three OH groups, and have a side chain that ends in a carboxyl group that is ionized at pH 7.0 (hence the name bile salt). The carboxyl group of the bile acids is often conjugated via an amide bond to either glycine (NH_2-CH_2-COOH) or taurine (NH_2–CH_2–CH_2–SO_3H) to form **glycocholic** or **taurocholic acid,** respectively. The structure of glycocholic acid is shown in Figure 10.42.

When the primary bile acids undergo chemical reactions by microorganisms in the gut, they give rise to secondary bile acids that also possess 24 carbon atoms. Examples of secondary bile acids are deoxycholic acid and lithocholic acid, which are derived from cholic acid and chenodeoxycholic acid, respectively, by the removal of one OH group (Figure 10.41). Transformation of cholesterol to bile acids requires: (1) epimerization of the 3β-OH group; (2) reduction of the C-5 double bond; (3) introduction of OH groups at C-7 (chenodeoxycholic acid) or at C-7 and C-12 (cholic acid); and (4) conversion of the C-27 side chain into a C-24 carboxylic acid by elimination of a propyl equivalent.

Bile acids are secreted into bile canaliculi, specialized channels formed by adjacent hepatocytes. Bile canaliculi unite with bile ductules, which in turn come together to form bile ducts. The bile acids are carried to the gallbladder for storage and ultimately to the small intestine where they are excreted. The capacity of liver to produce bile acids is insufficient to meet the physiological demands, so the body relies on an efficient **enterohepatic circulation** that carries the bile acids from the intestine back to the liver several times each day. The primary conjugated bile acids, after removal of the glycine or taurine residue in the gut, are reabsorbed by an active transport process from the intestine, primarily in the ileum, and returned to the liver by way of the portal vein. Bile acids that are not reabsorbed are acted on by bacteria in the gut and converted into secondary bile acids; a portion of secondary bile acids, primarily deoxycholic acid and lithocholic acid, are reabsorbed passively in the colon and

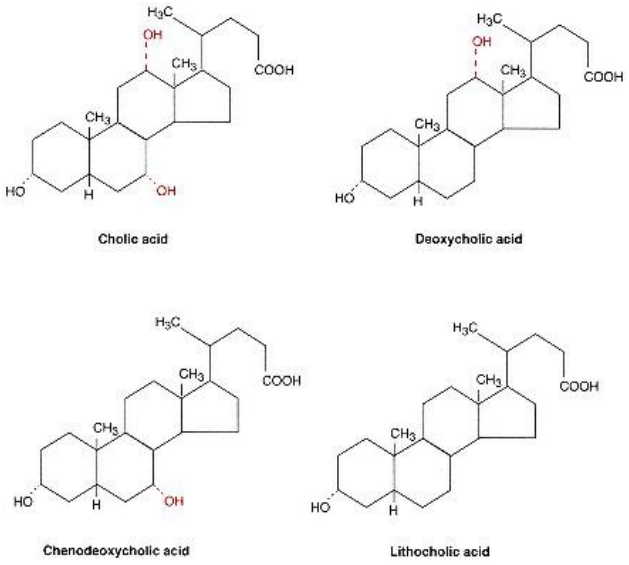


Figure 10.41
Structures of some common bile acids.

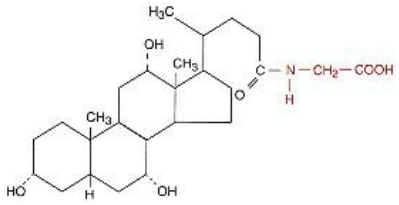


Figure 10.42
Structure of glycocholic acid, a conjugated bile acid.

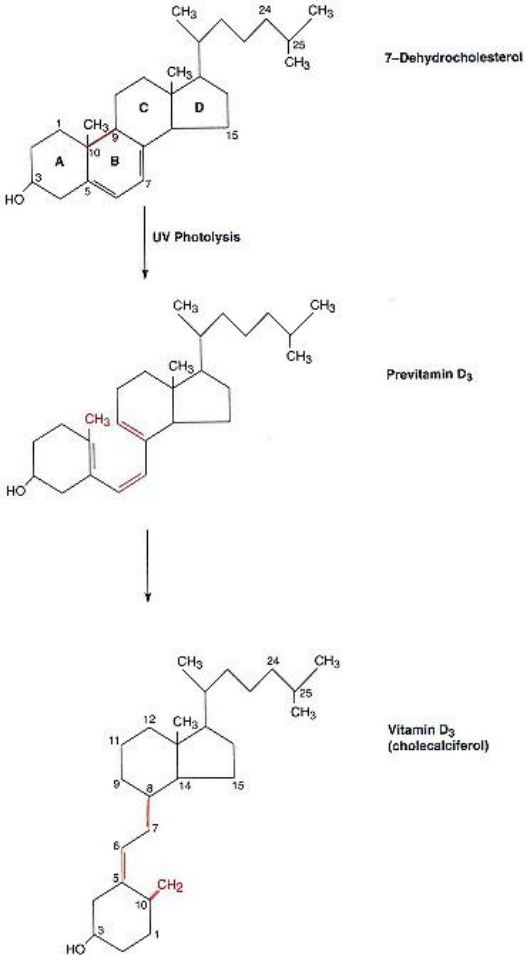


Figure 10.43
Photochemical conversion of 7-dehydrocholesterol to vitamin D_3 (cholecalciferol).

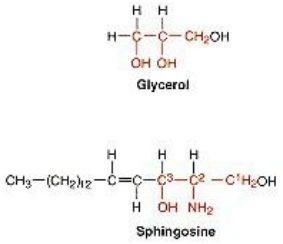


Figure 10.44
Comparison of the structures of glycerol and sphingosine (*trans*-1,3, dihydroxy-2- amino-4- octadecene).

returned to the liver where they are secreted into the gallbladder. Hepatic synthesis normally produces 0.2–0.6 g of bile acids per day to replace those lost in the feces. The gallbladder pool of bile acids is 2–4 g. Because the enterohepatic circulation recycles 6–12 times each day, the total amount of bile acids absorbed per day from the intestine corresponds to 12–32 g.

Bile acids are significant in medicine for several reasons. They represent the only significant way in which cholesterol can be excreted; the carbon skeleton of cholesterol is not oxidized to CO_2 and H_2O in humans but is excreted in bile as free cholesterol and bile acids. Bile acids prevent the precipitation of cholesterol out of solution in the gallbladder. Bile acids and phospholipids function to solubilize cholesterol in bile and act as emulsifying agents to prepare dietary triacylglycerols for hydrolysis by pancreatic lipase. Bile acids may also play a direct role in activating pancreatic lipase (see Chapter 25) and they facilitate the absorption of fat-soluble vitamins, particularly vitamin D, from the intestine.

Vitamin D Is Synthesized from an Intermediate of Cholesterol Biosynthesis

Cholesterol biosynthesis provides substrate for the photochemical production of **vitamin D_3** in skin. The metabolism and function of vitamin D_3 are discussed in Chapter 27. Vitamin D_3 is a secosteroid in which the 9,10 carbon bond of the B ring of the cholesterol nucleus has undergone fission (Figure 10.43). The most important supply of vitamin D_3 is that manufactured in the skin. **7-Dehydrocholesterol** is an intermediate in the pathway of cholesterol biosynthesis and is converted in the skin to provitamin D_3 by irradiation with UV rays of the sun (285–310 nm). Provitamin D_3 is biologically inert and labile and converted thermally and slowly (~36 h) to the double-bond isomer by a nonenzymatic reaction to the biologically active vitamin, cholecalciferol (vitamin D_3). As little as 10-min exposure each day of the hands and face to sunlight will satisfy the body's need for vitamin D. Photochemical action on the plant sterol ergosterol also provides a dietary precursor to a compound designated **vitamin D_2 (calciferol)** that can satisfy the vitamin D requirement.

10.4— Sphingolipids

Biosynthesis of Sphingosine

Sphingolipids are complex lipids whose core structure is provided by the long-chain amino alcohol **sphingosine** (Figure 10.44) (4-sphingenine or *trans*-1,3-dihydroxy-2-amino-4-octadecene). Sphingosine has two asymmetric carbon atoms (C-2 and C-3); of the four possible optical isomers, naturally occurring sphingosine is of the D-erythro form. The double bond of sphingosine has the trans configuration. The primary alcohol group at C-1 is a nucleophilic center that forms covalent bonds with sugars to form glycosphingolipids and phosphocholine to form sphingomyelin. The amino group at C-2 always bears a long-

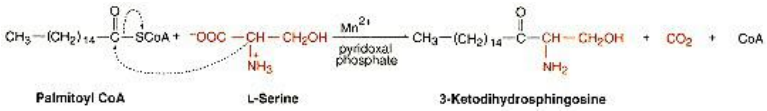


Figure 10.45
Formation of 3-ketodihydrosphingosine from serine and palmitoyl CoA.

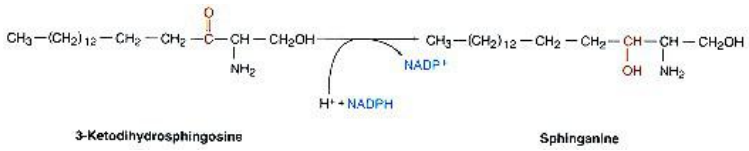


Figure 10.46
Conversion of 3-ketodihydrosphingosine to sphinganine.

chain fatty acid (usually C_{20}–C_{26}) in amide linkage. The secondary alcohol at C-3 is always free. It is useful to appreciate the structural similarity of a part of the sphingosine molecule to the glycerol moiety of the acylglycerols (Fig. 10.44).

Sphingolipids are present in blood and nearly all body tissues. The highest concentrations are found in the white matter of the central nervous system. Various sphingolipids are components of the plasma membrane of practically all cells.

Sphingosine is synthesized by way of **sphinganine** (dihydrosphingosine) in two steps from the precursors serine and palmitoyl CoA. Serine is the source of C-1, C-2, and the amino group of sphingosine, while palmitic acid provides the remaining carbon atoms. Condensation of serine and palmitoyl CoA is catalyzed by a pyridoxal phosphate-dependent enzyme, serine palmitoyltransferase. The driving force for the reaction is provided by both cleavage of the reactive, high-energy thioester bond of palmitoyl CoA and the release of CO_2 from serine (Figure 10.45). The next step involves the reduction of the carbonyl group in 3-ketodihydrosphingosine with reducing equivalents being derived from NADPH to produce sphinganine (Figure 10.46). The insertion of the double bond into sphinganine to produce sphingosine occurs at the level of ceramide (see below).

Ceramides Are Fatty Acid Amide Derivatives of Sphingosine

Sphingosine does not occur naturally. The core structure of the natural sphingolipids is **ceramide,** a long-chain fatty acid amide derivative of sphingosine. The long-chain fatty acid is attached to the 2-amino group of sphingosine through an amide bond (Figure 10.47). Most often the acyl group is **behenic acid,** a saturated C22 fatty acid, but other long-chain acyl groups can be used. There are two long-chain hydrocarbon domains in the ceramide molecule; these hydrophobic regions are responsible for the lipid character of sphingolipids.

Ceramide is synthesized from dihydrosphingosine and a molecule of long-chain fatty acyl CoA by a microsomal enzyme with dihydroceramide as an intermediate that is then oxidized by dehydrogenation at C-4 and C-5 (Figure 10.48). Free ceramide is not a component of membrane lipids but rather is an intermediate in the biosynthesis and catabolism of glycosphingolipids and sphingomyelin. Structures of prominent sphingolipids of humans are presented in Figure 10.49 in diagrammatic form.

Sphingomyelin Is the Only Sphingolipid Containing Phosphorus

Sphingomyelin, a major structural lipid of membranes of nervous tissue, is the only sphingolipid that is a phospholipid. In sphingomyelin the primary alcohol at C-1 of sphingosine is esterified to choline through a phosphodiester bridge of the kind that occurs in the acyl glycerophospholipids and the amino group of sphingosine is attached to a long-chain fatty acid by an amide bond. Sphingomyelin is therefore a ceramide phosphocholine. It contains one negative

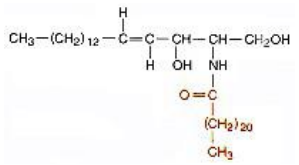


Figure 10.47
Structure of a ceramide
(*N*-acylsphingosine).

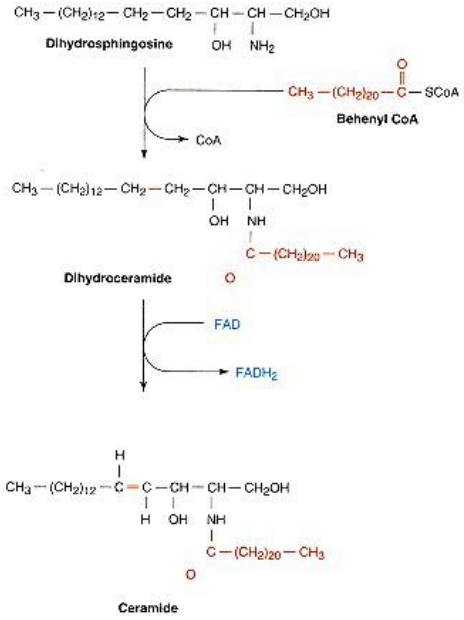


Figure 10.48
Formation of ceramide from dihydrosphingosine.

and one positive charge so that it is neutral at physiological pH (Figure 10.50). The most common fatty acids in sphingomyelin are palmitic (16:0), stearic (18:0), lignoceric (24:0), and nervonic acid (24:1). The sphingomyelin of myelin contains predominantly longer chain fatty acids, mainly lignoceric and nervonic, whereas that of gray matter contains largely stearic acid. Excessive accumulations of sphingomyelin occur in Niemann–Pick disease.

Sphingomyelin Is Synthesized from a Ceramide and Phosphatidylcholine

Conversion of ceramide to sphingomyelin involves transfer of a phosphocholine moiety from phosphatidylcholine (lecithin), not from CDP–choline as was suspected for many years; this reaction is catalyzed by **sphingomyelin synthase** (Figure 10.51).

Glycosphingolipids Usually Have a Galactose or Glucose Unit

The principal glycosphingolipid classes are cerebrosides, sulfatides, globosides, and gangliosides. In the glycolipid class of compounds the polar head group is attached to sphingosine via the glycosidic linkage of a sugar molecule rather than a phosphate ester bond, as in phospholipids.

Cerebrosides Are Glycosylceramides

Cerebrosides are ceramide monohexosides; the two most common are **galactocerebroside** and **glucocerebroside.** Unless specified otherwise, the term cerebroside usually refers to galactocerebroside, also called **"galactolipid."** In Figure 10.52 note that the monosaccharide units are attached at C-1 of the sugar

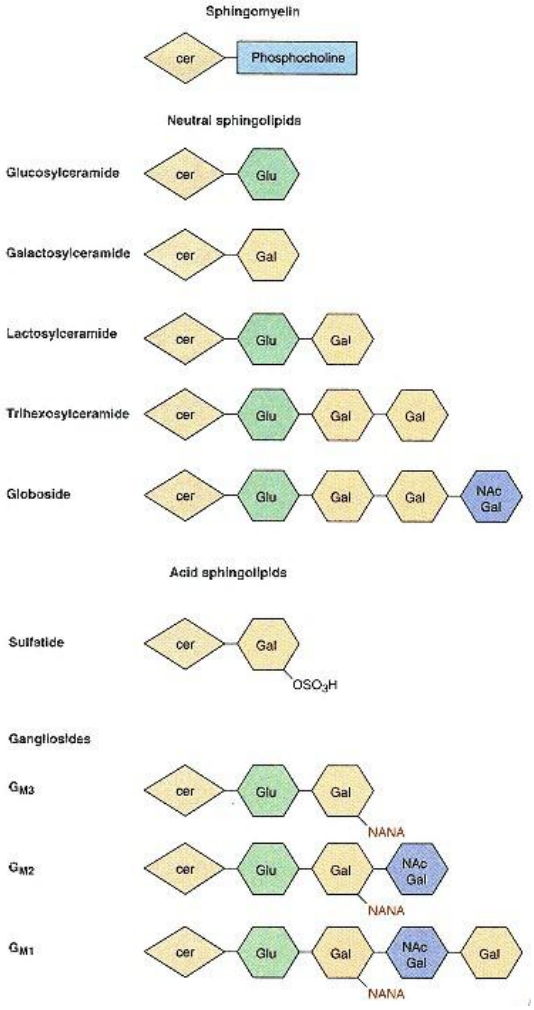


Figure 10.49
Structures of some common sphingolipids in diagrammatic form.
Cer, ceramide; Glu, glucose; Gal, galactose; NAcGal, *N*-acetyl-galactosamine; and NANA, *N*-acetylneuraminic acid (sialic acid).

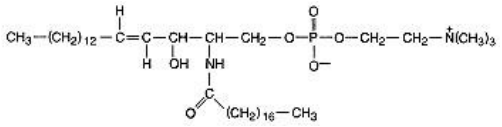


Figure 10.50
Structure of sphingomyelin.

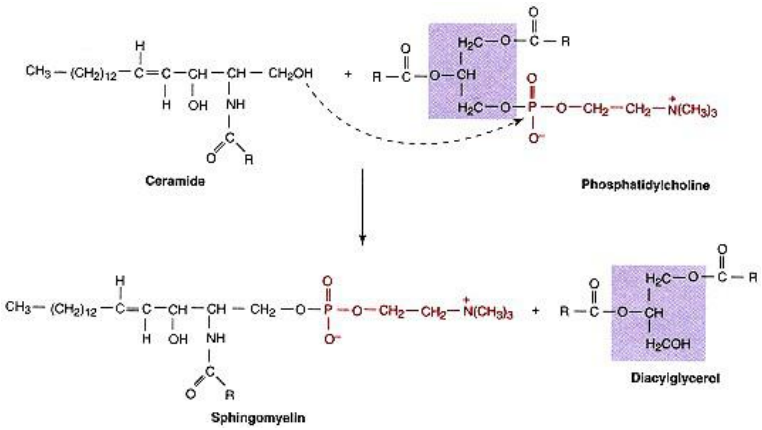


Figure 10.51
Sphingomyelin synthesis from ceramide and phosphatidylcholine.

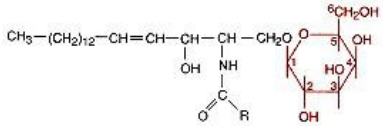


Figure 10.52
Structure of galactocerebroside (galactolipid).

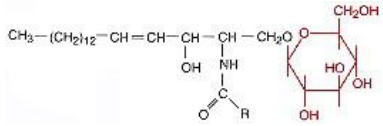


Figure 10.53
Structure of glucocerebroside.

Figure 10.54
Synthesis of galacto- and glucocerebrosides.

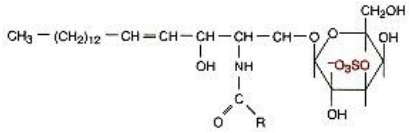


Figure 10.55
Structure of galactocerebroside sulfate (sulfolipid).

moiety to the C-1 position of ceramide, and the anomeric configuration of the glycosidic bond between ceramide and hexose in both galactocerebroside and glucocerebroside is β. The largest amount of galactocerebroside in healthy individuals is found in the brain. Moderately increased amounts of galactocerebroside accumulate in the white matter in Krabbe's disease, also called globoid leukodystrophy, due to a deficiency in the lysosomal enzyme galactocerebrosidase.

Glucocerebroside (glucosylceramide) is not normally a component of membranes but is an intermediate in the synthesis and degradation of more complex glycosphingolipids (see Figure 10.53). However, 100-fold increases or more in the glucocerebroside content of spleen and liver occur in the genetic lipid storage disorder called Gaucher's disease, which results from a deficiency of lysosomal glucocerebrosidase.

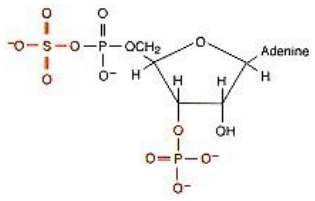


Figure 10.56
Structure of PAPS (3 -phosphoadenosine 5 -phosphosulfate).

Galactocerebroside and glucocerebroside are synthesized from ceramide and the activated nucleotide sugars UDP-galactose and UDP-glucose, respectively. The enzymes that catalyze these reactions, **glucosyl** and **galactosyl-transferases,** are associated with the endoplasmic reticulum (Figure 10.54). In some tissues, the synthesis of glucocerebroside (glucosylceramide) proceeds by glucosylation of sphingosine catalyzed by glucosyltransferase:

Sphingosine + UDP-glucose → glucosylsphingosine + UDP

followed by fatty acylation:

Glucosylsphingosine + stearoyl CoA → glucocerebroside + CoASH

Sulfatide Is a Sulfuric Acid Ester of Galactocerebroside

Sulfatide, or **sulfogalactocerebroside,** is a sulfuric acid ester of galactocerebroside. Galactocerebroside 3-sulfate is the major sulfolipid in brain and accounts for approximately 15% of the lipids of white matter (see Figure 10.55). Galactocerebroside sulfate is synthesized from galactocerebroside and 3 -phosphoadenosine 5 -phosphosulfate (PAPS) in a reaction catalyzed by sulfotransferase:

Galactocerebroside + PAPS → PAP + galactocerebroside 3-sulfate

The structure of PAPS, sometimes referred to as "activated sulfate," is indicated in Figure 10.56. Large quantities of sulfatide accumulate in the central nervous system in metachromatic leukodystrophy due to a deficiency of a specific lysosomal sulfatase.

Globosides Are Ceramide Oligosaccharides

Globosides are cerebrosides that contain two or more sugar residues, usually galactose, glucose, or *N*-acetylgalactosamine. The ceramide oligosaccharides are neutral compounds and contain no free amino groups. Lactosylceramide is a component of the erythrocyte membrane (Figure 10.57). Another prominent globoside is **ceramide trihexoside** or ceramide galactosyllactoside: ceramide-β-glc-(4 1)-β-gal(4 1)-α-gal. Note that the terminal galactose residue of this globoside has the α-anomeric configuration. Ceramide trihexoside accumulates in kidneys of patients with Fabry's disease who are deficient in lysosomal α-galactosidase A.

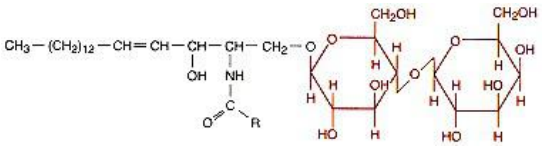


Figure 10.57
Structure of ceramide- -glc(4 1)- -gal (lactosylceramide).

Gangliosides Contain Sialic Acid

Ganglioside are sialic acid-containing glycosphingolipids highly concentrated in the ganglion cells of the central nervous system, particularly in the nerve endings. The central nervous system is unique among human tissues because more than one-half of the sialic acid is in ceramide–lipid bound form, with the remainder of the sialic acid occurring in the oligosaccharides of glycoproteins. Lesser amounts of gangliosides are present in the surface membranes of the cells of most extraneural tissues, where they account for less than 10% of the total sialic acid.

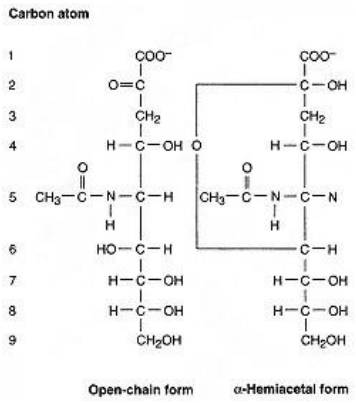


Figure 10.58
Structure of N-acetylneuraminic acid (NANA).

Neuraminic acid (abbreviated Neu) is present in gangliosides, glycoproteins, and mucins. The amino group of neuraminic acid occurs most often as the *N*-acetyl derivative, and the resulting structure is called ***N*-acetylneuraminic acid** or **sialic acid,** commonly abbreviated NANA (see Figure 10.58). The OH group on C-2 occurs most often in the α-anomeric configuration and the linkage between NANA and the oligosaccharide ceramide always involves the OH group on position 2 of *N*-acetylneuraminic acid. Structures of some common gangliosides are indicated in Table 10.1. The principal gangliosides in brain are $\mathbf{G_{M1}}$, $\mathbf{G_{D1a}}$, $\mathbf{G_{D1b}}$, and $\mathbf{G_{T1b}}$. Nearly all gangliosides of the body are derived from the family of compounds originating with glucosylceramide. In the nomenclature of

TABLE 10.1 Structures of Some Common Gangliosides

Code Name	*Chemical Structure*
G_{M3}	Galβ → 4Glcβ → Cer 3 ↑ αNANA
G_{M2}	GalNAcβ → 4Galβ → 4Glcβ → Cer 3 ↑ αNANA
G_{M1}	Galβ → 3GalNAcβ → 4Galβ → 4Glcβ → Cer 3 ↑ αNANA
G_{D1a}	Galβ → 3GalNAcβ → 4Galβ → 4Glcβ → Cer 3 3 ↑ ↑ αNANA αNANA
G_{D1b}	Galβ → 3GalNAcβ → 4Galβ → 4Glcβ → Cer 3 ↑ αNANA8 ← αNANA
G_{T1a}	Galβ → 3GalNAcβ → 4Galβ → 4Glcβ → Cer 3 3 ↑ ↑ αNANA8 ← αNANA αNANA
G_{T1b}	Galβ → 3GalNAcβ → 4Galβ → 4Glcβ → Cer 3 3 ↑ ↑ αNANA αNANA8 ← αNANA
G_{Q1b}	Galβ → 3GalNAcβ → 4Galβ → 4Glcβ → Cer 3 3 ↑ ↑ αNANA8 ← αNANA αNANA8 ← αNANA

the sialoglycosphingolipids, the letter G refers to the name ganglioside. The subscripts M, D, T, and Q indicate mono-, di-, tri-, and quatra (tetra)-sialic acid-containing gangliosides and subscripts 1, 2, and 3 designate the carbohydrate sequence that is attached to ceramide as indicated as follows: 1, Gal-GalNAc-Gal-Glc-ceramide; 2, GalNAc-Gal-Glc-ceramide; and 3, Gal-Glc-ceramide. Consider the nomenclature of the Tay–Sachs ganglioside; the designation G_{M2} denotes the ganglioside structure shown in Table 10.1.

A specific ganglioside on intestinal mucosal cells mediates the action of cholera toxin, a protein of mol wt 84,000, secreted by the pathogen *Vibrio cholerae*. The toxin stimulates the secretion of chloride ions into the gut lumen, resulting in the severe diarrhea characteristic of cholera. Two kinds of subunits, A and B, comprise the cholera toxin; there is one copy of the A subunit (28,000 Da) and five copies of the B subunit (~ 11,000 Da each). After binding to the cell surface membrane through a domain on the B subunit, the active subunit A passes into the cell. There it acts as an **ADP-ribosyltransferase** and transfers ADP-ribose of NAD^+ on to the G_{α_s} subunit of a G-protein on the cytoplasmic side of the cell membrane (see p. 859). This leads to activation of adenylate cyclase. The cAMP generated stimulates chloride ion transport and produces diarrhea. The choleragenoid domain, as the B subunits are called, binds to the **ganglioside G_{M1}** that has the structure shown in Table 10.1.

Gangliosides are also thought to be receptors for other toxins, such as tetanus toxin, and certain viruses, such as the influenza viruses. There is also speculation that gangliosides play an informational role in cell–cell interactions by providing specific recognition determinants on the surface of cells. There are several lipid storage disorders that involve the accumulation of sialic acid-containing glycosphingolipids. The two most common gangliosidoses involve the storage of the gangliosides G_{M1} (G_{M1} gangliosidosis) and G_{M2} (Tay–Sachs disease). G_{M1} gangliosidosis is an autosomal recessive metabolic disease characterized by impaired psychomotor function, mental retardation, hepatosplenomegaly, and death within the first few years of life. The massive cerebral and visceral accumulation of G_{M1} ganglioside is due to a marked deficiency of β-galactosidase.

Sphingolipidoses Are Lysosomal Storage Diseases with Defects in the Catabolic Pathway for Sphingolipids

Sphingolipids are normally degraded within lysosomes of phagocytic cells, particularly the histiocytes or macrophages of the reticuloendothelial system located primarily in liver, spleen, and bone marrow. Degradation of the sphingolipids by visceral organs begins with the engulfment of the membranes of white cells and erythrocytes that are rich in lactosylceramide (Cer-Glc-Gal) and hematoside (Cer-Glc-Gal-NANA). In the brain, the majority of the cerebroside-type lipids are gangliosides. Particularly during the neonatal period, ganglioside turnover in the central nervous system is extensive so that glycosphingolipids are rapidly being broken down and resynthesized. The pathway of sphingolipid catabolism is summarized in Figure 10.59. Note that among the various sphingolipids that comprise this pathway, there occurs a sulfate ester (in sulfolipid or sulfogalactolipid); *N*-acetylneuraminic acid groups (in the gangliosides); an α-linked galactose residue (in ceramide trihexoside); several β-galactosides (in galactocerebroside and G_{M1}); the ganglioside G_{M2}, which terminates in a β-linked *N*-acetylgalactosamine unit; and glucocerebroside, which is composed of a single glucose residue attached to ceramide through a β linkage. The phosphodiester bond in sphingomyelin is broken to produce ceramide, which in turn is converted in sphingosine by the cleavage of an amide bond to a long-chain fatty acid. This overall pathway of sphingolipid catabolism is composed of a series of enzymes that cleave specific bonds in the compounds including α- and β-galactosidases, a β-glucosidase, a neuraminidase, hexosaminidase, a

sphingomyelin-specific phosphodiesterase (sphingomyelinase), a sulfate esterase (sulfatase), and a ceramide-specific amidase (ceramidase). The important features of the sphingolipid catabolic pathway are as follows: (1) all the reactions take place within the lysosome; that is, the enzymes of the pathway are contained in lysosomes; (2) the enzymes are hydrolases; therefore one of the substrates in each reaction is water; (3) the pH optimum of each of the hydrolases is in the acid range, pH 3.5–5.5; (4) most of the enzymes are relatively stable and occur as isoenzymes; for example, **hexosaminidase** occurs in two forms: hexosaminidase A (HexA) and hexosaminidase B (HexB); (5) the hydrolases that comprise the sphingolipid pathway are glycoproteins and often occur firmly bound to the lysosomal membrane; and (6) the pathway is composed of intermediates that differ by only one sugar molecule, a sulfate group, or a fatty acid residue. The substrates are converted to products by the sequential, stepwise removal of constituents such as sugars and sulfate, by hydrolytic, irreversible reactions.

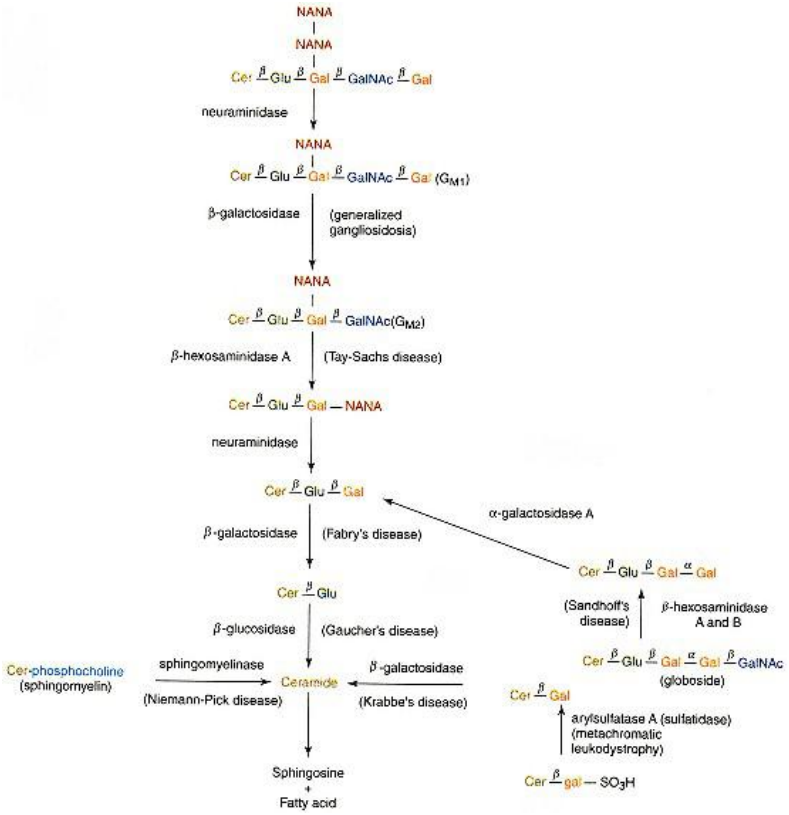


Figure 10.59
Summary of the pathways for catabolism of sphingolipids by lysosomal enzymes.
The genetically determined enzyme deficiency diseases are indicated in parentheses.

TABLE 10.2 Sphingolipid Storage Diseases of Humans

Disorder	*Principal Signs and Symptoms*	*Principal Storage Substance*	*Enzyme Deficiency*
1. Tay-Sachs disease	Mental retardation, blindness, cherry red spot on macula, death between second and third year	Ganglioside G_{M2}	Hexosaminidase A
2. Gaucher's disease	Liver and spleen enlargement, erosion of long bones and pelvis, mental retardation in infantile form only	Glucocerebroside	Glucocerebrosidase
3. Fabry's disease	Skin rash, kidney failure, pains in lower extremities	Ceramide trihexoside	-Galactosidase A
4. Niemann–Pick disease	Liver and spleen enlargement, mental retardation	Sphingomyelin	Sphingomyelinase
5. Globoid leukodystrophy (Krabbe's disease)	Mental retardation, absence of myelin	Galactocerebroside	Galactocerebrosidase
6. Metachromatic leukodystrophy	Mental retardation, nerves stain yellowish brown with cresyl violet dye (metachromasia)	Sulfatide	Arylsulfatase A
7. Generalized gangliosidosis	Mental retardation, liver enlargement, skeletal involvement	Ganglioside G_{M1}	G_{M1}ganglioside: β-galactosidase
8. Sandhoff–Jatzkewitz disease	Same as 1; disease has more rapidly progressing course	G_{M2} ganglioside, globoside	Hexosaminidase A and B
9. Fucosidosis	Cerebral degeneration, muscle spasticity, thick skin	Pentahexosylfucoglycolipid	-L-Fucosidase

In most cases, sphingolipid catabolism functions smoothly, and all of the various complex glycosphingolipids and sphingomyelin are degraded to the level of their basic building blocks, namely, sugars, sulfate, fatty acid, phosphocholine, and sphingosine. However, when the activity of one of the hydrolytic enzymes is markedly reduced due to a genetic error, then the substrate for the defective or missing enzyme accumulates and is deposited within the lysosomes of the tissue responsible for the catabolism of that sphingolipid. For most of the reactions in Figure 10.59, patients have been identified who lack the enzyme that normally catalyzes that reaction. These disorders, called **sphingolipidoses,** are summarized in Table 10.2.

We can generalize about some of the common features of **lipid storage diseases:** (1) usually only a single sphingolipid accumulates in the involved organs; (2) the ceramide portion is common to the various storage lipids; (3) the rate of biosynthesis of the accumulating lipid is normal; (4) a catabolic enzyme is missing in each of these disorders; and (5) the extent of the enzyme deficiency is the same in all tissues.

Diagnostic Enzyme Assays for Sphingolipidoses

Diagnosis of a given sphingolipidosis can be made from a biopsy of the involved organ, usually bone marrow, liver, or brain, on morphologic grounds on the basis of the highly characteristic appearance of the storage lipid within lysosomes. Assay of enzyme activity is used to confirm the diagnosis of a particular lipid storage disease. Of great practical value is the fact that, for most of the diseases, peripheral leukocytes, cultured skin fibroblasts, and chorionic villi express the relevant enzyme deficiency and can be used as a source of enzyme for diagnostic purposes. In some cases (e.g., Tay–Sachs disease) serum and even tears are a source of enzyme for the diagnosis of a lipid storage disorder. Sphingolipidoses, for the most part, are autosomal recessive, with the disease occurring only in homozygotes with a defect in both alleles. Enzyme assays can identify carriers or heterozygotes.

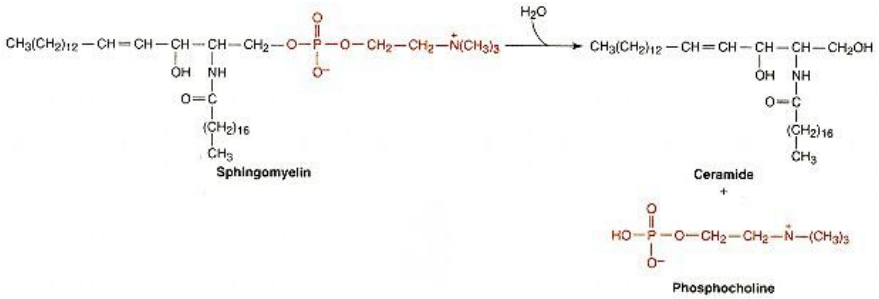


Figure 10.60
Sphingomyelinase reaction.

In **Niemann–Pick disease,** the deficient enzyme is **sphingomyelinase,** which normally catalyzes the reaction shown in Figure 10.60. Sphingomyelin, radiolabeled in the methyl groups of choline with carbon-14, provides a useful substrate for determining sphingomyelinase activity. Extracts of white blood cells from healthy, appropriate controls will hydrolyze the labeled substrate and produce the water-soluble product, phosphocholine. Extraction of the final incubation medium with an organic solvent such as chloroform will result in radioactivity in the upper, aqueous phase; the unused, lipid-like substrate sphingomyelin will be found in the chloroform phase. On the other hand, if the white blood cells were derived from a patient with Niemann–Pick disease, then after incubation with labeled substrate and extraction with chloroform, little or no radioactivity (i.e., phosphocholine) would be found in the aqueous phase and the diagnosis would be confirmed.

CLINICAL CORRELATION 10.4

Diagnosis of Gaucher's Disease in an Adult

Gaucher's disease is an inherited disease of lipid catabolism that results in deposition of glucocerebroside in macrophages of the reticuloendothelial system. Because of the large numbers of macrophages in spleen, bone marrow, and liver, hepatomegaly, splenomegaly and its sequelae (thrombocytopenia or anemia), and bone pain are the most common signs and symptoms of the disease.

Gaucher's disease results from a deficiency of glucocerebrosidase. Although this enzyme deficiency is inherited, different clinical patterns are observed. Some patients suffer severe neurologic deficits as infants, while others do not exhibit symptoms until adulthood. The diagnosis can be made by assaying leukocytes or fibroblasts for their ability to hydrolyze the β-glycosidic bond of artificial substrates (β-glucosidase activity) or of glucocerebroside (glucocerebrosidase activity). Gaucher's disease has been treated with regular infusions of purified glucocerebrosidase and the long-term efficacy of the therapy looks encouraging.

Brady, R. O., Kanfer, J. N., Bradley, R. M., and Shapiro, D. Demonstration of a deficiency of glucocerebroside-cleaving enzyme in Gaucher's disease. *J. Clin. Invest.* 45:1112, 1966.

Another disease that can be diagnosed by use of an artificial substrate is **Tay–Sachs disease,** the most common form of **G_{M2} gangliosidosis.** In this fatal disorder the ganglion cells of the cerebral cortex are swollen and the lysosomes are engorged with the acidic lipid, G_{M2} ganglioside. This results in a loss of ganglion cells, proliferation of glial cells, and demyelination of peripheral nerves. The pathognomonic finding is a cherry red spot on the macula caused by swelling and necrosis of ganglion cells in the eye. In Tay–Sachs disease, the commercially available artificial substrate 4-methylumbelliferyl-β-*N*-acetyl-glucosamine is used to confirm the diagnosis. The compound is hydrolyzed by hexosaminidase A, the deficient lysosomal hydrolase, to produce the intensely fluorescent product 4-methylumbelliferone (Figure 10.61). Unfortunately, the diagnosis may be confused by the presence of hexosaminidase B in tissue extracts and body fluids. This enzyme is not deficient in the Tay–Sachs patient and will hydrolyze the test substrate, thereby confusing the interpretation of results. The problem is usually resolved by taking advantage of the relative heat lability of hexosaminidase A and heat stability of hexosaminidase B. The tissue extract or serum specimen to be tested is first heated at 55°C for 1 h and then assayed for hexosaminidase activity. The amount of heat-labile activity is a measure of hexosaminidase A, and this value is used in making the diagnosis.

Enzyme assays of serum or extracts of tissues, peripheral leukocytes, and fibroblasts have proved useful in heterozygote detection. Once carriers of a lipid storage disease have been identified, or if there has been a previously affected child in a family, the pregnancies at risk for these diseases can be monitored. All nine of the lipid storage disorders are transmitted as recessive genetic abnormalities. In all but one the allele is carried on an autosomal chromosome. Fabry's disease is linked to the X chromosome. In all of these conditions statistically one of four fetuses will be homozygous (or hemizygous in Fabry's disease), two fetuses will be carriers, and one will be completely

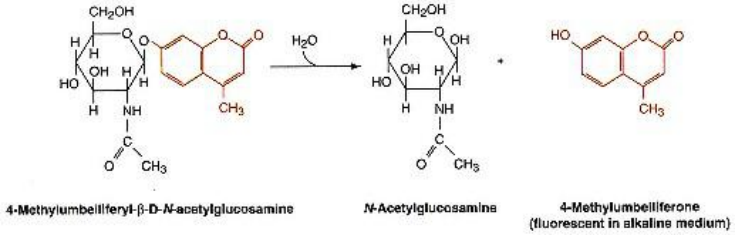


Figure 10.61
-Hexosaminidase reaction.

normal. The enzyme assays have been used to detect affected fetuses and carriers *in utero*, using cultured fibroblasts obtained by amniocentesis as a source of enzyme.

Except for Gaucher's disease, there is no therapy for the sphingolipidoses; the role of medicine at present is prevention through genetic counseling based on enzyme assays of the type discussed above. A discussion of the diagnosis and therapy of **Gaucher's disease** is presented in Clin. Corr. 10.4.

10.5— Prostaglandins and Thromboxanes

Prostaglandins and Thromboxanes Are Derivatives of Twenty-Carbon, Monocarboxylic Acids

In mammalian cells two major pathways of arachidonic acid metabolism produce important mediators of cellular and bodily functions: the **cyclooxygenase** and the **lipoxygenase pathways**. The substrate for both pathways is unesteri-

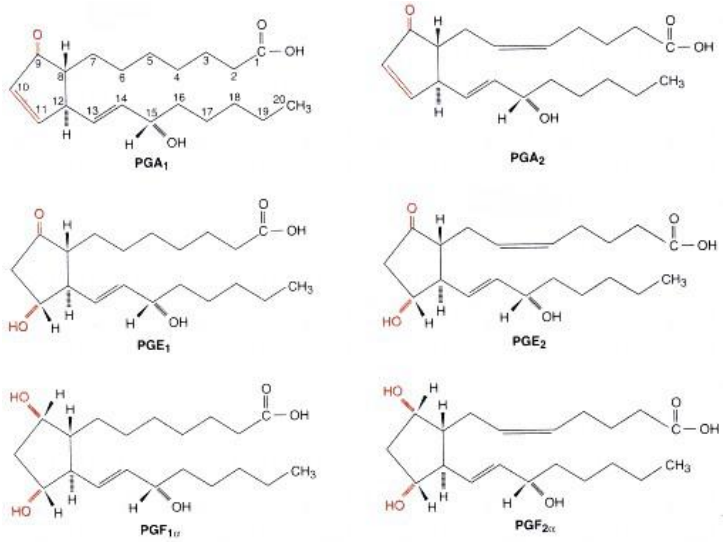


Figure 10.62
Structures of the major prostaglandins.

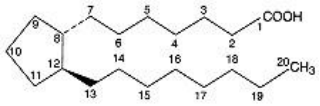


Figure 10.63
Structure of prostanoic acid.

fied arachidonic acid. The cyclooxygenase pathway leads to a series of compounds including prostaglandins and thromboxanes. Prostaglandins were discovered through their effects on smooth muscle, specifically their ability to promote the contraction of intestinal and uterine muscle and the lowering of blood pressure. Although the complexity of their structures and the diversity of their sometimes conflicting functions often create a sense of frustration, the potent pharmacological effects of the prostaglandins have afforded them an important place in human biology and medicine. With the exception of the red blood cell, the prostaglandins are produced and released by nearly all mammalian cells and tissues; they are not confined to specialized cells. Unlike most hormones, prostaglandins are not stored in cells but instead are synthesized and released immediately.

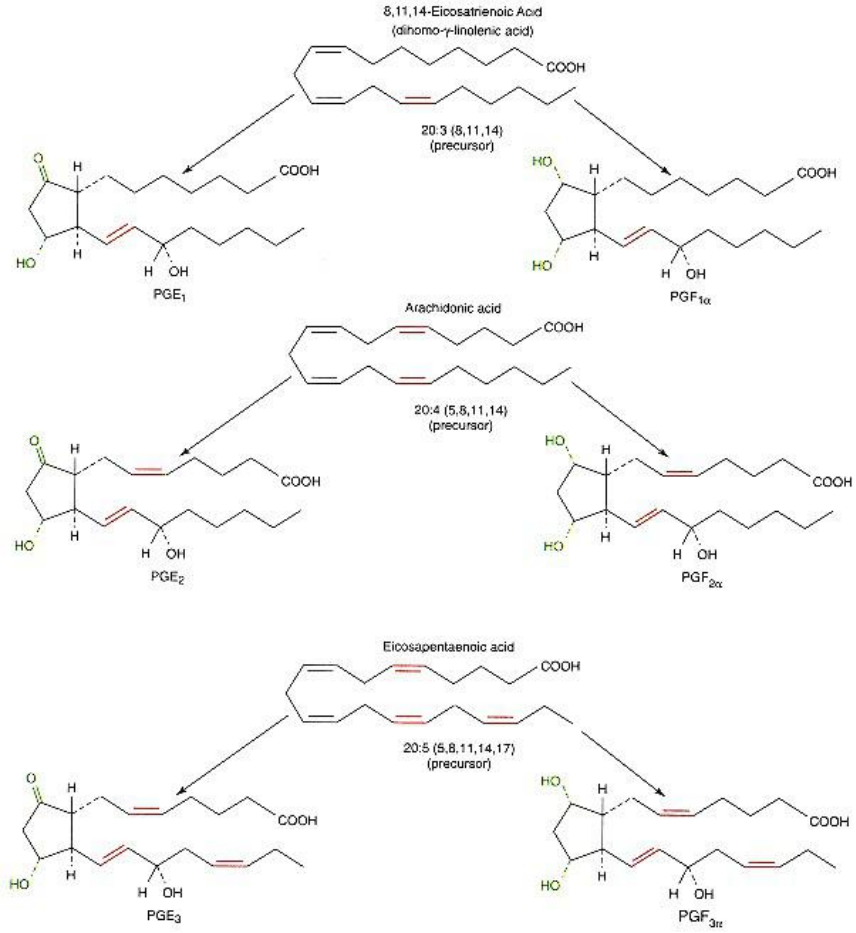


Figure 10.64
Synthesis of E and F prostaglandins from fatty acid precursors.

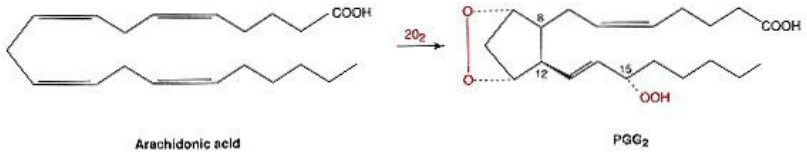


Figure 10.65
Cyclooxygenase reaction.

There are three major classes of primary **prostaglandins,** the **A, E,** and **F series.** The structures of the more common prostaglandins A, E, and F are shown in Figure 10.62 (p. 431). All are related to the hypothetical parent compound, prostanoic acid (Figure 10.63). Note that the prostaglandins contain a multiplicity of functional groups; for example, PGE_2 contains a carboxyl group, a β-hydroxyketone, a secondary alkylic alcohol, and two carbon–carbon double bonds. The three classes (A, E, and F) are distinguished on the basis of the functional groups about the cyclopentane ring (Figure 10.64): the E series contains a β-hydroxyketone, the F series are 1,3-diols, and those in the A series are $\alpha\beta$-unsaturated ketones. The subscript numerals 1, 2, and 3, refer to the number of double bonds in the side chains. The subscript α refers to the configuration of the C-9 OH group: an α-hydroxyl group projects "down" from the plane of the ring.

The most important dietary precursor of the prostaglandins is linoleic acid (18:2), which is an essential fatty acid. In adults linoleic acid is ingested daily in amounts of about 10 g. Only a very minor part of this total intake is converted by carbon chain elongation and desaturation in liver to arachidonic acid (eicosatetraenoic acid) and to some extent also to dihomo-γ-linolenic acid. Since the total daily excretion of prostaglandins and their metabolites is only about 1 mg, it is clear that the formation of prostaglandins is a quantitatively unimportant pathway in the overall metabolism of fatty acids. At the same time, however, the metabolism of prostaglandins is completely dependent on a regular and constant supply of linoleic acid. When the diet is deficient in linoleic acid, there is decreased production of prostaglandins. The diet also provides arachidonic acid.

Synthesis of Prostaglandins Involves a Cyclooxygenase

The immediate precursors to the prostaglandins are C_{20} polyunsaturated fatty acids containing 3, 4, and 5 carbon–carbon double bonds. Since **arachidonic acid** and most of its metabolites contain 20 carbon atoms, they are referred to as **eicosanoids.** During their transformation into various prostaglandins they are cyclized and take up oxygen. Dihomo-γ-linolenic acid (20:3(8,11,14)) is the precursor to PGE_1 and $PGF_{1\alpha}$; arachidonic acid (20:4(5,8,11,14)) is the precursor to PGE_2 and $PGF_{2\alpha}$; and eicosapentaenoic acid (20:4(5,8,11,14,17)) is the precursor to PGE_3 and $PGF_{3\alpha}$ (see Figure 10.64).

Compounds of the 2-series derived from arachidonic acid are the principal prostaglandins in humans and are of the greatest significance biologically. The

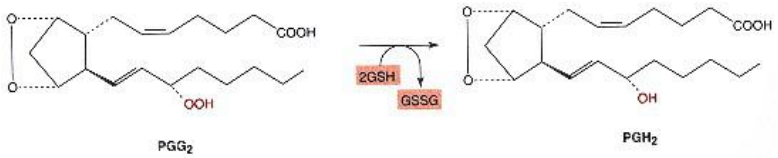


Figure 10.66
Conversion of PGG_2 to PGH_2; PG hydroperoxidase (PGH synthase) reaction.

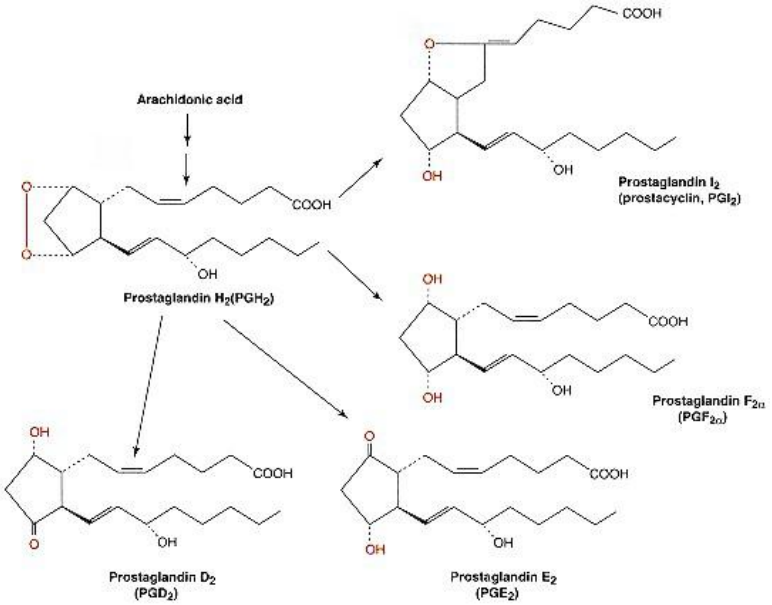


Figure 10.67
Major routes of prostaglandin biosynthesis.

central enzyme system in prostaglandin biosynthesis is the **prostaglandin synthase** (PGS) **complex,** which catalyzes oxidative cyclization of polyunsaturated fatty acids. Arachidonic acid is derived from membrane phospholipids by the action of the hydrolase **phospholipase A_2.** This cleavage step is the rate-limiting step in prostaglandin synthesis and some agents that stimulate prostaglandin production act by stimulating the activity of phospholipase A_2. Cholesterol esters containing arachidonic acid may also serve as a source of arachidonic acid substrate.

The **cyclooxygenase** component of the prostaglandin synthase complex catalyzes the cyclization of C-8–C-12 of arachidonic acid to form the cyclic 9,11-endoperoxide 15-hydroperoxide, PGG_2. The reaction requires two molecules of oxygen (Figure 10.65; see p. 433). PGG_2 is then converted to prostaglandin H_2 (PGH_2) by a reduced **glutathione** (GSH)-**dependent peroxidase** (PG hydroperoxidase) (Figure 10.66; see p. 433). Details of the additional steps leading to individual prostaglandins remain to be elucidated. Reactions that cyclize polyunsaturated fatty acids are found in the membranes of the endoplasmic reticulum. Major pathways of prostaglandin biosynthesis are summarized in Figure 10.67. Formation of primary prostaglandins of the D, E, and F series and of thromboxanes or prostacyclin (PGI_2) is mediated by different specific enzymes, whose presence varies depending on the cell type and tissue. This results in a degree of tissue specificity as to the type and quantity of prostaglandin produced. In kidney and spleen PGE_2 and $PGF_{2\alpha}$ are the major prostaglandins formed. In contrast, blood vessels produce mostly PGI_2 and $PGF_{2\alpha}$. In the heart PGE_2, $PGF_{2\alpha}$, and PGI_2 are formed in about equal amounts. Thromboxane A_2 (TXA_2) is the main prostaglandin endoperoxide formed in platelets.

There are two forms of cyclooxygenase (COX) or **prostaglandin synthase** (PGS). COX-1, or PGS-1, is a constitutive enzyme found in gastric mucosa,

platelets, vascular endothelium, and kidney. COX-2, or PGS-2, is inducible and is generated in response to inflammation. It is expressed mainly in activated macrophages and monocytes when they are stimulated by platelet-activating factor (PAF), interleukin-1, or bacterial lipopolysaccharide (LPS), and in smooth muscle cells, epithelial and endothelial cells, and neurons. PGS-2 induction is inhibited by glucocorticoids. The two forms of PGS catalyze both oxygenation of arachidonic acid to PGG_2 and the reduction of PGG_2 to PGH_2, which is the peroxidase reaction.

Prostaglandins have a very short half-life. Soon after release they are rapidly taken up by cells and inactivated either by oxidation of the 15-hydroxy group or by β-oxidation from the C_1-COOH end of the fatty acid chain. The lungs appear to play an important role in inactivating prostaglandins.

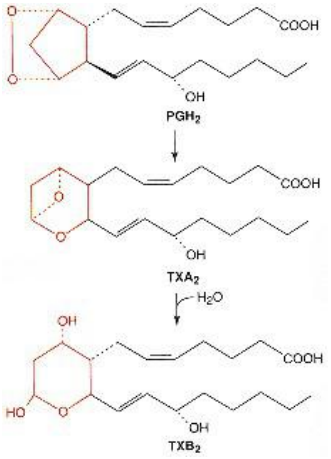


Figure 10.68
Synthesis of TXB_2 from PGH_2.

Thromboxanes are highly active metabolites of the PGG_2- and PGG_2-type prostaglandin endoperoxides that have the cyclopentane ring replaced by a six-membered oxygen-containing (oxane) ring. The term thromboxane is derived from the fact that these compounds have a thrombus-forming potential. **Thromboxane A synthase**, present in the endoplasmic reticulum, is abundant in lung and platelets and catalyzes conversion of endoperoxide PGH_2 to TXA_2. The half-life of TXA_2 is very short in water ($t_{1/2} \sim 1$ min) as the compound is transformed rapidly into inactive thromboxane B_2 (TXB_2) by the reaction shown in Figure 10.68.

Prostaglandin Production Is Inhibited by Steroidal and Nonsteroidal Anti-inflammatory Agents

Two types of drugs affect prostaglandin metabolism and are therapeutically useful. The **nonsteroidal, anti-inflammatory drugs (NSAIDs)**, such as aspirin (acetylsalicylic acid), indomethacin, and phenylbutazone, block prostaglandin production by inhibiting cyclooxygenase. In the case of aspirin, irreversible inhibition occurs by acetylation of the enzyme. Other NSAIDs inhibit cyclooxygenase but do so by binding noncovalently to the enzyme instead of acetylating it; they are called "non-aspirin NSAIDs." Certain NSAIDs inhibit COX-1 more than COX-2 and vice versa. These drugs are not without their undesirable side effects; aplastic anemia can result from phenylbutazone therapy. **Steroidal anti-inflammatory drugs** like hydrocortisone, prednisone, and betamethasone block prostaglandin release by inhibiting phospholipase A_2 activity so as to interfere with mobilization of arachidonic acid (see Figure 10.69). The rate-limiting step in the synthesis of prostaglandins is release of arachidonic acid from membrane phospholipid stores in response to phospholipase A_2 activation.

Factors that govern the biosynthesis of prostaglandins are poorly understood, but, in general, prostaglandin release seems to be triggered following hormonal or neural excitation or after muscular activity. For example, histamine stimulates an increase in the prostaglandin concentration in gastric perfusates. Also, prostaglandins are released during labor and after cellular injury (e.g., platelets exposed to thrombin, lungs irritated by dust).

Prostaglandins Exhibit Many Physiological Effects

Prostaglandins are natural mediators of **inflammation**. Inflammatory reactions most often involve the joints (rheumatoid arthritis), skin (psoriasis), and eyes, and inflammation of these sites is frequently treated with corticosteroids that inhibit prostaglandin synthesis. Administration of PGE_2 or PGE_1 induce the signs of inflammation that include redness and heat (due to arteriolar vasodilation) and swelling and edema resulting from increased capillary permeability. PGE_2 generated in immune tissues (e.g., macrophages, mast cells, B cells) evokes chemokinesis of T cells. PGE_2 in amounts that alone do not cause **pain**, prior to administration of the autocoids, histamine and bradykinin, enhance both the intensity and duration of pain caused by these two agents. It is thought that

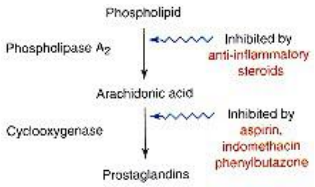


Figure 10.69
Site of action of inhibitors of prostaglandin synthesis.

pyrogens (**fever**-inducing agents) activate the prostaglandin biosynthetic pathway resulting in release of PGE_2 in the region of the hypothalamus where body temperature is regulated. Aspirin, which is an antipyretic drug, acts by inhibiting cyclooxygenase. The prostaglandins have been used extensively as drugs in **reproduction**. Both PGE_2 and PGF_2 have been used to induce parturition and for the termination of an unwanted pregnancy, specifically in the second trimester. There is also evidence that the PGE series of prostaglandins may play some role in infertility in males.

Synthetic prostaglandins have proved to be very effective in inhibiting **gastric acid secretion** in patients with **peptic ulcers**. The inhibitory effect of PGE compounds appears to be due to inhibition of cAMP formation in gastric mucosal cells. Prostaglandins also accelerate the healing of gastric ulcers. Prostaglandins play an important role in controlling blood vessel tone and arterial **blood pressure**. The vasodilator prostaglandins, PGE, PGA, and PGI_2, lower systemic arterial pressure, thereby increasing local blood flow and decreasing peripheral resistance. TXA_2 causes contraction of vascular smooth muscle and glomerular mesangium. There is hope that the prostaglandins may eventually prove useful in the treatment of hypertension. PGE_2 functions in the fetus to maintain the patency of the **ductus arteriosus** prior to birth. If the ductus remains open after birth, closure can be hastened by administration of the cyclooxygenase inhibitor indomethacin. In other situations it may be desirable to keep the ductus open. For example, in infants born with congenital abnormalities where the defect can be corrected surgically, infusion of prostaglandins will maintain blood flow through the ductus over this interim period.

Certain prostaglandins, especially PGI_2, inhibit **platelet aggregation**, whereas PGE_2 and TXA_2 promote this clotting process. TXA_2 is produced by platelets and accounts for the spontaneous aggregation that occurs when platelets contact some foreign surface, collagen, orthrombin. Endothelial cells lining blood vessels release PGI_2 and this may account for the lack of adherence of platelets to the healthy blood vessel wall. PGE_2 and PGD_2 dilate renal blood vessels and increase blood flow through the kidney. They also regulate sodium secretion and glomerular filtration rate.

10.6— Lipoxygenase and Oxy-Eicosatetraenoic Acids

Cyclooxygenase directs polyunsaturated fatty acids into the prostaglandin pathway. Another equally important arachidonic acid-oxygenating enzyme, called **lipoxygenase**, is a dioxygenase. Actually, there is a family of lipoxygenases that differ in the position of the double bond on the arachidonic acid molecule at which oxygen attack initially occurs (e.g., positions 5, 11, or 15). In humans the most important leukotrienes are the 5-lipoxygenase products that are involved in the mediation of inflammatory disorders.

Monohydroperoxyeicosatetraenoic Acids Are Products of Lipoxygenase Action

The products of the lipoxygenase reaction, which arise by addition of hydroperoxy groups to arachidonic acid, are designated **monohydroperoxyeicosatetraenoic acids** (HPETEs). Figure 10.70 shows the conversion of arachidonic acid to the three major HPETEs. Thus, in contrast to the cyclooxygenase of prostaglandin endoperoxide synthase, which catalyzes the bis-dioxygenation of unsaturated fatty acids to endoperoxides, lipoxygenases catalyze the monodioxygenation of unsaturated fatty acids to allylic hydroperoxides. Hydroperoxy substitution of arachidonic acid by lipoxygenases may occur at position 5, 12, or 15. 5-HPETE is the major lipoxygenase product in basophils, polymorphonuclear (PMN) leukocytes, macrophages, mast cells, and any organ undergoing

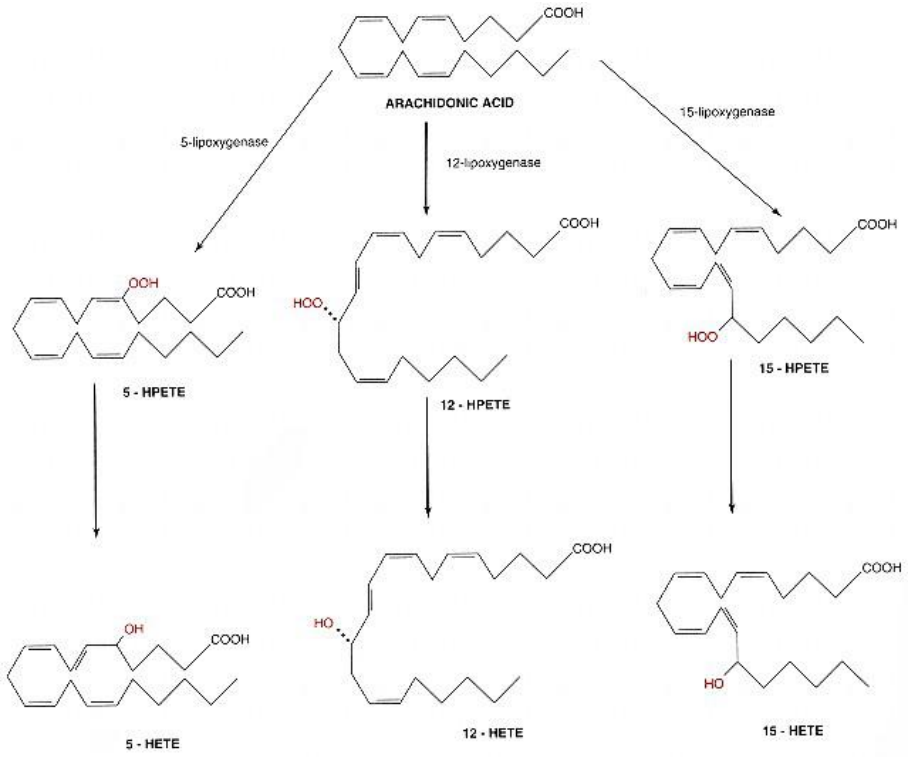


Figure 10.70
Lipoxygenase reaction and role of 5-hydroperoxyeicosatetraenoic acids (HPETEs) as precursors of hydroxyeicosatetraenoic acids (HETEs).

an inflammatory response; 12-HPETE predominates in platelets, pancreatic endocrine islet cells, vascular smooth muscle, and glomerular cells; 15-HPETE is the principal lipoxygenase product in reticulocytes, eosinophils, T-lymphocytes, and tracheal epithelial cells. The 5-, 12-, and 15-lipoxygenases occur mainly in the cytosol. Specific stimuli or signals determine which type of lipoxygenase product a given type of cell produces. The oxygenated carbon atom in HPETEs is asymmetric and there are two possible stereoisomers of the hydroperoxy acid, (*R*) or (*S*). All three major HPETEs are of the (*S*) configuration. 5-Lipoxygenase (5-LO) exhibits both a dioxygenase activity that converts arachidonic acid to 5-HPETE and a dehydrase activity that transforms 5-HPETE to LTA_4. 5-LO activity is restricted to a few cell types, including B lymphocytes but not T lymphocytes. It is activated by an accessory protein called 5-lipoxygenase activating protein.

Leukotrienes and Hydroxyeicosatetraenoic Acids Are Hormones Derived from HPETEs

HPETE-hydroperoxides are not hormones, but are highly reactive, unstable intermediates that are converted either to the analogous alcohol (hydroxy fatty

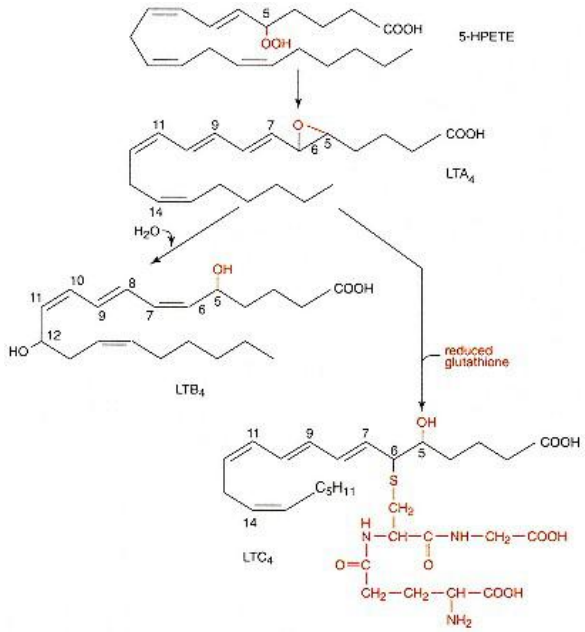


Figure 10.71
Conversion of 5-HPETE to LTB_4 and LTC_4 through LTA_4 as Intermediate.

acid) by reduction of the peroxide moiety or to leukotrienes. Leukotrienes are lipoxygenase products containing at least three conjugated double bonds. Figure 10.71 shows how 5-HPETE rearranges to the epoxide **leukotriene A_4** (LTA_4), which is then converted to LTB_4 or LTC_4, emphasizing that 5-HPETE occurs at an important branch point in the lipoxygenase pathway.

Peroxidative reduction of 5-HPETE to the stable **5-hydroxyeicosatetraenoic acid** (5-HETE) is illustrated in Figure 10.70. Note that the double bonds in 5-HETE occur at positions 6, 8, 11, and 14, and that they are unconjugated and that the geometry of the double bonds is trans, cis, cis, and cis, respectively. Two other common forms of HETE are 12- and 15-HETE. The HPETEs are reduced either spontaneously or by the action of peroxidases to the corresponding HETEs.

Leukotrienes are derived from the unstable precursor 5-HPETE by a reaction catalyzed by **LTA_4 synthase** that generates an epoxide called LTA_4. In the leukotriene series, the subscript indicates the number of double bonds. Thus, while double-bond rearrangement may occur, the number of double bonds in the leukotriene product is the same as in the original arachidonic acid. LTA_4 occurs at a branch point (Figure 10.71) and can be converted either to 5,12-dihydroxyeicosatetraenoic acid (designated leukotriene B_4 or LTB_4) or to LTC_4 and LTD_4.

Conversion of 5-HPETE to the diol LTB_4 (Figure 10.71) is catalyzed by a cytosolic enzyme, **LTB_4 synthase** (LTA_4 hydrolase), which adds water to the double bond between C-11 and C-12. The diversion of LTA_4 to leukotrienes LTC_4, LTD_4, and LTE_4 requires the participation of reduced glutathione that opens the epoxide ring in LTA_4 to produce LTC_4 (Figure 10.71). Sequential removal of glutamic acid and glycine residues by specific dipeptidases yields

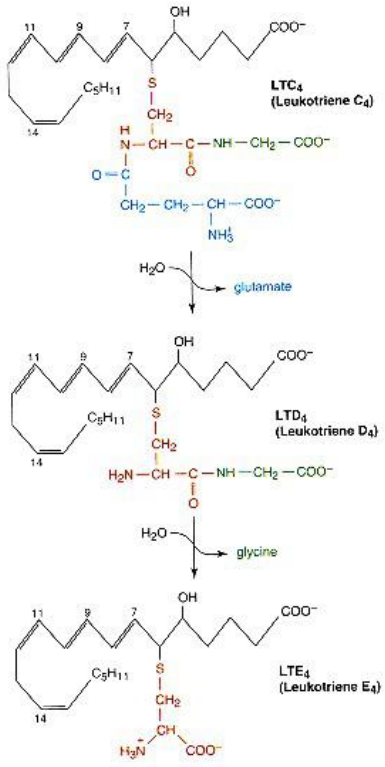


Figure 10.72
Conversion of LTC_4 to LTD_4 and LTE_4.

the leukotrienes LTD_4 and LTE_4 (Figure 10.72). The subscript 4 denotes the total number of double bonds.

Leukotrienes and HETEs Affect Several Physiological Processes

Leukotrienes persist for as long as 4 h in the body. Stepwise ω-oxidation of the methyl end and β-oxidation of the resulting COOH-terminated fatty acid chain are responsible for the inactivation and degradation of LTB_4 and LTE_4. These reactions occur in mitochondria and peroxisomes. The actions of the thionyl peptides LTC_4, LTD_4, and LTE_4 comprise the **slow-reacting substance of anaphylaxis** (SRS-A). They cause slowly evolving but protracted contraction of smooth muscles in the airways and gastrointestinal tract. LTC_4 is rapidly converted to LTD_4 and then slowly converted to LTE_4. These conversions are catalyzed by enzymes in plasma. LTB_4 and the sulfidopeptides LTC_4, LTD_4, and LTE_4 exert their biological actions through specific ligand–receptor interactions.

In general, HETEs (especially 5-HETE) and LTB_4 are involved mainly in regulating neutrophil and eosinophil function: they mediate chemotaxis, stimulate adenylate cyclase, and induce PMNs to degranulate and release lysosomal hydrolytic enzymes. In contrast, LTC_4 and LTD_4 are humoral agents that promote smooth muscle contraction, constriction of pulmonary airways, trachea, and

intestine, and increases in capillary permeability (edema). HETEs appear to exert their effects by being incorporated into the phospholipids of target cells. It is thought that the presence of fatty acyl chains containing a polar OH group disturbs the packing of lipids and thus the structure and function of the membrane. LTB_4 has immunosuppressive activity exerted through inhibition of $CD4^+$ cells and proliferation of suppressor $CD8^+$ cells. LTB_4 also promotes neutrophil–endothelial cell adhesion.

Monohydroxyeicosatetraenoic acids that comprise the lipoxygenase pathway are potent mediators of processes involved in allergy (hypersensitivity) and inflammation, secretion (e.g., insulin), cell movement, cell growth, and calcium fluxes. The initial allergic event, namely, the binding of IgE antibody to receptors on the surface of the mast cell, causes the release of substances, including leukotrienes, that are referred to as mediators of immediate hypersensitivity. Lipoxygenase products are usually produced within minutes after the stimulus. The leukotrienes LTC_4, LTD_4, and LTE_4 are much more potent than histamine in contracting nonvascular smooth muscles of bronchi and intestine. LTD_4 increases the permeability of the microvasculature. Mono-HETEs and LTB_4 stimulate migration (chemotaxis) of eosinophils and neutrophils, making them the principal mediators of PMN–leukocyte infiltration in inflammatory reactions.

Eicosatrienoic acids (e.g., dihomo-γ-linolenic acid) and **eicosapentaenoic acid** (Figure 10.64) also serve as lipoxygenase substrates. The content of these C20 fatty acids with three and five double bonds in tissues is less than that of arachidonic acid, but special diets can increase their levels. The lipoxygenase products of these tri- and pentaeicosanoids are usually less active than LTA_4 or LTB_4. It remains to be determined if fish oil diets rich in eicosapentaenoic acid are useful in the treatment of allergic and autoimmune diseases.

Pharmaceutical research into therapeutic uses of lipoxygenase and cyclo-oxygenase inhibitors and inhibitors and agonists of leukotrienes in treatment of inflammatory diseases such as asthma, psoriasis, rheumatoid arthritis, and ulcerative colitis is very active.

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Questions

C. N. Angstadt and J. Baggott

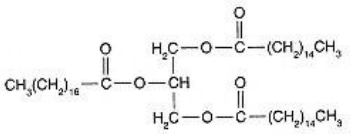
Refer to the following structures for Questions 1–3.

1. A plasmalogen.

2. A cardiolipin.

3. An acylglycerol that would likely be liquid at room temperature.

A



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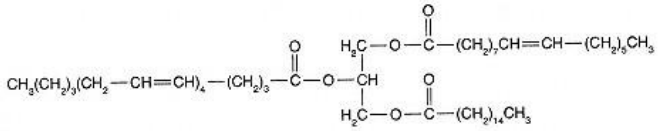

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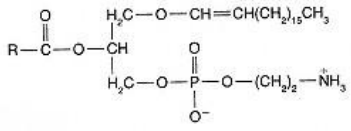

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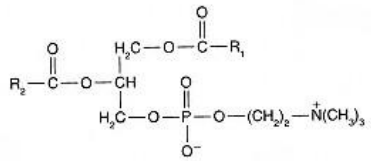
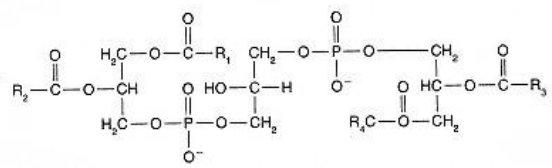


E



4. Roles of various phospholipids include all of the following EXCEPT:

A. cell–cell recognition.

B. a surfactant function in the lung.

C. activation of certain membrane enzymes.

D. signal transduction.

E. mediator of hypersensitivity and acute inflammatory reactions.

5. Which of the following represents a correct group of enzymes involved in phosphatidylcholine synthesis in adipose tissue?

A. choline phosphotransferase, glycerol kinase, phosphatidic acid phosphatase.

B. choline phosphotransferase, glycerol phosphate:acyltransferase, phosphatidylethanolamine *N*-methyltransferase

C. glycerol phosphate:acyltransferase, α-glycerol-phosphate dehydrogenase, phosphatidic acid phosphatase.

D. glycerol phosphate:acyltransferase, α-glycerol-phosphate dehydrogenase, glycerol kinase.

E. α-glycerol-phosphate dehydrogenase, glycerol kinase, phosphatidic acid phosphatase.

6. CDP-X (where X is the appropriate alcohol) reacts with 1,2-diacylglycerol in the primary synthetic pathway for:

A. phosphatidylcholine.

B. phosphatidylinositol.

C. phosphatidylserine.

D. all of the above.

E. none of the above.

7. Phospholipases A_1 and A_2:

A. have no role in phospholipid synthesis.

B. are responsible for the initial insertion of fatty acids in *sn*-1 and *sn*-2 positions during synthesis.

C. are responsible for base exchange in the interconversion of phosphatidylethanolamine and phosphatidylserine.

D. hydrolyze a phosphatidic acid to a diglyceride.

E. remove a fatty acid in an *sn*-1 or *sn*-2 position so it can be replaced by another in phospholipid synthesis.

8. In the biosynthesis of cholesterol:

A. 3-hydroxy-3-methyl glutaryl CoA (HMG CoA) is synthesized by mitochondrial HMG CoA synthase.

B. HMG CoA reductase catalyzes the rate-limiting step.

C. the conversion of mevalonic acid to farnesyl pyrophosphate proceeds via the condensation of three molecules of mevalonic acid.

D. the condensation of two farnesyl pyrophosphates to form squalene is a freely reversible reaction.

E. the conversion of squalene to lanosterol is initiated by formation of the fused ring system, followed by addition of oxygen.

9. The cholesterol present in LDL (low-density lipoprotein):

A. binds to a cell receptor and diffuses across the cell membrane.

B. when it enters a cell, suppresses the activity of ACAT (acyl CoA:cholesterol acyltransferase)

C. once in the cell is converted to cholesterol esters by LCAT (lecithin:cholesterol acyltransferase).

D. once it has accumulated in the cell, inhibits the replenishment of LDL receptors.

E. represents primarily cholesterol that is being removed from peripheral cells.

10. Primary bile acids.

A. are any bile acids that are found in the intestinal tract.

B. are any bile acids reabsorbed from the intestinal tract.

C. are synthesized in the intestinal tract by bacteria.

D. are synthesized in hepatocytes directly from cholesterol.

E. are converted to secondary bile acids by conjugation with glycine or taurine.

11. A ganglioside may contain all of the following EXCEPT:

A. a ceramide structure.

B. glucose or galactose.

C. phosphate.

D. one or more sialic acids.

E. sphingosine.

12. Sphingomyelins differ from the other sphingolipids in that they are:

A. not based on a ceramide core.

B. acidic rather than neutral at physiological pH.

C. the only types containing *N*-acetylneuraminic acid.

D. the only types that are phospholipids.

E. not amphipathic.

13. All of the following are true about the degradation of sphingolipids EXCEPT it:

A. occurs by hydrolytic enzymes contained in lysosomes.

B. terminates at the level of ceramides.

C. is a sequential, stepwise removal of constituents.

D. is inhibited in the types of diseases known as sphingolipidoses (lysosomal storage diseases).

E. is catalyzed by enzymes that are specific for a type of linkage rather than for a particular compound.

14. Structural features that are common to all prostaglandins include:

A. 20-carbon atoms.

B. an oxygen-containing internal heterocyclic ring.

C. a peroxide group at C-15.

D. two double bonds.

E. a ketone group.

15. The prostaglandin synthase complex:

A. catalyzes the rate-limiting step of prostaglandin synthesis.

B. is inhibited by anti-inflammatory steroids.

C. contains both a cyclooxygenase and a peroxidase component.

D. produces PGG_2 as the end product.

E. uses as substrate the pool of free arachidonic acid in the cell.

16. Thromboxane A_2:

A. is a long-lived prostaglandin.

B. is an inactive metabolite of PGE_2.

C. is the major prostaglandin produced in all cells.

D. does not contain a ring structure.

E. is synthesized from the intermediate PGH_2.

17. Hydroperoxy eicosatetraenoic acids (HPETEs):

A. are derived from arachidonic acid by a peroxidase reaction.

B. are mediators of hypersensitivity reactions.

C. are intermediates in the formation of leukotrienes.

D. are relatively stable compounds (persist for as long as 4 h).

E. are the inactivated forms of leukotrienes.

Answers

1. C Only one with an ether instead of an ester link at *sn*-1. D is a phosphatidylcholine (p. 397).

2. E Two phosphatidic acids connected by glycerol (p. 398).

3. B Note the two unsaturated fatty acids. A: With all saturated fatty acids, would likely be solid at room temperature.

4. A This function appears to be associated with complex glycosphingolipids (p. 427). B: Especially dipalmitoyllecithin (p. 398). C: For example, β-hydroxybutyrate dehydrogenase (p. 399). D: Especially the phosphatidylinositols (p. 400). E: Platelet activating factor (PAF) does this (p. 398).

5. C A, D, and E: Glycerol kinase is not present in adipose tissue, which must rely on the α-glycerol-phosphate dehydrogenase. This is a liver process only (p. 402).

6. A B: Phosphatidylinositol is formed from CDP-diglyceride reacting with *myo*-inositol (Figure 10.21, p. 406). C: This is formed by "base exchange" (Figure 10.20, p. 406).

7. E Phospholipases A_1 and A_2, as their names imply, hydrolyze a fatty acid from a phospholipid and so are part of phospholipid degradation. They are also important in synthesis, however, in assuring the asymmetric distribution of fatty acids that occurs in phospholipids (p. 406).

8. B A: Remember that cholesterol biosynthesis is cytosolic; mitochondrial biosynthesis of HMG CoA leads to ketone body formation. C: The rate-limiting step produces the isoprene pyrophosphates, which are the condensing units. D: Pyrophosphate is hydrolyzed, which prevents reversal. E: The process is initiated by epoxide formation (pp. 411–414).

9. D This is one of the ways to prevent overload in the cell. A: The LDL binds to the cell receptor and is endocytosed and then degraded in lysosome to release cholesterol. B: ACAT is activated to facilitate storage. C: LCAT is a plasma enzyme. E: The primary role of LDLs is to deliver cholesterol to peripheral tissues (pp. 415–417).

10. D The intestinal tract contains a mixture of primary and secondary bile acids, both of which can be reabsorbed. Secondary bile acids are formed by bacteria in the intestine by chemical reactions, such as the removal of the C-7 OH group (pp. 417 and 418).

11. C The glycosphingolipids do not contain phosphate. A and E: Ceramide, which is formed from sphingosine, is the base structure from which the glycosphingolipids are formed. D: By definition, gangliosides must contain sialic acid (p. 426).

12. D Sphingomyelins are not glycosphingolipids. They are formed from ceramides, are amphipathic, and are neutral. C is the definition of gangliosides (p. 421).

13. B Ceramides are hydrolyzed to sphingosine and the fatty acid. E: Many of the sphingolipids share the same types of bonds (e.g., a β-galactosidic bond), and one enzyme (e.g., β-galactosidase), will hydrolyze it whenever it occurs (p. 428, Figure 10.59).

14. A Prostaglandins are eicosanoids. B: This is true of thromboxanes but the prostaglandin ring contains only carbons. C: True only of the intermediate of synthesis, PGG_2. D: The number of double bonds is variable. E: True of the A and E series but not of the F series (Figures 10.64–10.68).

15. C A and B: The release of the precursor fatty acid by phospholipase A_2 is the rate-limiting step and the one inhibited by anti-inflammatory steroids. D: The peroxidase component converts the PGG_2 to PGH_2. E: Arachidonic acid is not free in the cell but is part of the membrane phospholipids (p. 433).

16. E TXA_2 is very active, has a very short half-life, contains a six-membered ring, and is the main prostaglandin in platelets but not all tissues (p. 435).

17. C A: The enzyme is a lipoxygenase. B–E: HPETEs themselves are not hormones but highly unstable intermediates that are converted to either HETEs (mediators of hypersensitivity) or leukotrienes (p. 436).