LIPIDS

Biological lipids are a chemically diverse group of compounds, the common and defining feature of which is their insolubility in water. The biological functions of the lipids are as diverse as their chemistry. Fats and oils are the principal stored forms of energy in many organisms. Phospholipids and sterols are major structural elements of biological membranes. Other lipids, although present in relatively small quantities, play crucial roles as enzyme cofactors, electron carriers, light-absorbing pigments, hydrophobic anchors for proteins, "chaperones" to help membrane proteins fold, emulsifying agents in the digestive tract, hormones, and intracellular messengers.

Storage Lipids

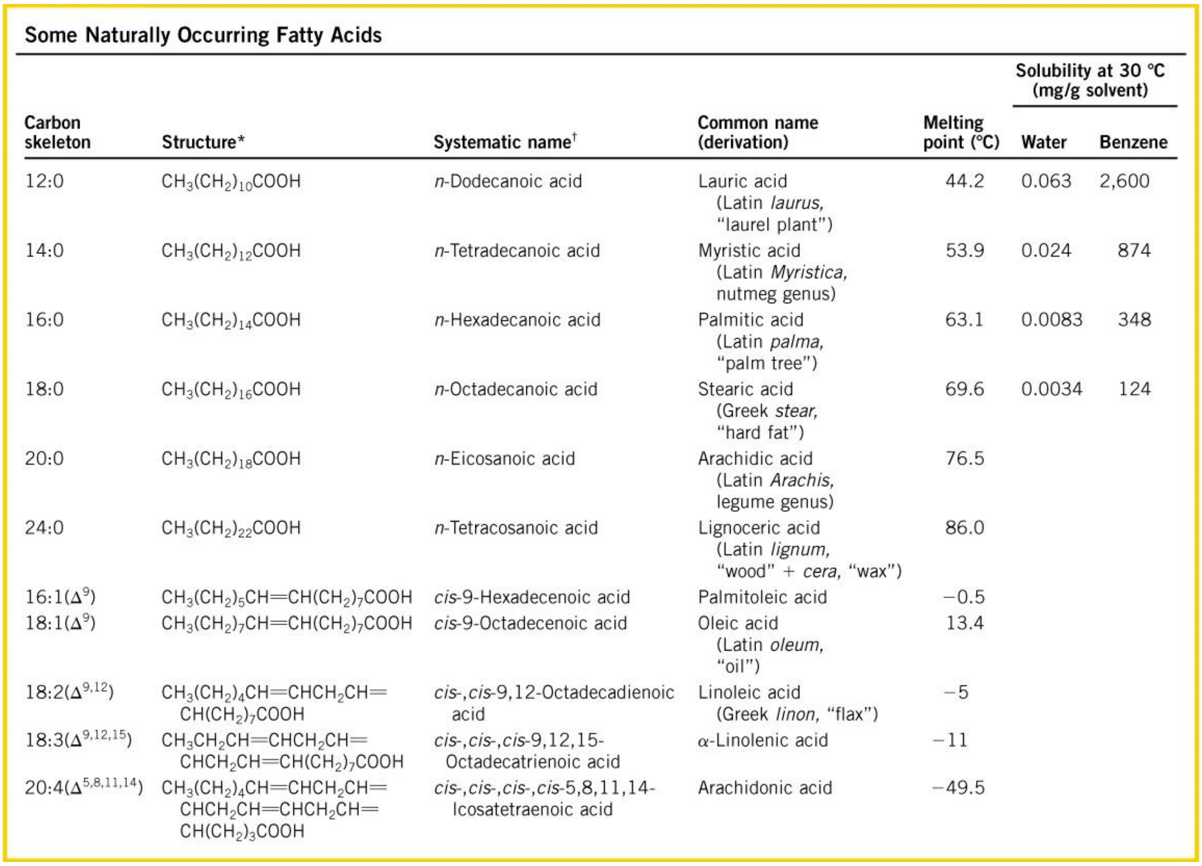
The fats and oils used almost universally as stored forms of energy in living organisms are derivatives of **fatty acids.**

Fatty Acids Are Hydrocarbon Derivatives

Fatty acids are carboxylic acids with hydrocarbon chains ranging from 4 to 36 carbons long (C4 to C36). In some fatty acids, this chain is unbranched and fully saturated (contains no double bonds); in others the chain contains one or more double bonds (Table 10–1). A few contain three-carbon rings, hydroxyl groups, or methyl group branches. A simplified nomenclature for these compounds specifies the chain length and number of double bonds, separated by a colon; for example, the 16-carbon saturated palmitic acid is abbreviated 16:0, and the 18-carbon oleic acid, with one double bond, is 18:1. The positions of any double bonds are specified by superscript numbers following Δ (delta); a 20-carbon fatty acid with one double bond between C-9 and C-10 (C-1 being the carboxyl carbon) and another between C-12 and C-13 is designated 20:2($\Delta^{9,12}$). The most commonly occurring fatty acids have even numbers of carbon atoms in an unbranched chain of 12 to 24 carbons (Table 10–1). The even number of carbons results from the mode of synthesis of these compounds, which involves condensation of two-carbon (acetate) units.

There is also a common pattern in the location of double bonds; in most monounsaturated fatty acids the double bond is between C-9 and C-10 (Δ^9), and the other double bonds of polyunsaturated fatty acids are generally Δ^{12} and Δ^{15}. (Arachidonic acid is an exception to this generalization.) The double bonds of polyunsaturated fatty acids are almost never conjugated (alternating single and double bonds, as in —CH=CH—CH=CH—), but are separated by a methylene group: —CH=CH—CH_2—CH=CH—. In nearly all naturally occurring unsaturated fatty acids, the double bonds are in the cis configuration. Trans fatty acids are produced by fermentation in the rumen of dairy animals and are obtained from dairy products and meat. They are also produced during hydrogenation of fish or vegetable oils. Because diets high in trans fatty acids correlate with increased blood levels of LDL (bad cholesterol) and decreased HDL (good cholesterol), it is generally recommended that one avoid large amounts of these fatty acids. Unfortunately, French fries, doughnuts, and cookies tend to be high in trans fatty acids.

TABLE 10-1

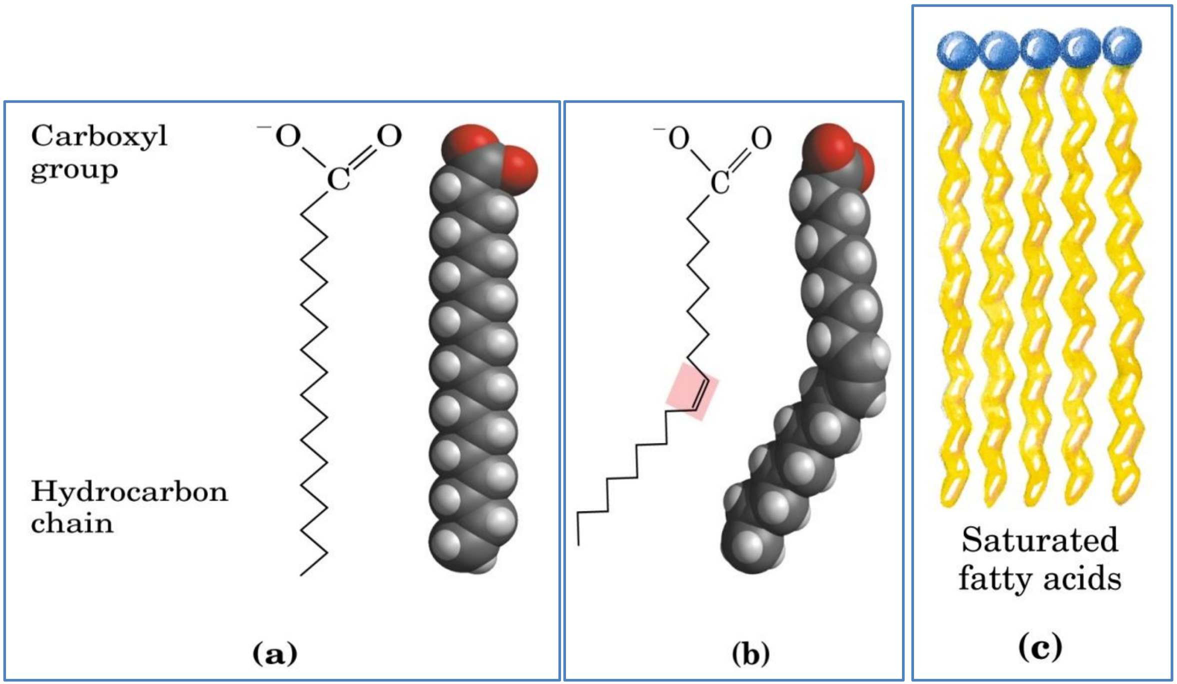


Some Naturally Occurring Fatty Acids

Carbon skeleton	Structure*	Systematic name†	Common name (derivation)	Melting point (°C)	Solubility at 30 °C (mg/g solvent)	
					Water	Benzene
12:0	$CH_3(CH_2)_{10}COOH$	*n*-Dodecanoic acid	Lauric acid (Latin *laurus*, "laurel plant")	44.2	0.063	2,600
14:0	$CH_3(CH_2)_{12}COOH$	*n*-Tetradecanoic acid	Myristic acid (Latin *Myristica*, nutmeg genus)	53.9	0.024	874
16:0	$CH_3(CH_2)_{14}COOH$	*n*-Hexadecanoic acid	Palmitic acid (Latin *palma*, "palm tree")	63.1	0.0083	348
18:0	$CH_3(CH_2)_{16}COOH$	*n*-Octadecanoic acid	Stearic acid (Greek *stear*, "hard fat")	69.6	0.0034	124
20:0	$CH_3(CH_2)_{18}COOH$	*n*-Eicosanoic acid	Arachidic acid (Latin *Arachis*, legume genus)	76.5		
24:0	$CH_3(CH_2)_{22}COOH$	*n*-Tetracosanoic acid	Lignoceric acid (Latin *lignum*, "wood" + *cera*, "wax")	86.0		
$16{:}1(\Delta^{9})$	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	*cis*-9-Hexadecenoic acid	Palmitoleic acid	−0.5		
$18{:}1(\Delta^{9})$	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	*cis*-9-Octadecenoic acid	Oleic acid (Latin *oleum*, "oil")	13.4		
$18{:}2(\Delta^{9,12})$	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	*cis*-,*cis*-9,12-Octadecadienoic acid	Linoleic acid (Greek *linon*, "flax")	−5		
$18{:}3(\Delta^{9,12,15})$	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	*cis*-,*cis*-,*cis*-9,12,15-Octadecatrienoic acid	α-Linolenic acid	−11		
$20{:}4(\Delta^{5,8,11,14})$	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOH$	*cis*-,*cis*-,*cis*-,*cis*-5,8,11,14-Icosatetraenoic acid	Arachidonic acid	−49.5		

The physical properties of the fatty acids, and of compounds that contain them, are largely determined by the length and degree of unsaturation of the hydrocarbon chain. The nonpolar hydrocarbon chain accounts for the poor solubility of fatty acids in water. The longer the fatty acyl chain and the fewer the double bonds, the lower is the solubility in water. The carboxylic acid group is polar (and ionized at neutral pH) and accounts for the slight solubility of short-chain fatty acids in water.

Melting points are also strongly influenced by the length and degree of unsaturation of the hydrocarbon chain. At room temperature (25 °C), the saturated fatty acids from 12:0 to 24:0 have a waxy consistency, whereas unsaturated fatty acids of these lengths are oily liquids. This difference in melting points is due to different degrees of packing of the fatty acid molecules (Fig. 10–1). In the fully saturated compounds, free rotation around each carbon–carbon bond gives the hydrocarbon chain great flexibility; the most stable conformation is the fully extended form, in which the steric hindrance of neighboring atoms is minimized. These molecules can pack together tightly in nearly crystalline arrays, with atoms all along their lengths in van der Waals contact with the atoms of neighboring molecules. In unsaturated fatty acids, a cis double bond forces a kink in the hydrocarbon chain. Fatty acids with one or several such kinks cannot pack together as tightly as fully saturated fatty acids, and their interactions with each other are therefore weaker. Because it takes less thermal energy to disorder these poorly ordered arrays of unsaturated fatty acids, they have markedly lower melting points than saturated fatty acids of the same chain length (Table 10–1).



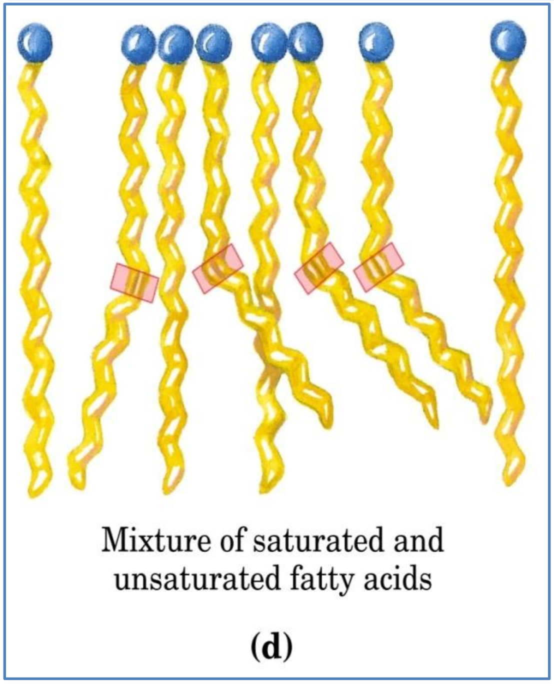


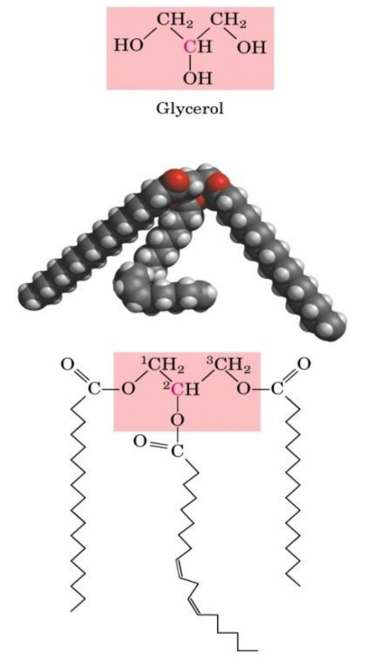
FIGURE 10–1 The packing of fatty acids into stable aggregates. The extent of packing depends on the degree of saturation. **(a)** Two representations of the fully saturated acid stearic acid (stearate at pH 7) in its usual extended conformation. Each line segment of the zigzag represents a single bond between adjacent carbons. **(b)** The cis double bond (shaded) in oleic acid (oleate) does not permit rotation and introduces a rigid bend in the hydrocarbon tail. All other bonds in the chain are free to rotate. **(c)** Fully saturated fatty acids in the extended form pack into nearly crystalline arrays, stabilized by many hydrophobic interactions. **(d)** The presence of one or more cis double bonds interferes with this tight packing and results in less stable aggregates.

In vertebrates, free fatty acids (unesterified fatty acids, with a free carboxylate group) circulate in the blood bound noncovalently to a protein carrier, serum albumin. However, fatty acids are present in blood plasma mostly as carboxylic acid derivatives such as esters or amides. Lacking the charged carboxylate group, these fatty acid derivatives are generally even less soluble in water than are the free fatty acids.

Triacylglycerols Are Fatty Acid Esters of Glycerol

The simplest lipids constructed from fatty acids are the **triacylglycerols,** also referred to as triglycerides, fats, or neutral fats. Triacylglycerols are composed of three fatty acids each in ester linkage with a single glycerol (Fig. 10–2). Those containing the same kind of fatty acid in all three positions are called simple triacylglycerols and are named after the fatty acid they contain. Simple triacylglycerols of 16:0, 18:0, and 18:1, for example, are tripalmitin, tristearin, and triolein, respectively. Most naturally occurring triacylglycerols are mixed; they contain two or more different fatty acids. To name these compounds unambiguously, the name and position of each fatty acid must be specified.

FIGURE 10–2 Glycerol and a triacylglycerol. The mixed triacylglycerol shown here has three different fatty acids attached to the glycerol backbone. When glycerol has two different fatty acids at C-1 and C-3, the C-2 is a chiral center



1-Stearoyl, 2-linoleoyl, 3-palmitoyl glycerol, a mixed triacylglycerol

Because the polar hydroxyls of glycerol and the polar carboxylates of the fatty acids are bound in ester linkages, triacylglycerols are nonpolar, hydrophobic molecules, essentially insoluble in water. Lipids have lower specific gravities than water, which explains why mixtures of oil and water have two phases: oil, with the lower specific gravity, floats on the aqueous phase.

Triacylglycerols Provide Stored Energy and Insulation

In most eukaryotic cells, triacylglycerols form a separate phase of microscopic, oily droplets in the aqueous cytosol, serving as depots of metabolic fuel. In vertebrates, specialized cells called adipocytes, or fat cells, store large amounts of triacylglycerols as fat droplets that nearly fill the cell. Triacylglycerols are also stored as oils in the seeds of many types of plants, providing energy and biosynthetic precursors during seed germination. Adipocytes and germinating seeds contain **lipases,** enzymes that catalyze the hydrolysis of stored triacylglycerols, releasing fatty acids for export to sites where they are required as fuel.

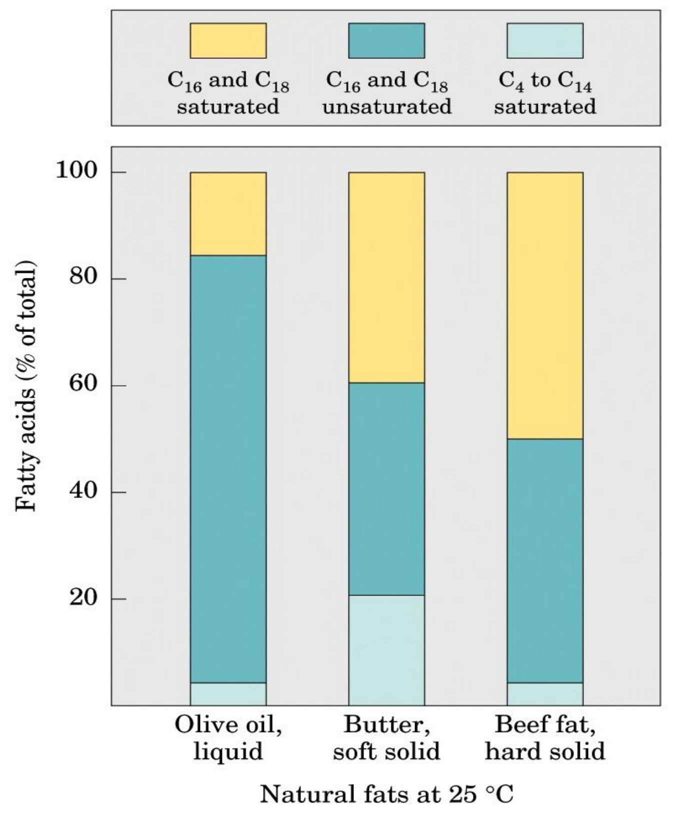
There are two significant advantages to using triacylglycerols as stored fuels, rather than polysaccharides such as glycogen and starch. First, because the carbon atoms of fatty acids are more reduced than those of sugars, oxidation of triacylglycerols yields more than twice as much energy, gram for gram, as the oxidation of carbohydrates. Second, because triacylglycerols are hydrophobic and therefore unhydrated, the organism that carries fat as fuel does not have to carry the extra weight of water of hydration that is associated with stored polysaccharides (2 g per gram of polysaccharide). Humans have fat tissue (composed primarily of adipocytes) under the skin, in the abdominal cavity, and in the mammary glands. Moderately obese people with 15 to 20 kg of triacylglycerols deposited in their adipocytes could meet their energy needs for months by drawing on their fat stores. In contrast, the human body can store less than a day's energy supply in the form of glycogen. Carbohydrates such as glucose and glycogen do offer certain advantages as quick sources of metabolic energy, one of which is their ready solubility in water.

In some animals, triacylglycerols stored under the skin serve not only as energy stores but as insulation against low temperatures. Seals, penguins, and other warm-blooded polar animals are amply padded with triacylglycerols. In hibernating animals (bears, for example), the huge fat reserves accumulated before hibernation serve the dual purposes of insulation and energy storage. The low density of triacylglycerols is the basis for another remarkable function of these compounds. In sperm whales, a store of triacylglycerols and waxes allows the animals to match the buoyancy of their bodies to that of their surroundings during deep dives in cold water.

Many Foods Contain Triacylglycerols

Most natural fats, such as those in vegetable oils, dairy products, and animal fat, are complex mixtures of simple and mixed triacylglycerols. These contain a variety of fatty acids differing in chain length and degree of saturation (Fig. 10–4). Vegetable oils such as corn (maize) and olive oil are composed largely of triacylglycerols with unsaturated fatty acids and thus are liquids at room temperature. They are converted industrially into solid fats by catalytic hydrogenation, which reduces some of their double bonds to single bonds and converts others to trans double bonds. Triacylglycerols containing only saturated fatty acids, such as tristearin, the major component of beef fat, are white, greasy solids at room temperature.

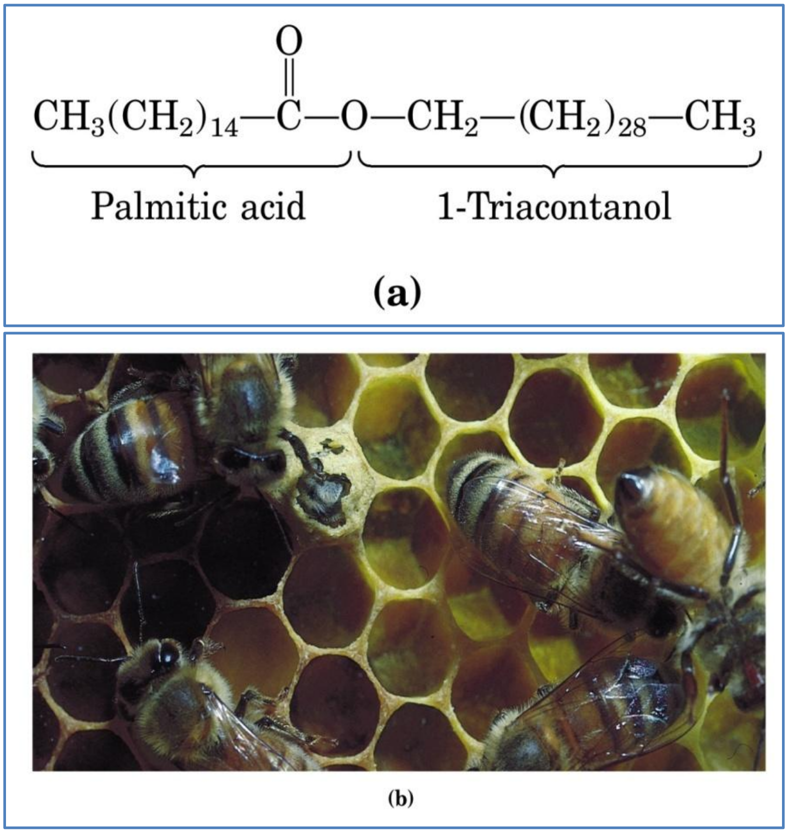
FIGURE 10–4 Fatty acid composition of three food fats. Olive oil, butter, and beef fat consist of mixtures of triacylglycerols, differing in their fatty acid composition. The melting points of these fats—and hence their physical state at room temperature (25 °C)—are a direct function of their fatty acid composition. Olive oil has a high proportion of long-chain (C16 and C18) unsaturated fatty acids, which accounts for its liquid state at 25 °C. The higher proportion of long-chain (C16 and C18) saturated fatty acids in butter increases its melting point, so butter is a soft solid at room temperature. Beef fat, with an even higher proportion of long-chain saturated fatty acids, is a hard solid.



When lipid-rich foods are exposed too long to the oxygen in air, they may spoil and become rancid. The unpleasant taste and smell associated with rancidity result from the oxidative cleavage of the double bonds in unsaturated fatty acids, which produces aldehydes and carboxylic acids of shorter chain length and therefore higher volatility.

Waxes Serve as Energy Stores and Water Repellents

Biological waxes are esters of long-chain (C14 to C36) saturated and unsaturated fatty acids with long-chain (C16 to C30) alcohols (Fig. 10–5). Their melting points (60 to 100 °C) are generally higher than those of triacylglycerols. In plankton, waxes are the chief storage form of metabolic fuel.



$$\underbrace{CH_3(CH_2)_{14}-\overset{O}{\overset{\|}{C}}}_{\text{Palmitic acid}}-\underbrace{O-CH_2-(CH_2)_{28}-CH_3}_{\text{1-Triacontanol}}$$

FIGURE 10–5 Biological wax. (a) Triacontanoylpalmitate, the major component of beeswax, is an ester of palmitic acid with the alcohol triacontanol. **(b)** A honeycomb, constructed of beeswax, is firm at 25 °C and completely impermeable to water.

Waxes also serve a diversity of other functions related to their water-repellent properties and their firm consistency. Certain skin glands of vertebrates secrete waxes to protect hair and skin and keep it flexible, lubricated, and waterproof. Birds, particularly waterfowl, secrete waxes from their glands to keep their feathers water-repellent. The shiny leaves of many tropical plants are coated with a thick layer of waxes, which prevents excessive evaporation of water and protects against parasites.

Structural Lipids in Membranes

The central architectural feature of biological membranes is a double layer of lipids, which acts as a barrier to the passage of polar molecules and ions. Membrane lipids are amphipathic: one end of the molecule is hydrophobic, the other hydrophilic. Their hydrophobic interactions with each other and their hydrophilic interactions with water direct their packing into sheets called membrane bilayers. Membrane lipids include glycerophospholipids, in which the hydrophobic regions are composed of two fatty acids joined to glycerol; galactolipids and sulfolipids, which also contain two fatty acids esterified to glycerol, but lack the characteristic phosphate of phospholipids; sphingolipids, in which a single fatty acid is joined to a fatty amine, sphingosine; and sterols, compounds characterized by a rigid system of four fused hydrocarbon rings.

The hydrophilic moieties in these amphipathic compounds may be as simple as a single —OH group at one end of the sterol ring system, or they may be much more complex. In glycerophospholipids and some sphingolipids, a polar head group is joined to the hydrophobic moiety by a phosphodiester linkage; these are the **phospholipids.** Other sphingolipids lack phosphate but have a simple sugar or complex oligosaccharide at their polar ends; these are the **glycolipids** (Fig. 10–6). Within these groups of membrane lipids, enormous diversity results from various combinations of fatty acid "tails" and polar "heads."

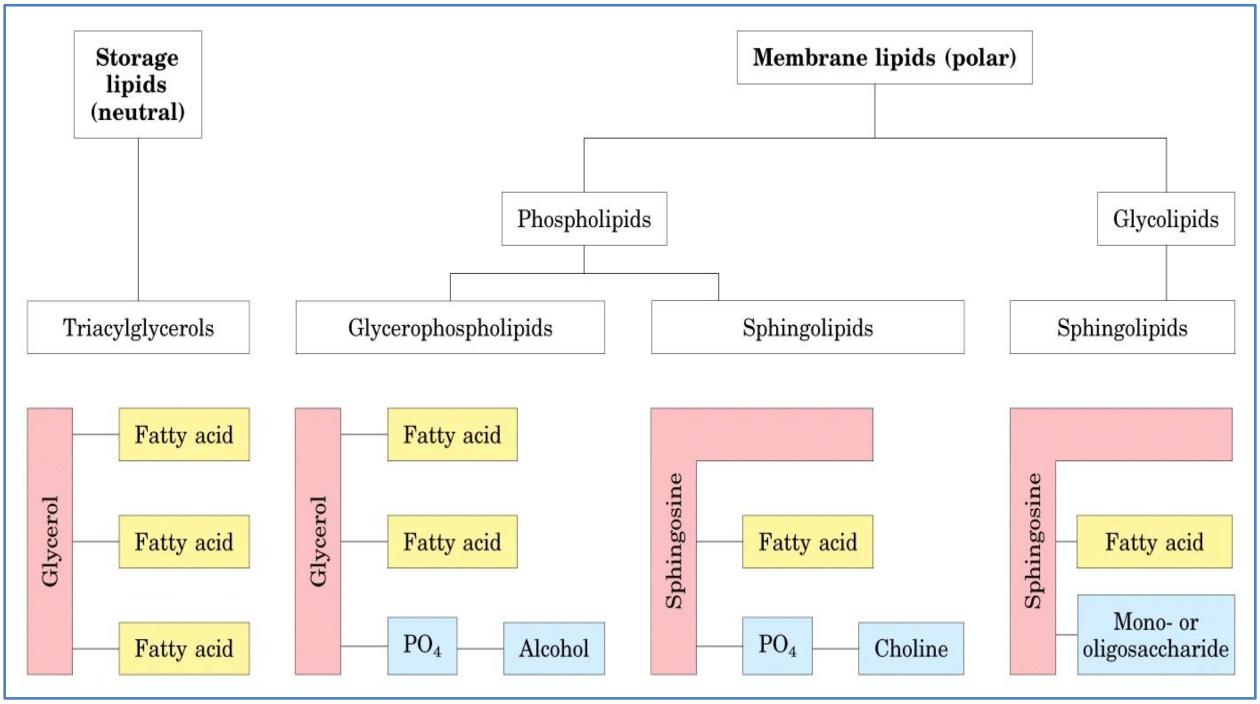
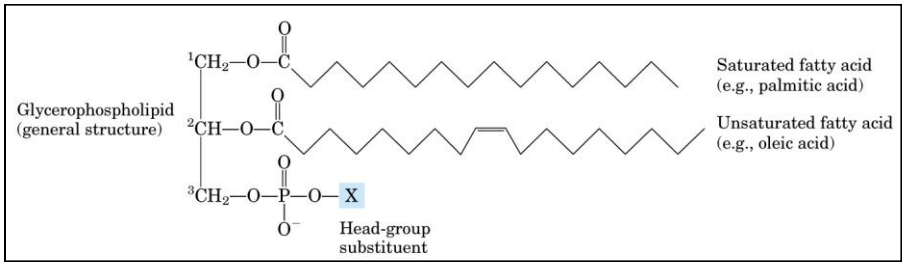


FIGURE 10–6 Some common types of storage and membrane lipids. All the lipid types shown here have either glycerol or sphingosine as the backbone (pink screen), to which are attached one or more long chain alkyl groups (yellow) and a polar head group (blue). In triacylglycerols, glycerophospholipids, galactolipids, and sulfolipids, the alkyl groups are fatty acids in ester linkage. Sphingolipids contain a single fatty acid, in amide linkage to the sphingosine backbone. In phospholipids the polar head group is joined through a phosphodiester, whereas glycolipids have a direct glycosidic linkage between the head-group sugar and the backbone glycerol.

Glycerophospholipids Are Derivatives of Phosphatidic Acid

Glycerophospholipids, also called phosphoglycerides, are membrane lipids in which two fatty acids are attached in ester linkage to the first and second carbons of glycerol, and a highly polar or charged group is attached through a phosphodiester linkage to the third carbon. Glycerophospholipids are named as derivatives of the parent compound, phosphatidic acid (Fig. 10–8), according to the polar alcohol in the head group. Phosphatidylcholine and phosphatidylethanolamine have choline and ethanolamine in their polar head groups, for example. In all these compounds, the head group is joined to glycerol through a phosphodiester bond, in which the phosphate group bears a negative charge at neutral pH. The polar alcohol may be negatively charged (as in phosphatidylinositol 4,5-bisphosphate), neutral (phosphatidylserine), or positively charged (phosphatidylcholine, phosphatidylethanolamine). These charges contribute greatly to the surface properties of membranes.



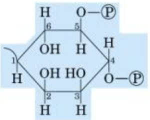
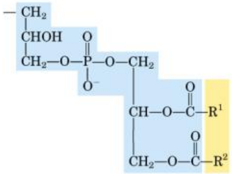
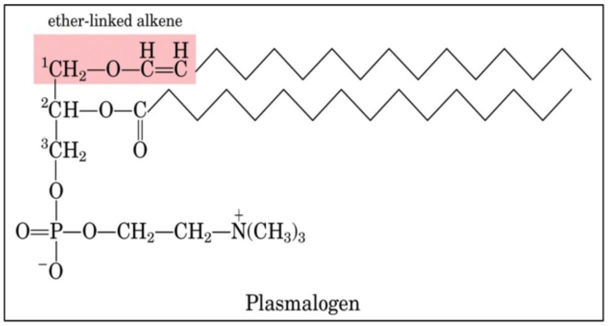
Name of glycerophospholipid	Name of X	Formula of X	Net charge (at pH 7)
Phosphatidic acid	—	$-H$	−1
Phosphatidylethanolamine	Ethanolamine	$-CH_2-CH_2-\overset{+}{N}H_3$	0
Phosphatidylcholine	Choline	$-CH_2-CH_2-\overset{+}{N}(CH_3)_3$	0
Phosphatidylserine	Serine	$-CH_2-\underset{COO^-}{CH}-\overset{+}{N}H_3$	−1
Phosphatidylglycerol	Glycerol	$-CH_2-\underset{OH}{CH}-CH_2-OH$	−1
Phosphatidylinositol 4,5-bisphosphate	*myo*-Inositol 4,5-bisphosphate		−4
Cardiolipin	Phosphatidyl-glycerol		−2

FIGURE 10–8 Glycerophospholipids. The common glycerophospholipids are diacylglycerols linked to head-group alcohols through a phosphodiester bond. Phosphatidic acid, a phosphomonoester, is the parent compound. Each derivative is named for the head-group alcohol (X), with the prefix "phosphatidyl-." In cardiolipin, two phosphatidic acids share a single glycerol.

The fatty acids in glycerophospholipids can be any of a wide variety, so a given phospholipid (phosphatidylcholine, for example) may consist of a number of molecular species, each with its unique complement of fatty acids. The distribution of molecular species is specific for different organisms, different tissues of the same organism, and different glycerophospholipids in the same cell or tissue. In general, glycerophospholipids contain a C16 or C18 saturated fatty acid at C-1 and a C18 to C20 unsaturated fatty acid at C-2. With few exceptions, the biological significance of the variation in fatty acids and head groups is not yet understood.

Some Phospholipids Have Ether-Linked Fatty Acids

Some animal tissues and some unicellular organisms are rich in **ether lipids,** in which one of the two acyl chains is attached to glycerol in ether, rather than ester, linkage. The ether-linked chain may be saturated, as in the alkyl ether lipids, or may contain a double bond between C-1 and C-2, as in **plasmalogens** (Fig. 10–9). Vertebrate heart tissue is uniquely enriched in ether lipids; about half of the heart phospholipids are plasmalogens. The membranes of halophilic bacteria, ciliated protists, and certain invertebrates also contain high proportions of ether lipids. The functional significance of ether lipids in these membranes is unknown; perhaps their resistance to the phospholipases that cleave ester-linked fatty acids from membrane lipids is important in some roles. At least one ether lipid, **platelet-activating factor,** is a potent molecular signal. It is released from leukocytes called basophils and stimulates platelet aggregation and the release of serotonin (a vasoconstrictor) from platelets. It also exerts a variety of effects on liver, smooth muscle, heart, uterine, and lung tissues and plays an important role in inflammation and the allergic response.



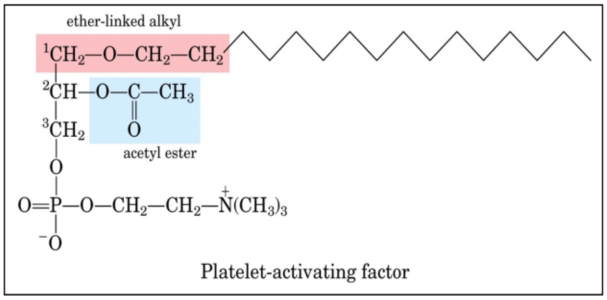
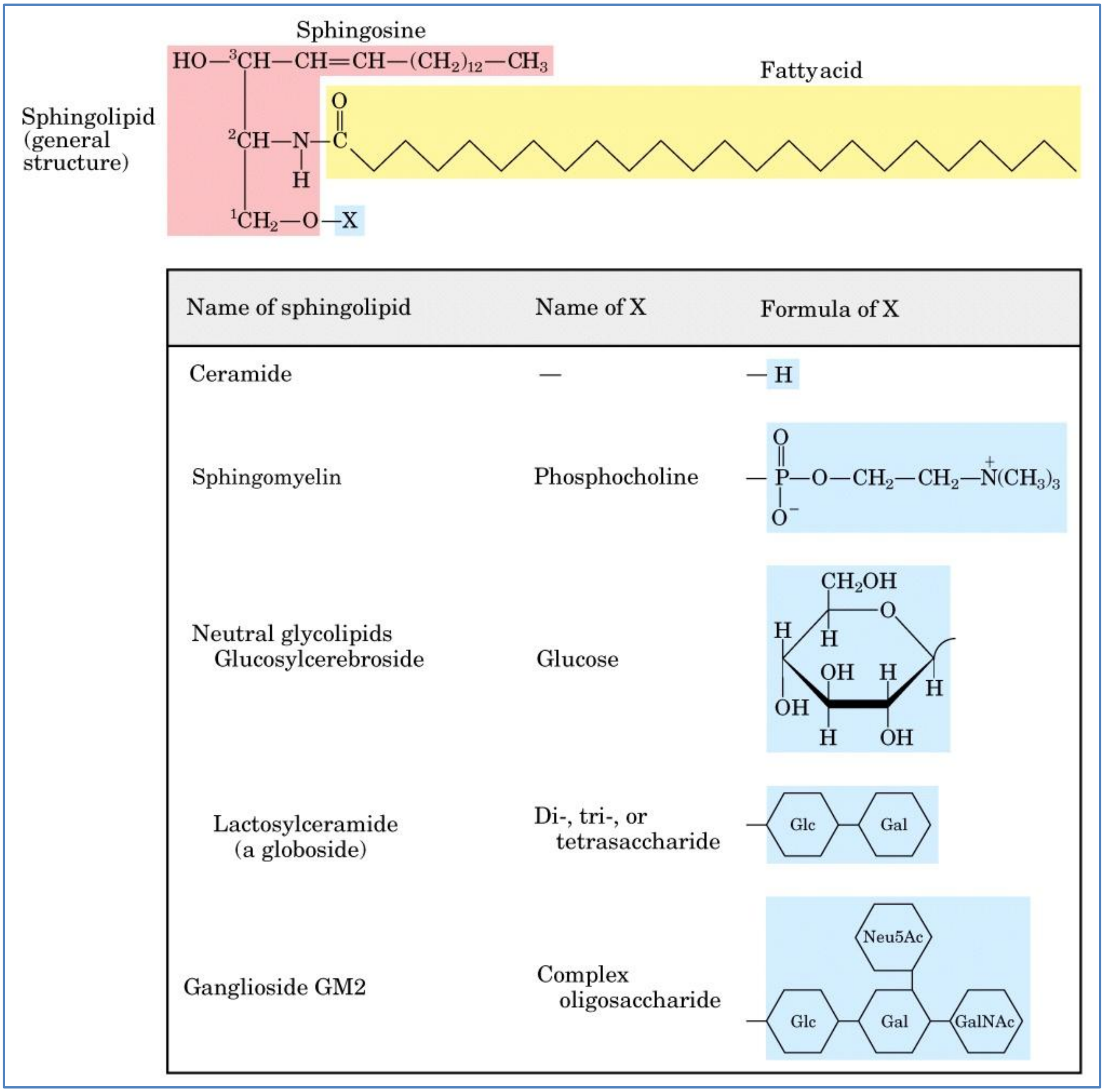


FIGURE 10–9 Ether lipids. Plasmalogens have an ether-linked alkenyl chain where most glycerophospholipids have an ester-linked fatty acid (compare Fig. 10–8). Platelet-activating factor has a long ether-linked alkyl chain at C-1 of glycerol, but C-2 is ester-linked to acetic acid, which makes the compound much more water-soluble than most glycerophospholipids and plasmalogens. The head-group alcohol is choline in plasmalogens and in platelet-activating factor.

Sphingolipids Are Derivatives of Sphingosine

Sphingolipids, the fourth large class of membrane lipids, also have a polar head group and two nonpolar tails, but unlike glycerophospholipids and galactolipids they contain no glycerol. Sphingolipids are composed of one molecule of the long-chain amino alcohol sphingosine or one of its derivatives, one molecule of a long-chain fatty acid, and a polar head group that is joined by a glycosidic linkage in some cases and by a phosphodiester in others (Fig. 10–12).

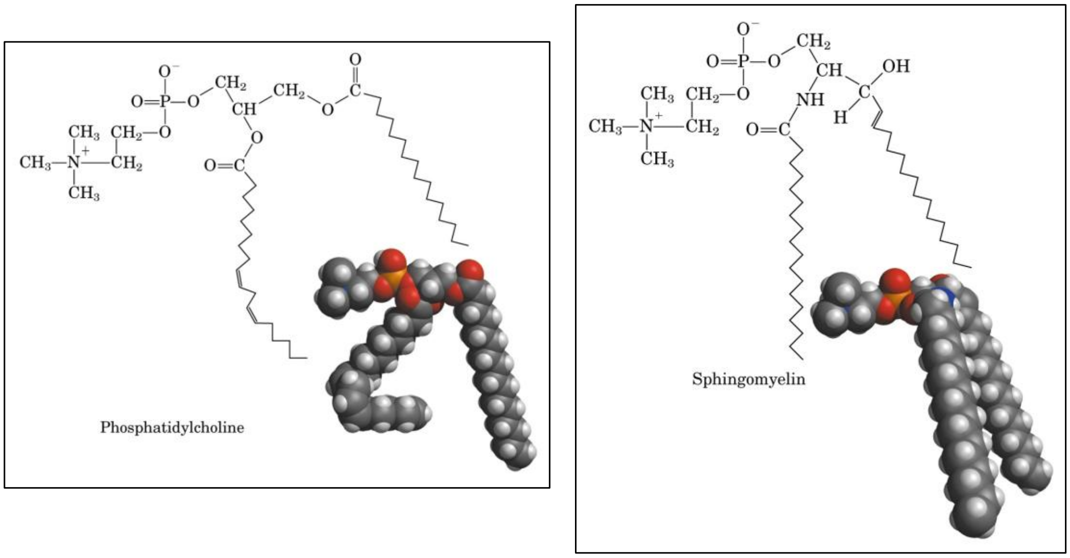
Carbons C-1, C-2, and C-3 of the sphingosine molecule are structurally analogous to the three carbons of glycerol in glycerophospholipids. When a fatty acid is attached in amide linkage to the —NH_2 on C-2, the resulting compound is a **ceramide,** which is structurally similar to a diacylglycerol. Ceramide is the structural parent of all sphingolipids.



Name of sphingolipid	Name of X	Formula of X
Ceramide	—	—H
Sphingomyelin	Phosphocholine	$-\overset{O}{\underset{O^-}{P}}-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3$
Neutral glycolipids Glucosylcerebroside	Glucose	
Lactosylceramide (a globoside)	Di-, tri-, or tetrasaccharide	—Glc—Gal
Ganglioside GM2	Complex oligosaccharide	—Glc—Gal(—Neu5Ac)—GalNAc

FIGURE 10–12 Sphingolipids. The first three carbons at the polar end of sphingosine are analogous to the three carbons of glycerol in glycerophospholipids. The amino group at C-2 bears a fatty acid in amide linkage. The fatty acid is usually saturated or monounsaturated, with 16, 18, 22, or 24 carbon atoms. Ceramide is the parent compound for this group. Other sphingolipids differ in the polar head group (X) attached at C-1. Gangliosides have very complex oligosaccharide head groups. Standard abbreviations for sugars are used in this figure: Glc, D-glucose; Gal, D-galactose; GalNAc, *N*-acetyl-D-galactosamine; Neu5Ac, *N*-acetylneuraminic acid (sialic acid).

There are three subclasses of sphingolipids, all derivatives of ceramide but differing in their head groups: sphingomyelins, neutral (uncharged) glycolipids, and gangliosides. **Sphingomyelins** contain phosphocholine or phosphoethanolamine as their polar head group and are therefore classified along with glycerophospholipids as phospholipids (Fig. 10–6). Indeed, sphingomyelins resemble phosphatidylcholines in their general properties and three-dimensional structure, and in having no net charge on their head groups (Fig. 10–13). Sphingomyelins are present in the plasma membranes of animal cells and are especially prominent in myelin, a membranous sheath that surrounds and insulates the axons of some neurons—thus the name "sphingomyelins."



Phosphatidylcholine

Sphingomyelin

FIGURE 10–13 The similarities in shape and in molecular structure of phosphatidylcholine (a glycerophospholipid) and sphingomyelin (a sphingolipid) are clear when their space-filling and structural formulas are drawn as here.

Glycosphingolipids, which occur largely in the outer face of plasma membranes, have head groups with one or more sugars connected directly to the -OH at C-1 of the ceramide moiety; they do not contain phosphate. **Cerebrosides** have a single sugar linked to ceramide; those with galactose are characteristically found in the plasma membranes of cells in neural tissue, and those with glucose in the plasma membranes of cells in nonneural tissues. Cerebrosides are sometimes called **neutral glycolipids,** as they have no charge at pH 7. **Gangliosides,** the most complex sphingolipids, have oligosaccharides as their polar head groups and one or more residues of *N*-acetylneuraminic acid (Neu5Ac), a sialic acid (often simply called "sialic acid"), at the termini. Sialic acid gives gangliosides the negative charge at pH 7. Gangliosides with one sialic acid residue are in the GM (M for mono-) series, those with two are in the GD (D for di-) series, and so on (GT, three sialic acid residues; GQ, four).

Sphingolipids at Cell Surfaces Are Sites of Biological Recognition

In humans, at least 60 different sphingolipids have been identified in cellular membranes. Many of these are especially prominent in the plasma membranes of neurons, and some are clearly recognition sites on the cell surface, but a specific function for only a few sphingolipids has been discovered thus far. The carbohydrate moieties of certain sphingolipids define the human blood groups and therefore determine the type of blood that individuals can safely receive in blood transfusions (Fig. 10–14).

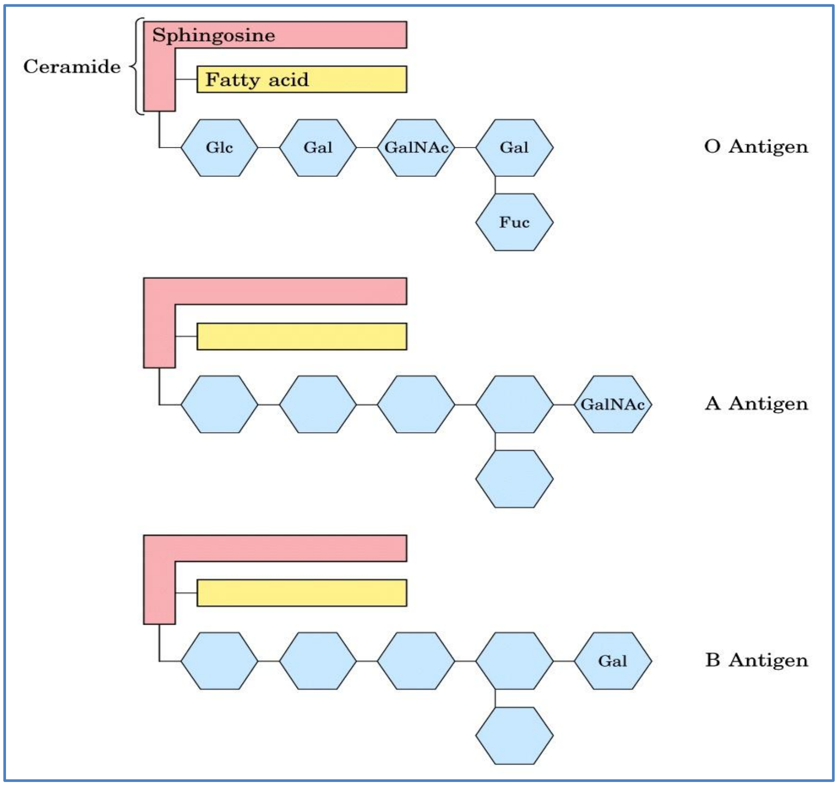


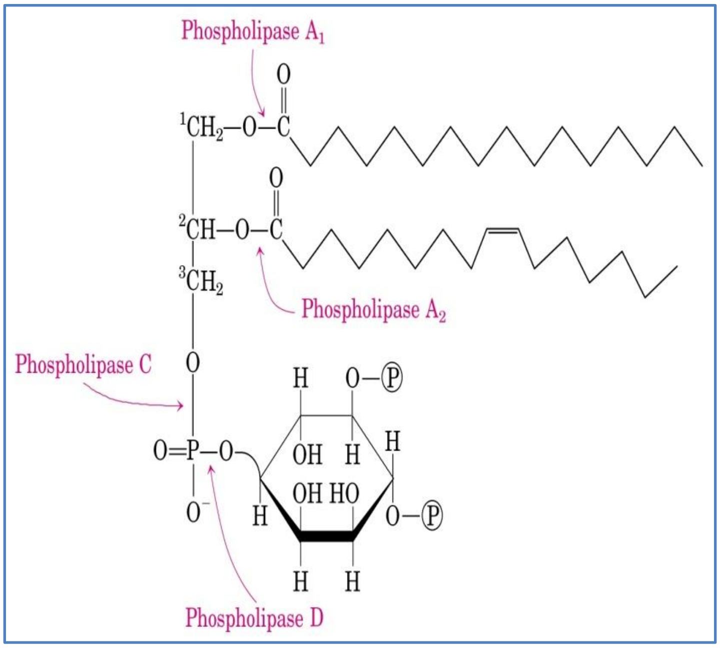
FIGURE 10–14 Glycosphingolipids as determinants of blood groups. The human blood groups (O, A, B) are determined in part by the oligosaccharide head groups (blue) of these glycosphingolipids. The same three oligosaccharides are also found attached to certain blood proteins of individuals of blood types O, A, and B, respectively. (Fuc represents the sugar fucose.)

Gangliosides are concentrated in the outer surface of cells, where they present points of recognition for extracellular molecules or surfaces of neighboring cells. The kinds and amounts of gangliosides in the plasma membrane change dramatically during embryonic development. Tumor formation induces the synthesis of a new complement of gangliosides, and very low concentrations of a specific ganglioside have been found to induce differentiation of cultured neuronal tumor cells.

Phospholipids and Sphingolipids Are Degraded in Lysosomes

Most cells continually degrade and replace their membrane lipids. For each hydrolyzable bond in a glycerophospholipid, there is a specific hydrolytic enzyme in the lysosome (Fig. 10–15). Phospholipases of the A type remove one of the two fatty acids, producing a lysophospholipid. (These esterases do not attack the ether link of plasmalogens.) Lysophospholipases remove the remaining fatty acid.

FIGURE 10–15 The specificities of phospholipases. Phospholipases A_1 and A_2 hydrolyze the ester bonds of intact glycerophospholipids at C-1 and C-2 of glycerol, respectively. Phospholipases C and D each split one of the phosphodiester bonds in the head group. Some phospholipases act on only one type of glycerophospholipid, such as phosphatidylinositol 4,5-bisphosphate (shown here) or phosphatidylcholine; others are less specific. When one of the fatty acids has been removed by a type A phospholipase, the second fatty acid is cleaved from the molecule by a lysophospholipase (not shown).



Gangliosides are degraded by a set of lysosomal enzymes that catalyze the stepwise removal of sugar units, finally yielding a ceramide. A genetic defect in any of these hydrolytic enzymes leads to the accumulation of gangliosides in the cell, with severe medical consequences.

Inherited Human Diseases Resulting from Abnormal Accumulations of Membrane Lipids

The polar lipids of membranes undergo constant metabolic turnover, the rate of their synthesis normally counterbalanced by the rate of breakdown. The breakdown of lipids is promoted by hydrolytic enzymes in lysosomes, each enzyme capable of hydrolyzing a specific bond. When sphingolipid degradation is impaired by a defect in one of these enzymes (Box 10-2 Fig. 1), partial breakdown products accumulate in the tissues, causing serious disease.

For example, Niemann-Pick disease is caused by a rare genetic defect in the enzyme sphingomyelinase, which cleaves phosphocholine from sphingomyelin. Sphingomyelin accumulates in the brain, spleen, and liver. The disease becomes evident in infants, and causes mental retardation and early death. More common is Tay-Sachs disease, in which ganglioside GM2 accumulates in the brain and spleen owing to lack of the enzyme hexosaminidase A. The symptoms of Tay-Sachs disease are progressive retardation in development, paralysis, blindness, and death by the age of 3 or 4 years. Tests on prospective parents can detect abnormal enzymes, then DNA testing can determine the exact nature of the defect and the risk it poses for offspring. Once a pregnancy occurs, fetal cells obtained by sampling a part of the placenta (chorionic villus sampling) or the fluid surrounding the fetus (amniocentesis) can be tested in the same way.

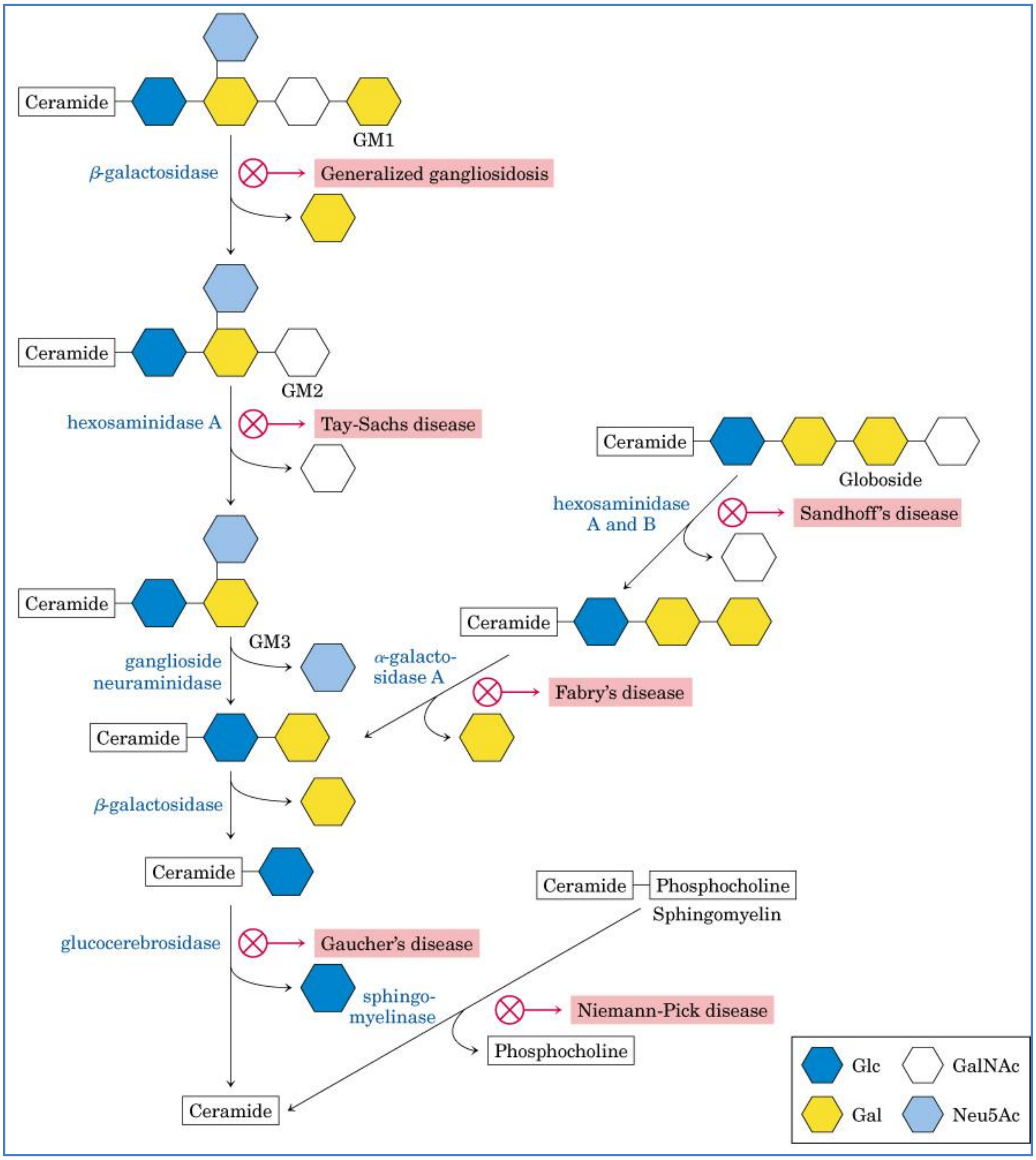


FIGURE 1 (BOX 10-2) Pathways for the breakdown of GM1, loboside, and sphingomyelin to ceramide. A defect in the enzyme hydrolyzing a particular step is indicated by ⊗, and the disease that results from accumulation of the partial breakdown product is noted.

Sterols Have Four Fused Carbon Rings

Sterols are structural lipids present in the membranes of most eukaryotic cells. The characteristic structure of this fifth group of membrane lipids is the steroid nucleus, consisting of four fused rings, three with six carbons and one with five (Fig. 10–16). The steroid nucleus is almost planar and is relatively rigid; the fused rings do not allow rotation about C—C bonds. **Cholesterol,** the major sterol in animal tissues, is amphipathic, with a polar head group (the hydroxyl group at C-3) and a nonpolar hydrocarbon body (the steroid nucleus and the hydrocarbon side chain at C-17), about as long as a 16- carbon fatty acid in its extended form. Similar sterols are found in other eukaryotes: stigmasterol in plants and ergosterol in fungi, for example. In addition to their roles as membrane constituents, the sterols serve as precursors for a variety of products with specific biological activities. Steroid hormones, for example, are potent biological signals that regulate gene expression. Bile acids are polar derivatives of cholesterol that act as detergents in the intestine, emulsifying dietary fats to make them more readily accessible to digestive lipases.

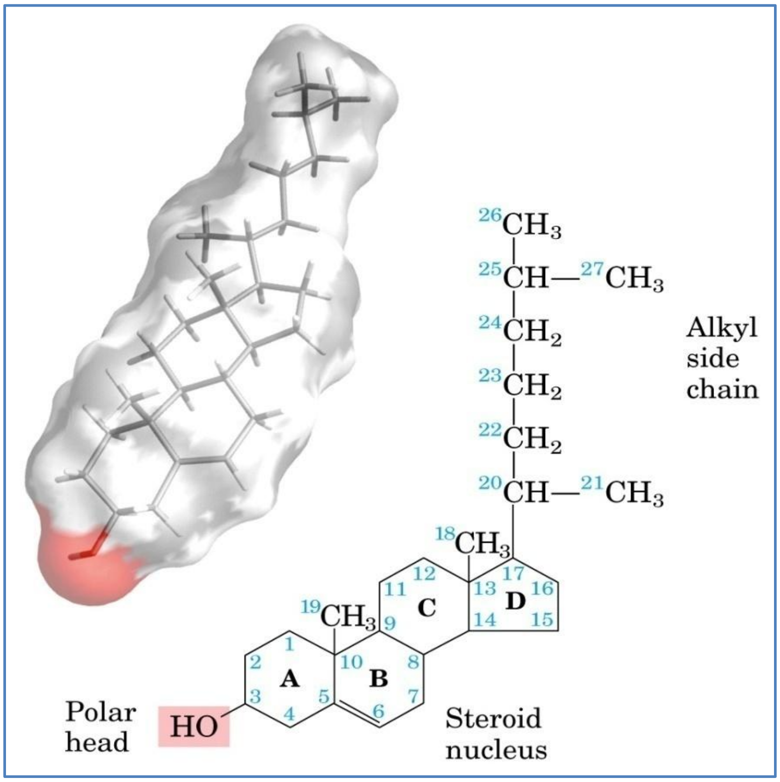


FIGURE 10–16 Cholesterol. The stick structure of cholesterol is visible through a transparent surface contour model of the molecule (from coordinates supplied by Dave Woodcock). In the chemical structure, the rings are labeled A through D to simplify reference to derivatives of the steroid nucleus, and the carbon atoms are numbered in blue. The C-3 hydroxyl group (pink in both representations) is the polar head group. For storage and transport of the sterol, this hydroxyl group condenses with a fatty acid to form a sterol ester.

Lipids as Signals, Cofactors, and Pigments

The two functional classes of lipids considered thus far (storage lipids and structural lipids) are major cellular components; membrane lipids make up 5% to 10% of the dry mass of most cells, and storage lipids more than 80% of the mass of an adipocyte. With some important exceptions, these lipids play a *passive* role in the cell; lipid fuels are stored until oxidized by enzymes, and membrane lipids form impermeable barriers around cells and cellular compartments. Another group of lipids, present in much smaller amounts, have *active* roles in the metabolic traffic as metabolites and messengers. Some serve as potent signals—as hormones, carried in the blood from one tissue to another, or as intracellular messengers generated in response to an extracellular signal (hormone or growth factor). Others function as enzyme cofactors in electron-transfer reactions in chloroplasts and mitochondria, or in the transfer of sugar moieties in a variety of glycosylation (addition of sugar) reactions. A third group consists of lipids with a system of conjugated double bonds: pigment molecules that absorb visible light. Some of these act as light-capturing pigments in vision and photosynthesis; others produce natural colorations, such as the orange of carrots and the yellow of canary feathers. Specialized lipids such as these are derived from lipids of the plasma membrane or from the fat-soluble vitamins A, D, E, and K.

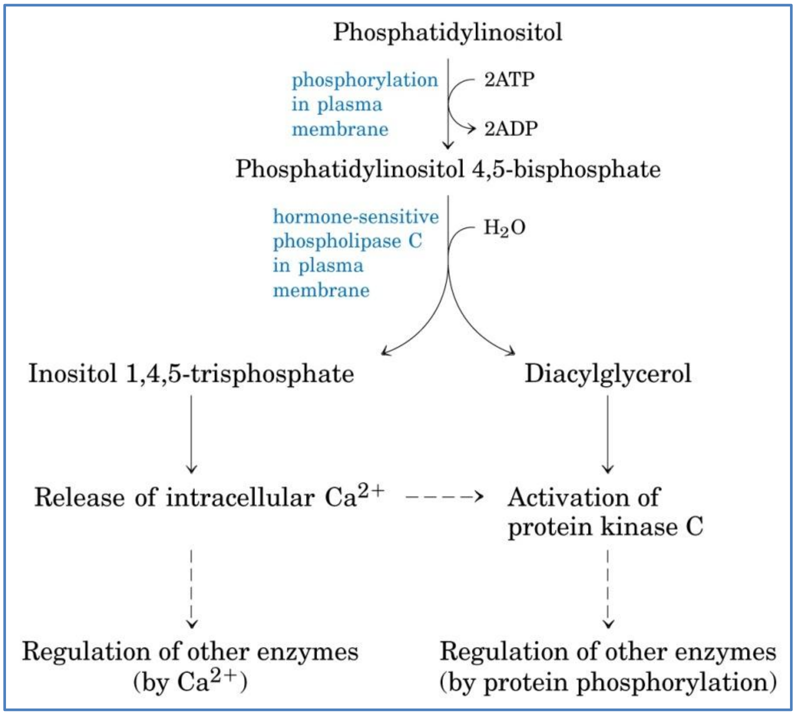


FIGURE 10–17 **Phosphatidylinositols in cellular regulation.** Phosphatidylinositol 4,5-bisphosphate in the plasma membrane is hydrolyzed by a specific phospholipase C in response to hormonal signals. Both products of hydrolysis act as intracellular messengers.

Phosphatidylinositols and Sphingosine Derivatives Act as Intracellular Signals

Phosphatidylinositol and its phosphorylated derivatives act at several levels to regulate cell structure and metabolism (Fig. 10–17). Phosphatidylinositol 4,5-bisphosphate (Fig. 10–8) in the cytoplasmic (inner) face of plasma membranes serves as a specific binding site for certain cytoskeletal proteins and for some soluble proteins involved in membrane fusion during exocytosis. It also serves as a reservoir of messenger molecules that are released inside the cell in response to extracellular signals interacting with specific receptors on the outer surface of the plasma membrane. The signals act through a series of steps (Fig. 10–17) that begins with enzymatic removal of a phospholipid head group and ends with activation of an enzyme (protein kinase C). For example, when the hormone vasopressin binds to plasma membrane receptors on the epithelial cells of the renal collecting duct, a specific phospholipase C is activated.

Phospholipase C hydrolyzes the bond between glycerol and phosphate in phosphatidylinositol 4,5-bisphosphate, releasing two products: inositol 1,4,5-trisphosphate (IP_3), which is water-soluble, and diacylglycerol, which remains associated with the plasma membrane. IP_3 triggers release of Ca^{2+} from the endoplasmic reticulum, and the combination of diacylglycerol and elevated cytosolic Ca^{2+} activates the enzyme protein kinase C. Membrane sphingolipids also can serve as sources of intracellular messengers. Both ceramide and sphingomyelin (Fig. 10–12) are potent regulators of protein kinases, and ceramide or its derivatives are known to be involved in the regulation of cell division, differentiation, migration, and programmed cell death (also called apoptosis).

Eicosanoids Carry Messages to Nearby Cells

Eicosanoids are paracrine hormones, substances that act only on cells near the point of hormone synthesis instead of being transported in the blood to act on cells in other tissues or organs. These fatty acid derivatives have a variety of dramatic effects on vertebrate tissues. They are known to be involved in reproductive function; in the inflammation, fever, and pain associated with injury or disease; in the formation of blood clots and the regulation of blood pressure; in gastric acid secretion; and in a variety of other processes important in human health or disease.

All eicosanoids are derived from arachidonic acid ($20{:}4\Delta^{5,8,11,14}$) (Fig. 10–18), the 20-carbon polyunsaturated fatty acid from which they take their general name (Greek *eikosi*, “twenty”). There are three classes of eicosanoids: prostaglandins, thromboxanes, and leukotrienes.

Prostaglandins (PG) contain a five-carbon ring originating from the chain of arachidonic acid. Their name derives from the prostate gland, the tissue from which they were first isolated. Two groups of prostaglandins were originally defined: PGE, for *e*ther-soluble, and PGF, for phosphate (*fosfat* in Swedish) buffer–soluble. Each group contains numerous subtypes, named PGE1, PGE2, and so forth. Prostaglandins act in many tissues by regulating the synthesis of the intracellular messenger 3',5'-cyclic AMP (cAMP). Because cAMP mediates the action of diverse hormones, the prostaglandins affect a wide range of cellular and tissue functions. Some prostaglandins stimulate contraction of the smooth muscle of the uterus during menstruation and labor. Others affect blood flow to specific organs, the wake-sleep cycle, and the responsiveness of certain tissues to hormones such as epinephrine and glucagon. Prostaglandins in a third group elevate body temperature (producing fever) and cause inflammation and pain.

The **thromboxanes** have a six-membered ring containing an ether. They are produced by platelets (also called thrombocytes) and act in the formation of blood clots and the reduction of blood flow to the site of a clot. The nonsteroidal antiinflammatory drugs (NSAIDs)— aspirin and ibuprofen, for example— were shown to inhibit the enzyme prostaglandin H2 synthase (also called cyclooxygenase or COX), which catalyzes an early step in the pathway from arachidonate to prostaglandins and thromboxanes (Fig. 10–18).

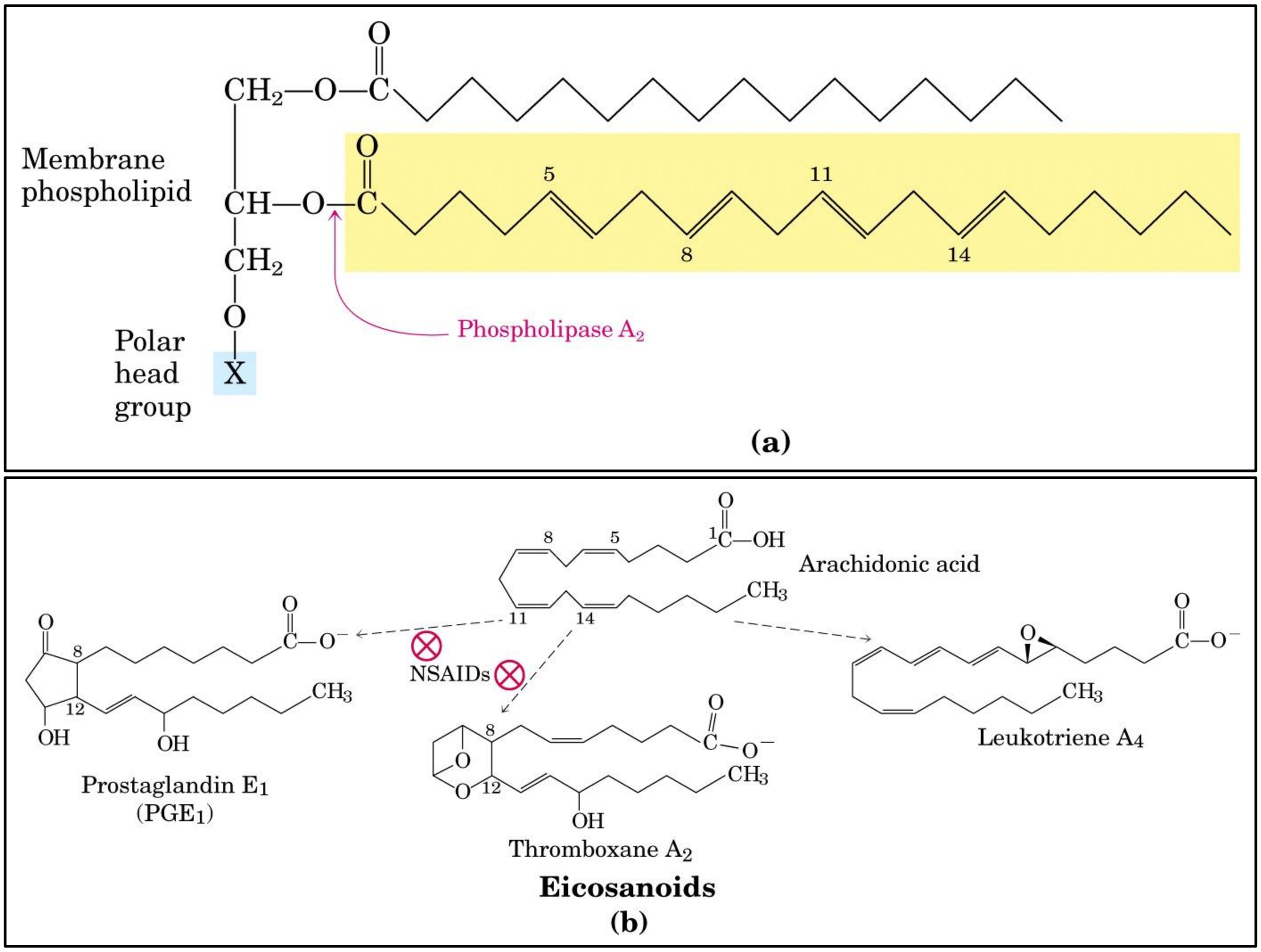


FIGURE 10–18 Arachidonic acid and some eicosanoid derivatives. (a) In response to hormonal signals, phospholipase A_2 cleaves arachidonic acid–containing membrane phospholipids to release arachidonic acid (arachidonate at pH 7), the precursor to various eicosanoids. **(b)** These compounds include prostaglandins such as PGE_1, in which C-8 and C-12 of arachidonate are joined to form the characteristic five-membered ring. In thromboxane A_2, the C-8 and C-12 are joined and an oxygen atom is added to form the sixmembered ring. Leukotriene A_4 has a series of three conjugated double bonds. Nonsteroidal antiinflammatory drugs (NSAIDs) such as aspirin and ibuprofen block the formation of prostaglandins and thromboxanes from arachidonate by inhibiting the enzyme cyclooxygenase (prostaglandin H_2 synthase).

Leukotrienes, first found in leukocytes, contain three conjugated double bonds. They are powerful biological signals. For example, leukotriene D4, derived from leukotriene A4, induces contraction of the muscle lining the airways to the lung. Overproduction of leukotrienes causes asthmatic attacks, and leukotriene synthesis is one target of antiasthmatic drugs such as prednisone. The strong contraction of the smooth muscles of the lung that occurs during anaphylactic shock is part of the potentially fatal allergic reaction in individuals hypersensitive to bee stings, penicillin, or other agents.

Steroid Hormones Carry Messages between Tissues

Steroids are oxidized derivatives of sterols; they have the sterol nucleus but lack the alkyl chain attached to ring D of cholesterol, and they are more polar than cholesterol. Steroid hormones move through the bloodstream (on protein carriers) from their site of production to target tissues, where they enter cells, bind to highly specific receptor proteins in the nucleus, and trigger changes in gene expression and metabolism. Because hormones have very high affinity for their receptors, very low concentrations of hormones (nanomolar or less) are sufficient to produce responses in target tissues. The major groups of steroid hormones are the male and female sex hormones and the hormones produced by the adrenal cortex, cortisol and aldosterone (Fig. 10–19). Prednisone and prednisolone are steroid drugs with potent antiinflammatory activities, mediated in part by the inhibition of arachidonate release by phospholipase A_2 (Fig. 10–18) and consequent inhibition of the synthesis of leukotrienes, prostaglandins, and thromboxanes. They have a variety of medical applications, including the treatment of asthma and rheumatoid arthritis.

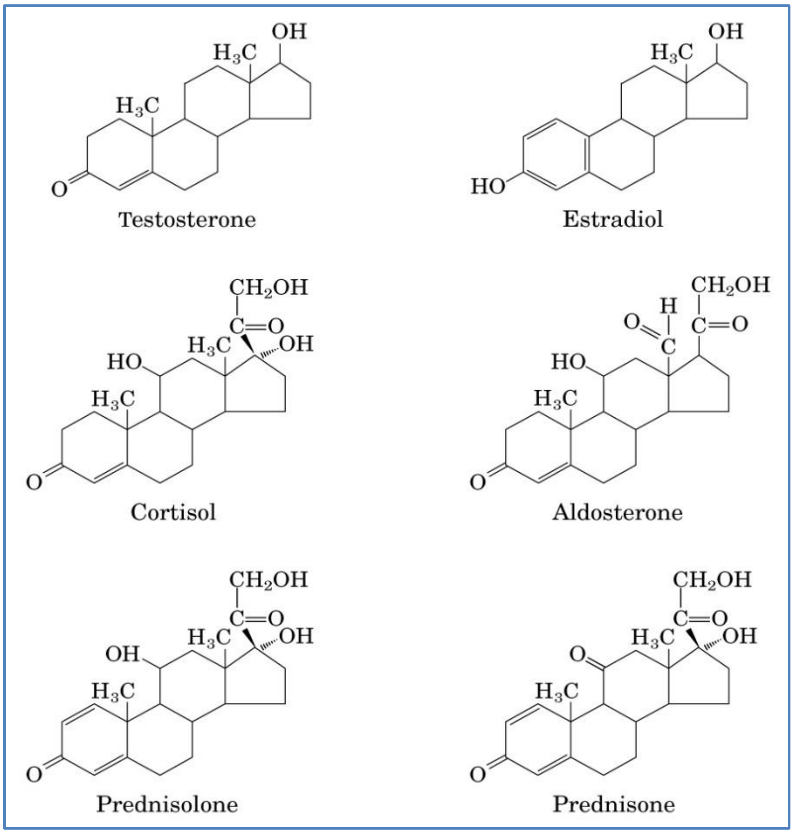


FIGURE 10–19 Steroids derived from cholesterol. Testosterone, the male sex hormone, is produced in the testes. Estradiol, one of the female sex hormones, is produced in the ovaries and placenta. Cortisol and aldosterone are hormones synthesized in the cortex of the adrenal gland; they regulate glucose metabolism and salt excretion, respectively. Prednisolone and prednisone are synthetic steroids used as antiinflammatory agents.

Plants Use Phosphatidylinositols, Steroids, and Eicosanoid-like Compounds in Signaling

Vascular plants contain phosphatidylinositol 4,5-bisphosphate, as well as the phospholipase that releases IP_3, and they use IP_3 to regulate the intracellular concentration of Ca^{2+}.

Vitamins A and D Are Hormone Precursors

Vitamins are compounds that are essential to the health of humans and other vertebrates but cannot be synthesized by these animals and must therefore be obtained in the diet. Early nutritional studies identified two general classes of such compounds: those soluble in nonpolar organic solvents (fat-soluble vitamins) and those that could be extracted from foods with aqueous solvents (water-soluble vitamins). Eventually the fat-soluble group was resolved into the four vitamin groups A, D, E, and K, Two of these (D and A) serve as hormone precursors.

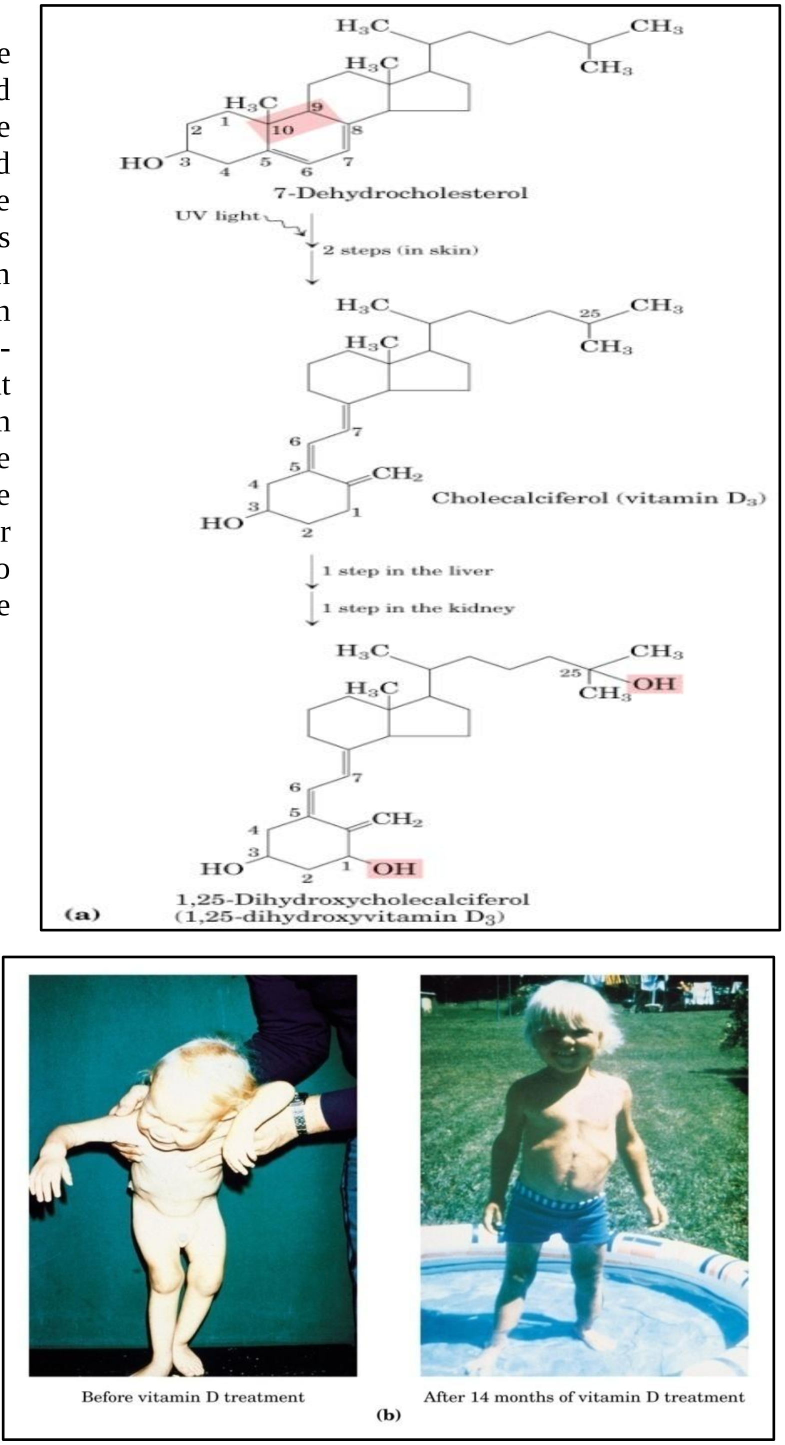


FIGURE 10–20 Vitamin D3 production and metabolism. (a) Cholecalciferol (vitamin D3) is produced in the skin by UV irradiation of 7-dehydro-cholesterol, which breaks the bond shaded pink. In the liver, a hydroxyl group is added at C-25 (pink); in the kidney, a second hydroxylation at C-1 (pink) produces the active hormone, 1,25-dihydroxycholecalciferol. This hormone regulates the metabolism of Ca^{2+} in kidney, intestine, and bone. **(b)** Dietary vitamin D prevents rickets, a disease once common in cold climates where heavy clothing blocks the UV component of sunlight necessary for the production of vitamin D3 in skin. On the left is a 2½-year-old boy with severe rickets; on the right, same boy at age 5, after 14 months of vitamin D therapy.

Vitamin D_3, also called **cholecalciferol,** is normally formed in the skin from 7-dehydrocholesterol in a photochemical reaction driven by the UV component of sunlight (Fig. 10–20). Vitamin D_3 is not itself biologically active, but it is converted by enzymes in the liver and kidney to 1,25-dihydroxycholecalciferol, a hormone that regulates calcium uptake in the intestine and calcium levels in kidney and bone. Deficiency of vitamin D leads to defective bone formation and the disease rickets, for which administration of vitamin D produces a dramatic cure. Vitamin D_2 (ergocalciferol) is a commercial product formed by UV irradiation of the ergosterol of yeast. Vitamin D_2 is structurally similar to D_3, with slight modification to the side chain attached to the sterol D ring. Both have the same biological effects, and D_2 is commonly added to milk and butter as a dietary supplement. Like steroid hormones, the product of vitamin D metabolism, 1,25-dihydroxycholecalciferol, regulates gene expression—for example, turning on the synthesis of an intestinal Ca^{2+}-binding protein.

Vitamin A (retinol) in its various forms functions as a hormone and as the visual pigment of the vertebrate eye (Fig. 10–21). Acting through receptor proteins in the cell nucleus, the vitamin A derivative retinoic acid regulates gene expression in the development of epithelial tissue, including skin. Retinoic acid is the active ingredient in the drug (Retin-A), used in the treatment of severe acne and wrinkled skin. The vitamin A derivative retinal is the pigment that initiates the response of rod and cone cells of the retina to light, producing a neuronal signal to the brain.

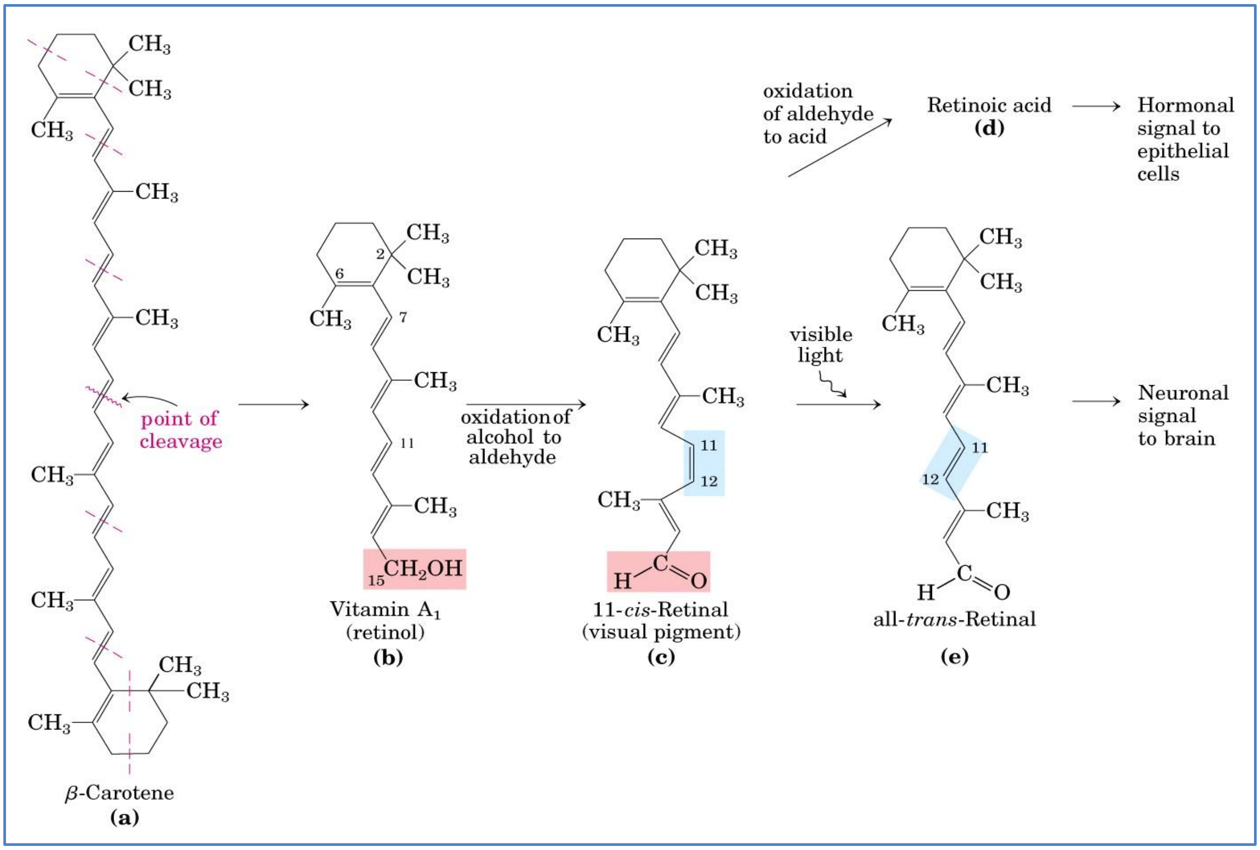


FIGURE 10–21 Vitamin A₁ and its precursor and derivatives. (a) β-Carotene is the precursor of vitamin A_1. Isoprene structural units are set off by dashed red lines. Cleavage of **β**-carotene yields two molecules of vitamin A_1 (retinol) **(b).** Oxidation at C-15 converts retinol to the aldehyde, retinal **(c),** and further oxidation produces retinoic acid **(d),** a hormone that regulates gene expression. Retinal combines with the protein opsin to form rhodopsin (not shown), a visual pigment widespread in nature. In the dark, retinal of rhodopsin is in the 11-*cis* form **(c).** When a rhodopsin molecule is excited by visible light, the 11-*cis*-retinal undergoes a series of photochemical reactions that convert it to all-*trans*-retinal **(e),** forcing a change in the shape of the entire rhodopsin molecule. This transformation in the rod cell of the vertebrate retina sends an electrical signal to the brain that is the basis of visual transduction.

Vitamin A was first isolated from fish liver oils; liver, eggs, whole milk, and butter are good dietary sources. In vertebrates, β-carotene, the pigment that gives carrots, sweet potatoes, and other yellow vegetables their characteristic color, can be enzymatically converted to vitamin A. Deficiency of vitamin A leads to a variety of symptoms in humans, including dryness of the skin, eyes, and mucous membranes; retarded development and growth; and night blindness, an early symptom commonly used in diagnosing vitamin A deficiency.

Vitamins E and K and the Lipid Quinones Are Oxidation-Reduction Cofactors

Vitamin E is the collective name for a group of closely related lipids called **tocopherols,** all of which contain a substituted aromatic ring and a long isoprenoid side chain (Fig. 10–22a). Because they are hydrophobic, tocopherols associate with cell membranes, lipid deposits, and lipoproteins in the blood. Tocopherols are biological antioxidants. The aromatic ring reacts with and destroys the most reactive forms of oxygen radicals and other free radicals, protecting unsaturated fatty acids from oxidation and preventing oxidative damage to membrane lipids, which can cause cell fragility. Tocopherols are found in eggs and vegetable oils and are especially abundant in wheat germ. Laboratory animals fed diets depleted of vitamin E develop scaly skin, muscular weakness and wasting, and sterility. Vitamin E deficiency in humans is very rare; the principal symptom is fragile erythrocytes.

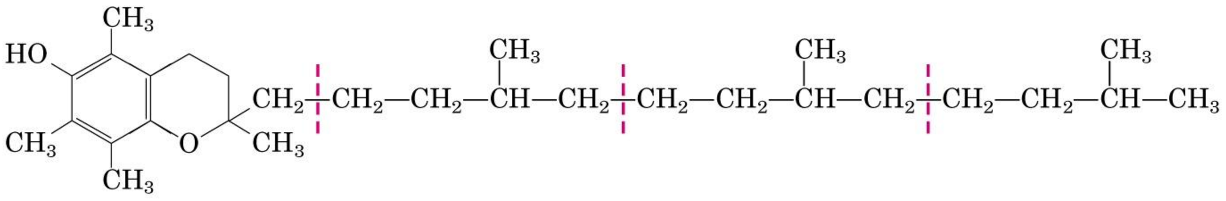
The aromatic ring of **vitamin K** (Fig. 10–22b) undergoes a cycle of oxidation and reduction during the formation of active prothrombin, a blood plasma protein essential in blood clot formation. Prothrombin is a proteolytic enzyme that splits peptide bonds in the blood protein fibrinogen to convert it to fibrin, the insoluble fibrous protein that holds blood clots together. Vitamin K deficiency slows blood clotting, which can be fatal. Vitamin K deficiency is very uncommon in humans, aside from a small percentage of infants who suffer from hemorrhagic disease of the newborn, a potentially fatal disorder. In the United States, newborns are routinely given a 1 mg injection of vitamin K. Vitamin K_1 (phylloquinone) is found in green plant leaves; a related form, vitamin K_2 (menaquinone), is formed by bacteria residing in the vertebrate intestine.

Warfarin (Fig. 10–22c) is a synthetic compound that inhibits the formation of active prothrombin. It is particularly poisonous to rats, causing death by internal bleeding. Ironically, this potent rodenticide is also an invaluable anticoagulant drug for treating humans at risk for excessive blood clotting, such as surgical patients and those with coronary thrombosis.

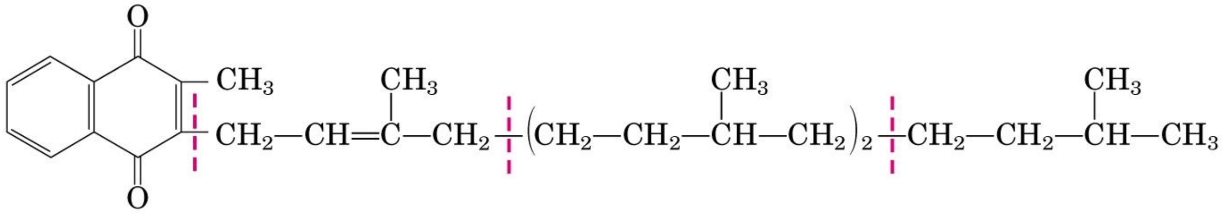
Ubiquinone (also called coenzyme Q) and plastoquinone (Fig. 10–22d, e) are isoprenoids that function as lipophilic electron carriers in the oxidation-reduction reactions that drive ATP synthesis in mitochondria and chloroplasts, respectively. Both ubiquinone and plastoquinone can accept either one or two electrons and either one or two protons.

Dolichols Activate Sugar Precursors for Biosynthesis

During assembly of the complex carbohydrates of bacterial cell walls, and during the addition of polysaccharide units to certain proteins (glycoproteins) and lipids (glycolipids) in eukaryotes, the sugar units to be added are chemically activated by attachment to isoprenoid alcohols called **dolichols** (Fig. 10–22f). These compounds have strong hydrophobic interactions with membrane lipids, anchoring the attached sugars to the membrane, where they participate in sugar-transfer reactions.

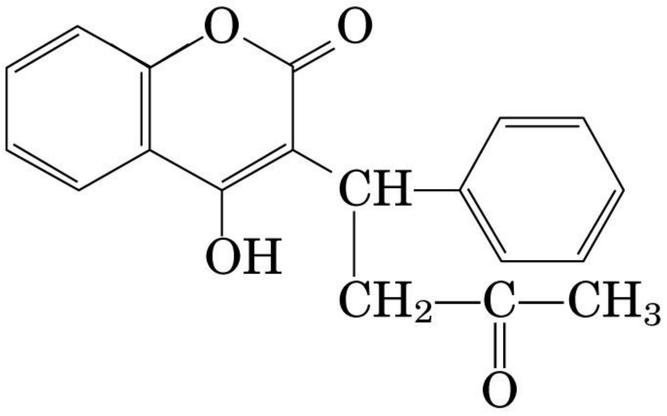


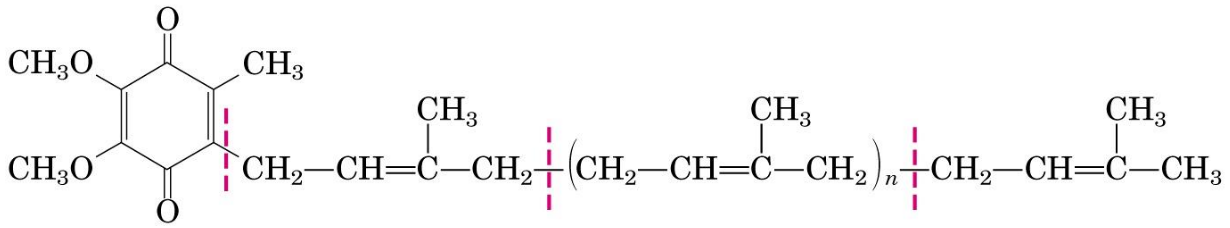
(a)
Vitamin E: an antioxidant



(b)
Vitamin K_1: a blood-clotting
cofactor (phylloquinone)

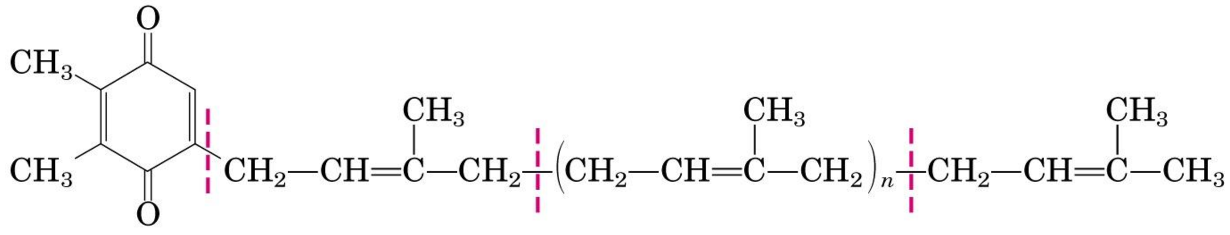
(c)
Warfarin: a blood
anticoagulant





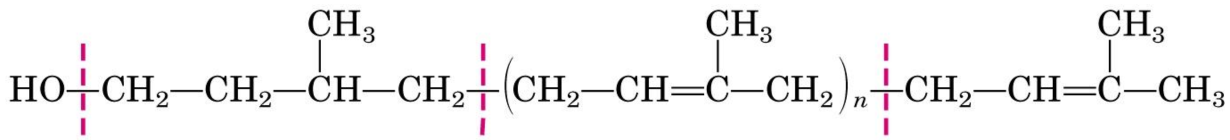
(d)

Ubiquinone: a mitochondrial
electron carrier (coenzyme Q)
(n = 4–8)



(e)

Plastoquinone: a chloroplast
electron carrier (n = 4–8)



(f)

Dolichol: a sugar carrier
(n = 9–22)

FIGURE 10–22 Some other biologically active isoprenoid compounds or derivatives. Isoprene structural units are set off by dashed red lines. In most mammalian tissues, ubiquinone (also called coenzyme Q) has 10 isoprene units. Dolichols of animals have 17 to 21 isoprene units (85 to 105 carbon atoms), bacterial dolichols have 11, and those of plants and fungi have 14 to 24.

Working with Lipids

In exploring the biological role of lipids in cells and tissues, it is essential to know which lipids are present and in what proportions. Because lipids are insoluble in water, their extraction and subsequent fractionation require the use of organic solvents and some techniques not commonly used in the purification of water-soluble molecules such as proteins and carbohydrates. In general, complex mixtures of lipids are separated by differences in the polarity or solubility of the components in nonpolar solvents. Lipids that contain ester- or amide-linked fatty acids can be hydrolyzed by treatment with acid or alkali or with highly specific hydrolytic enzymes (phospholipases, glycosidases) to yield their component parts for analysis. Some methods commonly used in lipid analysis are shown in Figure 10–23 and discussed below.

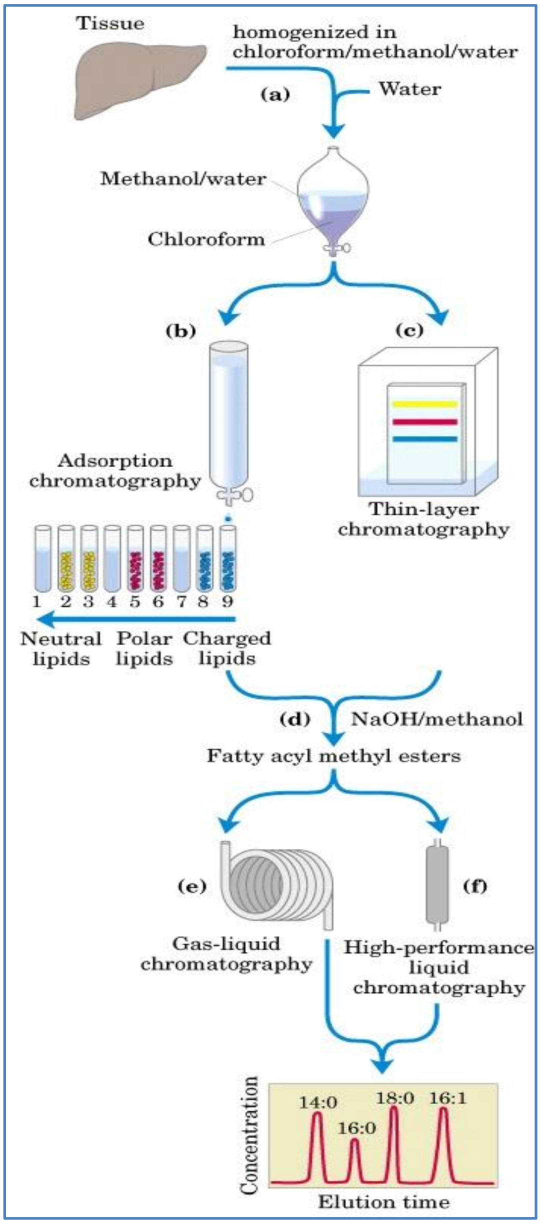


FIGURE 10–23 Common procedures in the extraction, separation, and identification of cellular lipids. (a) Tissue is homogenized in a chloroform/methanol/water mixture, which on addition of water and removal of unextractable sediment by centrifugation yields two phases. Different types of extracted lipids in the chloroform phase may be separated by **(b)** adsorption chromatography on a column of silica gel, through which solvents of increasing polarity are passed, or **(c)** thin layer chromatography (TLC), in which lipids are carried up a silica gel coated plate by a rising solvent front, less polar lipids traveling farther than more polar or charged lipids. TLC with appropriate solvents can also be used to separate closely related lipid species; for example, the charged lipids phosphatidylserine, phosphatidylglycerol, and phosphatidylinositol are easily separated by TLC. For the determination of fatty acid composition, a lipid fraction containing ester-linked fatty acids is transesterified in a warm aqueous solution of NaOH and methanol **(d),** producing a mixture of fatty acyl methyl esters. These methyl esters are then separated on the basis of chain length and degree of saturation by **(e)** gas-liquid chromatography (GLC) or **(f)** high-performance liquid chromatography (HPLC). Precise determination of molecular mass by mass spectrometry allows unambiguous identification of individual lipids.

Lipid Extraction Requires Organic Solvents

Neutral lipids (triacylglycerols, waxes, pigments, and so forth) are readily extracted from tissues with ethyl ether, chloroform, or benzene, solvents that do not permit lipid clustering driven by hydrophobic interactions. Membrane lipids are more effectively extracted by more polar organic solvents, such as ethanol or methanol, which reduce the hydrophobic interactions among lipid molecules while also weakening the hydrogen bonds and electrostatic interactions that bind membrane lipids to membrane proteins. A commonly used extractant is a mixture of chloroform, methanol, and water, initially in volume proportions (1:2:0.8) that are miscible, producing a single phase. After tissue is homogenized in this solvent to extract all lipids, more water is added to the resulting extract and the mixture separates into two phases, methanol/water (top phase) and chloroform (bottom phase). The lipids remain in the chloroform layer, and more polar molecules such as proteins and sugars partition into the methanol/water layer.

Adsorption Chromatography Separates Lipids of Different Polarity

Complex mixtures of tissue lipids can be fractionated by chromatographic procedures based on the different polarities of each class of lipid. In adsorption chromatography (Fig. 10–23b), an insoluble, polar material such as silica gel (a form of silicic acid, $Si(OH)_4$) is packed into a glass column, and the lipid mixture (in chloroform solution) is applied to the top of the column. (In high-performance liquid chromatography, the column is of smaller diameter and solvents are forced through the column under high pressure.) The polar lipids bind tightly to the polar silicic acid, but the neutral lipids pass directly through the column and emerge in the first chloroform wash. The polar lipids are then eluted, in order of increasing polarity, by washing the column with solvents of progressively higher polarity. Uncharged but polar lipids (cerebrosides, for example) are eluted with acetone, and very polar or charged lipids (such as glycerophospholipids) are eluted with methanol.

Thin-layer chromatography on silicic acid employs the same principle (Fig. 10–23c). A thin layer of silica gel is spread onto a glass plate, to which it adheres. A small sample of lipids dissolved in chloroform is applied near one edge of the plate, which is dipped in a shallow container of an organic solvent or solvent mixture—all of which is enclosed within a chamber saturated with the solvent vapor. As the solvent rises on the plate by capillary action, it carries lipids with it. The less polar lipids move farthest, as they have less tendency to bind to the silicic acid. The separated lipids can be detected by spraying the plate with a dye (rhodamine) that fluoresces when associated with lipids or by exposing the plate to iodine fumes. Iodine reacts reversibly with the double bonds in fatty acids, such that lipids containing unsaturated fatty acids develop a yellow or brown color. A number of other spray reagents are also useful in detecting specific lipids. For subsequent analysis, regions containing separated lipids can be scraped from the plate and the lipids recovered by extraction with an organic solvent.

Gas-Liquid Chromatography Resolves Mixtures of Volatile Lipid Derivatives

Gas-liquid chromatography separates volatile components of a mixture according to their relative tendencies to dissolve in the inert material packed in the chromatography column and to volatilize and move through the column, carried by a current of an inert gas such as helium. Some lipids are naturally volatile, but most must first be derivatized to increase their volatility (that is, lower their boiling point). For an analysis of the fatty acids in a sample of phospholipids, the lipids are first heated in a methanol/HCl or methanol/NaOH mixture, which converts fatty acids esterified to glycerol into their methyl esters (in a process of transesterification; Fig. 10–23d). These fatty acyl methyl esters are then loaded onto the gas-liquid chromatography column, and the column is heated to volatilize the compounds. Those fatty acyl esters most soluble in the column material partition into (dissolve in) that material; the less soluble lipids are carried by the stream of inert gas and emerge first from the column. The order of elution depends on the nature of the solid adsorbant in the column and on the boiling point of the components of the lipid mixture. Using these techniques, mixtures of fatty acids of various chain lengths and various degrees of unsaturation can be completely resolved (Fig. 10–23e).

Specific Hydrolysis Aids in Determination of Lipid Structure

Certain classes of lipids are susceptible to degradation under specific conditions. For example, all ester-linked fatty acids in triacylglycerols, phospholipids, and sterol esters are released by mild acid or alkaline treatment, and somewhat harsher hydrolysis conditions release amide-bound fatty acids from sphingolipids. Enzymes that specifically hydrolyze certain lipids are also useful in the determination of lipid structure. Phospholipases A, C, and D (Fig. 10–15) each split particular bonds in phospholipids and yield products with characteristic solubilities and chromatographic behaviors. Phospholipase C, for example, releases a water-soluble phosphoryl alcohol (such as phosphocholine from phosphatidylcholine) and a chloroform-soluble diacylglycerol, each of which can be characterized separately to determine the structure of the intact phospholipid. The combination of specific hydrolysis with characterization of the products by thin-layer, gas-liquid, or high-performance liquid chromatography often allows determination of a lipid structure.

Mass Spectrometry Reveals Complete Lipid Structure

To establish unambiguously the length of a hydrocarbon chain or the position of double bonds, mass spectral analysis of lipids or their volatile derivatives is invaluable. The chemical properties of similar lipids (for example, two fatty acids of similar length unsaturated at different positions, or two isoprenoids with different numbers of isoprene units) are very much alike, and their positions of elution from the various chromatographic procedures often do not distinguish between them. When the effluent from a chromatography column is sampled by mass spectrometry, however, the components of a lipid mixture can be simultaneously separated and identified by their unique pattern of fragmentation (Fig. 10–24).

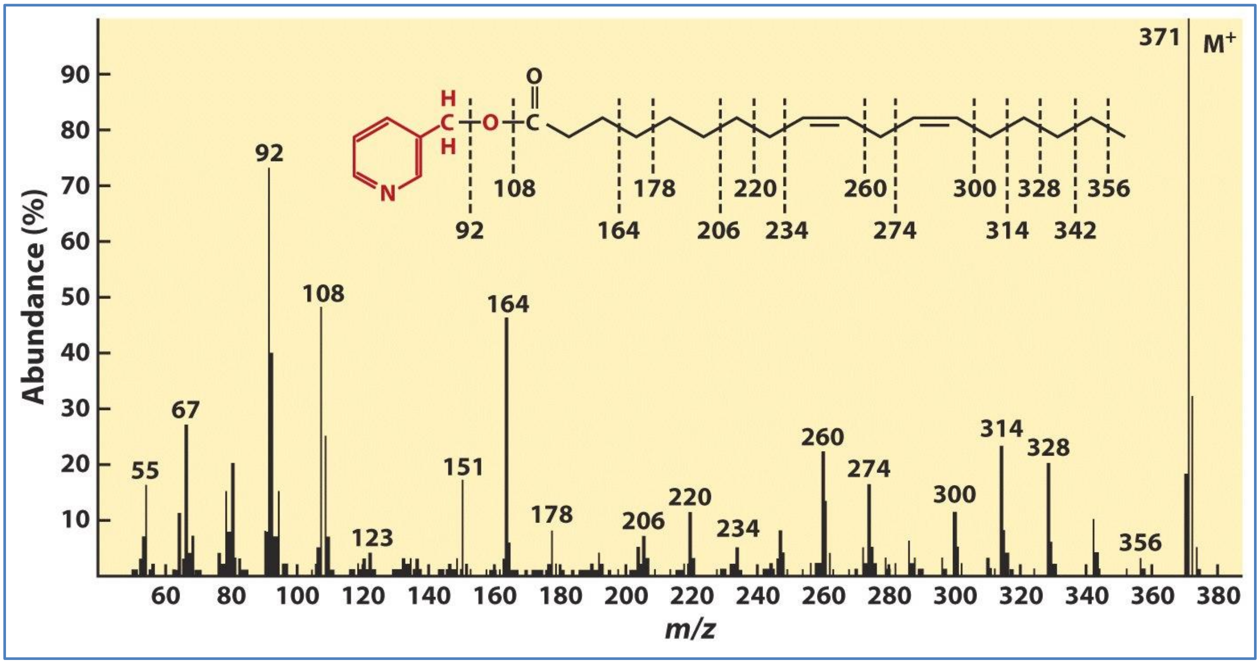


FIGURE 10–24 **Determination of the structure of a fatty acid by mass spectrometry.** The fatty acid is first converted to a derivative that minimizes migration of the double bonds when the molecule is fragmented by electron bombardment. The derivative shown here is a picolinyl ester of linoleic acid —18:2($\Delta^{9,12}$) (M_r 371)—in which the alcohol is picolinol (red). When bombarded with a stream of electrons, this molecule is volatilized and converted to a parent ion (M^+ ; M_r 371), in which the N atom bears the positive charge, and a series of smaller fragments produced by breakage of COC bonds in the fatty acid. The mass spectrometer separates these charged fragments according to their mass/charge ratio (*m/z*). The prominent ions at *m/z* = 92, 108, 151, and 164 contain the pyridine ring of the picolinol and various fragments of the carboxyl group, showing that the compound is indeed a picolinyl ester. The molecular ion (*m/z* = 371) confirms the presence of a C-18 fatty acid with two double bonds. The uniform series of ions 14 atomic mass units (amu) apart represents loss of each successive methyl and methylene group from the right end of the molecule (C-18 of the fatty acid), until the ion at *m/z* = 300 is reached. This is followed by a gap of 26 amu for the carbons of the terminal double bond, at *m/z* = 274; a further gap of 14 amu for the C-11 methylene group, at *m/z* = 260, and so forth. By this means the entire structure is determined, although these data alone do not reveal the configuration (cis or trans) of the double bonds.