Carbohydrate I

Biomedical Importance:

Carbohydrates are widely distributed in plants and animals; they have important structural and metabolic roles. In plants, glucose is synthesized from carbon dioxide and water by photosynthesis and stored as starch or used to synthesize the cellulose of the plant cell walls. Animals can synthesize carbohydrates from amino acids, but most animal carbohydrate is derived ultimately from plants. **Glucose** is the most important carbohydrate; most dietary carbohydrate is absorbed into the bloodstream as glucose, and other sugars are converted to glucose in the liver. Glucose is the major metabolic fuel of mammals (except ruminants) and a universal fuel of the fetus. It is the precursor for synthesis of all the other carbohydrates in the body, including **glycogen** for storage; **ribose** and **deoxyribose** in nucleic acids; **galactose** in lactose of milk, in glycolipids, and in combination with protein in glycoproteins and proteoglycans. Diseases associated with carbohydrate metabolism include **diabetes mellitus, galactosemia, glycogen storage diseases,** and **lactose intolerance.**

Carbohydrates Are Aldehyde or Ketone Derivatives of Polyhydric Alcohols:

Carbohydrates are classified as follows:

(1) Monosaccharides are those carbohydrates that cannot be hydrolyzed into simpler carbohydrates. They may be classified as **trioses, tetroses, pentoses, hexoses,** or **heptoses,** depending upon the number of carbon atoms; and as **aldoses** or **ketoses** depending upon whether they have an aldehyde or ketone group. Examples are listed in Table 14–1. In addition to aldehydes and ketones, the polyhydric alcohols (sugar alcohols or **polyols**), in which the aldehyde or ketone group has been reduced to an alcohol group, also occur naturally in foods. They are manufactured by reduction of monosaccharides for use in the manufacture of foods for weight reduction and for diabetics. They are poorly absorbed, and have about half the energy yield of sugars.

(2) Disaccharides are condensation products of two monosaccharide units; examples are maltose and sucrose.
(3) Oligosaccharides are condensation products of three to ten monosaccharides. Most are not digested by human enzymes.
(4) Polysaccharides are condensation products of more than ten monosaccharide units; examples are the starches and dextrins, which may be linear or branched polymers.

Polysaccharides are sometimes classified as hexosans or pentosans, depending on the identity of the constituent monosaccharides. In addition to starches and dextrins, foods contain a wide variety of other polysaccharides that are collectively known as nonstarch polysaccharides; they are not digested by human enzymes, and are the major component of dietary fiber. Examples are cellulose from plant cell walls (a glucose polymer) and inulin, the storage carbohydrate in some plants (a fructose polymer).

Table 14–1. Classification of Important Sugars.

	Aldoses	**Ketoses**
Trioses ($C_3H_6O_3$)	Glycerose (glyceraldehyde)	Dihydroxyacetone
Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
Pentoses ($C_5H_{10}O_5$)	Ribose	Ribulose
Hexoses ($C_6H_{12}O_6$)	Glucose	Fructose
Heptoses ($C_7H_{14}O_7$)	—	Sedoheptulose

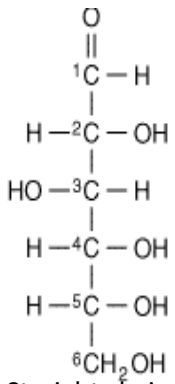
Biomedically, Glucose Is the Most Important Monosaccharide

The Structure of Glucose Can Be Represented in Three Ways:

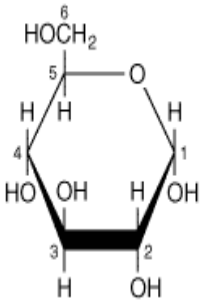
The straight-chain structural formula (aldohexose; Figure 14–1A) can account for some of the properties of glucose, but a cyclic structure (a **hemiacetal** formed by reaction between the aldehyde group and a hydroxyl group) is thermodynamically favored and accounts for other properties. The cyclic structure is normally drawn as shown in Fig 14-1B, the Haworth projection, in which the molecule is viewed from the side and above the plane of the ring; the bonds nearest to the viewer are bold and thickened, and the hydroxyl groups are above or below the plane of the ring. The six-membered ring containing one oxygen atom is actually in the form of a chair (Figure 14–1C).

Figure 14-1 :

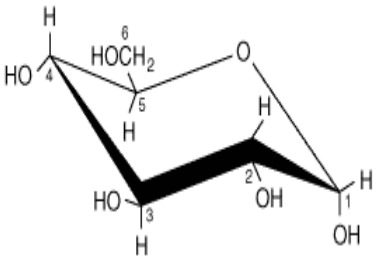
A



B



C



(A) Straight chain form. **(B)** -D-glucose; Haworth projection. **(C)** -D-glucose; chair form

Sugars Exhibit Various Forms of Isomerism:

Glucose, with four asymmetric carbon atoms, can form 16 isomers. The more important types of isomerism found with glucose are:

(1) D and L isomerism: The designation of a sugar isomer as the D form or of its mirror image as the L form is determined by its spatial relationship to the parent compound of the carbohydrates, the three-carbon sugar glycerose (glyceraldehyde). The L and D forms of this sugar, and of glucose, are shown in Figure 14–2. The orientation of the —H and —OH groups around the carbon atom adjacent to the terminal primary alcohol carbon (carbon 5 in glucose) determines whether the sugar belongs to the D or L series. When the —OH group on this carbon is on the right (as seen in Figure 14–2), the sugar is the D isomer; when it is on the left, it is the L isomer. Most of the monosaccharides occurring in mammals are D sugars, and the enzymes responsible for their metabolism are specific for this configuration.

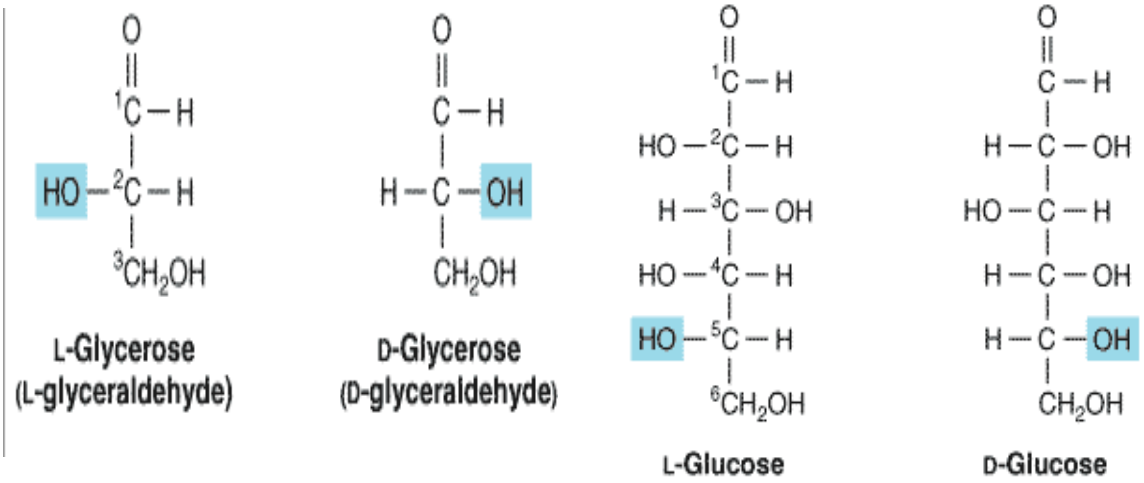
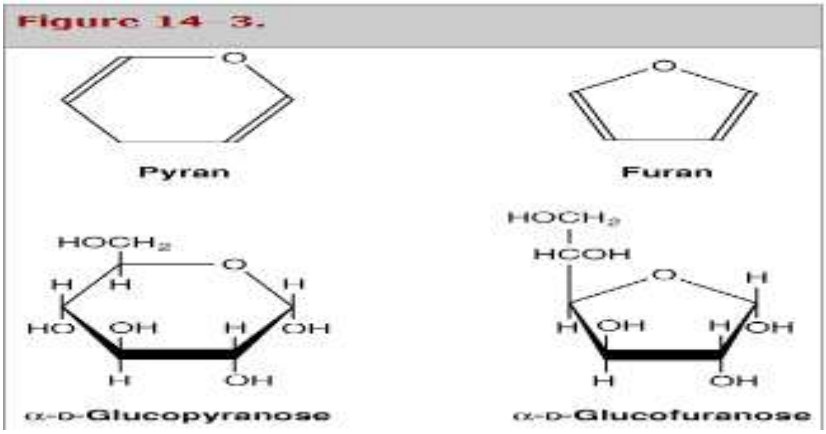
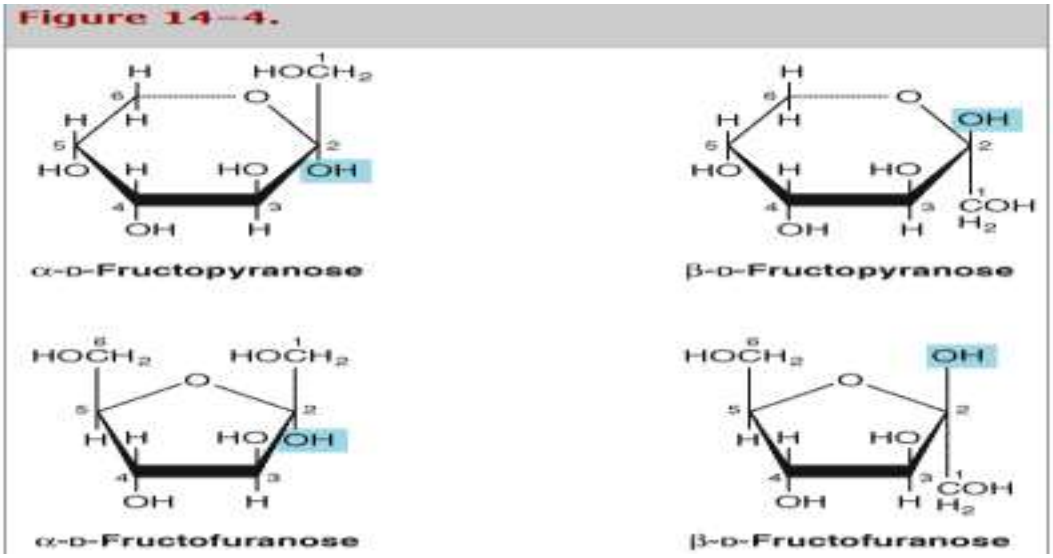


Figure 14-2

The presence of asymmetric carbon atoms also confers **optical activity** on the compound. When a beam of plane-polarized light is passed through a solution of an **optical isomer,** it rotates either to the right, dextrarotatory (+), or to the left, levorotatory (–). The direction of rotation of polarized light is independent of the stereochemistry of the sugar, so it may be designated D(–), D(+), L(–), or L(+). For example, the naturally occurring form of fructose is the D(–) isomer. In solution, glucose is dextrarotatory, and glucose solutions are sometimes known as **dextrose.**

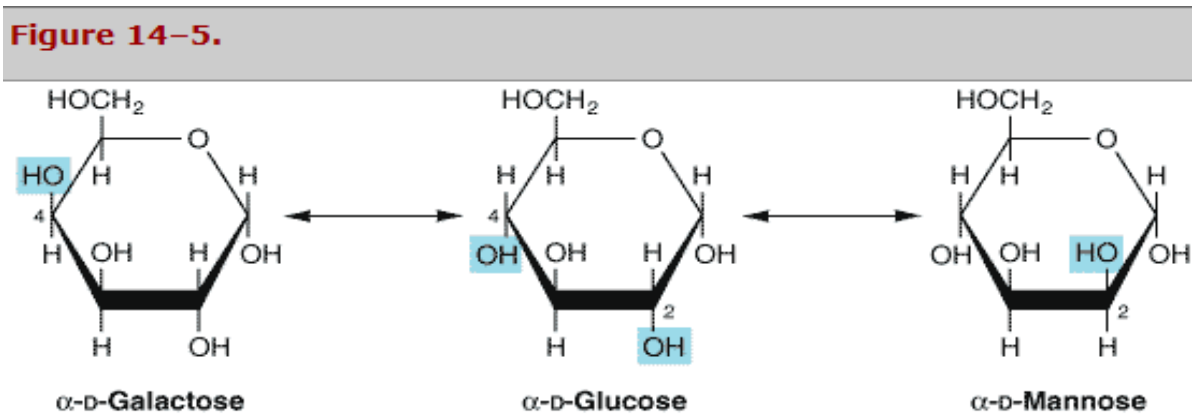
(2) Pyranose and furanose ring structures: The ring structures of monosaccharides are similar to the ring structures of either pyran (a six-membered ring) or furan (a five-membered ring) (Figures 14–3 and 14–4). For glucose in solution, more than 99% is in the pyranose form.





(3) Alpha and beta anomers: The ring structure of an aldose is a hemiacetal, since it is formed by combination of an aldehyde and an alcohol group. Similarly, the ring structure of a ketose is a hemiketal. Crystalline glucose is α-D-glucopyranose. The cyclic structure is retained in solution, but isomerism occurs about position 1, the carbonyl or **anomeric carbon atom,** to give a mixture of α-glucopyranose (38%) and β-glucopyranose (62%). Less than 0.3% is represented by α and β anomers of glucofuranose.

(4) Epimers: Isomers differing as a result of variations in configuration of the —OH and —H on carbon atoms 2, 3, and 4 of glucose are known as epimers. Biologically, the most important epimers of glucose are mannose and galactose, formed by epimerization at carbons 2 and 4, respectively (Figure 14–5).



(5) Aldose-ketose isomerism: Fructose has the same molecular formula as glucose but differs in its structural formula, since there is a potential keto group in position 2, the anomeric carbon of fructose (Figures 14–4 and 14–6), whereas there is a potential aldehyde group in position 1, the anomeric carbon of glucose (Figures 14–2 and 14–7).

Figure 14–6.

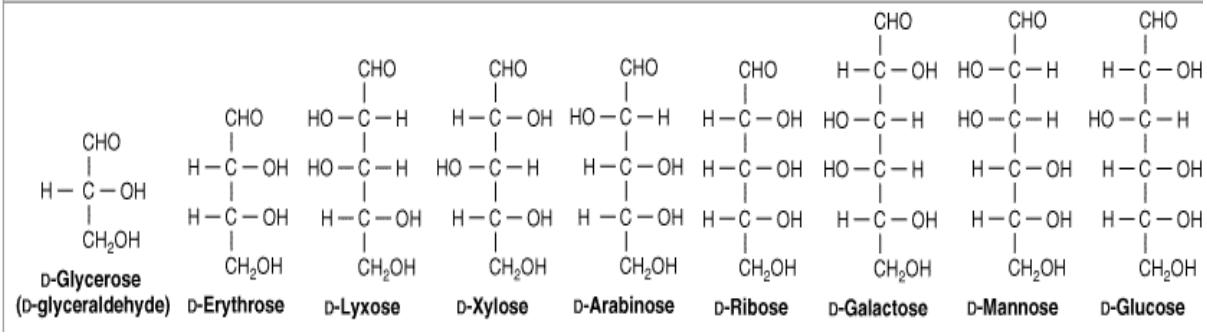
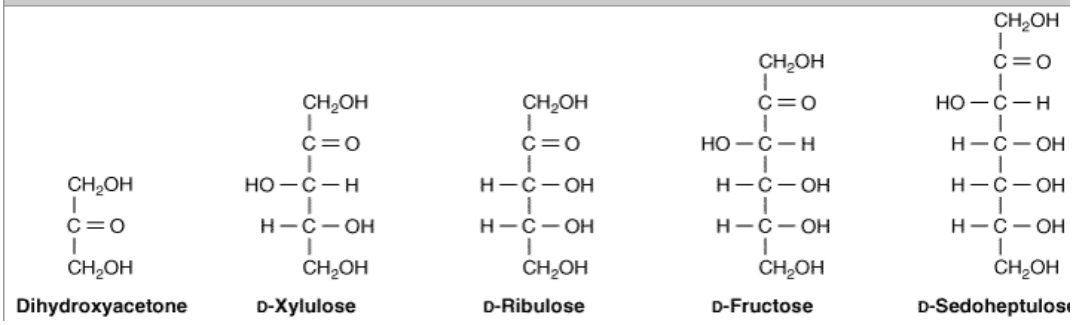


Figure 14–7.



Many Monosaccharides Are Physiologically Important:

Derivatives of trioses, tetroses, and pentoses and of a seven-carbon sugar (sedoheptulose) are formed as metabolic intermediates in glycolysis and the pentose phosphate pathway. Pentoses are important in nucleotides, nucleic acids, and several coenzymes (Table 14–2). Glucose, galactose, fructose, and mannose are physiologically the most important hexoses (Table 14–3). The biochemically important ketoses are shown in Figure 14–6, and important aldoses in Figure 14–7.

Table 14-2. Pentoses of Physiologic Importance.

Sugar	Source	Biochemical and Clinical Importance
D-Ribose	Nucleic acids and metabolic intermediate	Structural component of nucleic acids and coenzymes, including ATP, NAD(P), and flavin coenzymes
D-Ribulose	Metabolic intermediate	Intermediate in the pentose phosphate pathway
D-Arabinose	Plant gums	Constituent of glycoproteins
D-Xylose	Plant gums, proteoglycans, glycosaminoglycans	Constituent of glycoproteins
L-Xylulose	Metabolic intermediate	Excreted in the urine in essential pentosuria

Table 14-3. Hexoses of Physiologic Importance.

Sugar	Source	Biochemical Importance	Clinical Significance
D-Glucose	Fruit juices, hydrolysis of starch, cane or beet sugar, maltose and lactose	The main metabolic fuel for tissues; "blood sugar"	Excreted in the urine (glucosuria) in poorly controlled diabetes mellitus as a result of hyperglycemia
D-Fructose	Fruit juices, honey, hydrolysis of cane or beet sugar and inulin, enzymic isomerization of glucose syrups for food manufacture	Readily metabolized either via glucose or directly	Hereditary fructose intolerance leads to fructose accumulation and hypoglycemia
D-Galactose	Hydrolysis of lactose	Readily metabolized to glucose; synthesized in the mammary gland for synthesis of lactose in milk. A constituent of glycolipids and glycoproteins	Hereditary galactosemia as a result of failure to metabolize galactose leads to cataracts
D-Mannose	Hydrolysis of plant mannan gums	Constituent of glycoproteins	

Carbohydrate II

Sugars Form Glycosides with Other Compounds & with Each Other

Glycosides are formed by condensation between the hydroxyl group of the anomeric carbon of a monosaccharide, and a second compound that may or may not (in the case of an **aglycone**) be another monosaccharide. If the second group is a hydroxyl, the O-glycosidic bond is an **acetal** link because it results from a reaction between a hemiacetal group (formed from an aldehyde and an —OH group) and another —OH group. If the hemiacetal portion is glucose, the resulting compound is a **glucoside;** if galactose, a **galactoside;** and so on. If the second group is an amine, an *N*-glycosidic bond is formed, eg, between adenine and ribose in nucleotides such as ATP (Figure 11–4).

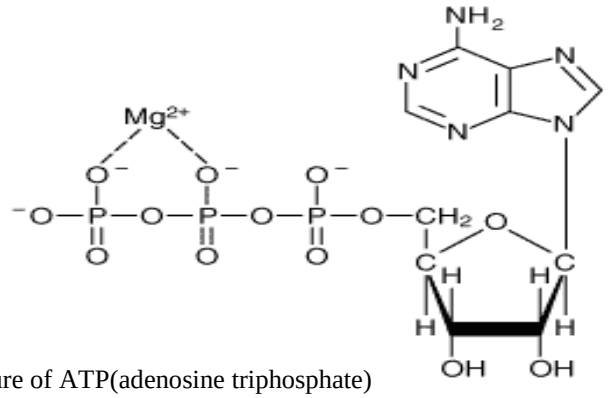


Figure 11-4: structure of ATP(adenosine triphosphate)

Glycosides are widely distributed in nature; the aglycone may be methanol, glycerol, a sterol, a phenol, or a base such as adenine. The glycosides that are important in medicine because of their action on the heart **(cardiac glycosides)** all contain steroids as the aglycone. These include derivatives of digitalis and strophanthus such as **ouabain,** an inhibitor of the Na^+-K^+ ATPase of cell membranes. Other glycosides include antibiotics such as **streptomycin.**

Deoxy Sugars Lack an Oxygen Atom:

Deoxy sugars are those in which one hydroxyl group has been replaced by hydrogen. An example is **deoxyribose** (Figure 14–9) in DNA. 2-deoxyglucose is used experimentally as an inhibitor of glucose

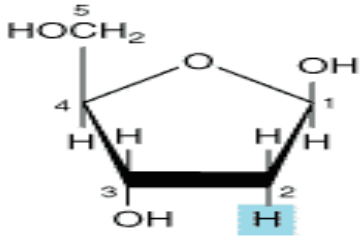


Figure 14–9: 2-Deoxy-D-ribofuranose (β-form).

Amino Sugars (Hexosamines) Are Components of Glycoproteins, Gangliosides, & Glycosaminoglycans :

The amino sugars include D-glucosamine, a constituent of hyaluronic acid (Figure 14–10), D-galactosamine (also known as chondrosamine), a constituent of chondroitin and D-mannosamine. Several **antibiotics** (eg, erythromycin) contain amino sugars, which are important for their antibiotic activity.

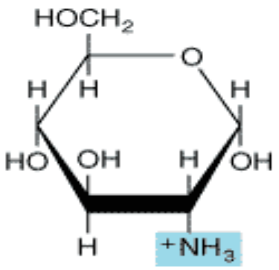


Figure 14–10: Glucosamine (2-amino-D-glucopyranose). Galactosamine is 2-amino-D-galactopyranose. Both glucosamine and galactosamine occur as *N*-acetyl derivatives in more complex carbohydrates, eg, glycoproteins.

Maltose, Sucrose, & Lactose Are Important Disaccharides:

The disaccharides are sugars composed of two monosaccharide residues linked by a glycoside bond (Figure 14–11). The physiologically important disaccharides are maltose, sucrose, and lactose (Table 14–4). Hydrolysis of sucrose yields a mixture of glucose and fructose called "invert sugar" because fructose is strongly levorotatory and changes (inverts) the weaker dextrarotatory action of sucrose.

Table 14–4. Disaccharides of Physiologic Importance.

Sugar	Composition	Source	Clinical Significance
Isomaltose	O-α-D-glucopyranosyl-(1→6)-α-D-glucopyranose	Enzymic hydrolysis of starch (the branch points in amylopectin)	
Maltose	O-α-D-glucopyranosyl-(1→4)-α-D-glucopyranose	Enzymic hydrolysis of starch (amylase); germinating cereals and malt	
Lactose	O-α-D-galactopyranosyl-(1→4)-β-D-glucopyranose	Milk (and many pharmaceutical preparations as a filler)	Lack of lactase (alactasia) leads to lactose intolerance—diarrhea and flatulence; may be excreted in the urine in pregnancy
Lactulose	O-α-D-galactopyranosyl-(1→4)-β-D-fructofuranose	Heated milk (small amounts), mainly synthetic	Not hydrolyzed by intestinal enzymes, but fermented by intestinal bacteria; used as a mild osmotic laxative
Sucrose	O-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside	Cane and beet sugar, sorghum and some fruits and vegetables	Rare genetic lack of sucrase leads to sucrose intolerance—diarrhea and flatulence
Trehalose	O-α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside	Yeasts and fungi; the main sugar of insect hemolymph	

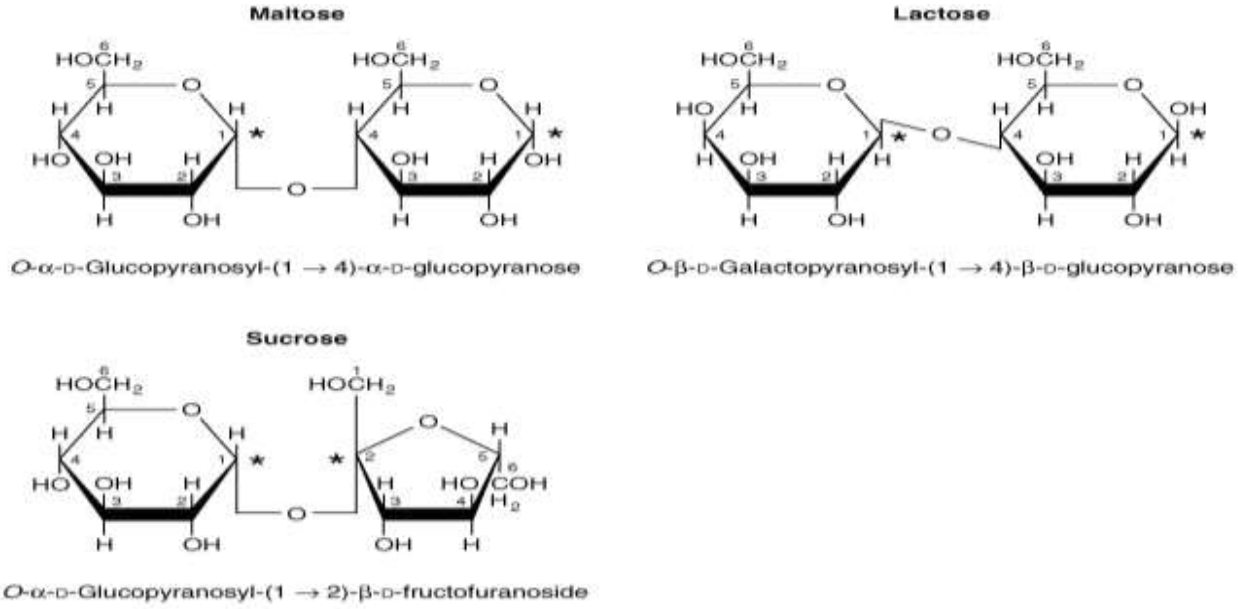


Figure 14–11: Structures of important disaccharides. The α and β refer to the configuration at the anomeric carbon atom (*). When the anomeric carbon of the second residue takes part in the formation of the glycosidic bond, as in sucrose, the residue becomes a glycoside known as a furanoside or a pyranoside. As the disaccharide no longer has an anomeric carbon with a free potential aldehyde or ketone group, it no longer exhibits reducing properties. The configuration of the β-fructofuranose residue in sucrose results from turning the β-fructofuranose molecule depicted in Figure 14–4 through 180 degrees and inverting it.

Polysaccharides Serve Storage & Structural Functions:

Polysaccharides include the following physiologically important carbohydrates:

Starch is a homopolymer of glucose forming an α-glucosidic chain, called a **glucosan** or **glucan.** It is the most important dietary source of carbohydrate in cereals, potatoes, legumes, and other vegetables. The two main constituents are **amylose** (13–20%), which has a nonbranching helical structure, and **amylopectin** (80–85%), which consists of branched chains composed of 24–30 glucose residues united by α1 → 4 linkages in the chains and by α1 → 6 linkages at the branch points (Figure 14–12).

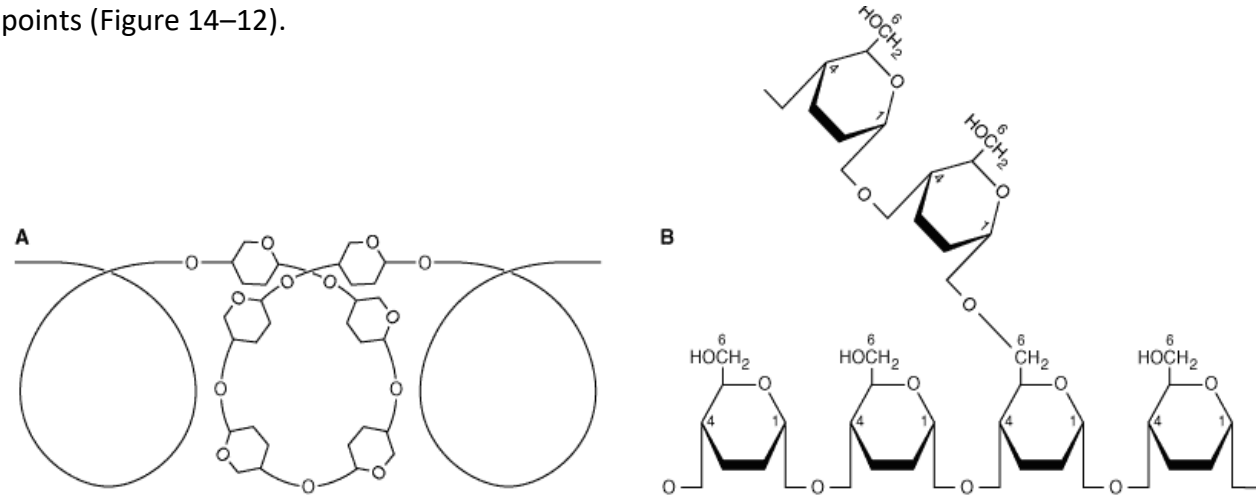


Figure 14–12: Structure of starch. **(A)** Amylose, showing helical coil structure. **(B)** Amylopectin, showing α 1 → 6 branch point.

The extent to which starch in foods is hydrolyzed by amylase is determined by its structure, the degree of crystallization or hydration (the result of cooking), and whether it is enclosed in intact (and indigestible) plant cells walls. The **glycemic index** of a starchy food is a measure of its digestibility, based on the extent to which it raises the blood concentration of glucose compared with an equivalent amount of glucose or a reference food such as white bread or boiled rice.

Glycogen (Figure 14–13) is the storage polysaccharide in animals and is sometimes called animal starch. It is a more highly branched structure than amylopectin with chains of 12–14 α-D-glucopyranose residues (in α1→4 glucosidic linkage) with branching by means of α1→6 glucosidic bonds.

Inulin is a polysaccharide of fructose (and hence a fructosan) found in tubers and roots of dahlias, artichokes, and dandelions. It is readily soluble in water and is used to determine the glomerular filtration rate, but it is not hydrolyzed by intestinal enzymes.

Dextrins are intermediates in the hydrolysis of starch.

Cellulose is the chief constituent of plant cell walls. It is insoluble and consists of β-D-glucopyranose units linked by β 1→4 bonds to form long, straight chains strengthened by cross-linking hydrogen bonds. Mammals lack any enzyme that hydrolyzes the β 1→4 bonds, and so cannot digest cellulose. It is an important source of "bulk" in the diet, and the major component of dietary fiber. Microorganisms in the gut of ruminants and other herbivores can hydrolyze the β linkage and ferment the products to short-chain fatty acids as a major energy source. There is some bacterial metabolism of cellulose in the human colon.

Chitin is a structural polysaccharide in the exoskeleton of crustaceans and insects, and also in mushrooms. It consists of *N*-acetyl-D-glucosamine units joined by β1→4 glycosidic bonds (Figure 14–14).

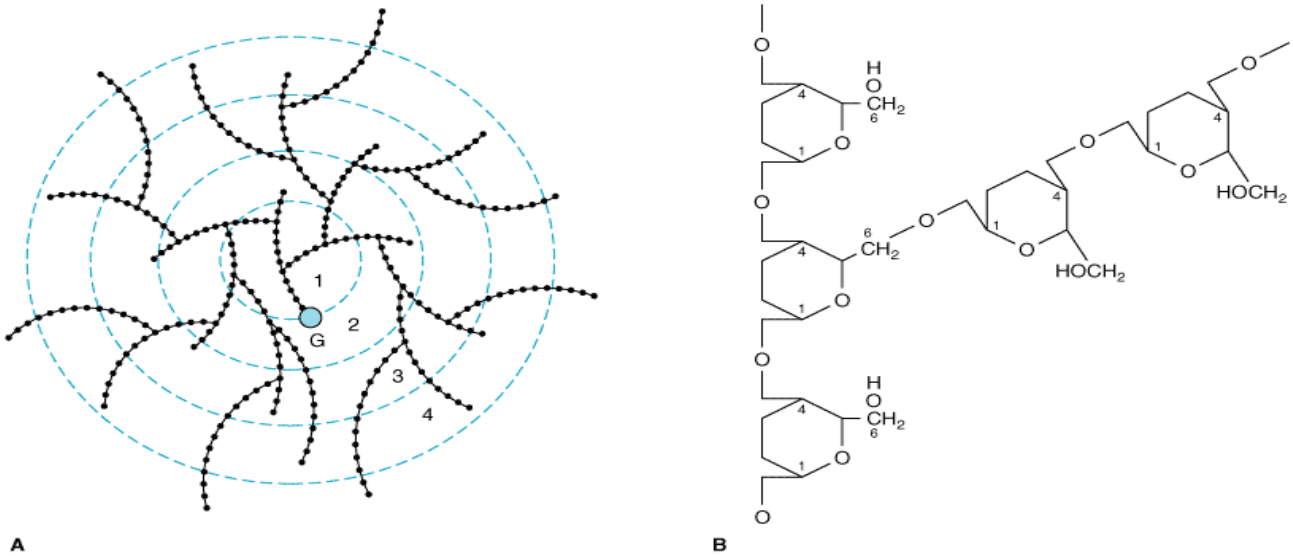


Figure 14–13: The glycogen molecule. **(A)** General structure. **(B)** Enlargement of structure at a branch point. The molecule is a sphere approximately 21 nm in diameter that can be seen in electron micrographs. It has a molecular mass

of 10^7 Da and consists of polysaccharide chains, each containing about 13 glucose residues. The chains are either branched or unbranched and are arranged in 12 concentric layers (only four are shown in the figure). The branched chains (each has two branches) are found in the inner layers and the unbranched chains in the outer layer. (G, glycogenin, the primer molecule for glycogen synthesis.)

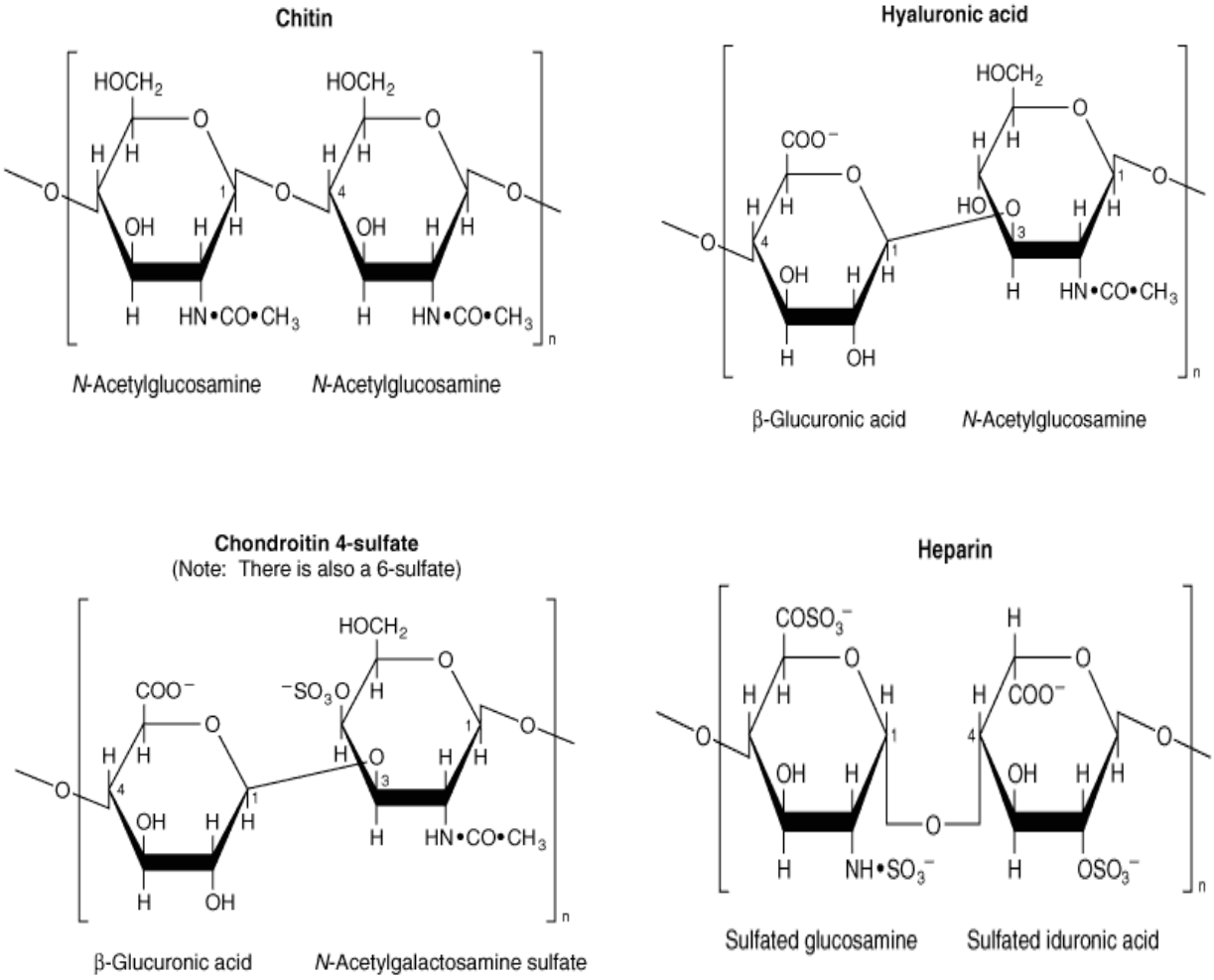


Figure 14–14: Structure of some complex polysaccharides and glycosaminoglycans.

Glycosaminoglycans (mucopolysaccharides) are complex carbohydrates containing **amino sugars** and **uronic acids.** They may be attached to a protein molecule to form a **proteoglycan.** Proteoglycans provide the ground or packing substance of connective tissue. They hold large quantities of water and occupy space, thus cushioning or lubricating other structures, because of the large number of —OH groups and negative charges on the molecule which, by repulsion, keep the carbohydrate chains apart. Examples are **hyaluronic acid, chondroitin sulfate,** and **heparin** (Figure 14–16).

Glycoproteins (also known as mucoproteins) are proteins containing branched or unbranched oligosaccharide chains (Table 14–5); they occur in cell membranes (Chapters 40 and 46) and many other situations; serum albumin is a glycoprotein. The **sialic acids** are *N*- or *O*-acyl derivatives of

neuraminic acid (Figure 14–16). **Neuraminic acid** is a nine-carbon sugar derived from mannosamine (an epimer of glucosamine) and pyruvate. Sialic acids are constituents of both **glycoproteins** and **gangliosides**. Gangliosides are also glycolipids.

Table 14–5. Carbohydrates Found in Glycoproteins.	
Hexoses	Mannose (Man), galactose (Gal)
Acetyl hexosamines	*N*-Acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc)
Pentoses	Arabinose (Ara), Xylose (Xyl)
Methyl pentose	L-Fucose (Fuc, see Fig. 14–15)
Sialic acids	*N*-Acyl derivatives of neuraminic acid; the predominant sialic acid is *N*-acetylneuraminic acid (NeuAc, see Fig. 14–16)

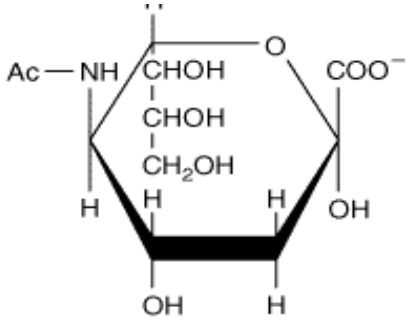


Figure 14–16: Structure of *N*-acetylneuraminic acid, a sialic acid (Ac = CH_3—CO—).

Lipids I

Biomedical Importance:

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, that are related more by their physical than by their chemical properties. They have the common property of being (1) relatively **insoluble in water** and (2) **soluble in nonpolar solvents** such as ether and chloroform. They are important dietary constituents not only because of their high energy value, but also because of the fat-soluble vitamins and the essential fatty acids contained in the fat of natural foods. Fat is stored in **adipose tissue,** where it also serves as a thermal insulator in the subcutaneous tissues and around certain organs. Nonpolar lipids act as **electrical insulators,** allowing rapid propagation of depolarization waves along **myelinated nerves.** Combinations of lipid and protein (lipoproteins) are important cellular constituents, occurring both in the cell **membrane** and in the mitochondria, and serving also as the means of **transporting lipids** in the blood. Knowledge of lipid biochemistry is necessary in understanding many important biomedical areas, eg, **obesity, diabetes mellitus, atherosclerosis,** and the role of various **polyunsaturated fatty acids** in nutrition and health.

Lipids Are Classified as Simple or Complex:

1. **Simple lipids:** Esters of fatty acids with various alcohols.

   a. **Fats:** Esters of fatty acids with glycerol. **Oils** are fats in the liquid state.

   b. **Waxes:** Esters of fatty acids with higher molecular weight monohydric alcohols.
2. **Complex lipids:** Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.

   a. **Phospholipids:** Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They frequently have nitrogen-containing bases and other substituents, eg, in **glycerophospholipids** the alcohol is glycerol and in **sphingophospholipids** the alcohol is sphingosine.

   b. **Glycolipids (glycosphingolipids):** Lipids containing a fatty acid, sphingosine, and carbohydrate.

   c. **Other complex lipids:** Lipids such as sulfolipids and aminolipids. Lipoproteins may also be placed in this category.
3. **Precursor and derived lipids:** These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies (Chapter 22), hydrocarbons, lipid-soluble vitamins, and hormones.

Because they are uncharged, acylglycerols (glycerides), cholesterol, and cholesteryl esters are termed **neutral lipids.**

Fatty Acids Are Aliphatic Carboxylic Acids:

Fatty acids occur mainly as esters in natural fats and oils but do occur in the unesterified form as **free fatty acids,** a transport form found in the plasma. Fatty acids that occur in natural fats are usually straight-chain derivatives containing an even number of carbon atoms. The chain may be **saturated** (containing no double bonds) or **unsaturated** (containing one or more double bonds).

Fatty Acids Are Named after Corresponding Hydrocarbons:

The most frequently used systematic nomenclature names the fatty acid after the hydrocarbon with the same number and arrangement of carbon atoms, with **-oic** being substituted for the final **-e** (Genevan system). Thus, saturated acids end in **-anoic,** eg, octanoic acid, and unsaturated acids with double bonds end in **-enoic,** eg, octadecenoic acid (oleic acid).

Carbon atoms are numbered from the carboxyl carbon (carbon No. 1). The carbon atoms adjacent to the carboxyl carbon (Nos. 2, 3, and 4) are also known as the α, β, and γ carbons, respectively, and the terminal methyl carbon is known as the ω or n-carbon.

Various conventions use Δ for indicating the number and position of the double bonds (Figure 15–1); eg, Δ^9 indicates a double bond between carbons 9 and 10 of the fatty acid; ω9 indicates a double bond on the ninth carbon counting from the ω-carbon. In animals, additional double bonds are introduced only between the existing double bond (eg, ω9, ω6, or ω3) and the carboxyl carbon, leading to three series of fatty acids known as the ω9, ω6, and ω3 families, respectively.

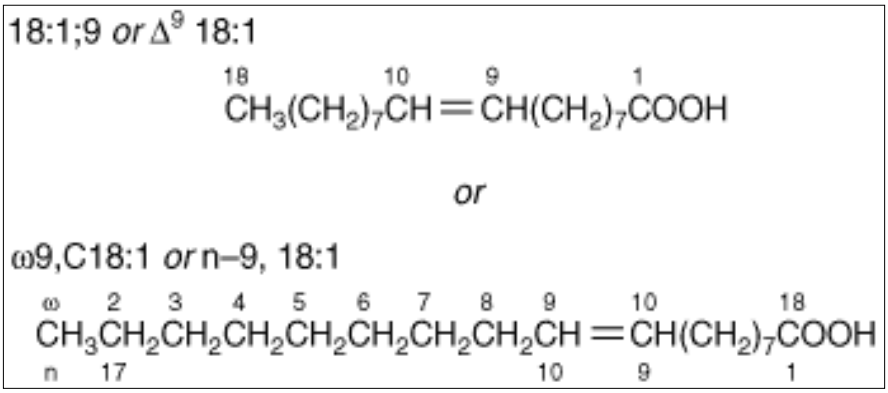


Figure 15–1: Oleic acid. n - 9 (n minus 9) is equivalent to ω9.

Saturated Fatty Acids Contain No Double Bonds:

Saturated fatty acids may be envisaged as based on acetic acid (CH_3 —COOH) as the first member of the series in which —CH_2 — is progressively added between the terminal CH_3 — and —COOH groups. Examples are shown in Table 15–1. Other higher members of the series are known to occur, particularly in waxes. A few branched-chain fatty acids have also been isolated from both plant and animal sources.

Table 15–1. Saturated Fatty Acids.		
Common Name	**Number of C Atoms**	
Acetic	2	Major end product of carbohydrate fermentation by rumen organisms
Butyric	4	In certain fats in small amounts (especially butter). An end product of carbohydrate fermentation by rumen organisms[1]
Valeric	5	
Caproic	6	
Lauric	12	Spermaceti, cinnamon, palm kernel, coconut oils, laurels, butter
Myristic	14	Nutmeg, palm kernel, coconut oils, myrtles, butter
Palmitic	16	Common in all animal and plant fats
Stearic	18	

Unsaturated Fatty Acids Contain One or More Double Bonds:

Unsaturated fatty acids (Table 15–2) may be further subdivided as follows:

Table 15–2. Unsaturated Fatty Acids of Physiologic and Nutritional Significance.				
Number of C Atoms and Number and Position of Double Bonds	**Family**	**Common Name**	**Systematic Name**	**Occurrence**
Monoenoic acids (one double bond)				
16:1;9	ω7	Palmitoleic	cis-9-Hexadecenoic	In nearly all fats.
18:1;9	ω9	Oleic	cis-9-Octadecenoic	Possibly the most common fatty acid in natural fats.
18:1;9	ω9	Elaidic	trans-9-Octadecenoic	Hydrogenated and ruminant fats.
Dienoic acids (two double bonds)				
18:2;9,12	ω6	Linoleic	all-cis-9,12-Octadecadienoic	Corn, peanut, cottonseed, soybean, and many plant oils.
Trienoic acids (three double bonds)				
18:3;6,9,12	ω6	γ-Linolenic	all-cis-6,9,12-Octadecatrienoic	Some plants, eg, oil of evening primrose, borage oil; minor fatty acid in animals.
18:3;9,12,15	ω3	α-Linolenic	all-cis-9,12,15-Octadecatrienoic	Frequently found with linoleic acid but particularly in linseed oil.
Tetraenoic acids (four double bonds)				
20:4;5,8,11,14	ω6	Arachidonic	all-cis-5,8,11,14-Eicosatetraenoic	Found in animal fats; important component of phospholipids in animals.
Pentaenoic acids (five double bonds)				
20:5;5,8,11,14,17	ω3	Timnodonic	all-cis-5,8,11,14,17-Eicosapentaenoic	Important component of fish oils, eg, cod liver, mackerel, menhaden, salmon oils.
Hexaenoic acids (six double bonds)				
22:6;4,7,10,13,16,19	ω3	Cervonic	all-cis-4,7,10,13,16,19-Docosahexaenoic	Fish oils, phospholipids in brain.

(1) Monounsaturated (monoethenoid, monoenoic) acids, containing one double bond.
(2) Polyunsaturated (polyethenoid, polyenoic) acids, containing two or more double bonds.
(3) Eicosanoids: These compounds, derived from eicosa (20-carbon) polyenoic fatty acids, comprise the **prostanoids, leukotrienes (LTs),** and **lipoxins (LXs).** Prostanoids include **prostaglandins (PGs), prostacyclins (PGIs),** and **thromboxanes (TXs).**

Prostaglandins exist in virtually every mammalian tissue, acting as local hormones; they have important physiologic and pharmacologic activities. They are synthesized in vivo by cyclization of the center of the carbon chain of 20-carbon (eicosanoic) polyunsaturated fatty acids (eg, arachidonic acid) to form a cyclopentane ring (Figure 15–2). A related series of compounds, the **thromboxanes,** have the cyclopentane ring interrupted with an oxygen atom (oxane ring) (Figure 15–3). Three different eicosanoic fatty acids give rise to three groups of eicosanoids characterized by the number of double bonds in the side chains, eg, PG_1, PG_2, PG_3. Different substituent groups attached to the rings give rise to series of prostaglandins and thromboxanes, labeled A, B, etc—eg, the "E" type of prostaglandin (as in PGE_2) has a keto group in position 9, whereas the "F" type has a hydroxyl group in this position. The **leukotrienes** and **lipoxins** are a third group of eicosanoid derivatives formed via the lipoxygenase pathway (Figure 15–4). They are characterized by the presence of three or four conjugated double bonds, respectively. Leukotrienes cause bronchoconstriction as well as being potent proinflammatory agents and play a part in **asthma.**

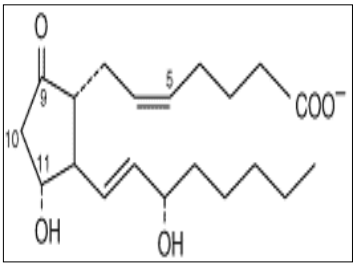


Figure 15–2: Prostaglandin E_2 (PGE_2).

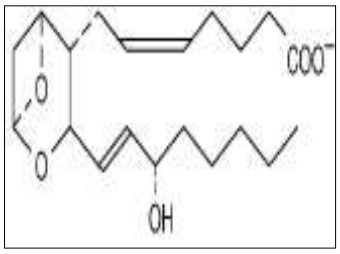


Figure 15–3: Thromboxane A_2 (TXA_2).

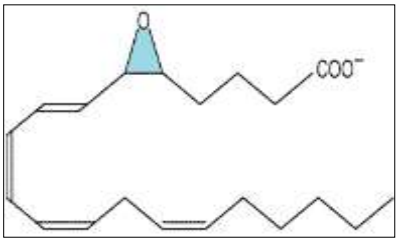


Figure 15–4: Leukotriene A4(LTA4).

Most Naturally Occurring Unsaturated Fatty Acids Have Cis Double Bonds:

The carbon chains of saturated fatty acids form a zigzag pattern when extended, as at low temperatures. At higher temperatures, some bonds rotate, causing chain shortening, which explains why biomembranes become thinner with increases in temperature. A type of **geometric isomerism** occurs in unsaturated fatty acids, depending on the orientation of atoms or groups around the axes of double bonds, which do not allow rotation. If the acyl chains are on the same side of the bond, it is *cis-,* as in oleic acid; if on opposite sides, it is *trans-,* as in elaidic acid, the *trans* isomer of oleic acid (Figure 15–5). Naturally occurring unsaturated long-chain fatty acids are nearly all of the *cis* configuration, the molecules being "bent" 120 degrees at the double bond. Thus, oleic acid has an L shape, whereas elaidic acid remains "straight." Increase in the number of *cis* double bonds in a fatty acid leads to a variety of possible spatial configurations of the molecule—eg, arachidonic acid, with four *cis* double bonds, has "kinks" or a U shape. This has profound significance for molecular packing in membranes and on the positions occupied by fatty acids in more complex molecules such as phospholipids. *Trans* double bonds alter these spatial relationships. *Trans* fatty acids are present in certain foods, arising as a by-product of the saturation of fatty acids during hydrogenation, or "hardening," of natural oils in the

manufacture of margarine. An additional small contribution comes from the ingestion of ruminant fat that contains *trans* fatty acids arising from the action of microorganisms in the rumen.

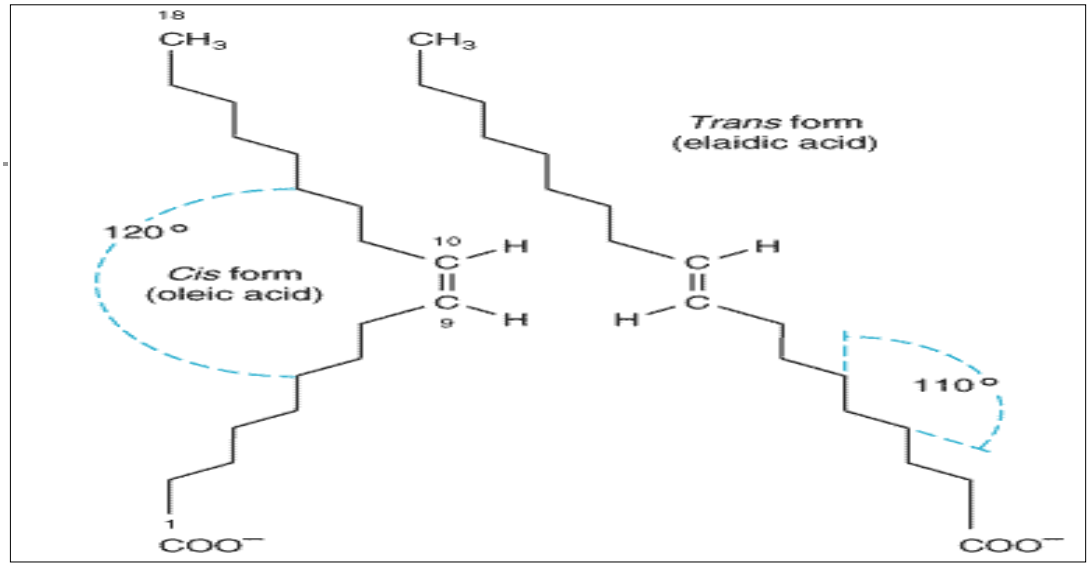


Figure 15–5: Geometric isomerism of Δ^9, 18:1 fatty acids (oleic and elaidic acids).

Physical and Physiologic Properties of Fatty Acids Reflect Chain Length and Degree of Unsaturation:

The melting points of even-numbered carbon fatty acids increase with chain length and decrease according to unsaturation. A triacylglycerol containing three saturated fatty acids of 12 carbons or more is solid at body temperature, whereas if the fatty acid residues are 18:2, it is liquid to below 0 °C. In practice, natural acylglycerols contain a mixture of fatty acids tailored to suit their functional roles. The membrane lipids, which must be fluid at all environmental temperatures, are more unsaturated than storage lipids. Lipids in tissues that are subject to cooling, eg, in hibernators or in the extremities of animals, are more unsaturated.

Triacylglycerols (Triglycerides) Are the Main Storage Forms of Fatty Acids* :

The triacylglycerols (Figure 15–6) are esters of the trihydric alcohol glycerol and fatty acids. Mono- and diacylglycerols, wherein one or two fatty acids are esterified with glycerol, are also found in the tissues. These are of particular significance in the synthesis and hydrolysis of triacylglycerols.

* According to the Standardized Terminology of the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB), the Monoglycerides, Diglycerides, and Triglycerides Should Be Designated Monoacylglycerols, Diacylglycerols, and Triacylglycerols, Respectively. However, the Older Terminology Is Still Widely Used, Particularly in Clinical Medicine.

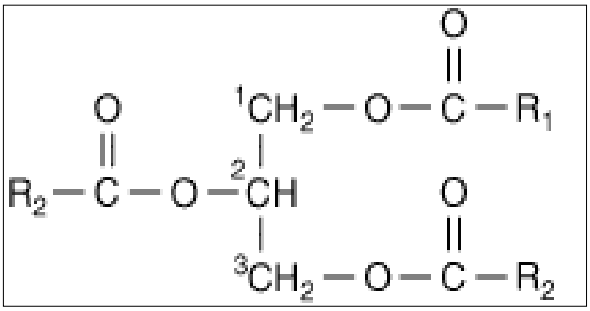


Figure 15–6: Triacylglycerol.

Carbons 1 & 3 of Glycerol Are Not Identical :

To number the carbon atoms of glycerol unambiguously, the -*sn* (stereochemical numbering) system is used. It is important to realize that carbons 1 and 3 of glycerol are not identical when viewed in three dimensions (shown as a projection formula in Figure 15–7). Enzymes readily distinguish between them and are nearly always specific for one or the other carbon; eg, glycerol is always phosphorylated on *sn*-3 by glycerol kinase to give glycerol 3-phosphate and not glycerol 1-phosphate.

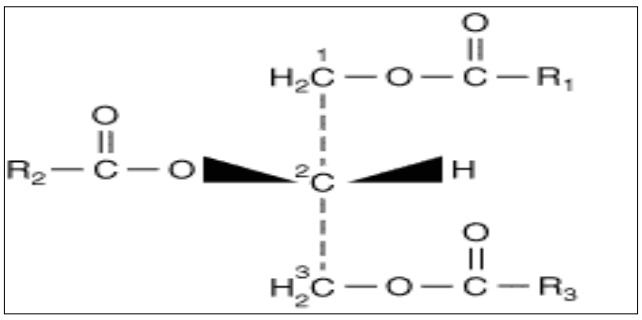
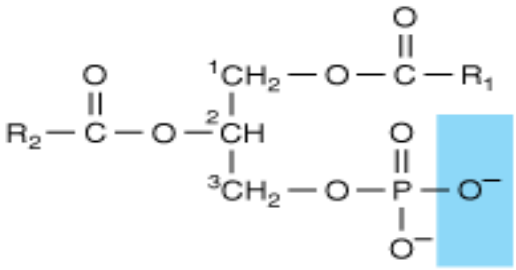


Figure 15–7: Triacyl-*sn*-glycerol.

Lipid II

Phospholipids Are the Main Lipid Constituents of Membranes :

Phospholipids may be regarded as derivatives of **phosphatidic acid** (Figure 15–8), in which the phosphate is esterified with the —OH of a suitable alcohol. Phosphatidic acid is important as an intermediate in the synthesis of triacylglycerols as well as phosphoglycerols but is not found in any great quantity in tissues.



Phosphatidic acid

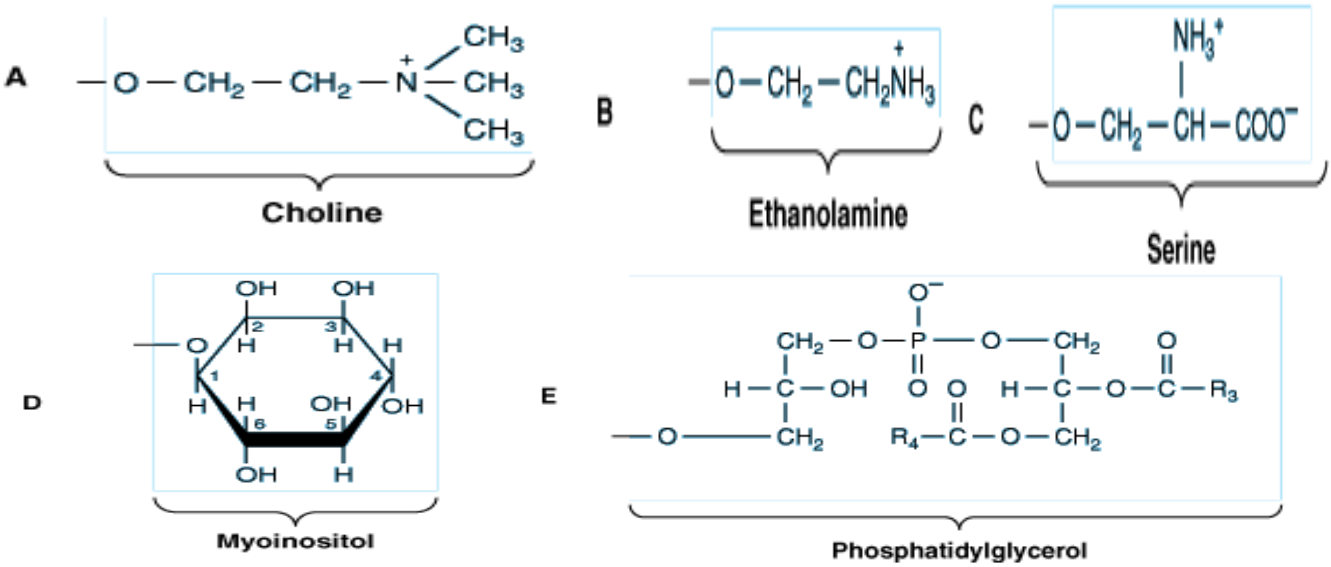


Figure 15–8: Phosphatidic acid and its derivatives. The O^- shown shaded in phosphatidic acid is substituted by the substituents shown to form in **(A)** 3-phosphatidylcholine, **(B)** 3-phosphatidylethanolamine, **(C)** 3-phosphatidylserine, **(D)** 3-phosphatidylinositol, and **(E)** cardiolipin (diphosphatidylglycerol).

Phosphatidylcholines (Lecithins) Occur in Cell Membranes :

Phosphoacylglycerols containing choline (Figure 15–8) are the most abundant phospholipids of the cell membrane and represent a large proportion of the body's store of choline. Choline is important in nervous transmission, as acetylcholine, and as a store of labile methyl groups. **Dipalmitoyl lecithin** is a very effective surface-

active agent and a major constituent of the **surfactant** preventing adherence, due to surface tension, of the inner surfaces of the lungs. Its absence from the lungs of premature infants causes **respiratory distress syndrome.** Most phospholipids have a saturated acyl radical in the *sn*-1 position but an unsaturated radical in the *sn*-2 position of glycerol.

Phosphatidylethanolamine (cephalin) and **phosphatidylserine**: (found in most tissues) differ from phosphatidylcholine only in that ethanolamine or serine, respectively, replaces choline (Figure 15–8).

Phosphatidylinositol Is a Precursor of Second Messengers :

The inositol is present in **phosphatidylinositol** as the stereoisomer, myoinositol (Figure 15–8). **Phosphatidylinositol 4,5-bisphosphate** is an important constituent of cell membrane phospholipids; upon stimulation by a suitable hormone agonist, it is cleaved into **diacylglycerol** and **inositol trisphosphate,** both of which act as internal signals or second messengers. **Cardiolipin:** Is a Major Lipid of Mitochondrial Membranes, Phosphatidic acid is a precursor of **phosphatidylglycerol** which, in turn, gives rise to **cardiolipin** (Figure 15–8).

Lysophospholipids Are Intermediates in the Metabolism of Phosphoglycerols :

These are phosphoacylglycerols containing only one acyl radical, eg, **lysophosphatidylcholine (lysolecithin),** important in the metabolism and interconversion of phospholipids (Figure 15–9). It is also found in oxidized lipoproteins and has been implicated in some of their effects in promoting **atherosclerosis.**

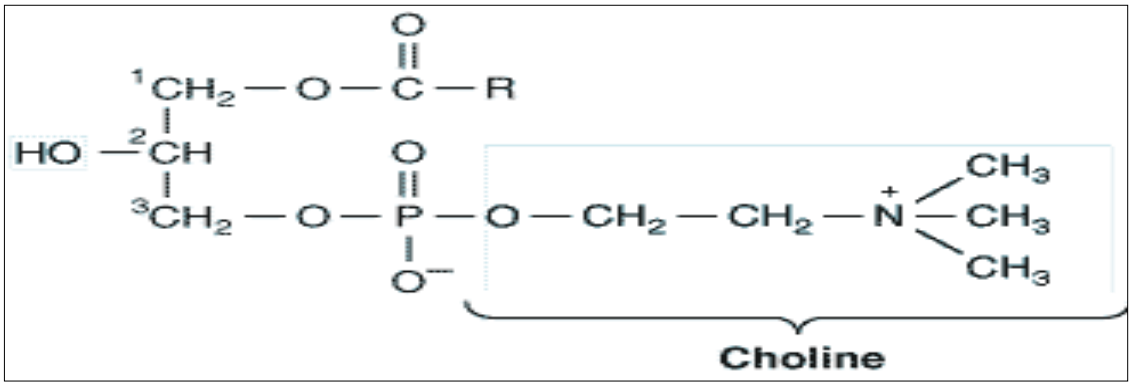


Figure 15–9: Lysophosphatidylcholine (lysolecithin).

Plasmalogens Occur in Brain & Muscle:

These compounds constitute as much as 10% of the phospholipids of brain and muscle. Structurally, the plasmalogens resemble phosphatidylethanolamine but

possess an ether link on the *sn*-1 carbon instead of the ester link found in acylglycerols. Typically, the alkyl radical is an unsaturated alcohol (Figure 15–10). In some instances, choline, serine, or inositol may be substituted for ethanolamine.

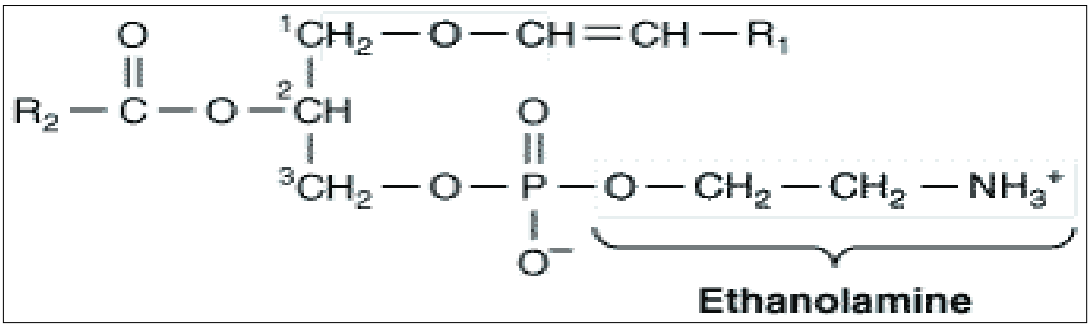


Figure 15–10: Plasmalogen

Sphingomyelins Are Found in the Nervous System :

Sphingomyelins are found in large quantities in brain and nerve tissue. On hydrolysis, the sphingomyelins yield a fatty acid, phosphoric acid, choline, and a complex amino alcohol, **sphingosine** (Figure 15–11). No glycerol is present. The combination of sphingosine plus fatty acid is known as **ceramide,** a structure also found in the glycosphingolipids (see below).

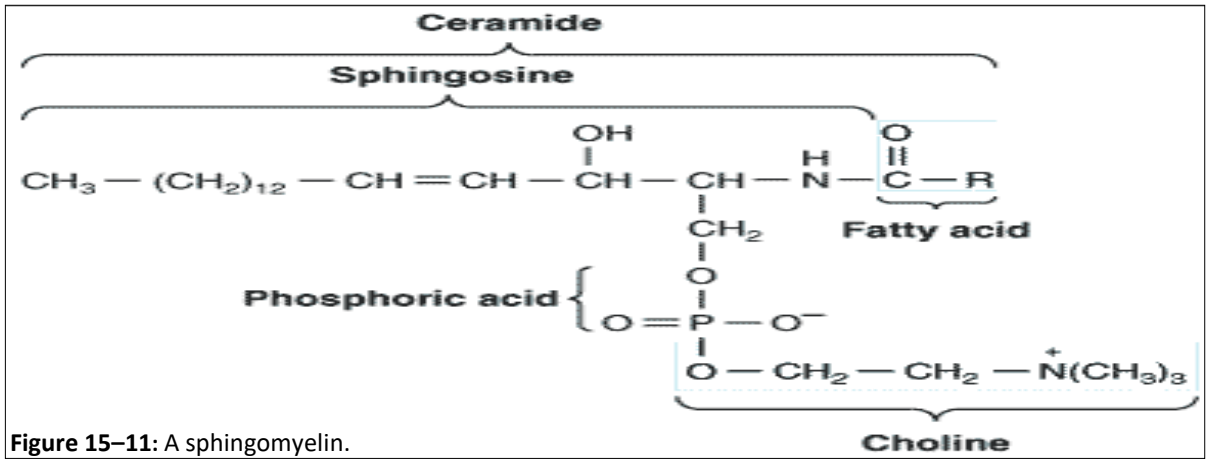


Figure 15–11: A sphingomyelin.

Glycolipids (Glycosphingolipids) Are Important in Nerve Tissues & in the Cell Membrane :

Glycolipids are widely distributed in every tissue of the body, particularly in nervous tissue such as brain. They occur particularly in the outer leaflet of the plasma membrane, where they contribute to **cell surface carbohydrates.**

The major glycolipids found in animal tissues are glycosphingolipids. They contain ceramide and one or more sugars. **Galactosylceramide** is a major glycosphingolipid of brain and other nervous tissue, found in relatively low amounts elsewhere. It

contains a number of characteristic C_{24} fatty acids, eg, cerebronic acid. Galactosylceramide (Figure 15–12) can be converted to sulfogalactosylceramide **(sulfatide),** present in high amounts in myelin. Glucosylceramide is the predominant simple glycosphingolipid of extraneural tissues, also occurring in the brain in small amounts. **Gangliosides** are complex glycosphingolipids derived from glucosylceramide that contain in addition one or more molecules of a **sialic acid.** Neuraminic acid (NeuAc; see Chapter 14) is the principal sialic acid found in human tissues. Gangliosides are also present in nervous tissues in high concentration. They appear to have receptor and other functions. The simplest ganglioside found in tissues is G_{M3}, which contains ceramide, one molecule of glucose, one molecule of galactose, and one molecule of NeuAc. In the shorthand nomenclature used, G represents ganglioside; M is a monosialo-containing species; and the subscript 3 is a number assigned on the basis of chromatographic migration. G_{M1} (Figure 15–13), a more complex ganglioside derived from G_{M3}, is of considerable biologic interest, as it is known to be the receptor in human intestine for cholera toxin. Other gangliosides can contain anywhere from one to five molecules of sialic acid, giving rise to di-, trisialogangliosides, etc.

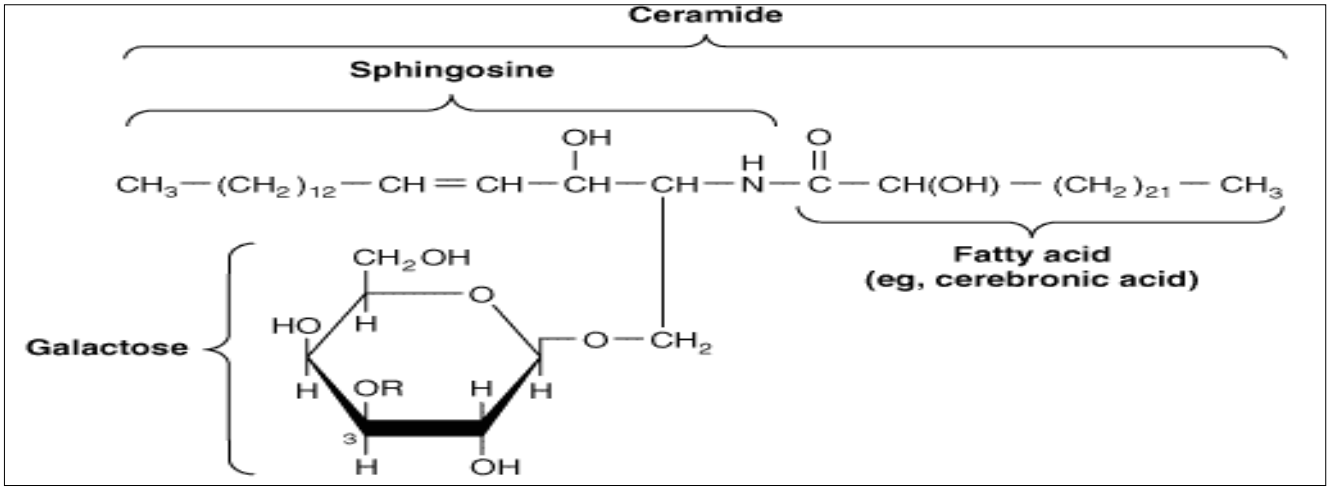


Figure 15–12: galactosylceramide (galactocerebroside, and sulfogalactosylceramide (a sulfatide, R = SO_4^{2-}).

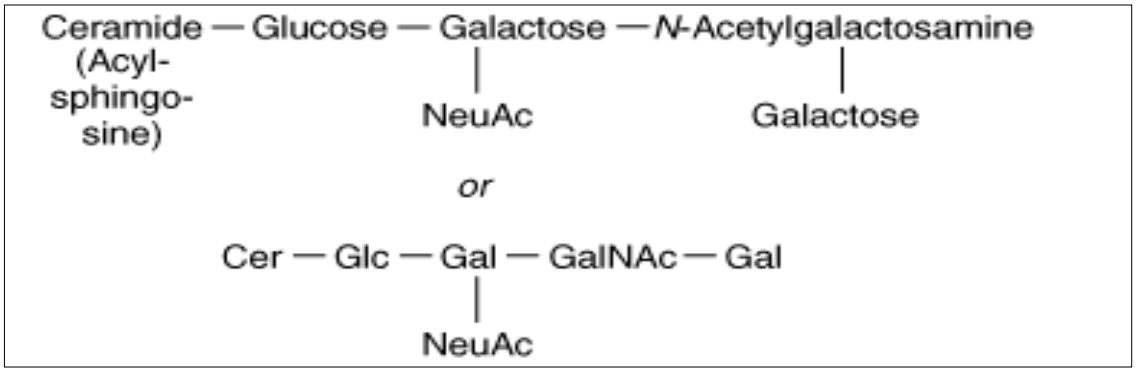


Figure 15–13: G_{M1} ganglioside, a monosialoganglioside, the receptor in human intestine for cholera toxin.

Lipid III

Steroids Play Many Physiologically Important Roles

Cholesterol is probably the best known steroid because of its association with **atherosclerosis** and heart disease. However, biochemically it is also of significance because it is the precursor of a large number of equally important steroids that include the bile acids, adrenocortical hormones, sex hormones, D vitamins, cardiac glycosides, sitosterols of the plant kingdom, and some alkaloids.

All steroids have a similar cyclic nucleus resembling phenanthrene (rings A, B, and C) to which a cyclopentane ring (D) is attached. The carbon positions on the steroid nucleus are numbered as shown in Figure 15–14. It is important to realize that in structural formulas of steroids, a simple hexagonal ring denotes a completely saturated six-carbon ring with all valences satisfied by hydrogen bonds unless shown otherwise; ie, it is not a benzene ring. All double bonds are shown as such. Methyl side chains are shown as single bonds unattached at the farther (methyl) end. These occur typically at positions 10 and 13 (constituting C atoms 19 and 18). A side chain at position 17 is usual (as in cholesterol). If the compound has one or more hydroxyl groups and no carbonyl or carboxyl groups, it is a **sterol,** and the name terminates in -ol.

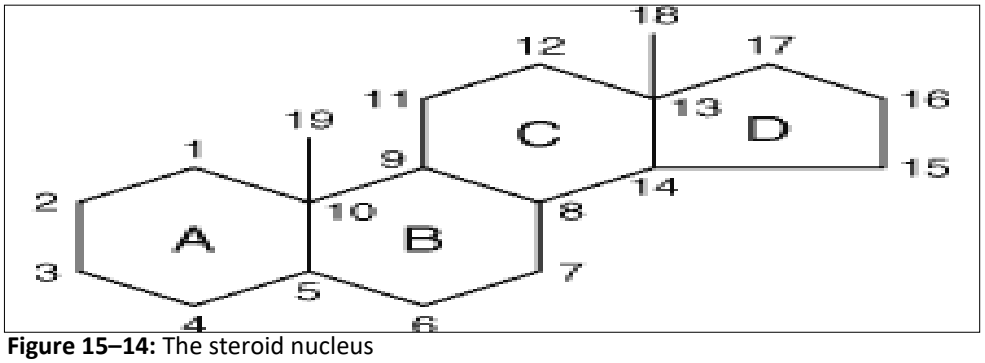


Figure 15–14: The steroid nucleus

Cholesterol Is a Significant Constituent of Many Tissues:

Cholesterol (Figure 15–17) is widely distributed in all cells of the body but particularly in nervous tissue. It is a major constituent of the plasma membrane and of plasma lipoproteins. It is often found as **cholesteryl**

ester, where the hydroxyl group on position 3 is esterified with a long-chain fatty acid. It occurs in animals but not in plants or bacteria.

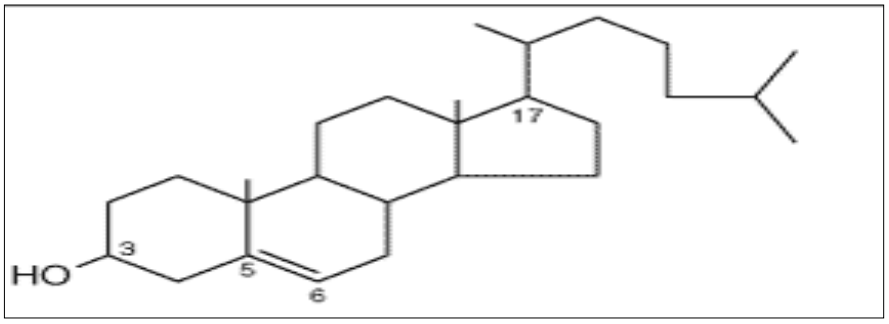


Figure 15–17: Cholesterol, 3-hydroxy-5,6-cholestene

Ergosterol Is a Precursor of Vitamin D:

Ergosterol occurs in plants and yeast and is important as a precursor of vitamin D (Figure 15–18). When irradiated with ultraviolet light, it acquires antirachitic properties consequent to the opening of ring B.

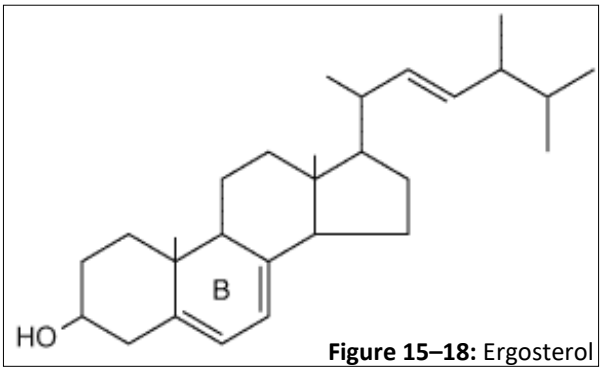


Figure 15–18: Ergosterol

Polyprenoids Share the Same Parent Compound as Cholesterol:

Although not steroids, these compounds are related because they are synthesized,like cholesterol (Figure 26–2), from five-carbon isoprene units (Figure 15–19). They include **ubiquinone** (Chapter 13), which participates in the respiratory chain in mitochondria, and the long-chain alcohol **dolichol** (Figure 15–20), which takes part in glycoprotein synthesis by transferring carbohydrate residues to asparagine residues of the polypeptide (Chapter 46). Plant-derived isoprenoid compounds include rubber, camphor, the fat-soluble vitamins A, D, E, and K, and β-carotene (provitamin A).

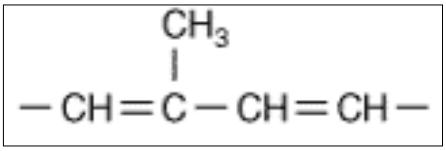


Figure 15–19: Isoprene unit

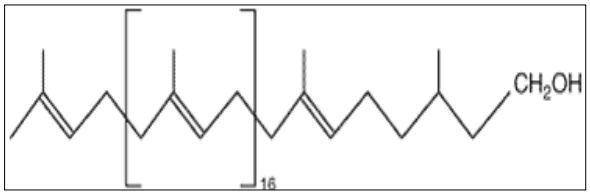


Figure 15–20: Dolichol—a C_{95} alcohol

Lipid Peroxidation Is a Source of Free Radicals:

Peroxidation **(auto-oxidation)** of lipids exposed to oxygen is responsible not only for deterioration of foods **(rancidity)** but also for damage to tissues in vivo, where it may be a cause of cancer, inflammatory diseases, atherosclerosis, and aging. The deleterious effects are considered to be caused by free radicals ($ROO^{\bullet}$, $RO^{\bullet}$, $OH^{\bullet}$) produced during peroxide formation from fatty acids containing methylene-interrupted double bonds, ie, those found in the naturally occurring polyunsaturated fatty acids (Figure 15–21). Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation. The whole process can be depicted as follows:

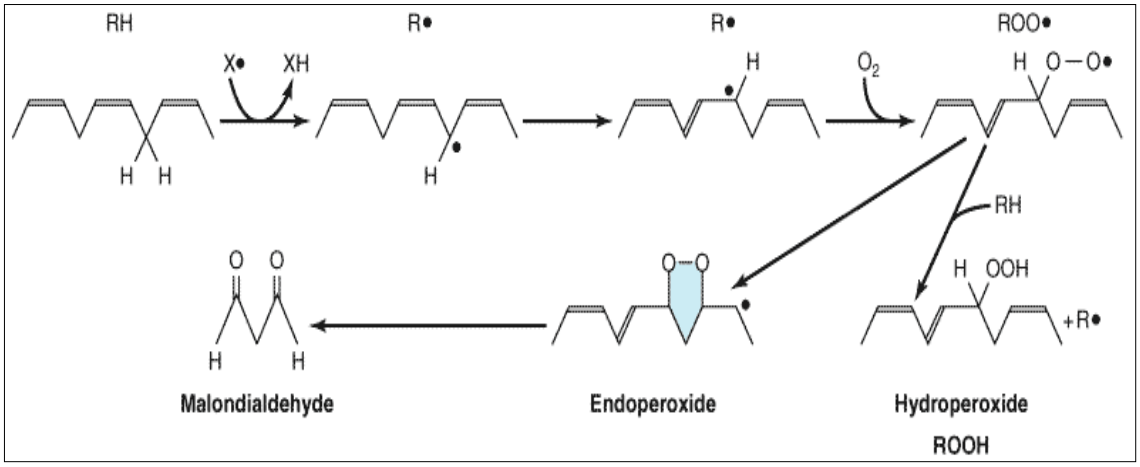


Figure 15–21: Lipid peroxidation. The reaction is initiated by an existing free radical (X•), by light, or by metal ions. Malondialdehyde is only formed by fatty acids with three or more double bonds and is used as a measure of lipid peroxidation together with ethane from the terminal two carbons of ω3 fatty acids and pentane from the terminal five carbons of ω6 fatty acids.

(1) Initiation:

$$ROOH + Metal^{(n)+} \rightarrow ROO^{\bullet} + Metal^{(n-1)+} + H^{+}$$

$$X^{\bullet} + RH \rightarrow R^{\bullet} + XH$$

(2) Propagation:

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}, \text{ etc}$$

(3) Termination:

$$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOR + O_2$$

$$ROO^{\bullet} + R^{\bullet} \rightarrow ROOR$$

$$R^{\bullet} + R^{\bullet} \rightarrow RR$$

Since the molecular precursor for the initiation process is generally the hydroperoxide product ROOH, lipid peroxidation is a chain reaction with potentially devastating effects. To control and reduce lipid peroxidation, both humans in their activities and nature invoke the use of **antioxidants.** Propyl gallate, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are antioxidants used as food additives. Naturally occurring antioxidants include vitamin E (tocopherol), which is lipid-soluble, and urate and vitamin C, which are water-soluble. Beta-carotene is an antioxidant at low PO_2. Antioxidants fall into two classes: (1) preventive antioxidants, which reduce the rate of chain initiation; and (2) chain-breaking antioxidants, which interfere with chain propagation. Preventive antioxidants include catalase and other peroxidases such as glutathione peroxidase that react with ROOH; selenium, which is an essential component of glutathione peroxidase and regulates its activity and chelators of metal ions such as EDTA (ethylenediaminetetraacetate) and DTPA (diethylenetriaminepentaacetate). In vivo, the principal chain-breaking antioxidants are superoxide dismutase, which acts in the aqueous phase to trap superoxide free radicals (O_2-); urate; and vitamin E, which acts in the lipid phase to trap ROO• radicals (Figure 44–6).

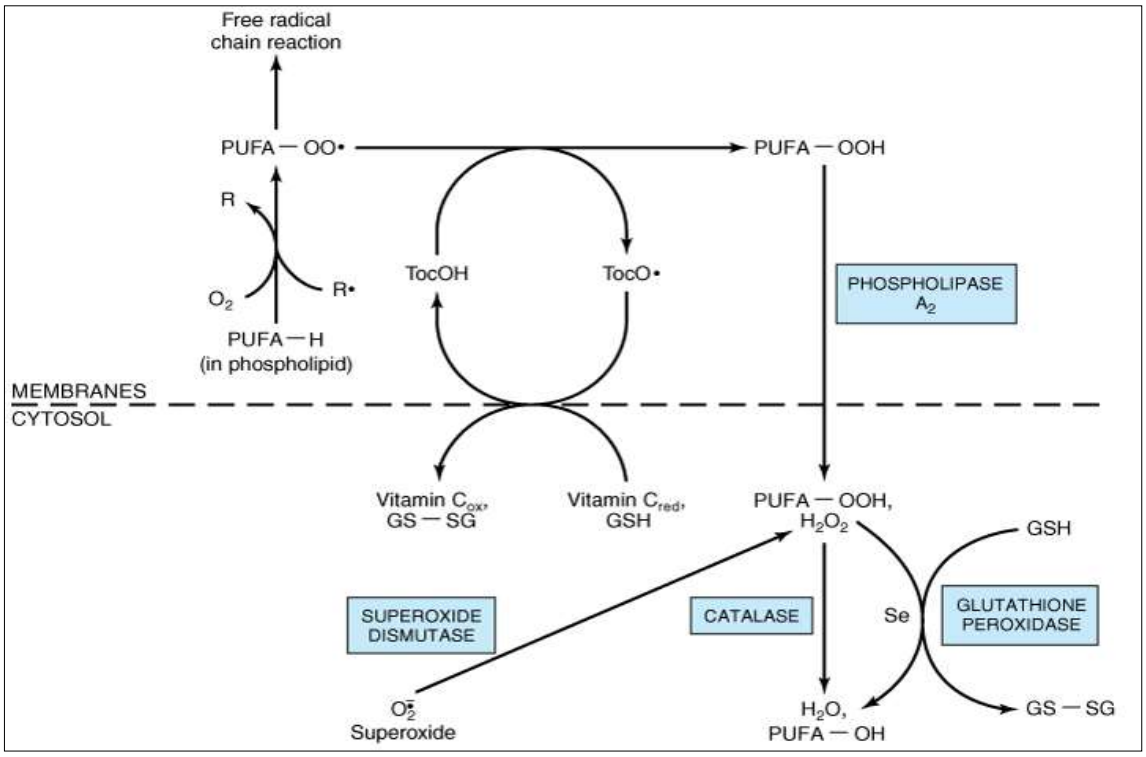


Figure 44–6: Interaction between antioxidants in the lipid phase (cell membranes) and the aqueous phase (cytosol). (R•, free radical; PUFA-OO•, peroxyl radical of polyunsaturated fatty acid in membrane phospholipid; PUFA-OOH, hydroxyperoxy polyunsaturated fatty acid in membrane phospholipid, released into the cytosol as hydroxyperoxy polyunsaturated fatty acid by the action of

phospholipase A_2; PUFA-OH, hydroxy polyunsaturated fatty acid; Toc-OH vitamin E (α-tocopherol); TocO•, tocopheroxyl radical; Se, selenium; SSH, reduced glutathione; GS-SG, oxidized glutathione, which is reduced to GSH after reaction with NADPH, catalyzed by glutathione reductase; PUFA-H, polyunsaturated fatty acid).

Peroxidation is also catalyzed in vivo by heme compounds and by **lipoxygenases** found in platelets and leukocytes. Other products of auto-oxidation or enzymic oxidation of physiologic significance include **oxysterols** (formed from cholesterol) and **isoprostanes** (prostanoids).

Amphipathic Lipids Self-Orient at Oil:Water Interfaces

They Form Membranes, Micelles, Liposomes, & Emulsions:

In general, lipids are insoluble in water since they contain a predominance of nonpolar (hydrocarbon) groups. However, fatty acids, phospholipids, sphingolipids, bile salts, and, to a lesser extent, cholesterol contain polar groups. Therefore, part of the molecule is **hydrophobic,** or water-insoluble; and part is **hydrophilic,** or water-soluble. Such molecules are described as **amphipathic** (Figure 15–22). They become oriented at oil:water interfaces with the polar group in the water phase and the nonpolar group in the oil phase. A bilayer of such amphipathic lipids is the basic structure in biologic membranes (Chapter 40). When a critical concentration of these lipids is present in an aqueous medium, they form **micelles.** Liposomes may be formed by sonicating an amphipathic lipid in an aqueous medium. They consist of spheres of lipid bilayers that enclose part of the aqueous medium. Aggregations of bile salts into micelles and liposomes and the formation of mixed micelles with the products of fat digestion are important in facilitating absorption of lipids from the intestine. Liposomes are of potential clinical use—particularly when combined with tissue-specific antibodies—as carriers of drugs in the circulation, targeted to specific organs, eg, in cancer therapy. In addition, they are used for gene transfer into vascular cells and as carriers for topical and transdermal delivery of drugs and cosmetics. **Emulsions** are much larger particles, formed usually by nonpolar lipids in an aqueous medium. These are stabilized by emulsifying agents such as amphipathic lipids (eg, lecithin), which form a surface layer separating the main bulk of the nonpolar material from the aqueous phase (Figure 15–22).

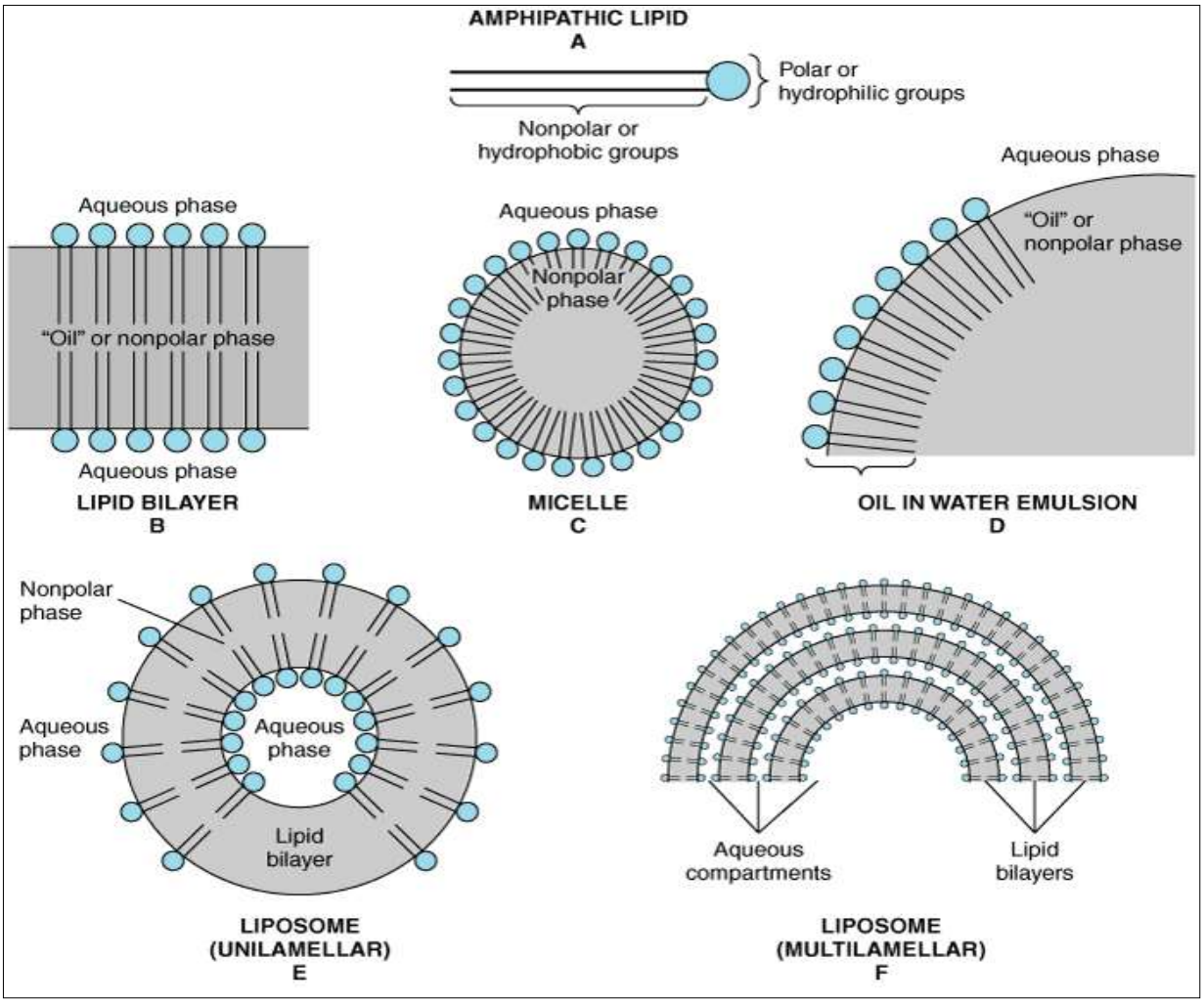


Figure 15–22: Formation of lipid membranes, micelles, emulsions, and liposomes from amphipathic lipids, eg, phospholipids

The Diversity of the Endocrine System I

Biomedical Importance:

The survival of multicellular organisms depends on their ability to adapt to a constantly changing environment. Intercellular communication mechanisms are necessary requirements for this adaptation. The nervous system and the endocrine system provide this intercellular, organism-wide communication. The nervous system was originally viewed as providing a fixed communication system, whereas the endocrine system supplied hormones, which are mobile messages. In fact, there is a remarkable convergence of these regulatory systems. For example, neural regulation of the endocrine system is important in the production and secretion of some hormones; many neurotransmitters resemble hormones in their synthesis, transport, and mechanism of action; and many hormones are synthesized in the nervous system. The word "hormone" is derived from a Greek term that means to arouse to activity. As classically defined, a hormone is a substance that is synthesized in one organ and transported by the circulatory system to act on another tissue. However, this original description is too restrictive because hormones can act on adjacent cells (paracrine action) and on the cell in which they were synthesized (autocrine action) without entering the systemic circulation. A diverse array of hormones—each with distinctive mechanisms of action and properties of biosynthesis, storage, secretion, transport, and metabolism—has evolved to provide homeostatic responses. This biochemical diversity is the topic of this chapter.

The Target Cell Concept:

There are about 200 types of differentiated cells in humans. Only a few produce hormones, but virtually all of the 75 trillion cells in a human are targets of one or more of the over 50 known hormones. The concept of the target cell is a useful way of looking at hormone action. It was thought that hormones affected a single cell type—or only a few kinds of cells—and that a hormone elicited a unique biochemical or physiologic action. We now know that a given hormone can affect several different

cell types; that more than one hormone can affect a given cell type; and that hormones can exert many different effects in one cell or in different cells. With the discovery of specific cell-surface and intracellular hormone receptors, the definition of a target has been expanded to include any cell in which the hormone (ligand) binds to its receptor, whether or not a biochemical or physiologic response has yet been determined.

Several factors determine the response of a target cell to a hormone. These can be thought of in two general ways: (1) as factors that affect the concentration of the hormone at the target cell (see Table 41–1) and (2) as factors that affect the actual response of the target cell to the hormone (see Table 41–2).

Table 41–2. Determinants of the Target Cell Response.
The number, relative activity, and state of occupancy of the specific receptors on the plasma membrane or in the cytoplasm or nucleus.
The metabolism (activation or inactivation) of the hormone in the target cell.
The presence of other factors within the cell that are neces- sary for the hormone response.
Up- or down-regulation of the receptor consequent to the interaction with the ligand.
Postreceptor desensitzation of the cell, including down-regulation of the receptor.

Table 41–1. Determinants of the Concentration of a Hormone at the Target Cell.
The rate of synthesis and secretion of the hormones.
The proximity of the target cell to the hormone source (dilution effect).
The dissociation constants of the hormone with specific plasma transport proteins (if any).
The conversion of inactive or suboptimally active forms of the hormone into the fully active form.
The rate of clearance from plasma by other tissues or by digestion, metabolism, or excretion.

Hormone Receptors Are of Central Importance

Receptors Discriminate Precisely:

One of the major challenges faced in making the hormone-based communication system work is illustrated in Figure 41–1. Hormones are present at very low concentrations in the extracellular fluid, generally in the range of 10^{-15} to 10^{-9} mol/L. This concentration is much lower than

that of the many structurally similar molecules (sterols, amino acids, peptides, proteins) and other molecules that circulate at concentrations in the 10^{-5} to 10^{-3} mol/L range. Target cells, therefore, must distinguish not only between different hormones present in small amounts but also between a given hormone and the 10^6- to 10^9-fold excess of other similar molecules. This high degree of discrimination is provided by cell-associated recognition molecules called receptors. Hormones initiate their biologic effects by binding to specific receptors, and since any effective control system also must provide a means of stopping a response, hormone-induced actions generally terminate when the effector dissociates from the receptor.

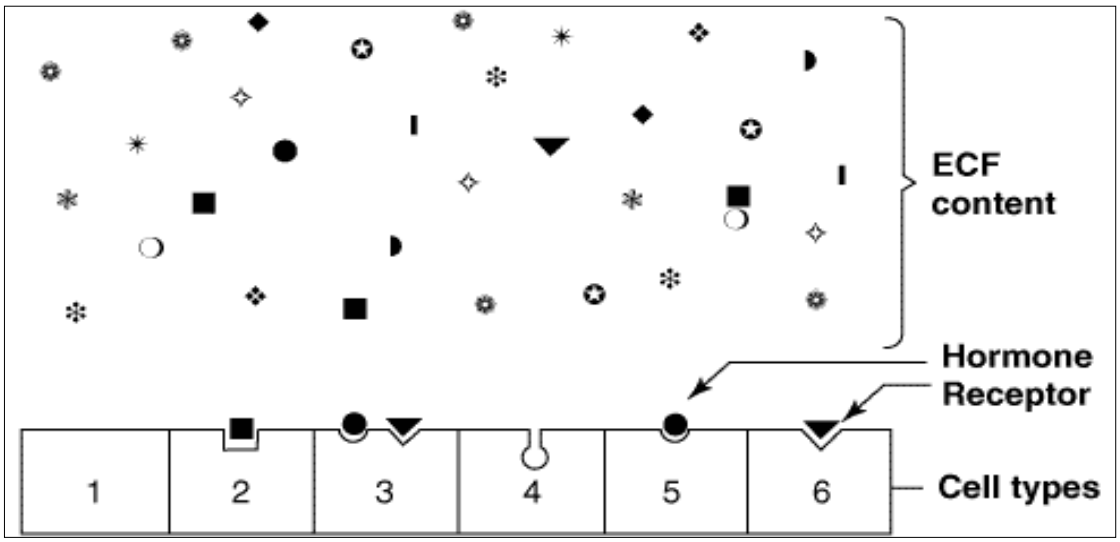


Figure 41–1: Specificity and selectivity of hormone receptors. Many different molecules circulate in the extracellular fluid (ECF), but only a few are recognized by hormone receptors. Receptors must select these molecules from among high concentrations of the other molecules. This simplified drawing shows that a cell may have no hormone receptors (1), have one receptor (2+5+6), have receptors for several hormones (3), or have a receptor but no hormone in the vicinity (4).

A target cell is defined by its ability to selectively bind a given hormone to its cognate receptor. Several biochemical features of this interaction are important in order for hormone-receptor interactions to be physiologically relevant: (1) binding should be specific, ie, displaceable by agonist or antagonist; (2) binding should be saturable; and (3) binding should occur within the concentration range of the expected biologic response.

Both Recognition & Coupling Domains Occur on Receptors:

All receptors have at least two functional domains. A recognition domain binds the hormone ligand and a second region generates a signal that couples hormone recognition to some intracellular function. Coupling (signal transduction) occurs in two general ways. Polypeptide and protein hormones and the catecholamines bind to receptors located in the plasma membrane and thereby generate a signal that regulates various intracellular functions, often by changing the activity of an enzyme. In contrast, steroid, retinoid, and thyroid hormones interact with intracellular receptors, and it is this ligand-receptor complex that directly provides the signal, generally to specific genes whose rate of transcription is thereby affected.

The domains responsible for hormone recognition and signal generation have been identified in the protein polypeptide and catecholamine hormone receptors. Steroid, thyroid, and retinoid hormone receptors have several functional domains: one site binds the hormone; another binds to specific DNA regions; a third is involved in the interaction with other coregulator proteins that result in the activation (or repression) of gene transcription; and a fourth may specify binding to one or more other proteins that influence the intracellular trafficking of the receptor.

The dual functions of binding and coupling ultimately define a receptor, and it is the coupling of hormone binding to signal transduction—so-called **receptor-effector coupling**—that provides the first step in amplification of the hormonal response. This dual purpose also distinguishes the target cell receptor from the plasma carrier proteins that bind hormone but do not generate a signal (see Table 41–6).

Table 41–6. Comparison of Receptors with Transport Proteins.

Feature	Receptors	Transport Proteins
Concentration	Very low (thousands/cell)	Very high (billions/μL)
Binding affinity	High (pmol/L to nmol/L range)	Low (μmol/L range)
Binding specificity	Very high	Low
Saturability	Yes	No
Reversibility	Yes	Yes
Signal transduction	Yes	No

Receptors Are Proteins:

Several classes of peptide hormone receptors have been defined. For example, the insulin receptor is a heterotetramer ($\alpha_2\beta_2$) linked by multiple disulfide bonds in which the extracellular α subunit binds insulin and the membrane-spanning β subunit transduces the signal through the tyrosine protein kinase domain located in the cytoplasmic portion of this polypeptide. The receptors for insulin-like growth factor I (IGF-I) and epidermal growth factor (EGF) are generally similar in structure to the insulin receptor. The growth hormone and prolactin receptors also span the plasma membrane of target cells but do not contain intrinsic protein kinase activity. Ligand binding to these receptors, however, results in the association and activation of a completely different protein kinase pathway, the Jak-Stat pathway. Polypeptide hormone and catecholamine receptors, which transduce signals by altering the rate of production of cAMP through G-proteins, are characterized by the presence of seven domains that span the plasma membrane. Protein kinase activation and the generation of cyclic AMP (cAMP, 3'5'-adenylic acid; see Figure 19–5) is a downstream action of this class of receptor (see Chapter 42 for further details).

A comparison of several different steroid receptors with thyroid hormone receptors revealed a remarkable conservation of the amino acid sequence in certain regions, particularly in the DNA-binding domains. This led to the realization that receptors of the steroid or thyroid type are members of a large superfamily of nuclear receptors. Many related members of this family have no known ligand at present and thus are called orphan receptors. The nuclear receptor superfamily plays a critical role in the regulation of gene transcription by hormones, as described in Chapter 42.

Hormones Can Be Classified in Several Ways:

Hormones can be classified according to chemical composition, solubility properties, location of receptors, and the nature of the signal used to mediate hormonal action within the cell. A classification based on the last two properties is illustrated in Table 41–3, and general features of each group are illustrated in Table 41–4.

Table 41–4. General Features of Hormone Classes.		
	Group I	**Group II**
Types	Steroids, iodothyronines, calcitriol, retinoids	Polypeptides, proteins, glycoproteins, catecholamines
Solubility	Lipophilic	Hydrophilic
Transport proteins	Yes	No
Plasma half-life	Long (hours to days)	Short (minutes)
Receptor	Intracellular	Plasma membrane
Mediator	Receptor-hormone complex	cAMP, cGMP, Ca^{2+}, metabolites of complex phosphoinositols, kinase cascades

Table 41–3. Classification of Hormones by Mechanism of Action.
I. Hormones that bind to intracellular receptors
Androgens
Calcitriol ($1,25[OH]_2$-D_3)
Estrogens
Glucocorticoids
Mineralocorticoids
Progestins
Retinoic acid
Thyroid hormones (T_3 and T_4)
II. Hormones that bind to cell surface receptors
A. The second messenger is cAMP
α_2-Adrenergic catecholamines
β-Adrenergic catecholamines
Adrenocorticotropic hormone
Antidiuretic hormone
Calcitonin
Chorionic gonadotropin, human
Corticotropin-releasing hormone
Follicle-stimulating hormone
Glucagon
Lipotropin
Luteinizing hormone
Melanocyte-stimulating hormone
Parathyroid hormone
Somatostatin
Thyroid-stimulating hormone

B. The second messenger is cGMP
Atrial natriuretic factor
Nitric oxide
C. The second messenger is calcium or phosphatidylinositols (or both)
Acetylcholine (muscarinic)
α_1-Adrenergic catecholamines
Angiotensin II
Antidiuretic hormone (vasopressin)
Cholecystokinin
Gastrin
Gonadotropin-releasing hormone
Oxytocin
Platelet-derived growth factor
Substance P
Thyrotropin-releasing hormone
D. The second messenger is a kinase or phosphatase cascade
Adiponectin
Chorionic somatomammotropin
Epidermal growth factor
Erythropoietin
Fibroblast growth factor
Growth hormone
Insulin
Insulin-like growth factors I and II
Leptin
Nerve growth factor
Platelet-derived growth factor
Prolactin

The hormones in group I are lipophilic. After secretion, these hormones associate with plasma transport or carrier proteins, a process that circumvents the problem of solubility while prolonging the plasma half-life of the hormone. The relative percentages of bound and free hormone are determined by the binding affinity and binding capacity of the transport protein. The free hormone, which is the biologically active form, readily traverses the lipophilic plasma membrane of all cells and encounters receptors in either the cytosol or nucleus of target cells. The ligand-receptor complex is assumed to be the intracellular messenger in this group.

The second major group consists of water-soluble hormones that bind to the plasma membrane of the target cell. Hormones that bind to the surfaces of cells communicate with intracellular metabolic processes through intermediary molecules called **second messengers** (the

hormone itself is the first messenger), which are generated as a consequence of the ligand-receptor interaction. The second messenger concept arose from an observation that epinephrine binds to the plasma membrane of certain cells and increases intracellular cAMP. This was followed by a series of experiments in which cAMP was found to mediate the effects of many hormones. Hormones that clearly employ this mechanism are shown in group II.A of Table 41–3. To date, only one hormone, atrial natriuretic factor (ANF), uses cGMP as its second messenger, but other hormones will probably be added to group II.B. Several hormones, many of which were previously thought to affect cAMP, appear to use ionic calcium (Ca^{2+}) or metabolites of complex phosphoinositides (or both) as the intracellular signal. These are shown in group II.C of the table. The intracellular messenger for group II.D is a protein kinase-phosphatase cascade. Several of these have been identified, and a given hormone may use more than one kinase cascade. A few hormones fit into more than one category, and assignments change as new information is brought forward.

Diversity of the Endocrine System:

Hormones Are Synthesized in a Variety of Cellular Arrangements:

Hormones are synthesized in discrete organs designed solely for this specific purpose, such as the thyroid (triiodothyronine), adrenal (glucocorticoids and mineralocorticoids), and the pituitary (TSH, FSH, LH, growth hormone, prolactin, ACTH). Some organs are designed to perform two distinct but closely related functions. For example, the ovaries produce mature oocytes and the reproductive hormones estradiol and progesterone. The testes produce mature spermatozoa and testosterone. Hormones are also produced in specialized cells within other organs such as the small intestine (glucagon-like peptide), thyroid (calcitonin), and kidney (angiotensin II). Finally, the synthesis of some hormones requires the parenchymal cells of more than one organ—eg, the skin, liver, and kidney are required for the production of $1,25(OH)_2$-D_3 (calcitriol). Examples of this diversity in the approach to hormone synthesis, each of which has evolved to fulfill a specific purpose, are discussed below.

Hormones Are Chemically Diverse:

Hormones are synthesized from a wide variety of chemical building blocks. A large series is derived from cholesterol. These include the glucocorticoids, mineralocorticoids, estrogens, progestins, and $1,25(OH)_2$-D_3 (see Figure 41–2). In some cases, a steroid hormone is the precursor molecule for another hormone. For example, progesterone is a hormone in its own right but is also a precursor in the formation of glucocorticoids, mineralocorticoids, testosterone, and estrogens. Testosterone is an obligatory intermediate in the biosynthesis of estradiol and in the formation of dihydrotestosterone (DHT). In these examples, described in detail below, the final product is determined by the cell type and the associated set of enzymes in which the precursor exists.

The amino acid tyrosine is the starting point in the synthesis of the catecholamines and of the thyroid hormones tetraiodothyronine (thyroxine; T_4) and triiodothyronine (T_3) (Figure 41–2). T_3 and T_4 are unique in that they require the addition of iodine (as I^-) for bioactivity. Because dietary iodine is very scarce in many parts of the world, an intricate mechanism for accumulating and retaining I^- has evolved.

Many hormones are polypeptides or glycoproteins. These range in size from thyrotropin-releasing hormone (TRH), a tripeptide, to single-chain polypeptides like adrenocorticotropic hormone (ACTH; 39 amino acids), parathyroid hormone (PTH; 84 amino acids), and growth hormone (GH; 191 amino acids) (Figure 41–2). Insulin is an AB chain heterodimer of 21 and 30 amino acids, respectively. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and chorionic gonadotropin (CG) are glycoprotein hormones of αβ heterodimeric structure. The α chain is identical in all of these hormones, and distinct β chains impart hormone uniqueness. These hormones have a molecular mass in the range of 25–30 kDa depending on the degree of glycosylation and the length of the β chain.

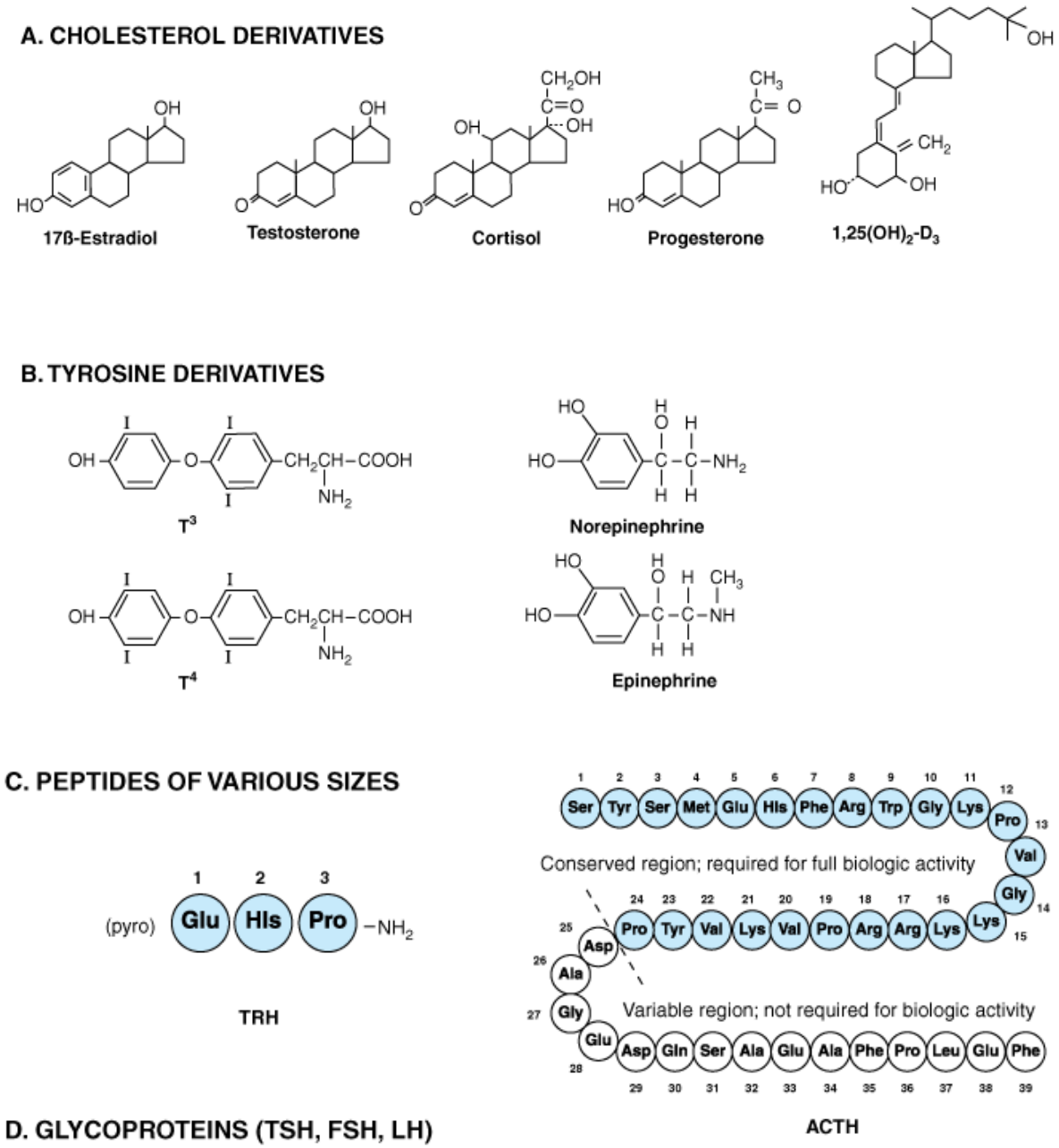


Figure 41–2: Chemical diversity of hormones. **(A)** Cholesterol derivatives. **(B)** Tyrosine derivatives. **(C)** Peptides of various sizes. **(D)** Glycoproteins (TSH, FSH, LH) with common α subunits and unique β subunits

The Diversity of the Endocrine System II

Many Hormones Are Made from Cholesterol

1- Adrenal Steroidogenesis:

The adrenal steroid hormones are synthesized from cholesterol. Cholesterol is mostly derived from the plasma, but a small portion is synthesized in situ from acetyl-CoA via mevalonate and squalene. Much of the cholesterol in the adrenal is esterified and stored in cytoplasmic lipid droplets. Upon stimulation of the adrenal by ACTH, an esterase is activated, and the free cholesterol formed is transported into the mitochondrion, where a **cytochrome P450 side chain cleavage enzyme (P450scc)** converts cholesterol to pregnenolone. Cleavage of the side chain involves sequential hydroxylations, first at C_{22} and then at C_{20}, followed by side chain cleavage (removal of the six-carbon fragment isocaproaldehyde) to give the 21-carbon steroid (Figure 41–3, top). An ACTH-dependent **steroidogenic acute regulatory (StAR) protein** is essential for the transport of cholesterol to P450scc in the inner mitochondrial membrane.

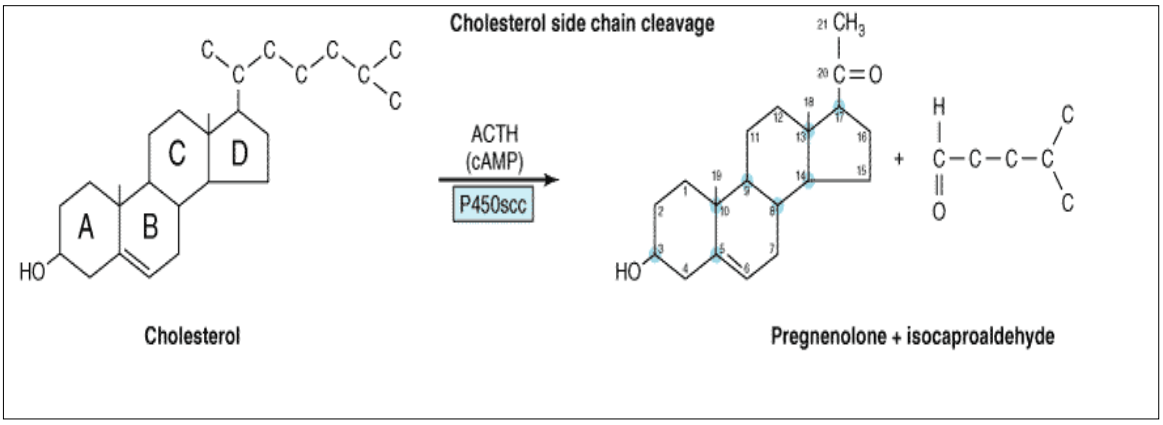
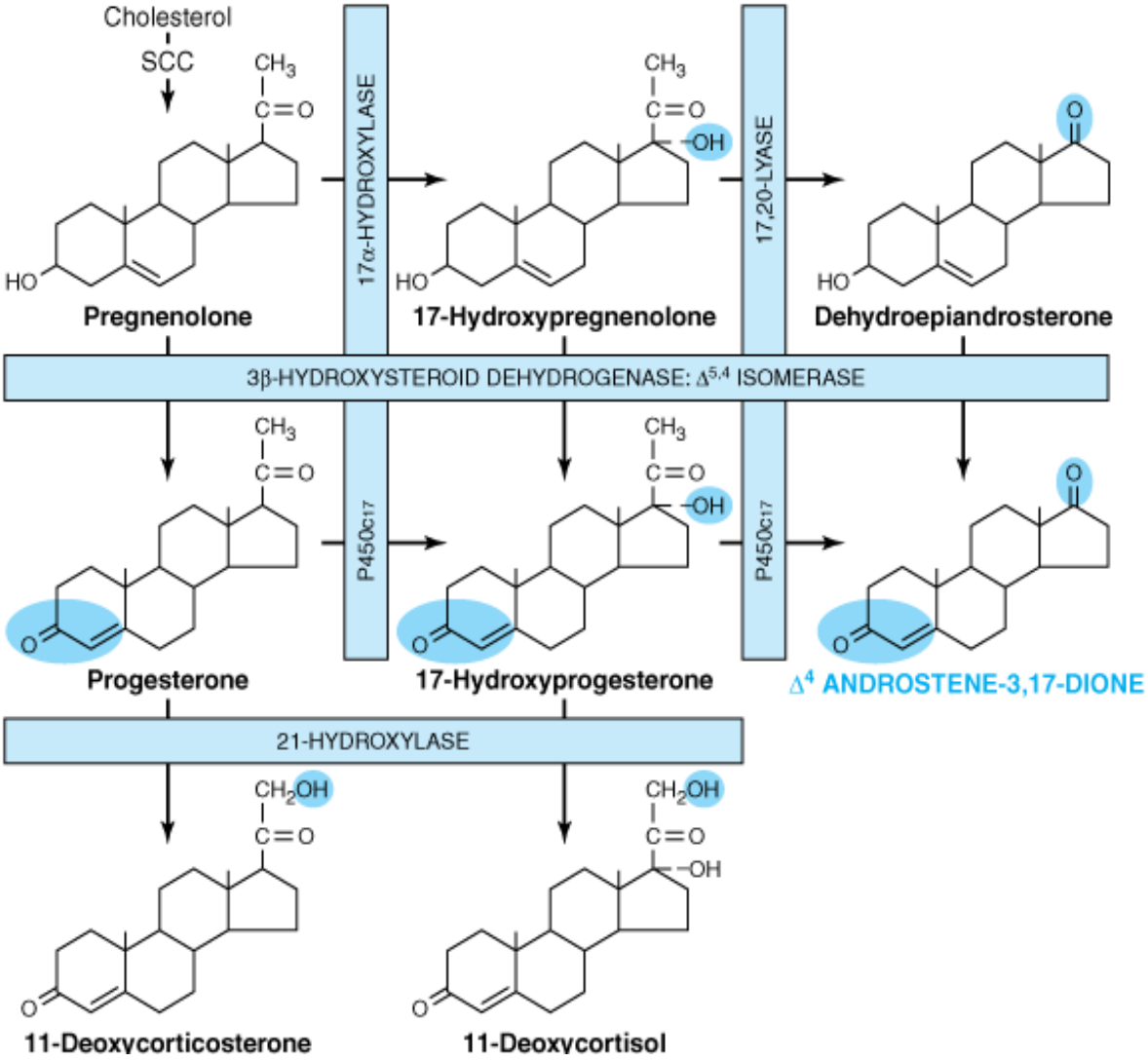


Figure 41–3: Cholesterol side-chain cleavage .

All mammalian steroid hormones are formed from cholesterol via pregnenolone through a series of reactions that occur in either the mitochondria or endoplasmic reticulum of the producing cell. Hydroxylases that require molecular oxygen and NADPH are essential, and dehydrogenases, an isomerase, and a lyase reaction are also necessary for certain steps. There is cellular specificity in adrenal steroidogenesis. For instance, 18-hydroxylase and 19-hydroxysteroid dehydrogenase, which are required for aldosterone synthesis, are found only in the zona glomerulosa cells (the outer region of the adrenal cortex), so that the biosynthesis of this mineralocorticoid is confined to this region. A schematic representation of the pathways involved in the synthesis of the three major classes of adrenal steroids is presented in Figure 41–4. The enzymes are shown in the rectangular boxes, and the modifications at each step are shaded.



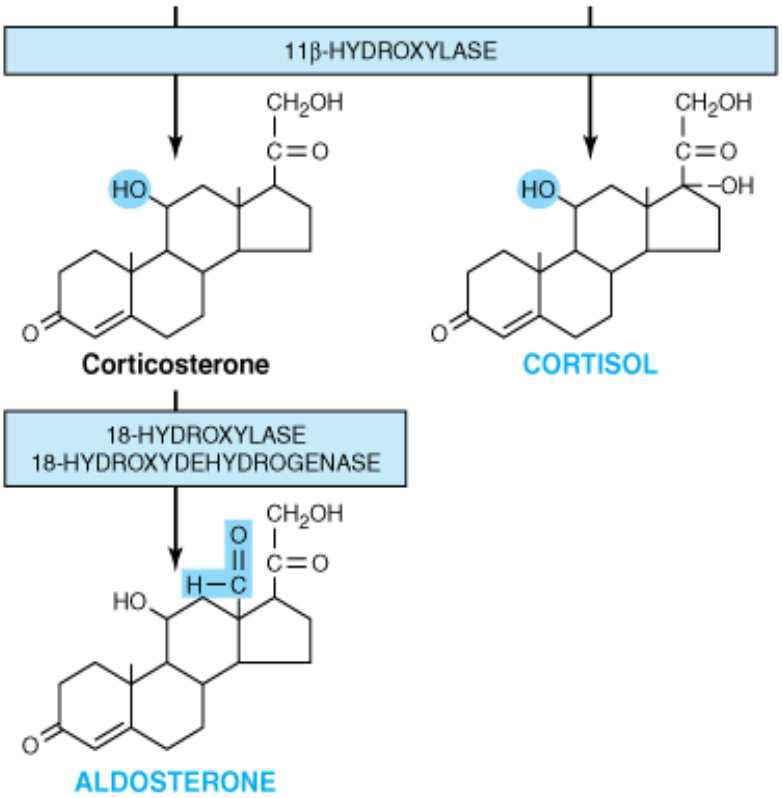


Figure 41–4: Pathways involved in the synthesis of the three major classes of adrenal steroids (mineralocorticoids, glucocorticoids, and androgens). Enzymes are shown in the rectangular boxes, and the modifications at each step are shaded. Note that the 17α-hydroxylase and 17,20-lyase activities are both part of one enzyme, designated P450c17.

a- Mineralocorticoid Synthesis:

Synthesis of aldosterone follows the mineralocorticoid pathway and occurs in the zona glomerulosa. Pregnenolone is converted to progesterone by the action of two smooth endoplasmic reticulum enzymes, **3β-hydroxysteroid dehydrogenase (3β-OHSD)** and **$\Delta^{5,4}$-isomerase.** Progesterone is hydroxylated at the C_{21} position to form 11-deoxycorticosterone (DOC), which is an active (Na^+-retaining) mineralocorticoid. The next hydroxylation, at C_{11}, produces corticosterone, which has glucocorticoid activity and is a weak mineralocorticoid (it has less than 5% of the potency of aldosterone). In some species (eg, rodents), it is the most potent glucocorticoid. C_{21} hydroxylation is necessary for both mineralocorticoid and glucocorticoid activity, but most steroids with a C_{17} hydroxyl group have more glucocorticoid and less mineralocorticoid action. In the zona glomerulosa, which does not have the smooth endoplasmic reticulum enzyme 17α-hydroxylase, a mitochondrial 18-hydroxylase is present. The **18-hydroxylase (aldosterone synthase)** acts on corticosterone to form 18-hydroxycorticosterone, which is changed to aldosterone by

conversion of the 18-alcohol to an aldehyde. This unique distribution of enzymes and the special regulation of the zona glomerulosa by K^+ and angiotensin II have led some investigators to suggest that, in addition to the adrenal being two glands, the adrenal cortex is actually two separate organs.

b- Glucocorticoid Synthesis:

Cortisol synthesis requires three hydroxylases located in the fasciculata and reticularis zones of the adrenal cortex that act sequentially on the C_{17}, C_{21}, and C_{11} positions. The first two reactions are rapid, while C_{11} hydroxylation is relatively slow. If the C_{11} position is hydroxylated first, the action of **17α-hydroxylase** is impeded and the mineralocorticoid pathway is followed (forming corticosterone or aldosterone, depending on the cell type). 17α-Hydroxylase is a smooth endoplasmic reticulum enzyme that acts upon either progesterone or, more commonly, pregnenolone. 17α-Hydroxyprogesterone is hydroxylated at C_{21} to form 11-deoxycortisol, which is then hydroxylated at C_{11} to form cortisol, the most potent natural glucocorticoid hormone in humans. 21-Hydroxylase is a smooth endoplasmic reticulum enzyme, whereas 11β-hydroxylase is a mitochondrial enzyme. Steroidogenesis thus involves the repeated shuttling of substrates into and out of the mitochondria.

c- Androgen Synthesis:

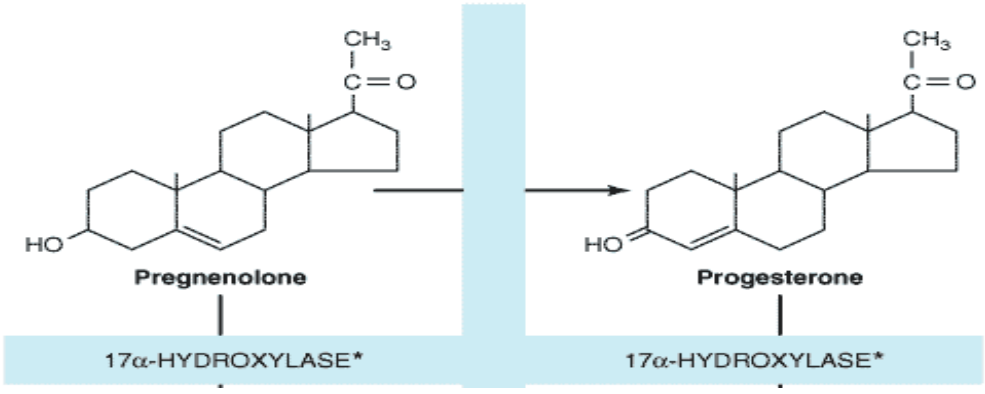
The major androgen or androgen precursor produced by the adrenal cortex is dehydroepiandrosterone (DHEA). Most 17-hydroxypregnenolone follows the glucocorticoid pathway, but a small fraction is subjected to oxidative fission and removal of the two-carbon side chain through the action of 17,20-lyase. The lyase activity is actually part of the same enzyme (P450c17) that catalyzes 17α-hydroxylation. This is therefore a **dual function protein.** The lyase activity is important in both the adrenals and the gonads and acts exclusively on 17α-hydroxy-containing molecules. Adrenal androgen production increases markedly if glucocorticoid biosynthesis is impeded by the lack of one of the hydroxylases **(adrenogenital syndrome).** DHEA is really a prohormone, since the actions of 3β-OHSD and $\Delta^{5,4}$-isomerase convert the weak androgen DHEA into the more potent **androstenedione.** Small amounts of androstenedione are also formed in the adrenal by the action of the lyase on 17α-hydroxyprogesterone. Reduction of androstenedione at the C_{17} position results in the formation of

testosterone, the most potent adrenal androgen. Small amounts of testosterone are produced in the adrenal by this mechanism, but most of this conversion occurs in the testes.

2- Testicular Steroidogenesis:

Testicular androgens are synthesized in the interstitial tissue by the Leydig cells. The immediate precursor of the gonadal steroids, as for the adrenal steroids, is cholesterol. The rate-limiting step, as in the adrenal, is delivery of cholesterol to the inner membrane of the mitochondria by the transport protein StAR. Once in the proper location, cholesterol is acted upon by the side chain cleavage enzyme P450scc. The conversion of cholesterol to pregnenolone is identical in adrenal, ovary, and testis. In the latter two tissues, however, the reaction is promoted by LH rather than ACTH.

The conversion of pregnenolone to testosterone requires the action of five enzyme activities contained in three proteins: (1) 3β-hydroxysteroid dehydrogenase (3β-OHSD) and $\Delta^{5,4}$-isomerase; (2) 17α-hydroxylase and 17,20-lyase; and (3) 17β-hydroxysteroid dehydrogenase (17β-OHSD). This sequence, referred to as the **progesterone (or Δ^4) pathway,** is shown on the right side of Figure 41–5. Pregnenolone can also be converted to testosterone by the **dehydroepiandrosterone (or Δ^5) pathway,** which is illustrated on the left side of Figure 41–5. The Δ^5 route appears to be most used in human testes.



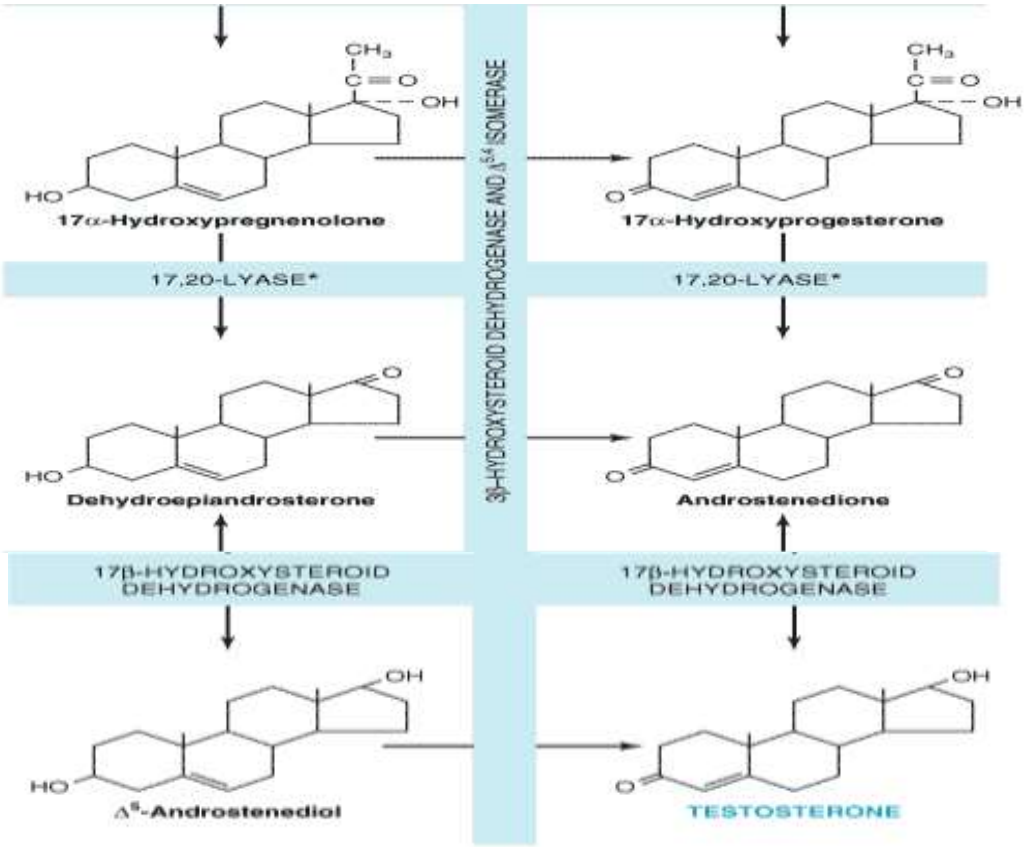


Figure 41–5: Pathways of testosterone biosynthesis. The pathway on the left side of the figure is called the Δ^5 or dehydroepiandrosterone pathway; the pathway on the right side is called the Δ^4 or progesterone pathway. The asterisk indicates that the 17α-hydroxylase and 17,20-lyase activities reside in a single protein, P450c17.

The five enzyme activities are localized in the microsomal fraction in rat testes, and there is a close functional association between the activities of 3β-OHSD and $^{\Delta 5,4}$-isomerase and between those of a 17α-hydroxylase and 17,20-lyase. These enzyme pairs, both contained in a single protein, are shown in the general reaction sequence in Figure 41–5.

Dihydrotestosterone Is Formed from Testosterone in Peripheral Tissues:

Testosterone is metabolized by two pathways. One involves oxidation at the 17 position, and the other involves reduction of the A ring double bond and the 3-ketone. Metabolism by the first pathway occurs in many tissues, including liver, and produces 17-ketosteroids that are generally inactive or less active than the parent compound. Metabolism by the second pathway, which is less efficient, occurs primarily in target tissues and produces the potent metabolite dihydrotestosterone (DHT).

The most significant metabolic product of testosterone is DHT, since in many tissues, including prostate, external genitalia, and some areas of the skin, this is the active form of the hormone. The plasma content of DHT in the adult male is about one-tenth that of testosterone, and approximately 400 µg of DHT is produced daily as compared with about

5 mg of testosterone. About 50–100 µg of DHT are secreted by the testes. The rest is produced peripherally from testosterone in a reaction catalyzed by the NADPH-dependent **5α-reductase** (Figure 41–6). Testosterone can thus be considered a prohormone, since it is converted into a much more potent compound (dihydrotestosterone) and since most of this conversion occurs outside the testes. Some estradiol is formed from the peripheral aromatization of testosterone, particularly in males.

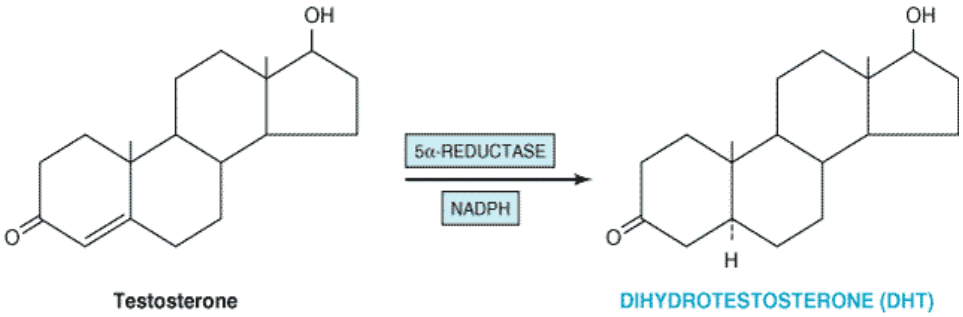


Figure 41–6: Dihydrotestosterone is formed from testosterone through action of the enzyme 5α-reductase.

3- Ovarian Steroidogenesis

The estrogens are a family of hormones synthesized in a variety of tissues. 17β-Estradiol is the primary estrogen of ovarian origin. In some species, estrone, synthesized in numerous tissues, is more abundant. In pregnancy, relatively more estriol is produced, and this comes from the placenta. The general pathway and the subcellular localization of the enzymes involved in the early steps of estradiol synthesis are the same as those involved in androgen biosynthesis. Features unique to the ovary are illustrated in Figure 41–7.

Estrogens are formed by the aromatization of androgens in a complex process that involves three hydroxylation steps, each of which requires O_2 and NADPH. The **aromatase enzyme complex** is thought to include a P450 monooxygenase. Estradiol is formed if the substrate of this enzyme complex is testosterone, whereas estrone results from the aromatization of androstenedione.

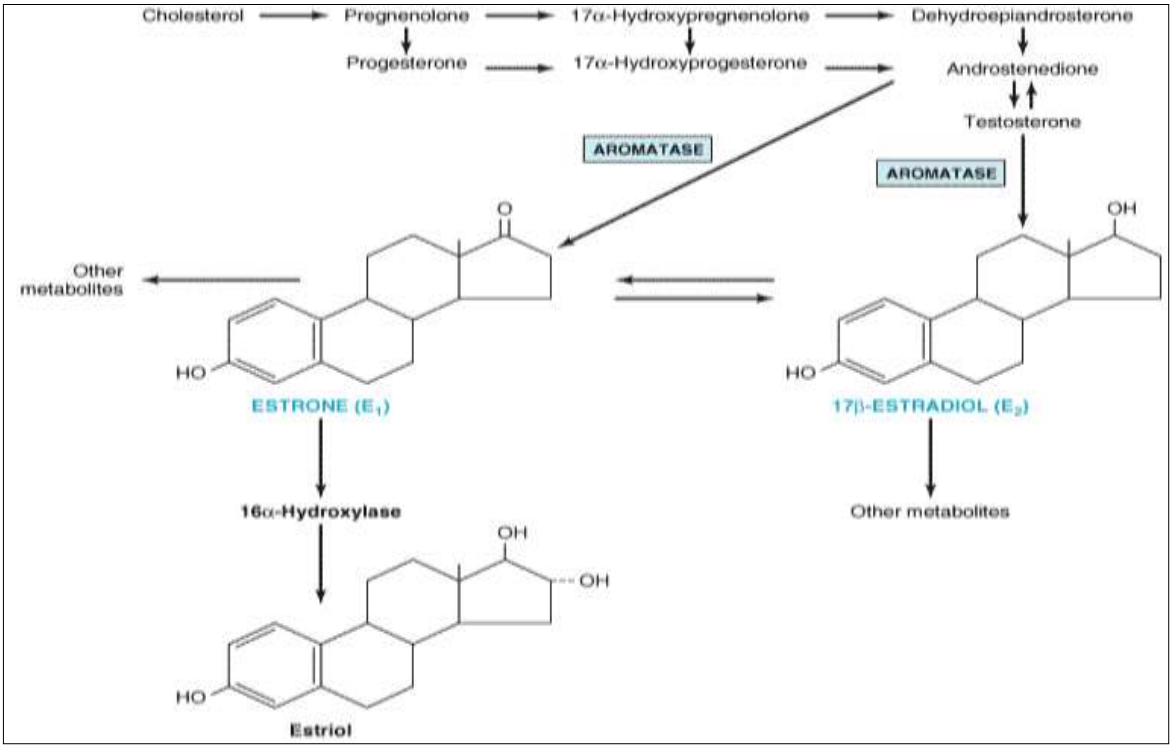


Figure 41–7: Biosynthesis of estrogens.

The cellular source of the various ovarian steroids has been difficult to unravel, but a transfer of substrates between two cell types is involved. Theca cells are the source of androstenedione and testosterone. These are converted by the aromatase enzyme in granulosa cells to estrone and estradiol, respectively. Progesterone, a precursor for all steroid hormones, is produced and secreted by the corpus luteum as an end-product hormone because these cells do not contain the enzymes necessary to convert progesterone to other steroid hormones (Figure 41–8).

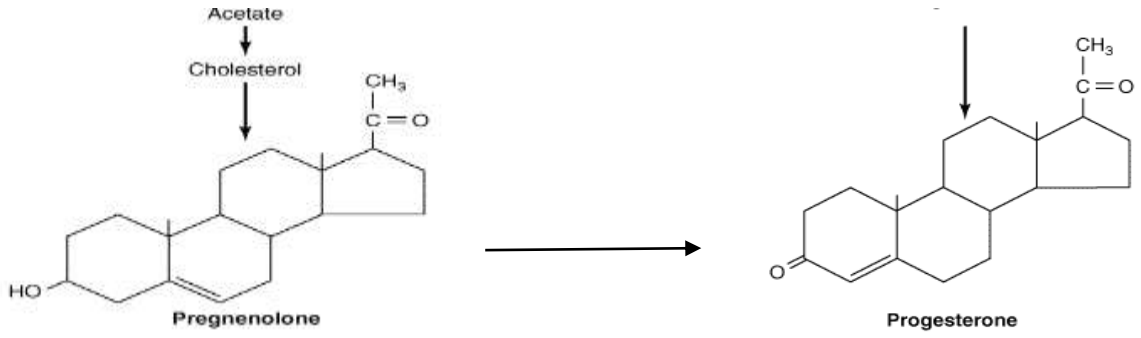


Figure 41–8: Biosynthesis of progesterone in the corpus luteum.

Significant amounts of estrogens are produced by the peripheral aromatization of androgens. In human males, the peripheral

aromatization of testosterone to estradiol (E_2) accounts for 80% of the production of the latter. In females, adrenal androgens are important substrates, since as much as 50% of the E_2 produced during pregnancy comes from the aromatization of androgens. Finally, conversion of androstenedione to estrone is the major source of estrogens in postmenopausal women. Aromatase activity is present in adipose cells and also in liver, skin, and other tissues. Increased activity of this enzyme may contribute to the "estrogenization" that characterizes such diseases as cirrhosis of the liver, hyperthyroidism, aging, and obesity. Aromatase inhibitors show promise as therapeutic agents in breast cancer and possibly in other female reproductive tract malignancies.

4- 1,25(OH)$_2$-D$_3$ (Calcitriol) Is Synthesized from a Cholesterol Derivative

1,25(OH)$_2$-D$_3$ is produced by a complex series of enzymatic reactions that involve the plasma transport of precursor molecules to a number of different tissues (Figure 41–9). One of these precursors is vitamin D—really not a vitamin, but this common name persists. The active molecule, 1,25(OH)$_2$-D$_3$, is transported to other organs where it activates biologic processes in a manner similar to that employed by the steroid hormones.

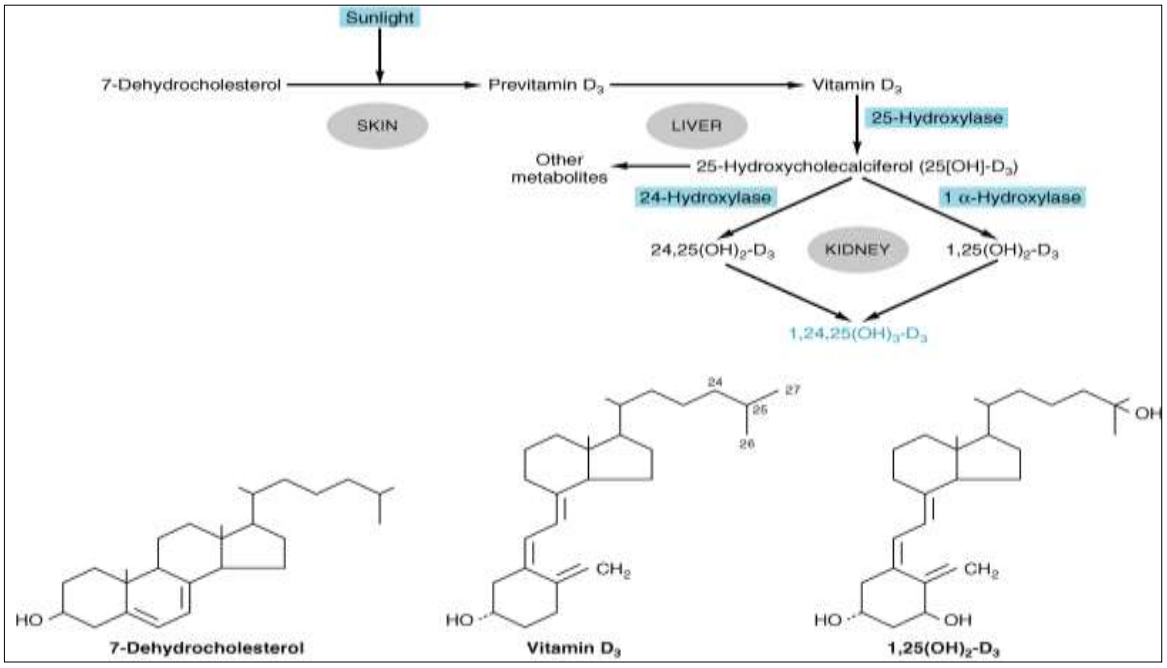


Figure 41–9: Formation and hydroxylation of vitamin D_3. 25-Hydroxylation takes place in the liver, and the other hydroxylations occur in the kidneys. 25,26(OH)$_2$-D$_3$ and 1,25,26(OH)$_3$-D$_3$ are probably formed as well. The formulas of 7-dehydrocholesterol, vitamin D_3, and 1,25(OH)$_2$-D$_3$ are also shown.

Skin

Small amounts of the precursor for $1,25(OH)_2$-D_3 synthesis are present in food (fish liver oil, egg yolk), but most of the precursor for $1,25(OH)_2$-D_3 synthesis is produced in the malpighian layer of the epidermis from 7-dehydrocholesterol in an ultraviolet light-mediated, nonenzymatic **photolysis** reaction. The extent of this conversion is related directly to the intensity of the exposure and inversely to the extent of pigmentation in the skin. There is an age-related loss of 7-dehydrocholesterol in the epidermis that may be related to the negative calcium balance associated with old age.

Liver

A specific transport protein called the **vitamin D-binding protein** binds vitamin D_3 and its metabolites and moves vitamin D_3 from the skin or intestine to the liver, where it undergoes 25-hydroxylation, the first obligatory reaction in the production of $1,25(OH)_2$-D_3. 25-Hydroxylation occurs in the endoplasmic reticulum in a reaction that requires magnesium, NADPH, molecular oxygen, and an uncharacterized cytoplasmic factor. Two enzymes are involved: an NADPH-dependent cytochrome P450 reductase and a cytochrome P450. This reaction is not regulated, and it also occurs with low efficiency in kidney and intestine. The $25(OH)_2$-D_3 enters the circulation, where it is the major form of vitamin D found in plasma, and is transported to the kidney by the vitamin D-binding protein.

Kidney

$25(OH)_2$-D_3 is a weak agonist and must be modified by hydroxylation at position C_1 for full biologic activity. This is accomplished in mitochondria of the renal proximal convoluted tubule by a three-component monooxygenase reaction that requires NADPH, Mg^{2+}, molecular oxygen, and at least three enzymes: (1) a flavoprotein, renal ferredoxin reductase; (2) an iron sulfur protein, renal ferredoxin; and (3) cytochrome P450. This system produces $1,25(OH)_2$-D_3, which is the most potent naturally occurring metabolite of vitamin D.

The Diversity of the Endocrine System III

Catecholamines & Thyroid Hormones Are Made from Tyrosine

1- Catecholamines Are Synthesized in Final Form & Stored in Secretion Granules

Three amines—dopamine, norepinephrine, and epinephrine—are synthesized from tyrosine in the chromaffin cells of the adrenal medulla. The major product of the adrenal medulla is epinephrine. This compound constitutes about 80% of the catecholamines in the medulla, and it is not made in extramedullary tissue. In contrast, most of the norepinephrine present in organs innervated by sympathetic nerves is made in situ (about 80% of the total), and most of the rest is made in other nerve endings and reaches the target sites via the circulation. Epinephrine and norepinephrine may be produced and stored in different cells in the adrenal medulla and other chromaffin tissues.
The conversion of tyrosine to epinephrine requires four sequential steps: (1) ring hydroxylation; (2) decarboxylation; (3) side chain hydroxylation to form norepinephrine; and (4) N-methylation to form epinephrine. The biosynthetic pathway and the enzymes involved are illustrated in Figure 41–10.

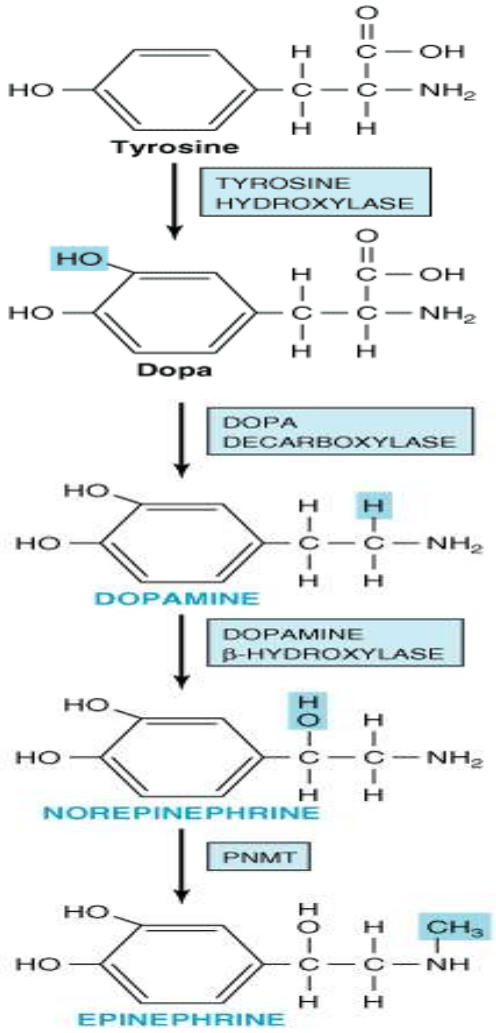


Figure 41–10: Biosynthesis of catecholamines. (PNMT, phenylethanolamine-*N*-methyltransferase.)

Tyrosine Hydroxylase Is Rate-Limiting for Catecholamine Biosynthesis

Tyrosine is the immediate precursor of catecholamines, and **tyrosine hydroxylase** is the rate-limiting enzyme in catecholamine biosynthesis. Tyrosine hydroxylase is found in both soluble and particle-bound forms only in tissues that synthesize catecholamines; it functions as an oxidoreductase, with tetrahydropteridine as a cofactor, to convert L-tyrosine to L-dihydroxyphenylalanine **(L-dopa).** As the rate-limiting enzyme, tyrosine hydroxylase is regulated in a variety of ways. The most important mechanism involves feedback inhibition by the catecholamines, which compete with the enzyme for the pteridine cofactor. Catecholamines cannot cross the blood-brain barrier; hence, in the brain they must be synthesized locally. In certain central nervous system diseases (eg, Parkinson's disease), there is a local deficiency of dopamine synthesis. L-Dopa, the precursor of dopamine, readily crosses the blood-brain barrier and so is an important agent in the treatment of Parkinson's disease.

Dopa Decarboxylase Is Present in All Tissues

This soluble enzyme requires pyridoxal phosphate for the conversion of L-dopa to 3,4-dihydroxyphenylethylamine **(dopamine).** Compounds that resemble L-dopa, such as α-methyldopa, are competitive inhibitors of this reaction. α-Methyldopa is effective in treating some kinds of hypertension.

Dopamine β-Hydroxylase (DBH) Catalyzes the Conversion of Dopamine to Norepinephrine

DBH is a monooxygenase and uses ascorbate as an electron donor, copper at the active site, and fumarate as modulator. DBH is in the particulate fraction of the medullary cells, probably in the secretion granule; thus, the conversion of dopamine to **norepinephrine** occurs in this organelle.

Phenylethanolamine-N-Methyltransferase (PNMT) Catalyzes the Production of Epinephrine

PNMT catalyzes the N-methylation of norepinephrine to form **epinephrine** in the epinephrine-forming cells of the adrenal medulla. Since PNMT is soluble, it is assumed that norepinephrine-to-epinephrine conversion occurs in the cytoplasm. The synthesis of PNMT is induced by glucocorticoid hormones that reach the medulla via the intra-adrenal portal system. This special system provides for a 100-fold steroid concentration gradient over systemic arterial blood, and this high intra-adrenal concentration appears to be necessary for the induction of PNMT.

2- T_3 & T_4 Illustrate the Diversity in Hormone Synthesis:

The formation of **triiodothyronine (T_3)** and **tetraiodothyronine (thyroxine; T_4)** (see Figure 41–2) illustrates many of the principles of diversity discussed in this chapter. These hormones require a rare element (iodine) for bioactivity; they are synthesized as part of a very large precursor molecule (thyroglobulin); they are stored in an intracellular reservoir (colloid); and there is peripheral conversion of T_4 to T_3, which is a much more active hormone.

The thyroid hormones T_3 and T_4 are unique in that iodine (as iodide) is an essential component of both. In most parts of the world, iodine is a scarce component of soil, and for that reason there is little in food. A complex mechanism has evolved to acquire and retain this crucial element and to

convert it into a form suitable for incorporation into organic compounds. At the same time, the thyroid must synthesize thyronine from tyrosine, and this synthesis takes place in thyroglobulin (Figure 41–11).

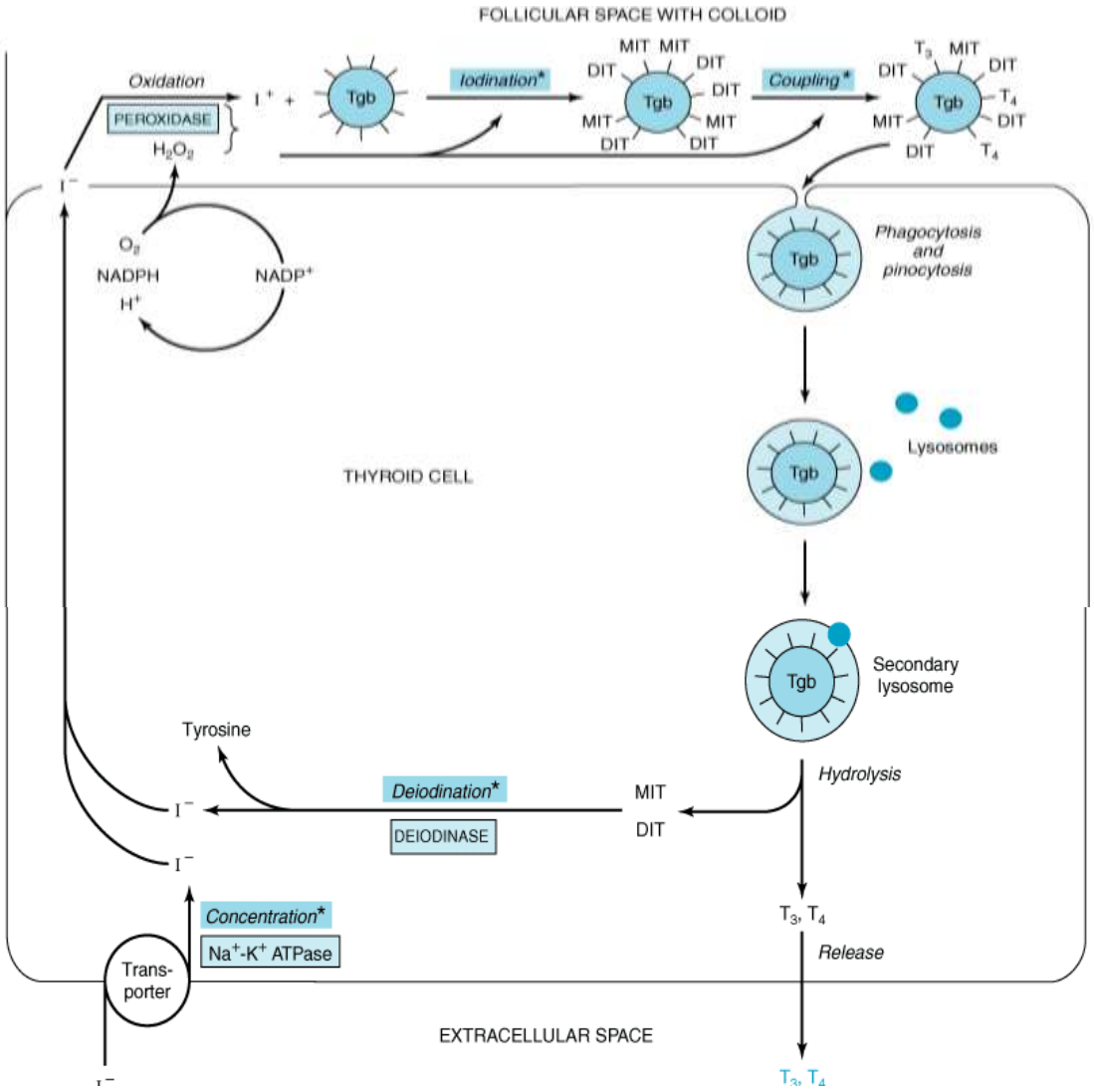


Figure 41–11: Model of iodide metabolism in the thyroid follicle. A follicular cell is shown facing the follicular lumen (top) and the extracellular space (at bottom). Iodide enters the thyroid primarily through a transporter (bottom left). Thyroid hormone synthesis occurs in the follicular space through a series of reactions, many of which are peroxidase-mediated. Thyroid hormones, stored in the colloid in the follicular space, are released from thyroglobulin by hydrolysis inside the thyroid cell. (Tgb, thyroglobulin; MIT, monoiodotyrosine; DIT, diiodotyrosine; T_3, triiodothyronine; T_4, tetraiodothyronine.) Asterisks indicate steps or processes that are inherited enzyme deficiencies that cause congenital goiter and often result in hypothyroidism.

Thyroglobulin is the precursor of T_4 and T_3. It is a large iodinated, glycosylated protein with a molecular mass of 660 kDa. Carbohydrate accounts for 8–10% of the weight of thyroglobulin and iodide for about 0.2–1%, depending upon the iodine content in the diet. Thyroglobulin is composed of two large subunits. It contains 115 tyrosine residues, each of which is a potential site of iodination. About 70% of the iodide in thyroglobulin exists in the inactive precursors, **monoiodotyrosine (MIT)** and **diiodotyrosine (DIT),** while 30% is in the **iodothyronyl residues,** T_4 and T_3. When iodine supplies are sufficient, the T_4:T_3 ratio is about 7:1. In **iodine deficiency,** this ratio decreases, as does the DIT:MIT ratio. Thyroglobulin, a large molecule of about 5000 amino acids, provides the conformation required for tyrosyl coupling and iodide organification necessary in the formation of the diaminoacid thyroid hormones. It is synthesized in the basal portion of the

cell and moves to the lumen, where it is a storage form of T_3 and T_4 in the colloid; several weeks' supply of these hormones exist in the normal thyroid. Within minutes after stimulation of the thyroid by TSH, colloid reenters the cell and there is a marked increase of phagolysosome activity. Various acid proteases and peptidases hydrolyze the thyroglobulin into its constituent amino acids, including T_4 and T_3, which are discharged from the basal portion of the cell (see Figure 41–11). Thyroglobulin is thus a very large prohormone.

Several Hormones Are Made from Larger Peptide Precursors

Formation of the critical disulfide bridges in insulin requires that this hormone be first synthesized as part of a larger precursor molecule, proinsulin. This is conceptually similar to the example of the thyroid hormones, which can only be formed in the context of a much larger molecule. Several other hormones are synthesized as parts of large precursor molecules, not because of some special structural requirement but rather as a mechanism for controlling the available amount of the active hormone. PTH and angiotensin II are examples of this type of regulation.

Insulin Is Synthesized as a Preprohormone & Modified Within the β Cell

Insulin has an AB heterodimeric structure with one intrachain (A6–A11) and two interchain disulfide bridges (A7–B7 and A20–B19) (Figure 41–12). The A and B chains could be synthesized in the laboratory, but attempts at a biochemical synthesis of the mature insulin molecule yielded very poor results. The reason for this became apparent when it was discovered that insulin is synthesized as a **preprohormone** (molecular weight approximately 11,500), which is the prototype for peptides that are processed from larger precursor molecules. The hydrophobic 23-amino-acid pre-, or leader, sequence directs the molecule into the cisternae of the endoplasmic reticulum and then is removed. This results in the 9000-MW proinsulin molecule, which provides the conformation necessary for the proper and efficient formation of the disulfide bridges. As shown in Figure 41–12, the sequence of proinsulin, starting from the amino terminal, is B chain—connecting (C) peptide—A chain. The proinsulin molecule undergoes a series of site-specific peptide cleavages that result in the formation of equimolar amounts of mature insulin and C peptide. These enzymatic cleavages are summarized in Figure 41–12.

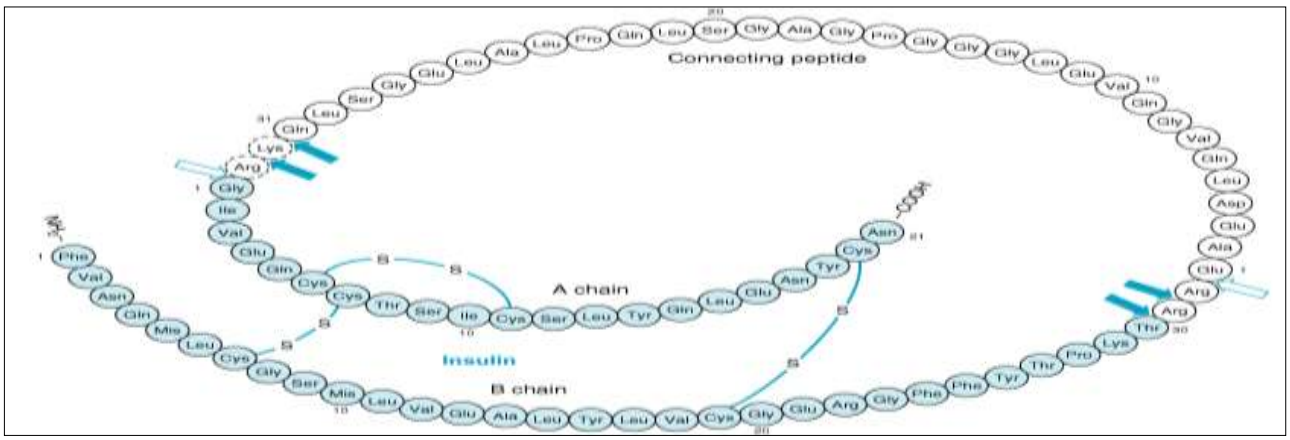


Figure 41–12: Structure of human proinsulin. Insulin and C-peptide molecules are connected at two sites by dipeptide links. An initial cleavage by a trypsin-like enzyme (open arrows) followed by several cleavages by a carboxypeptidase-like enzyme (solid arrows) results in the production of the heterodimeric (AB) insulin molecule (light color) and the C-peptide.

1- Parathyroid Hormone (PTH) Is Secreted as an 84-Amino-Acid Peptide:

The immediate precursor of PTH is **proPTH,** which differs from the native 84-amino-acid hormone by having a highly basic hexapeptide amino terminal extension. The primary gene product and the immediate precursor for proPTH is the 115-amino-acid **preproPTH.** This differs from proPTH by having an additional 25-amino-acid amino terminal extension that, in common with the other leader or signal sequences characteristic of secreted proteins, is hydrophobic. The complete structure of preproPTH and the sequences of proPTH and PTH are illustrated in Figure 41–13. $PTH_{1–34}$ has full biologic activity, and the region 25–34 is primarily responsible for receptor binding.

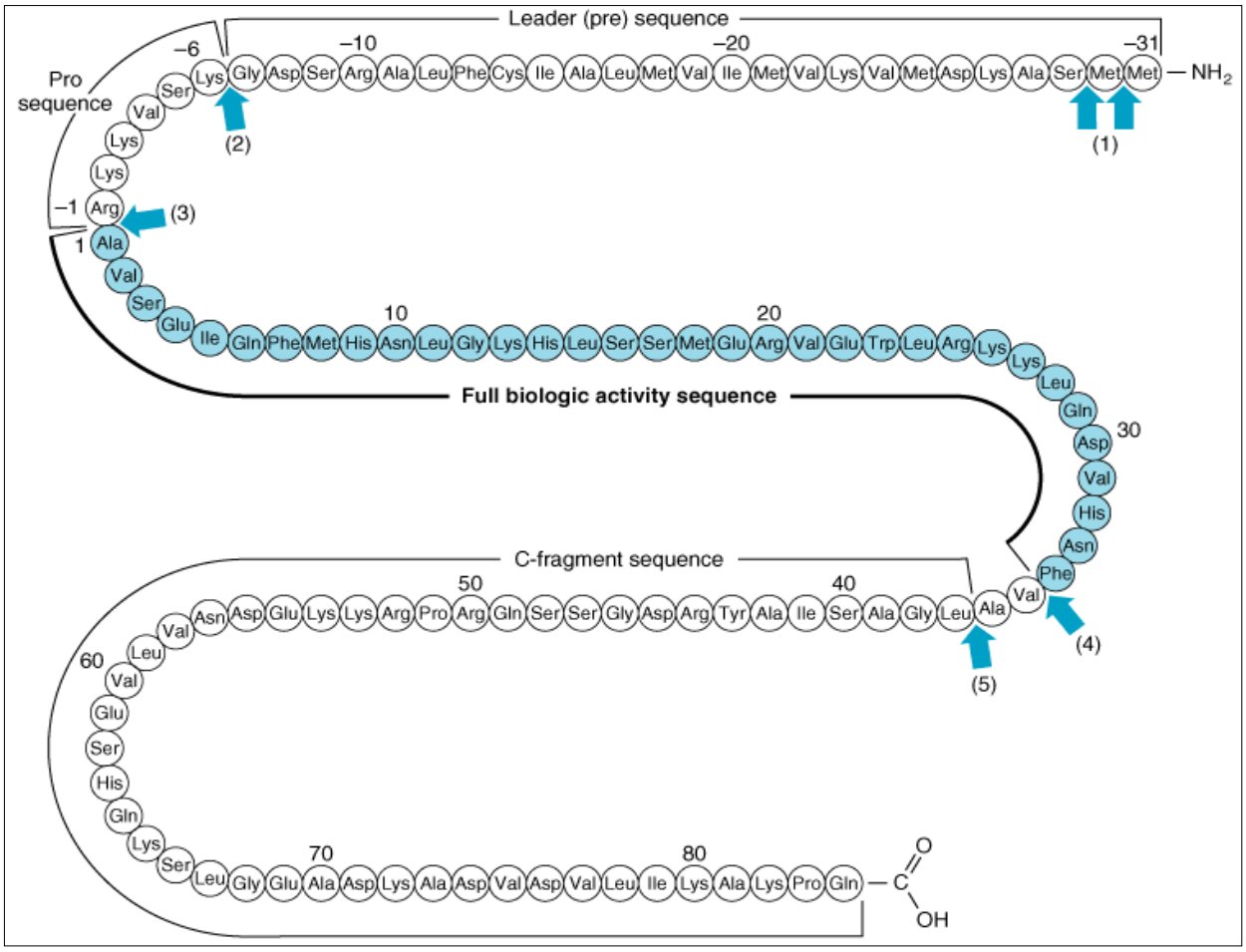


Figure 41–13: Structure of bovine preproparathyroid hormone. Arrows indicate sites cleaved by processing enzymes in the parathyroid gland (1–5) and in the liver after secretion of the hormone (4–5). The biologically active region of the molecule is flanked by sequence not required for activity on target receptors.

The biosynthesis of PTH and its subsequent secretion are regulated by the plasma ionized calcium (Ca^{2+}) concentration through a complex process. An acute decrease of Ca^{2+} results in a marked increase of PTH mRNA, and this is followed by an increased rate of PTH synthesis and secretion. However, about 80–90% of the proPTH synthesized cannot be accounted for as intact PTH in cells or in the incubation medium of experimental systems. This finding led to the conclusion that most of the proPTH synthesized is quickly degraded. It was later discovered that this rate of degradation decreases when Ca^{2+} concentrations are low, and it increases when Ca^{2+} concentrations are high. A Ca^{2+} receptor on the surface of the parathyroid cell mediates these effects. Very specific fragments of PTH are generated during its proteolytic digestion (Figure 41–13). A number of proteolytic enzymes, including cathepsins B and D, have been identified in parathyroid tissue. Cathepsin B

cleaves PTH into two fragments: $PTH_{1–36}$ and $PTH_{37–84}$. $PTH_{37–84}$ is not further degraded; however, $PTH_{1–36}$ is rapidly and progressively cleaved into di- and tripeptides. Most of the proteolysis of PTH occurs within the gland, but a number of studies confirm that PTH, once secreted, is proteolytically degraded in other tissues, especially the liver, by similar mechanisms.

2- Angiotensin II Is Also Synthesized from a Large Precursor:

The renin-angiotensin system is involved in the regulation of blood pressure and electrolyte metabolism (through production of aldosterone). The primary hormone involved in these processes is angiotensin II, an octapeptide made from angiotensinogen (Figure 41–14). Angiotensinogen, a large α_2-globulin made in liver, is the substrate for renin, an enzyme produced in the juxtaglomerular cells of the renal afferent arteriole. The position of these cells makes them particularly sensitive to blood pressure changes, and many of the physiologic regulators of renin release act through renal baroreceptors. The juxtaglomerular cells are also sensitive to changes of Na^+ and Cl^- concentration in the renal tubular fluid; therefore, any combination of factors that decreases fluid volume (dehydration, decreased blood pressure, fluid or blood loss) or decreases NaCl concentration stimulates renin release. Renal sympathetic nerves that terminate in the juxtaglomerular cells mediate the central nervous system and postural effects on renin release independently of the baroreceptor and salt effects, a mechanism that involves the β-adrenergic receptor. Renin acts upon the substrate angiotensinogen to produce the decapeptide angiotensin I.

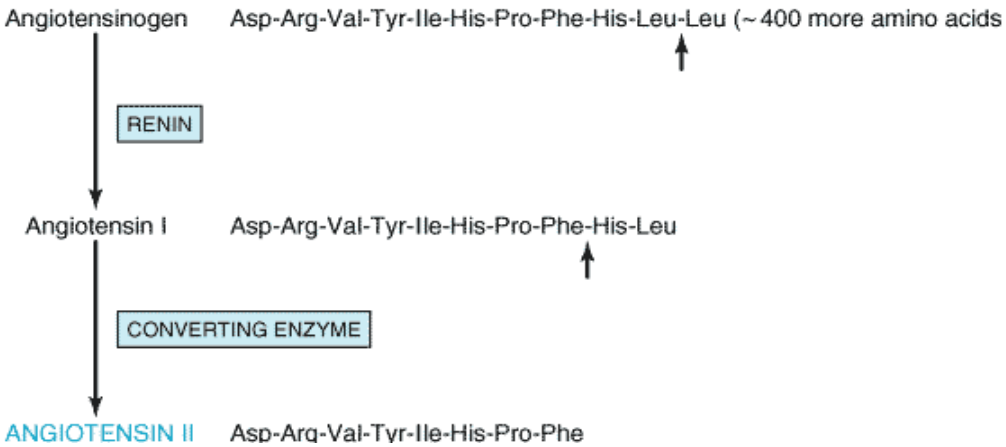


Figure 41–14: Formation and metabolism of angiotensins. Small arrows indicate cleavage sites.

Angiotensin-converting enzyme, a glycoprotein found in lung, endothelial cells, and plasma, removes two carboxyl terminal amino acids from the decapeptide angiotensin I to form angiotensin II in a step that is not thought to be rate-limiting. Various nonapeptide analogs of angiotensin I and other compounds act as competitive inhibitors of converting enzyme and are used to treat renin-dependent hypertension. These are referred to as **angiotensin-converting enzyme (ACE) inhibitors.** Angiotensin II increases blood pressure by causing vasoconstriction of the arteriole and is a very potent vasoactive substance. It inhibits renin release from the juxtaglomerular cells and is a potent stimulator of aldosterone production. This results in Na^+ retention, volume expansion, and increased blood pressure.

In some species, angiotensin II is converted to the heptapeptide angiotensin III (Figure 41–14), an equally potent stimulator of aldosterone production. In humans, the plasma level of angiotensin II is four times greater than that of angiotensin III, so most effects are exerted by the octapeptide. Angiotensins II and III are rapidly inactivated by angiotensinases.

Some Hormones Have Plasma Transport Proteins

The class I hormones are hydrophobic in chemical nature and thus are not very soluble in plasma. These hormones, principally the steroids and thyroid hormones, have specialized plasma transport proteins that serve several purposes. First, these proteins circumvent the solubility problem and thereby deliver the hormone to the target cell. They also provide a circulating reservoir of the hormone that can be substantial, as in the case of the thyroid hormones. Hormones, when bound to the transport proteins, cannot be metabolized, thereby prolonging their plasma half-life ($t_{1/2}$). The binding affinity of a given hormone to its transporter determines the bound versus free ratio of the hormone. This is important because only the free form of a hormone is biologically active. In general, the concentration of free hormone in plasma is very low, in the range of 10^{-15} to 10^{-9} mol/L. It is important to distinguish between plasma transport proteins and hormone receptors. Both bind hormones but with very different characteristics (Table 41–6).

1- Thyroid Hormones Are Transported by Thyroid-Binding Globulin:

Many of the principles discussed above are illustrated in a discussion of thyroid-binding proteins. One-half to two-thirds of T_4 and T_3 in the body is in an extrathyroidal reservoir. Most of this circulates in bound form, ie, bound to a specific binding protein, **thyroxine-binding globulin (TBG).** TBG, a glycoprotein with a molecular mass of 50 kDa, binds T_4 and T_3 and has the capacity to bind 20 µg/dL of plasma. Under normal circumstances, TBG binds—noncovalently—nearly all of the T_4 and T_3 in plasma, and it binds T_4 with greater affinity than T_3 (Table 41–7). The plasma half-life of T_4 is correspondingly four to five times that of T_3. The small, unbound (free) fraction is responsible for the biologic activity. Thus, in spite of the great difference in total amount, the free fraction of T_3 approximates that of T_4, and given that T_3 is intrinsically more active than T_4, most biologic activity is attributed to T_3. TBG does not bind any other hormones.

Table 41–7. Comparison of T_4 and T_3 in Plasma.

	Total Hormone (µg/dL)	Free Hormone			$t_{1/2}$ in Blood (days)
		Percentage of Total	ng/dL	Molarity	
T_4	8	0.03	~2.24	3.0×10^{-11}	6.5
T_3	0.15	0.3	~0.4	$\sim 0.6 \times 10^{-11}$	1.5

2- Glucocorticoids Are Transported by Corticosteroid-Binding Globulin:

Hydrocortisone (cortisol) also circulates in plasma in protein-bound and free forms. The main plasma binding protein is an α-globulin called **transcortin,** or **corticosteroid-binding globulin (CBG).** CBG is produced in the liver, and its synthesis, like that of TBG, is increased by estrogens. CBG binds most of the hormone when plasma cortisol levels are within the normal range; much smaller amounts of cortisol are bound to albumin. The avidity of binding helps determine the biologic half-lives of various glucocorticoids. Cortisol binds tightly to CBG and has a $t_{1/2}$ of 1.5–2 hours, while corticosterone, which binds less tightly, has a $t_{1/2}$ of less than 1 hour (Table 41–8).

Table 41–8. Approximate Affinities of Steroids for Serum-Binding Proteins.		
	SHBG[1]	**CBG[1]**
Dihydrotestosterone	1	> 100
Testosterone	2	> 100
Estradiol	5	>10
Estrone	> 10	> 100
Progesterone	> 100	~ 2
Cortisol	> 100	~ 3
Corticosterone	> 100	~ 5

[1]Affinity expressed as K_d (nmol/L).

3- Gonadal Steroids Are Transported by Sex Hormone-Binding Globulin:

Most mammals, humans included, have a plasma β-globulin that binds testosterone with specificity, relatively high affinity, and limited capacity (Table 41–8). This protein, usually called **sex hormone-binding globulin (SHBG)** or testosterone-estrogen-binding globulin (TEBG), is produced in the liver. Its production is increased by estrogens (women have twice the serum concentration of SHBG as men), certain types of liver disease, and hyperthyroidism; it is decreased by androgens, advancing age, and hypothyroidism. Many of these conditions also affect the production of CBG and TBG. Since SHBG and albumin bind 97–99% of circulating testosterone, only a small fraction of the hormone in circulation is in the free (biologically active) form. The primary function of SHBG may be to restrict the free concentration of testosterone in the serum. Testosterone binds to SHBG with higher affinity than does estradiol (Table 41–8). Therefore, a change in the level of SHBG causes a greater change in the free testosterone level than in the free estradiol level.

Hormone Action and Signal Transduction I

Biomedical Importance:

The homeostatic adaptations an organism makes to a constantly changing environment are in large part accomplished through alterations of the activity and amount of proteins. Hormones provide a major means of facilitating these changes. A hormone-receptor interaction results in generation of an intracellular signal that can either regulate the activity of a select set of genes, thereby altering the amount of certain proteins in the target cell, or affect the activity of specific proteins, including enzymes and transporter or channel proteins. The signal can influence the location of proteins in the cell and can affect general processes such as protein synthesis, cell growth, and replication, perhaps through effects on gene expression. Other signaling molecules—including cytokines, interleukins, growth factors, and metabolites—use some of the same general mechanisms and signal transduction pathways. Excessive, deficient, or inappropriate production and release of hormones and of these other regulatory molecules are major causes of disease. Many pharmacotherapeutic agents are aimed at correcting or otherwise influencing the pathways discussed in this chapter.

Hormones Transduce Signals to Affect Homeostatic Mechanisms:

The general steps involved in producing a coordinated response to a particular stimulus are illustrated in Figure 42–1. The stimulus can be a challenge or a threat to the organism, to an organ, or to the integrity of a single cell within that organism. Recognition of the stimulus is the first step in the adaptive response. At the organismic level, this generally involves the nervous system and the special senses (sight, hearing, pain, smell, touch). At the organismic or cellular level, recognition involves physicochemical factors such as pH, O_2 tension, temperature, nutrient supply, noxious metabolites, and osmolarity. Appropriate recognition results in the release of one or more hormones that will govern generation of the necessary adaptive response. For purposes of this discussion, the hormones are categorized as described in Chapter 41, ie, based on the location of their specific cellular receptors and the type of signals generated. Group I hormones interact with an intracellular receptor and group II hormones with receptor recognition sites located on the extracellular surface of the plasma membrane of target cells. The cytokines, interleukins, and growth factors should also be considered in this latter category. These molecules, of critical importance in homeostatic adaptation, are hormones in the sense that they are produced in specific cells, have the equivalent of autocrine, paracrine, and endocrine actions, bind to cell surface receptors, and activate many of the same signal transduction pathways employed by the more traditional hormones.

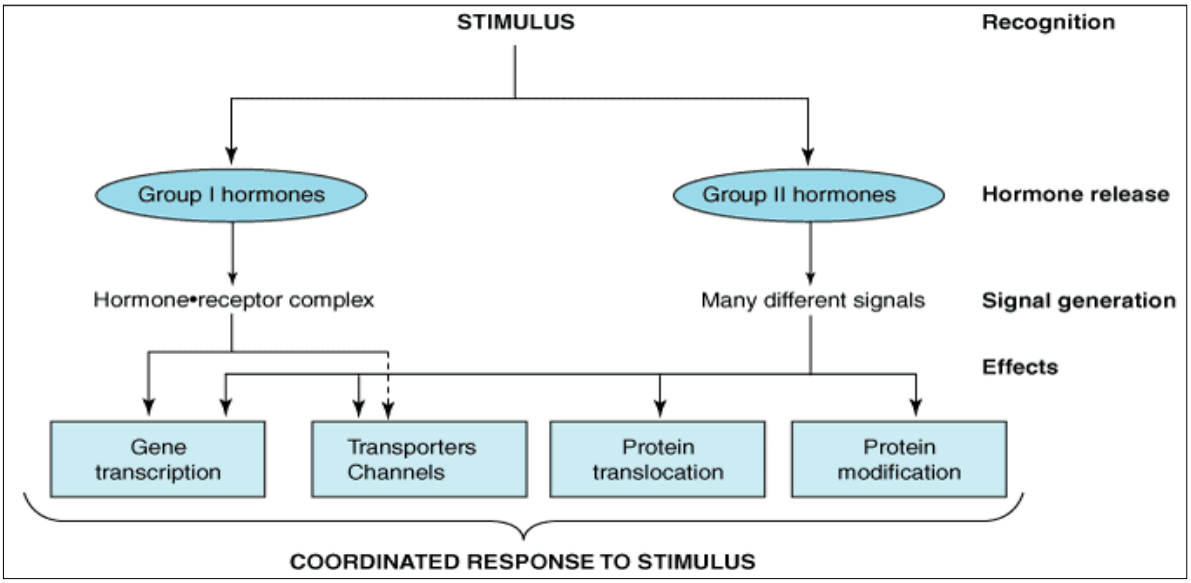


Figure 42–1: Hormonal involvement in responses to a stimulus. A challenge to the integrity of the organism elicits a response that includes the release of one or more hormones. These hormones generate signals at or within target cells, and these signals regulate a variety of biologic processes which provide for a coordinated response to the stimulus or challenge. See Figure 42–8 for a specific example.

Signal Generation

The Ligand-Receptor Complex Is the Signal for Group I Hormones:

The lipophilic group I hormones diffuse through the plasma membrane of all cells but only encounter their specific, high-affinity intracellular receptors in target cells. These receptors can be located in the cytoplasm or in the nucleus of target cells. The hormone-receptor complex first undergoes an **activation reaction.** As shown in Figure 42–2, receptor activation occurs by at least two mechanisms. For example, glucocorticoids diffuse across the plasma membrane and encounter their cognate receptor in the cytoplasm of target cells. Ligand-receptor binding results in the dissociation of heat shock protein 90 (hsp90) from the receptor. This step appears to be necessary for subsequent nuclear localization of the glucocorticoid receptor. This receptor also contains nuclear localization sequences that assist in the translocation from cytoplasm to nucleus. The now activated receptor moves into the nucleus (Figure 42–2) and binds with high affinity to a specific DNA sequence called the **hormone response element (HRE).** In the case illustrated, this is a glucocorticoid response element, or GRE. Consensus sequences for HREs are shown in Table 42–1. The DNA-bound, liganded receptor serves as a high-affinity binding site for one or more coactivator proteins, and accelerated gene transcription typically ensues when this occurs. By contrast, certain hormones such as the thyroid hormones and retinoids diffuse from the extracellular fluid across the plasma membrane and go

directly into the nucleus. In this case, the cognate receptor is already bound to the HRE (the thyroid hormone response element [TRE], in this example). However, this DNA-bound receptor fails to activate transcription because it exists in a corepressor complex. Indeed, this receptor-corepressor complex serves as an active repressor of gene transcription. The association of ligand with these receptors results in dissociation of the corepressor(s). The liganded receptor is now capable of binding one or more coactivators with high affinity, resulting in the activation of gene transcription. The relationship of hormone receptors to other nuclear receptors and to coregulators is discussed in more detail below.

Table 42–1. The DNA Sequences of Several Hormone Response Elements (HREs).[1]

Hormone or Effector	HRE	DNA Sequence
Glucocorticoids	GRE	GGTACA NNN TGTTCT ⟵ ⟶
Progestins	PRE	
Mineralocorticoids	MRE	
Androgens	ARE	
Estrogens	ERE	AGGTCA --- TGA/TCCT ⟵ ⟶
Thyroid hormone	TRE	AGGTCA N3,4,5 AGGTCA ⟶ ⟶
Retinoic acid	RARE	
Vitamin D	VDRE	
cAMP	CRE	TGACGTCA

[1]Letters indicate nucleotide; N means any one of the four can be used in that position. The arrows pointing in opposite directions illustrate the slightly imperfect inverted palindromes present in many HREs; in some cases these are called "half binding sites" because each binds one monomer of the receptor. The GRE, PRE, MRE, and ARE consist of the same DNA sequence. Specificity may be conferred by the intracellular concentration of the ligand or hormone receptor, by flanking DNA sequences not included in the consensus, or by other accessory elements. A second group of HREs includes those for thyroid hormones, estrogens, retinoic acid, and vitamin D. These HREs are similar except for the orientation and spacing between the half palindromes. Spacing determines the hormone specificity. VDRE (N = 3), TRE (N = 4), and RARE (N = 5) bind to direct repeats rather than to inverted repeats. Another member of the steroid receptor superfamily, the retinoid X receptor (RXR), forms heterodimers with VDR, TR, and RARE, and these constitute the *trans*-acting factors. cAMP affects gene transcription through the CRE.

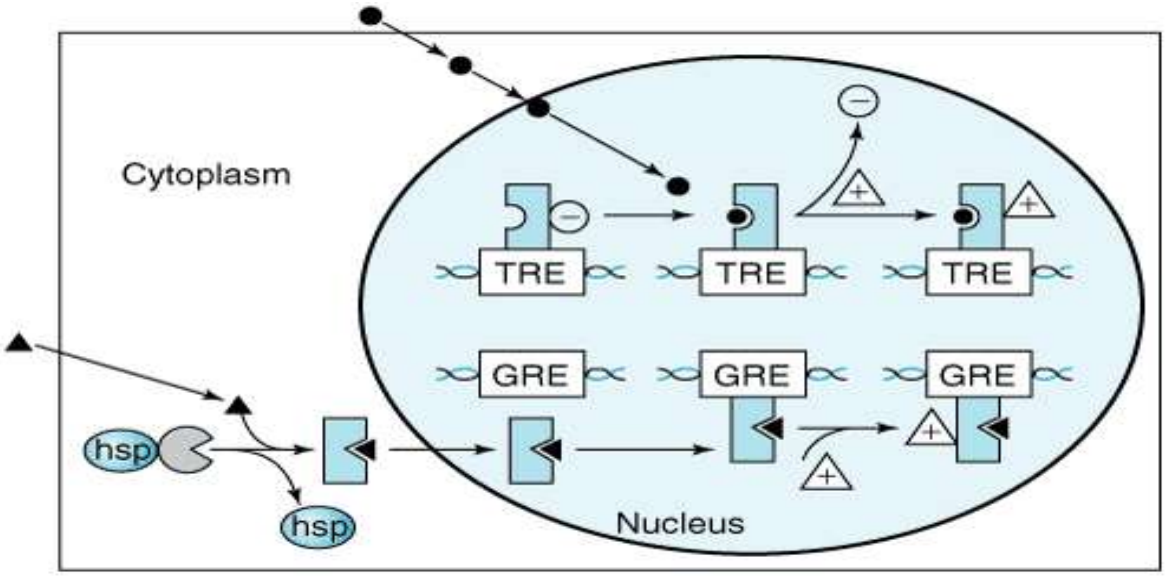


Figure 42–2: Regulation of gene expression by class I hormones. Steroid hormones readily gain access to the cytoplasmic compartment of target cells. Glucocorticoid hormones (solid triangles) encounter their cognate receptor in the cytoplasm, where it exists in a complex with heat shock protein 90 (hsp). Ligand binding causes dissociation of hsp and a conformational change of the receptor. The receptor•ligand complex then traverses the nuclear membrane and binds to DNA with specificity and high affinity at a glucocorticoid response element (GRE). This event triggers the assembly of a number of transcription coregulators (Δ), and enhanced transcription ensues. By contrast, thyroid hormones and retinoic acid (●) directly enter the nucleus, where their cognate receptors are already bound to the appropriate response elements with an associated transcription repressor complex (O). This complex, which consists of molecules such as N-CoR or SMRT (see Table 42–6) in the absence of ligand, actively inhibits transcription. Ligand binding results in dissociation of the repressor complex from the receptor, allowing an activator complex to assemble. The gene is then actively transcribed.

By selectively affecting gene transcription and the consequent production of appropriate target mRNAs, the amounts of specific proteins are changed and metabolic processes are influenced. The influence of each of these hormones is quite specific; generally, the hormone affects less than 1% of the genes, mRNA, or proteins in a target cell; sometimes only a few are affected. The nuclear actions of steroid, thyroid, and retinoid hormones are quite well defined. Most evidence suggests that these hormones exert their dominant effect on modulating gene transcription, but they—and many of the hormones in the other classes discussed below—can act at any step of the "information pathway" illustrated in Figure 42–3. Direct actions of steroids in the cytoplasm and on various organelles and membranes have also been described.

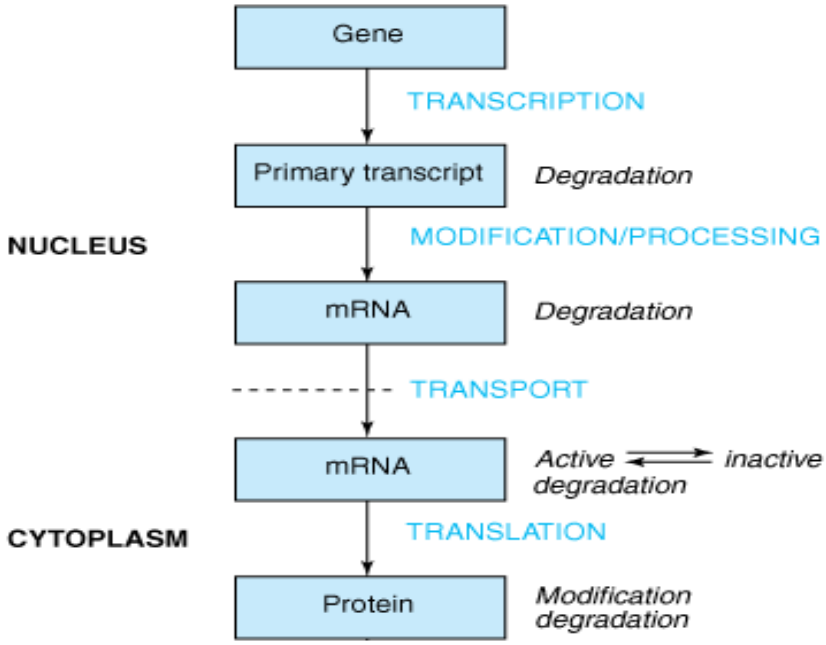


Figure 42–3: The "information pathway." Information flows from the gene to the primary transcript to mRNA to protein. Hormones can affect any of the steps involved and can affect the rates of processing, degradation, or modification of the various products.

Hormone Action & signal Transduction II

Group II (Peptide & Catecholamine) Hormones Have Membrane Receptors & Use Intracellular Messengers:

Many hormones are water-soluble, have no transport proteins (and therefore have a short plasma half-life), and initiate a response by binding to a receptor located in the plasma membrane (see Tables 41–3 and 41–4). The mechanism of action of this group of hormones can best be discussed in terms of the **intracellular signals** they generate. These signals include cAMP (cyclic AMP; 3',5'-adenylic acid; see Figure 19–5), a nucleotide derived from ATP through the action of adenylyl cyclase; cGMP, a nucleotide formed by guanylyl cyclase; Ca^{2+}; and phosphatidylinositides. Many of these second messengers affect gene transcription, as described in the previous paragraph; but they also influence a variety of other biologic processes, as shown in Figure 42–1.

G Protein-Coupled Receptors (GPCR):

Many of the group II hormones bind to receptors that couple to effectors through a GTP-binding protein intermediary. These receptors typically have seven hydrophobic plasma membrane-spanning domains. This is illustrated by the seven interconnected cylinders extending through the lipid bilayer in Figure 42–4. Receptors of this class, which signal through guanine nucleotide-bound protein intermediates, are known as **G protein-coupled receptors,** or **GPCRs.** To date, over 130 G protein-linked receptor genes have been cloned from various mammalian species. A wide variety of responses are mediated by the GPCRs.

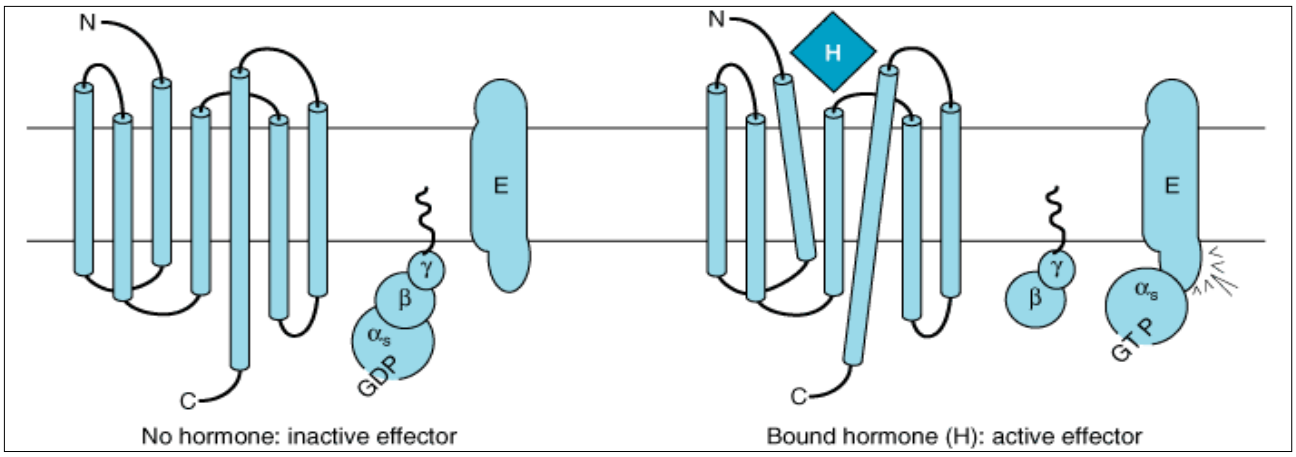


Figure 42–4: Components of the hormone receptor–G protein effector system. Receptors that couple to effectors through G proteins (GPCR) typically have seven membrane-spanning domains. In the absence of hormone (left), the heterotrimeric G-protein complex(α, β,γ) is in an inactive guanosine diphosphate (GDP)-bound form and is probably not associated with the receptor. This complex is anchored to the plasma membrane through prenylated groups on the βγ subunits (wavy lines) and perhaps by myristoylated groups on α subunits (not shown). On binding of hormone (H) to the receptor, there is a presumed conformational change of the receptor—as indicated by the tilted membrane spanning domains—and activation of the G-protein complex. This results from the exchange of GDP with guanosine triphosphate (GTP) on the α subunit, after which α and βγ dissociate. The α subunit binds to and activates the effector (E). E can be adenylyl cyclase, Ca^{2+}, Na^+, or Cl^- channels (α_s), or it could be a K^+ channel (α_i), phospholipase Cβ (α_q), or cGMP phosphodiesterase (α_t). The βγsubunit can also have direct actions on E.

cAMP Is the Intracellular Signal for Many Responses:

Cyclic AMP was the first intracellular signal identified in mammalian cells. Several components comprise a system for the generation, degradation, and action of cAMP.

Adenylyl Cyclase:

Different peptide hormones can either stimulate (s) or inhibit (i) the production of cAMP from adenylyl cyclase, which is encoded by at least nine different genes (Table 42–2). Two parallel systems, a stimulatory (s) one and an inhibitory (i) one, converge upon a catalytic molecule (C). Each consists of a receptor, R_s or R_i, and a regulatory complex, G_s and G_i. G_s and G_i are each trimers composed of α, β, and γ subunits. Because the α subunit in G_s differs from that in G_i, the proteins, which are distinct gene products, are designated α_s and α_i. The α subunits bind guanine nucleotides. The β and γ subunits are always associated (βγ) and appear to function as a heterodimer. The binding of a hormone to R_s or R_i results in a receptor-mediated activation of G, which entails the exchange of GDP by GTP on α and the concomitant dissociation of βγ from α.

Table 42–2. Subclassification of Group II.A Hormones.

Hormones That Stimulate Adenylyl Cyclase (H_S)	Hormones That Inhibit Adenylyl Cyclase (H_I)
ACTH	Acetylcholine
ADH	α_2-Adrenergics
β-Adrenergics	Angiotensin II
Calcitonin	Somatostatin
CRH	
FSH	
Glucagon	
hCG	
LH	
LPH	
MSH	
PTH	
TSH	

The α_s protein has intrinsic GTPase activity. The active form, α_s●GTP, is inactivated upon hydrolysis of the GTP to GDP; the trimeric G_s complex (αβγ) is then re-formed and is ready for another cycle of activation. Cholera and pertussis toxins catalyze the ADP-ribosylation of α_s and $\alpha_{i\text{-}2}$ (see Table 42–3), respectively. In the case of α_s, this modification disrupts the intrinsic GTP-ase activity; thus, α_s cannot reassociate with βγ and is therefore irreversibly activated. ADP-ribosylation of $\alpha_{i\text{-}2}$ prevents the dissociation of $\alpha_{i\text{-}2}$ from βγ, and free $\alpha_{i\text{-}2}$ thus cannot be formed. α_s activity in such cells is therefore unopposed.

Table 42–3. Classes and Functions of Selected G Proteins.[1,2]			
Class or Type	**Stimulus**	**Effector**	**Effect**
G_s			
α_s	Glucagon, β-adrenergics	↑Adenylyl cydase	Gluconeogenesis, lipolysis, glycogenolysis
		↑Cardiac Ca^{2+}, Cl^-, and Na^+ channels	
α_{olf}	Odorant	↑Adenylyl cydase	Olfaction
G_i			
$\alpha_{i-1,2,3}$	Acetylcholine, α_2-adrenergics	↓ Adenylyl cydase	Slowed heart rate
		↑Potassium channels	
	M_2 cholinergics	↓ Calcium channels	
α_o	Opioids, endorphins	↑Potassium channels	Neuronal electrical activity
α_t	Light	↑cGMP phosphodiesterase	Vision
G_q			
α_q	M_1 cholinergics		
	α_1-Adrenergics	↑Phospholipase C-β1	↑Muscle contraction and ↑Blood pressure
α_{11}	α_1-Adrenergics	↑Phospholipase c-β2	
G_{12}			

There is a large family of G proteins, and these are part of the superfamily of GTPases. The G protein family is classified according to sequence homology into four subfamilies, as illustrated in Table 42–3. There are 21α, 5β, and 8 γsubunit genes. Various combinations of these subunits provide a large number of possible αβγ and cyclase complexes.

The α subunits and the βγ complex have actions independent of those on adenylyl cyclase (see Figure 42–4 and Table 42–3). Some forms of α_i stimulate K^+ channels and inhibit Ca^{2+} channels, and some α_s molecules have the opposite effects. Members of the G_q family activate the phospholipase C group of enzymes. The βγ complexes have been associated with K^+ channel stimulation and phospholipase C activation. G proteins are involved in many important biologic processes in addition to hormone action. Notable examples include olfaction (α_{OLF}) and vision (α_t). Some examples are listed in Table 42–3. GPCRs are implicated in a number of diseases and are major targets for pharmaceutical agents.

Protein Kinase:

In prokaryotic cells, cAMP binds to a specific protein called catabolite regulatory protein (CRP) that binds directly to DNA and influences gene expression. In eukaryotic cells, cAMP binds to a protein kinase called **protein kinase A (PKA)** that is a heterotetrameric molecule consisting of two regulatory subunits (R) and two catalytic subunits (C). cAMP binding results in the following reaction:

$$4\ \text{cAMP} + R_2C_2 \rightleftarrows R_2 \cdot (4\ \text{cAMP}) + 2C$$

The R_2C_2 complex has no enzymatic activity, but the binding of cAMP by R dissociates R from C, thereby activating the latter (Figure 42–5). The active C subunit catalyzes the transfer of the γ phosphate of ATP to a serine or threonine residue in a variety of proteins. The consensus phosphorylation sites are -Arg-Arg/Lys-X-Ser/Thr- and -Arg-Lys-X-X-Ser-, where X can be any amino acid.

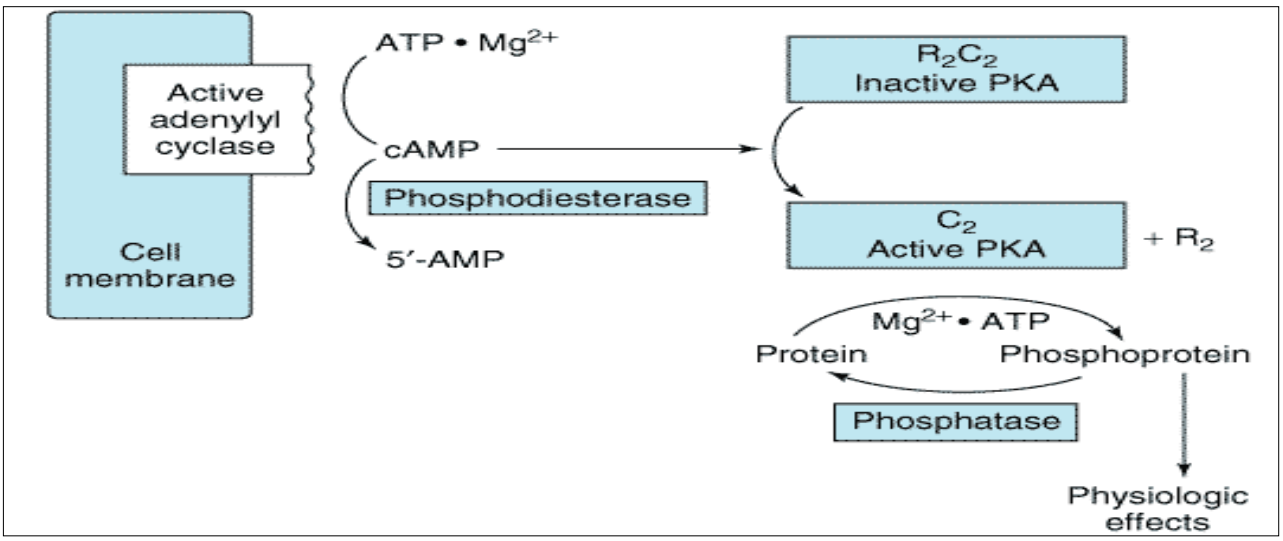


Figure 42–5: Hormonal regulation of cellular processes through cAMP-dependent protein kinase (PKA). PKA exists in an inactive form as an R_2C_2 heterotetramer consisting of two regulatory and two catalytic subunits. The cAMP generated by the action of adenylyl cyclase (activated as shown in Figure 42–4) binds to the regulatory (R) subunit of PKA. This results in dissociation of the regulatory and catalytic subunits and activation of the latter. The active catalytic subunits phosphorylate a number of target proteins on serine and threonine residues. Phosphatases remove phosphate from these residues and thus terminate the physiologic response. A phosphodiesterase can also terminate the response by converting cAMP to 5'-AMP.

Protein kinase activities were originally described as being "cAMP-dependent" or "cAMP-independent." This classification has changed, as protein phosphorylation is now recognized as being a major regulatory mechanism. Several hundred protein kinases have now been described. The kinases are related in sequence and structure within the catalytic domain, but each is a unique molecule with considerable variability with respect to subunit composition, molecular weight, autophosphorylation, K_m for ATP, and substrate specificity.

Phosphoproteins:

The effects of cAMP in eukaryotic cells are all thought to be mediated by protein phosphorylation-dephosphorylation, principally on serine and threonine residues. The control of any of the effects of cAMP, including such diverse processes as steroidogenesis, secretion, ion transport, carbohydrate and fat metabolism, enzyme induction, gene regulation, synaptic transmission, and cell growth and replication, could be conferred by a specific protein kinase, by a specific phosphatase, or by specific substrates for phosphorylation. These substrates help define a target tissue and are involved in defining the extent of a particular response within a given cell. For example, the effects of cAMP on gene transcription are mediated by the protein **cyclic AMP response element binding protein (CREB).** CREB binds to a cAMP responsive element (CRE) (see Table 42–1) in its nonphosphorylated state and is a weak activator of transcription. When phosphorylated by PKA, CREB binds the coactivator **CREB-binding protein CBP/ p300** (see below) and as a result is a much more potent transcription activator.

Phosphodiesterases:

Actions caused by hormones that increase cAMP concentration can be terminated in a number of ways, including the hydrolysis of cAMP to 5'-AMP by phosphodiesterases (see Figure 42–5). The presence of these hydrolytic enzymes ensures a rapid turnover of the signal (cAMP) and hence a rapid termination of the biologic process once the hormonal stimulus is removed. There are at least 11 known members of the phosphodiesterase family of enzymes. These are subject to regulation by their substrates, cAMP and cGMP; by hormones; and by intracellular messengers such as calcium, probably acting through calmodulin. Inhibitors of phosphodiesterase, most notably methylated xanthine derivatives such as caffeine, increase intracellular cAMP and mimic or prolong the actions of hormones through this signal.

Phosphoprotein Phosphatases:

Given the importance of protein phosphorylation, it is not surprising that regulation of the protein dephosphorylation reaction is another important control mechanism (see Figure 42–5). The phosphoprotein phosphatases are themselves subject to regulation by phosphorylation-dephosphorylation reactions and by a variety of other mechanisms, such as protein-protein interactions. In fact, the substrate specificity of the phosphoserine-phosphothreonine phosphatases may be dictated by distinct regulatory subunits whose binding is regulated hormonally. The best-studied role of regulation by the dephosphorylation of proteins is that of glycogen metabolism in muscle. Two major types of phosphoserine-phosphothreonine phosphatases have been described. Type I preferentially dephosphorylates the β subunit of phosphorylase kinase, whereas type II dephosphorylates the α subunit. Type I phosphatase is implicated in the regulation of glycogen synthase, phosphorylase, and phosphorylase kinase. This phosphatase is itself regulated by phosphorylation of certain of its subunits, and these reactions are reversed by the action of one of the type II phosphatases. In addition, two heat-stable protein inhibitors regulate type I phosphatase activity. Inhibitor-1 is phosphorylated and activated by cAMP-dependent protein kinases; and inhibitor-2, which may be a subunit of the inactive phosphatase, is also phosphorylated, possibly by glycogen synthase kinase-3. Phosphatases that attack phosphotyrosine are also important in signal transduction (see Figure 42–8).

Hormone Action & signal Transduction III

cGMP Is Also an Intracellular Signal

Cyclic GMP is made from GTP by the enzyme gua-nylyl cyclase, which exists in soluble and membrane-bound forms. Each of these isozymes has unique physiologic properties. The atriopeptins, a family of peptides produced in cardiac atrial tissues, cause natriuresis, diuresis, vasodilation, and inhibition of aldosterone secretion. These peptides (eg, atrial natriuretic factor) bind to and activate the membrane-bound form of guanylyl cyclase. This results in an increase of cGMP by as much as 50-fold in some cases, and this is thought to mediate the effects mentioned above. Other evidence links cGMP to vasodilation. A series of compounds, including nitroprusside, nitroglycerin, nitric oxide, sodium nitrite, and sodium azide, all cause smooth muscle relaxation and are potent vasodilators. These agents increase cGMP by activating the soluble form of guanylyl cyclase, and inhibitors of cGMP phosphodiesterase (the drug sildenafil [Viagra], for example) enhance and prolong these responses. The increased cGMP activates cGMP-dependent protein kinase (PKG), which in turn phosphorylates a number of smooth muscle proteins. Presumably, this is involved in relaxation of smooth muscle and vasodilation.

Several Hormones Act through Calcium or Phosphatidylinositols

Ionized calcium is an important regulator of a variety of cellular processes, including muscle contraction, stimulus-secretion coupling, the blood clotting cascade, enzyme activity, and membrane excitability. It is also an intracellular messenger of hormone action.

Calcium Metabolism

The extracellular calcium (Ca^{2+}) concentration is about 5 mmol/L and is very rigidly controlled. Although substantial amounts of calcium are associated with intracellular organelles such as mitochondria and the endoplasmic reticulum, the intracellular concentration of free or ionized calcium (Ca^{2+}) is very low: 0.05–10 µmol/L. In spite of this large concentration gradient and a favorable transmembrane electrical gradient, Ca^{2+} is restrained from entering the cell. A considerable amount of energy is expended to ensure that the intracellular Ca^{2+} is controlled, as a prolonged elevation of Ca^{2+} in the cell is very toxic. A Na^{+}/Ca^{2+} exchange mechanism that has a high capacity but low affinity pumps Ca^{2+} out of cells. There also is a Ca^{2+}/proton ATPase-dependent pump that extrudes Ca^{2+} in exchange for H^{+}. This has a high affinity for Ca^{2+} but a low capacity and is probably responsible for fine-tuning cytosolic Ca^{2+}. Furthermore, Ca^{2+} ATPases pump Ca^{2+} from the cytosol to the lumen of the endoplasmic reticulum. There are three ways of changing cytosolic Ca^{2+}: (1) Certain hormones (class II.C, Table 41–3) by binding to receptors that are themselves Ca^{2+} channels, enhance membrane permeability to Ca^{2+} and thereby increase Ca^{2+} influx. (2) Hormones also indirectly promote Ca^{2+} influx by modulating the membrane potential at the plasma membrane. Membrane depolarization opens voltage-gated Ca^{2+} channels and allows for Ca^{2+} influx. (3) Ca^{2+} can be mobilized from the endoplasmic reticulum, and possibly from mitochondrial pools.

An important observation linking Ca^{2+} to hormone action involved the definition of the intracellular targets of Ca^{2+} action. The discovery of a Ca^{2+}-dependent regulator of phosphodiesterase activity provided the basis for a broad understanding of how Ca^{2+} and cAMP interact within cells.

Calmodulin

The calcium-dependent regulatory protein is calmodulin, a 17-kDa protein that is homologous to the muscle protein troponin C in structure and function. Calmodulin has four Ca^{2+} binding sites, and full occupancy of these sites leads to a marked conformational change, which allows calmodulin to activate enzymes and ion channels. The interaction of Ca^{2+} with calmodulin (with the resultant change of activity of the latter) is conceptually similar to the binding of cAMP to PKA and the subsequent activation of this molecule. Calmodulin can be one of numerous subunits of complex proteins and is particularly involved in regulating various kinases and enzymes of cyclic nucleotide generation and degradation. A partial list of the enzymes regulated directly or indirectly by Ca^{2+}, probably through calmodulin, is presented in Table 42–4.

Table 42–4. Enzymes and Proteins Regulated by Calcium or Calmodulin.
Adenylyl cyclase
Ca^{2+}-dependent protein kinases
Ca^{2+}-Mg^{2+} ATPase
Ca^{2+}-phospholipid-dependent protein kinase
Cyclic nucleotide phosphodiesterase
Some cytoskeletal proteins
Some ion channels (eg, L-type calcium channels)
Nitric oxide synthase
Phosphorylase kinase
Phosphoprotein phosphatase 2B
Some receptors (eg, NMDA-type glutamate receptor)

In addition to its effects on enzymes and ion transport, Ca^{2+}/calmodulin regulates the activity of many structural elements in cells. These include the actin-myosin complex of smooth muscle, which is under α-adrenergic control, and various microfilament-mediated processes in noncontractile cells, including cell motility, cell conformation changes, mitosis, granule release, and endocytosis.

Calcium Is a Mediator of Hormone Action

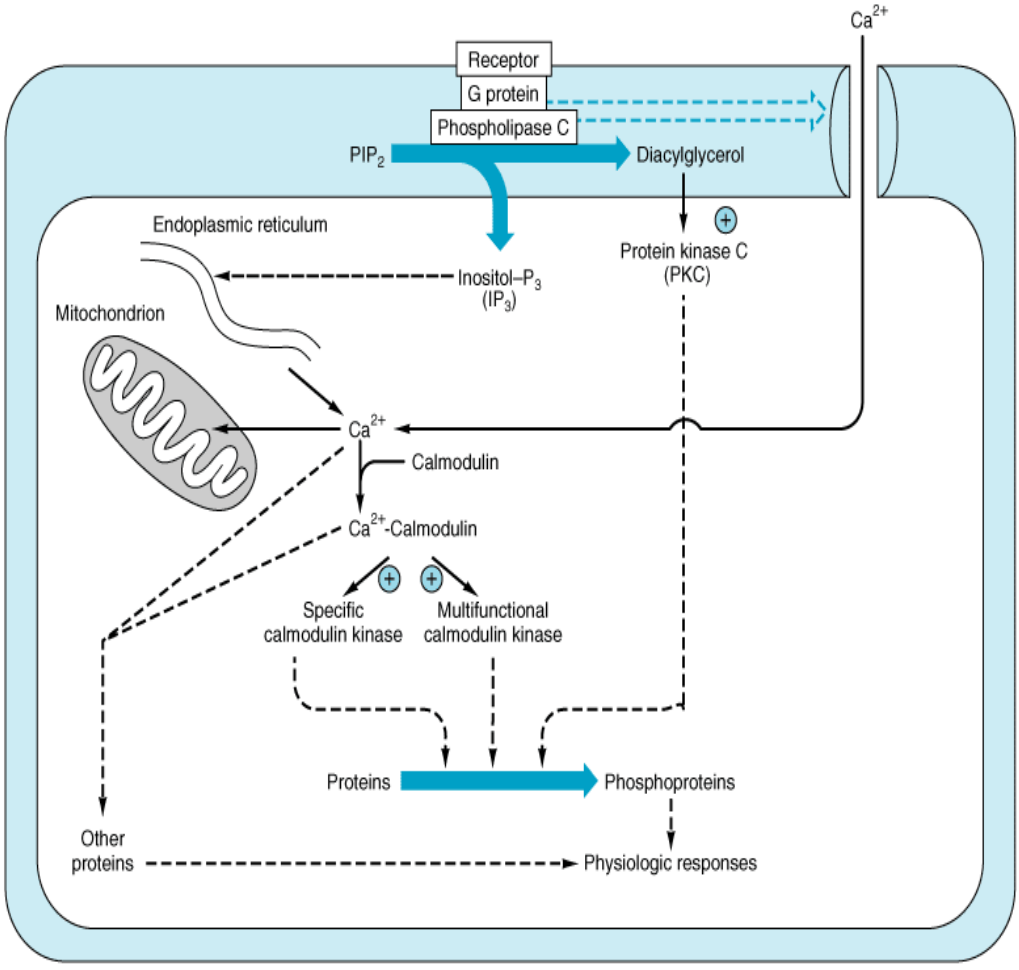
A role for Ca^{2+} in hormone action is suggested by the observations that the effect of many hormones is (1) blunted by Ca^{2+}-free media or when intracellular calcium is depleted; (2) can be mimicked by agents that increase cytosolic Ca^{2+}, such as the Ca^{2+} ionophore A23187; and (3) influences cellular calcium flux. The regulation of glycogen metabolism in liver by vasopressin and β -adrenergic catecholamines provides a good example. This is shown schematically in Figures 19–6 and 19–7.

A number of critical metabolic enzymes are regulated by Ca^{2+}, phosphorylation, or both, including glycogen synthase, pyruvate kinase, pyruvate carboxylase, glycerol-3-phosphate dehydrogenase, and pyruvate dehydrogenase.

Phosphatidylinositide Metabolism Affects Ca^{2+}-Dependent Hormone Action

Some signal must provide communication between the hormone receptor on the plasma membrane and the intracellular Ca^{2+} reservoirs. This is accomplished by products of phosphatidylinositol metabolism. Cell surface receptors such as those for acetylcholine, antidiuretic hormone, and α_1-type catecholamines are, when occupied by their respective ligands, potent activators of phospholipase C. Receptor binding and activation of phospholipase C are coupled by the G_q isoforms (Table 42–3 and Figure 42–6). Phospholipase C catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol trisphosphate (IP_3) and 1,2-diacylglycerol (Figure 42–7). Diacylglycerol is itself capable of activating **protein kinase C (PKC),** the activity of which also depends upon Ca^{2+}. IP_3, by interacting with a specific intracellular receptor, is an effective releaser of Ca^{2+} from intracellular storage sites in the endoplasmic reticulum. Thus, the hydrolysis of phosphatidylinositol 4,5-bisphosphate leads to activation of PKC and promotes an increase of cytoplasmic Ca^{2+}. As shown in Figure 42–4, the activation of G proteins can also have a direct action on Ca^{2+} channels. The resulting elevations of cytosolic Ca^{2+} activate Ca^{2+}–calmodulin-dependent kinases and many other Ca^{2+}–calmodulin-dependent enzymes.

Figure 42–6.



Certain hormone-receptor interactions result in the activation of phospholipase C. This appears to involve a specific G protein, which also may activate a calcium channel. Phospholipase C results in generation of inositol trisphosphate (IP_3), which liberates stored intracellular Ca^{2+}, and diacylglycerol (DAG), a potent activator of protein kinase C (PKC). In this scheme, the activated PKC phosphorylates specific substrates, which then alter physiologic processes. Likewise, the Ca^{2+}-calmodulin complex can activate specific kinases, two of which are shown here. These actions result in phosphorylation of substrates, and this leads to altered physiologic responses. This figure also shows that Ca^{2+} can enter cells through voltage- or ligand-gated Ca^{2+} channels. The intracellular Ca^{2+} is also regulated through storage and release by the mitochondria and endoplasmic reticulum.

Steroidogenic agents—including ACTH and cAMP in the adrenal cortex; angiotensin II, K^+, serotonin, ACTH, and cAMP in the zona glomerulosa of the adrenal; LH in the ovary; and LH and cAMP in the Leydig cells of the testes—have been associated with increased amounts of phosphatidic acid, phosphatidylinositol, and polyphosphoinositides (see Chapter 15) in the respective target tissues. Several other examples could be cited.

The roles that Ca^{2+} and polyphosphoinositide breakdown products might play in hormone action are presented in Figure 42–6. In this scheme the activated protein kinase C can phosphorylate specific substrates, which then alter physiologic processes. Likewise, the Ca^{2+}-calmodulin complex can activate specific kinases. These then modify substrates and thereby alter physiologic responses.