26.3.4. The LDL Receptor Is a Transmembrane Protein Having Five Different Functional Regions

The amino acid sequence of the human LDL receptor reveals the mosaic structure of this 115-kd protein, which is composed of six different types of domain (Figure 26.18). The amino-terminal region of the mature receptor consists of a cysteine-rich sequence of about 40 residues that is repeated, with some variation, seven times to form the LDL-binding domain (Figure 26.19). A set of conserved acidic side chains in this domain bind calcium ion; this metal ion lies at the center of each domain and, along with disulfide bonds formed from the conserved cysteine residues, stabilizes the three-dimensional structure. Protonation of these glutamate and aspartate side chains of the receptor in lysosomes leads to the release of calcium and hence to structural disruption and the release of LDL from its receptor. A second region of the LDL receptor includes two types of recognizable domains, three domains homologous to epidermal growth factor and six repeats that are similar to the blades of the transducin β subunit (Section 15.2.2). The six repeats form a propeller-like structure that packs against one of the EGF-like domains (Figure 26.20). An aspartate residue forms hydrogen bonds that hold each blade to the rest of the structure. These interactions, too, would most likely be disrupted at the low pH in the lysosome.

The third region contains a single domain that is very rich in serine and threonine residues and contains *O*-linked sugars. These oligosaccharides may function as struts to keep the receptor extended from the membrane so that the LDL-binding domain is accessible to LDL. The fourth region contains the fifth type of domain, which consists of 22 hydrophobic residues that span the plasma membrane. The final region contains the sixth type of domain; it consists of 50 residues and emerges on the cytosolic side of the membrane, where it controls the interaction of the receptor with coated pits and participates in endocytosis. The gene for the LDL receptor consists of 18 exons, which correspond closely to the structural units of the protein. *The LDL receptor is a striking example of a mosaic protein encoded by a gene that was assembled by exon shuffling.*

26.3.5. The Absence of the LDL Receptor Leads to Hypercholesteremia and Atherosclerosis

The results of Brown and Goldstein's pioneering studies of *familial hypercholesterolemia* revealed the physiologic importance of the LDL receptor. The total concentration of cholesterol and LDL in the plasma is markedly elevated in this genetic disorder, which results from a mutation at a single autosomal locus. The cholesterol level in the plasma of homozygotes is typically 680 mg dl^{-1}, compared with 300 mg dl^{-1} in heterozygotes (clinical assay results are often expressed in milligrams per deciliter, which is equal to milligrams per 100 milliliters). A value of < 200 mg dl^{-1} is regarded as desirable, but many people have higher levels. *In familial hypercholesterolemia, cholesterol is deposited in various tissues because of the high concentration of LDL cholesterol in the plasma.* Nodules of cholesterol called *xanthomas* are prominent in skin and tendons. Of particular concern is the oxidation of the excess blood LDL to form oxidized LDL (oxLDL). The oxLDL is taken up by immune-system cells called macrophages, which become engorged to form foam cells. These foam cells become trapped in the walls of the blood vessels and contribute to the formation of atherosclerotic plaques that cause arterial narrowing and lead to heart attacks (Figure 26.21). In fact, *most homozygotes die of coronary artery disease in childhood.* The disease in heterozygotes (1 in 500 people) has a milder and more variable clinical course. A serum esterase that degrades oxidized lipids is found in association with HDL. Possibly, the HDL-associated protein destroys the oxLDL, accounting for HDL's ability to protect against coronary disease.

The molecular defect in most cases of familial hypercholesterolemia is an absence or deficiency of functional receptors for LDL. Receptor mutations that disrupt each of the stages in the endocytotic pathway have been identified. Homozygotes have almost no functional receptors for LDL, whereas heterozygotes have about half the normal number. Consequently, the entry of LDL into liver and other cells is impaired, leading to an increased plasma level of LDL. Furthermore, less IDL enters liver cells because IDL entry, too, is mediated by the LDL receptor. Consequently, IDL stays in the blood longer in familial hypercholesterolemia, and more of it is converted into LDL than in normal people. All deleterious consequences of an absence or deficiency of the LDL receptor can be attributed to the ensuing elevated level of LDL cholesterol in the blood.

26.3.6. The Clinical Management of Cholesterol Levels Can Be Understood at a Biochemical Level

Homozygous familial hypercholesterolemia can be treated only by a liver transplant. A more generally applicable therapy is available for heterozygotes and others with high levels of cholesterol. *The goal is to reduce the amount of cholesterol in the blood by stimulating the single normal gene to produce more than the customary number of LDL receptors.* We have already observed that the production of LDL receptors is controlled by the cell's need for cholesterol. Therefore, in essence, the strategy is to deprive the cell of ready sources of cholesterol. When cholesterol is required, the amount of mRNA for the LDL receptor rises and more receptor is found on the cell surface. This state can be induced by a two-pronged approach. First, the intestinal reabsorption of bile salts is inhibited. Bile salts are cholesterol derivatives that promote the absorption of dietary cholesterol and dietary fats (Section 22.1.1). Second, de novo synthesis of cholesterol is blocked.

The reabsorption of bile is impeded by oral administration of positively charged polymers, such as cholestyramine, that bind negatively charged bile salts and are not themselves absorbed. Cholesterol synthesis can be effectively blocked by a class of compounds called *statins* (e.g., lovastatin, which is also called mevacor; Figure 26.22). These compounds are potent competitive inhibitors (K_i < 1 nM) of HMG-CoA reductase, the essential control point in the biosynthetic pathway. Plasma cholesterol levels decrease by 50% in many patients given both lovastatin and inhibitors of bile-salt reabsorption. Lovastatin and other inhibitors of HMG-CoA reductase are widely used to lower the plasma cholesterol level in people who have atherosclerosis, which is the leading cause of death in industrialized societies.

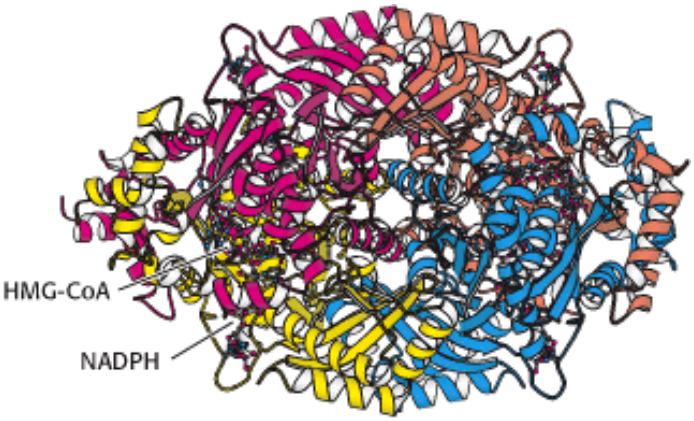


Figure 26.14. HMG-CoA Reductase. The structure of a portion of the tetrameric enzyme is shown.

Table 26.1. Properties of plasma lipoproteins

Lipoproteins	Major core lipids	Apoproteins	Mechanism of lipid delivery
Chylomicron	Dietary triacylglycerols	B-48, C, E	Hydrolysis by lipoprotein lipase
Chylomicron remnant	Dietary cholesterol esters	B-48, E	Receptor-mediated endocytosis by liver
Very low density lipoprotein (VLDL)	Endogenous triacylglycerols	B-100, C, E	Hydrolysis by lipoprotein lipase

Intermediate-density lipoprotein (IDL)	Endogenous cholesterol esters	B-100, E	Receptor-mediated endocytosis by liver and conversion into LDL
Low-density lipoprotein (LDL)	Endogenous cholesterol esters	B-100	Receptor-mediated endocytosis by liver and other tissues
High-density lipoprotein (HDL)	Endogenous cholesterol esters	A	Transfer of cholesterol esters to IDL and LDL

Source: After M. S. Brown and J. L. Goldstein, *The Pharmacological Basis of Therapeutics.* 7th ed., A. G. Gilman, L. S. Goodman, T. W. Rall, and F. Murad, Eds. (Macmillan, 1985), p. 828.

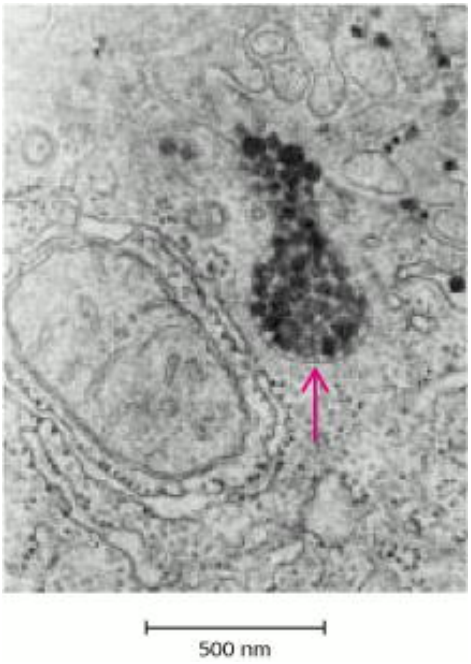


Figure 26.15. Site of Cholesterol Synthesis. Electron micrograph of a part of a liver cell actively engaged in the synthesis and secretion of very low density lipoprotein (VLDL). The arrow points to a vesicle that is releasing its content of VLDL particles. [Courtesy of Dr. George Palade.]

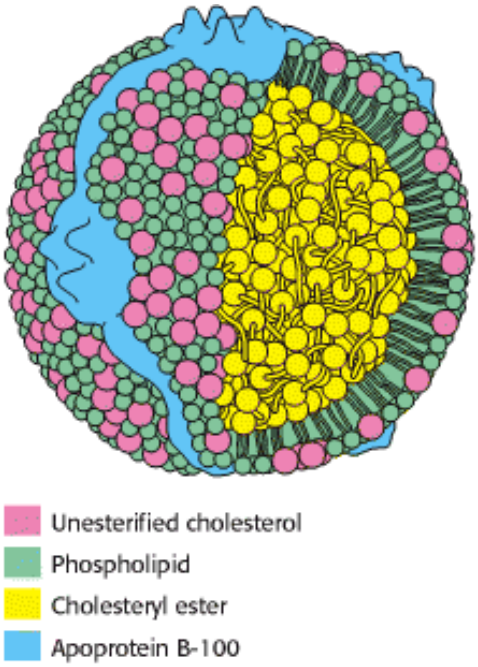


Figure 26.16. Schematic Model of Low-Density Lipoprotein. The LDL particle is approximately 22 nm (220 Å) in diameter.

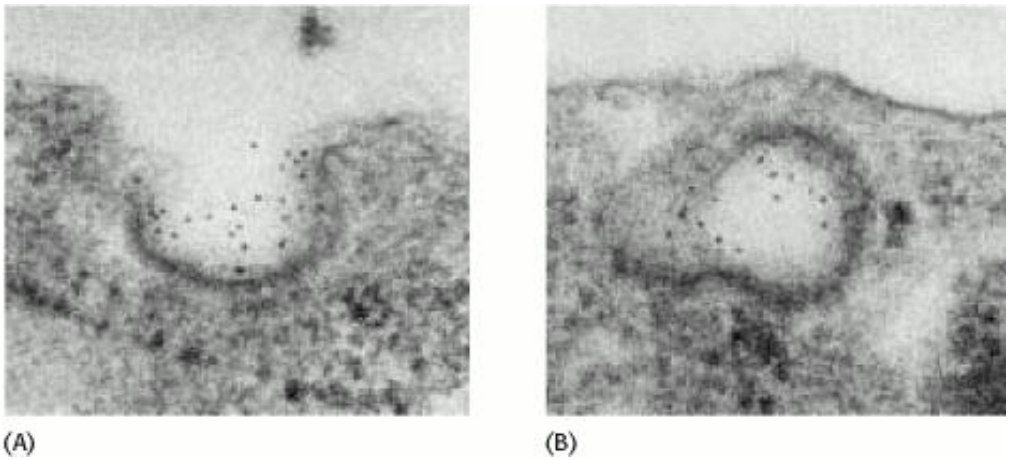


Figure 26.17. Endocytosis of LDL Bound to Its Receptor. (A) Electron micrograph showing LDL (conjugated to ferritin for visualization, dark spots) bound to a coated-pit region on the surface of a cultured human fibroblast cell. (B) Micrograph showing this region invaginating and fusing to form an endocytic vesicle [From R. G. W. Anderson, M. S. Brown, and J. L. Goldstein. *Cell* 10 (1977): 351.]

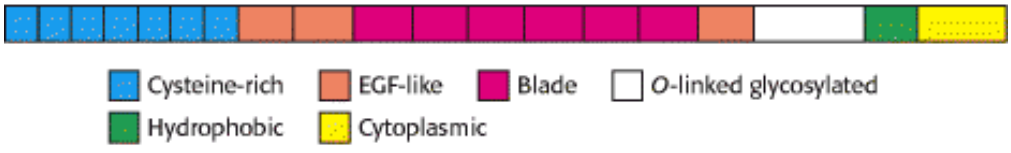


Figure 26.18. LDL Receptor Domains. A schematic representation of the amino acid sequence of the LDL receptor showing six types of domain.

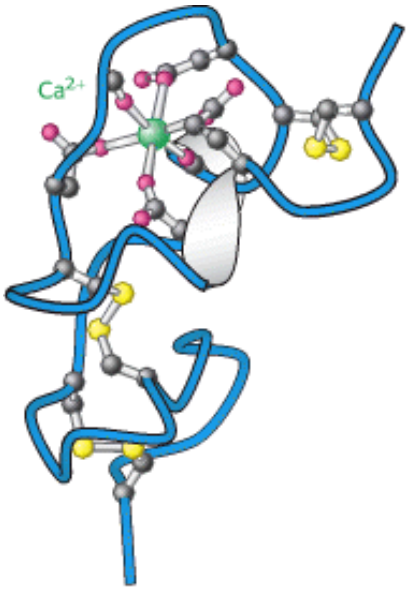


Figure 26.19. Structure of Cysteine-Rich Domain. This calcium-binding cysteine-rich domain is repeated seven times at the amino terminus of the LDL receptor.

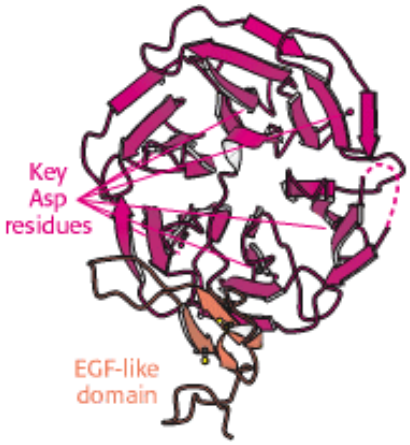


Figure 26.20. Structure of Propeller Domain. The six-bladed propeller domain and an adjacent EGF-like domain of the LDL receptor.

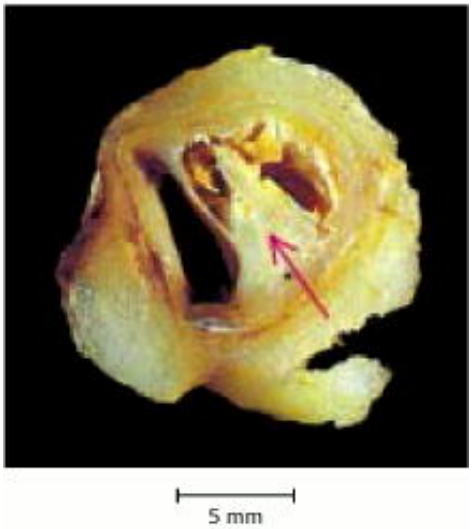


Figure 26.21. An Atherosclerotic Plaque. A plaque (marked by an arrow) blocks most of the lumen of this blood vessel. The plaque is rich in cholesterol. [Courtesy of Dr. Jeffrey Sklar.]

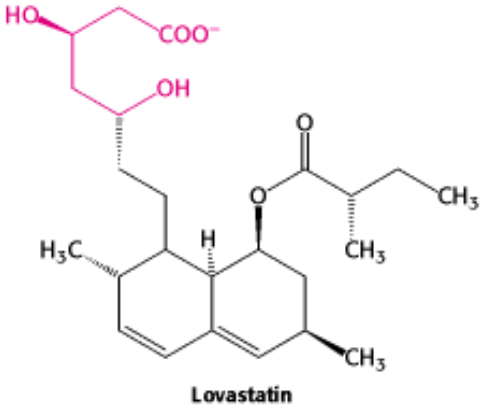


Figure 26.22. Lovastatin, a Competitive Inhibitor of HMG-CoA Reductase. The part of the structure that resembles the 3-hydroxy-3-methylglutaryl moiety is shown in red.

26.4. Important Derivatives of Cholesterol Include Bile Salts and Steroid Hormones

Cholesterol is a precursor for other important steroid molecules: the bile salts, steroid hormones, and vitamin D.

Bile Salts.

As polar derivatives of cholesterol, *bile salts* are highly effective *detergents* because they contain both polar and nonpolar regions. Bile salts are synthesized in the liver, stored and concentrated in the gall bladder, and then released into the small intestine. Bile salts, the major constituent of bile, *solubilize dietary lipids* (Section 22.1.1). Solubilization increases in the effective surface area of lipids with two consequences: more surface area is exposed to the digestive action of lipases and lipids are more readily absorbed by the intestine. Bile salts are also the major breakdown products of cholesterol.

Cholesterol is converted into trihydroxycoprostanoate and then into *cholyl CoA*, the activated intermediate in the synthesis of most bile salts (Figure 26.23). The activated carboxyl carbon of cholyl CoA then reacts with the amino group of glycine to form *glycocholate* or it reacts with the amino group of taurine ($H_2NCH_2CH_2SO_3^-$), derived from cysteine, to form *taurocholate. Glycocholate is the major bile salt.*

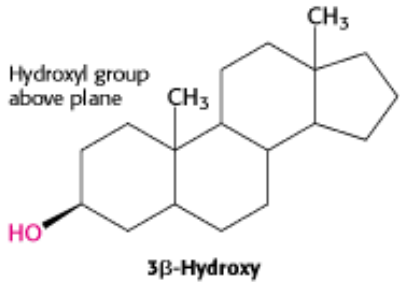
Steroid Hormones.

Cholesterol is the precursor of the five major classes of *steroid hormones:* progestagens, glucocorticoids, mineralocorticoids, androgens, and estrogens (Figure 26.24). These hormones are powerful signal molecules that regulate a host of organismal functions. *Progesterone,* a *progestagen,* prepares the lining of the uterus for implantation of an ovum. Progesterone is also essential for the maintenance of pregnancy. *Androgens* of male secondary sex characteristics, whereas *estrogens* (such as *estrone*) are required for the development of female secondary sex characteristics. Estrogens, along with progesterone, also participate in the ovarian cycle. *Glucocorticoids* (such as *cortisol*) promote gluconeogenesis and the formation of glycogen, enhance the degradation of fat and protein, and inhibit the inflammatory response. They enable animals to respond to stress—indeed, the absence of glucocorticoids can be fatal. *Mineralocorticoids* (primarily *aldosterone*) act on the distal tubules of the kidney to increase the reabsorption of Na^+ and the excretion of K^+ and H^+, which leads to an increase in blood volume and blood pressure. The major sites of synthesis of these classes of hormones are the corpus luteum, for progestagens; the ovaries, for estrogens; the testes, for androgens; and the adrenal cortex, for glucocorticoids and mineralocorticoids.

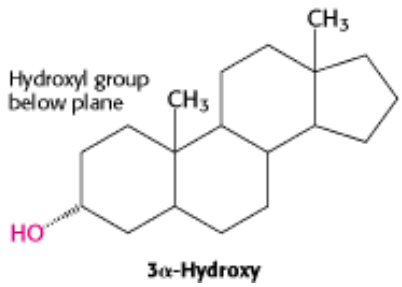
Steroid hormones bind to and activate receptor molecules that serve as transcription factors to regulate gene expression (Section 31.3.1). These small, relatively similar molecules are able to have greatly differing effects because the slight structural differences among them allow interactions with specific receptor molecules.

26.4.1. The Nomenclature of Steroid Hormones

Carbon atoms in steroids are numbered as shown for cholesterol in (Figure 26.25). The rings in steroids are denoted by the letters A, B, C, and D. Cholesterol contains two angular methyl groups: the C-19 methyl group is attached to C-10, and the C-18 methyl group is attached to C-13. The C-18 and C-19 methyl groups of cholesterol lie *above* the plane containing the four rings. A substituent that is above the plane is termed β *oriented,* whereas a substituent that is below the plane is α *oriented.*

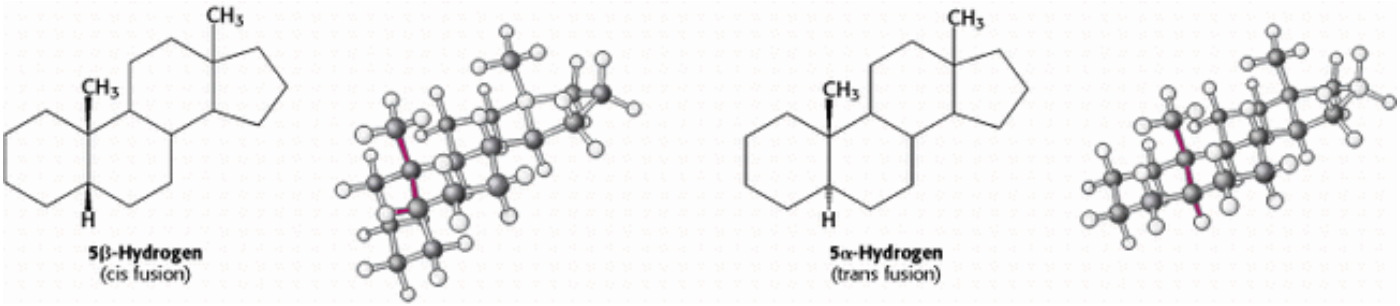


3β-Hydroxy



3α-Hydroxy

If a hydrogen atom is attached to C-5, it can be either α or β oriented. The A and B steroid rings are fused in a *trans* conformation if the C-5 hydrogen is < *oriented,* and *cis* if it is < *oriented.* The absence of a Greek letter for the C-5 hydrogen atom on the steroid nucleus implies a trans fusion. The C-5 hydrogen atom is α oriented in all steroid hormones that contain a hydrogen atom in that position. In contrast, bile salts have a β-oriented hydrogen atom at C-5. Thus, *a cis fusion is characteristic of the bile salts, whereas a trans fusion is characteristic of all steroid hormones that possess a hydrogen atom at C-5.* A trans fusion yields a nearly planar structure, whereas a cis fusion gives a buckled structure.



26.4.2. Steroids Are Hydroxylated by Cytochrome P450 Monooxygenases That Utilize NADPH and O_2

Hydroxylation reactions play a very important role in the synthesis of cholesterol from squalene and in the conversion of cholesterol into steroid hormones and bile salts. All these hydroxylations require *NADPH and* O_2. The oxygen atom of the incorporated hydroxyl group comes from O_2 rather than from H_2O. While one oxygen atom of the O_2 molecule goes into the substrate, the other is reduced to water. The enzymes catalyzing these reactions are called *monooxygenases* (or *mixed-function oxygenases*). Recall that a monooxygenase also participates in the hydroxylation of aromatic amino acids (Section 23.5.7).

$$RH + O_2 + NADPH + H^+ \longrightarrow ROH + H_2O + NADP^+$$

Hydroxylation requires the activation of oxygen. In the synthesis of steroid hormones and bile salts, activation is accomplished by a cytochrome P450, a family of cytochromes that absorb light maximally at 450 nm when complexed in vitro with exogenous carbon monoxide. These membraneanchored proteins (~50 kd) contain a heme prosthetic group. Because the hydroxylation reactions promoted by P450 enzymes are oxidation reactions, it is at first glance surprising that they also consume the reductant NADPH. NADPH transfers its high-potential electrons to a flavoprotein, which transfers them, one at a time, to *adrenodoxin*, a nonheme iron protein. Adrenodoxin transfers one electron to reduce the ferric (Fe^{3+}) form of P450 to the ferrous (Fe^{2+}) form (Figure 26.26). Without the addition of this electron, P450 will not bind oxygen. Recall that only the ferrous form of hemoglobin binds oxygen (Section 10.2.1). The binding of O_2 to the heme is followed by the acceptance of a second electron from adrenodoxin. The acceptance of this second electron leads to cleavage of the O–O bond. One of the oxygen atoms is then protonated and released as water. The remaining oxygen atom forms a highly reactive ferryl ($Fe{=}O$) intermediate. This intermediate abstracts a hydrogen atom from the substrate RH to form $R^{\bullet}$. This transient free radical captures the OH group from the iron atom to form ROH, the hydroxylated product, returning the iron atom to the ferric state.

26.4.3. The Cytochrome P450 System Is Widespread and Performs a Protective Function

The cytochrome P450 system, which in mammals is located primarily in the endoplasmic reticulum of the liver and small intestine, is also important in the *detoxification of foreign substances* (xenobiotic compounds) by oxidative metabolism. For example, the hydroxylation of phenobarbital, a barbiturate, *increases its solubility* and *facilitates its excretion.* Likewise, polycyclic aromatic hydrocarbons are hydroxylated by P450, providing sites for conjugation with highly polar units (e.g., glucuronate or sulfate), which markedly increase the solubility of the modified aromatic molecule. One of the most relevant functions of the cytochrome P450 system to human beings is its role in drug metabolism. Drugs such as caffeine and ibuprofen are oxidatively metabolized by these monooxygenases. Indeed, the duration of action of many medications depends on their rate of inactivation by the P450 system. Despite its general protective role in the removal of foreign chemicals, the action of the P450 system is not always beneficial. *Some of the most powerful carcinogens are generated from harmless compounds by the P450 system in vivo* in the process of *metabolic activation.* In plants, the cytochrome P450 system plays a role in the synthesis of toxic compounds as well as