Chapter 23— Biotransformations: The Cytochromes P450

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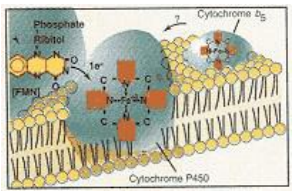

23.1— Overview

The term **cytochrome P450** refers to a family of **heme proteins** present in all mammalian cell types, except mature red blood cells and skeletal muscle cells, which catalyze oxidation of a wide variety of structurally diverse compounds. Cytochrome P450 also occurs in prokaryotes. Substrates for this enzyme system include endogenously synthesized compounds, such as steroids and fatty acids (including prostaglandins and leukotrienes), and exogenous compounds, such as drugs, food additives, or industrial by-products that enter the body through food sources, injection, inhalation from the air, or absorption through the skin. The cytochrome P450 system has far-reaching effects in medicine. It is involved in (1) inactivation or activation of therapeutic agents; (2) conversion of chemicals to highly reactive molecules, which may produce unwanted cellular damage, cell death, or mutations; (3) production of steroid hormones; and (4) metabolism of fatty acids and their derivatives. Other heme-binding, cysteine thiolate-containing proteins also exist, including thromboxane, prostacyclin, and allene oxide synthases, as well as the **nitric oxide synthases.** This chapter will address the cytochromes P450 in detail and will introduce the isoforms of nitric oxide synthase. Clinical implications of these oxygenation systems will be presented.

23.2— Cytochromes P450: Nomenclature and Overall Reaction

Designation of a particular protein as a cytochrome P450 originated from its spectral properties before its catalytic function was known. This group of proteins has a unique absorbance spectrum that is obtained by adding a reducing agent, such as sodium dithionite, to a suspension of **endoplasmic reticulum** vesicles, frequently referred to as **microsomes,** followed by bubbling of carbon monoxide gas into the solution. Carbon monoxide is bound to the reduced heme protein and produces an absorbance spectrum with a peak at approximately 450 nm (Figure 23.1); thus the name P450 for a pigment with an absorbance at 450 nm. Specific forms of cytochrome P450 differ in their maximum absorbance wavelengths, with a range between 446 and 452 nm. The many forms of cytochrome P450 are classified, according to their sequence similarities, into various **gene subfamilies;** this system of **nomenclature** is being adopted almost universally. Individual cytochrome P450 forms are given an Arabic number to designate a specific family, followed by a capital letter to identify its subfamily, followed by another Arabic number designating the individual P450 form, for example, 1A2 or 2D6. The term **CYP,** which represents the first two letters of *cy*tochrome and the first letter in *P*450, is used as a preface to designate a gene or protein as a cytochrome P450 form. Thus cytochromes P450 1A2 and P450 2D6 are designated CYP1A2 and CYP2D6 in this nomenclature system. Members of the same family share at least 40% amino acid sequence homology and members of the same subfamily share at least 55% sequence homology. Table 23.1 lists several human cytochrome P450 forms. In certain families several subfamilies have been identified such as in CYP2 (CYP2A and CYP2B) and CYP4 (CYP4A and CYP4B), whereas in others only a single gene has been reported (CYP17, CYP19, and CYP21).

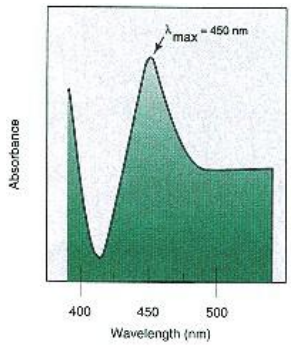

Figure 23.1
Absorbance spectrum of the carbon monoxide-bound cytochrome P450. The reduced form of this heme protein binds carbon monoxide to produce a maximum absorbance at approximately 450 nm. Hence this cytochrome was designated P450.

The general reaction catalyzed by cytochrome P450 is written as follows:

$$NADPH + H^+ + O_2 + SH \rightarrow NADP^+ + H_2O + SOH$$

where the substrate (S) may be a steroid, fatty acid, drug, or other chemical that has an alkane, alkene, aromatic ring, or heterocyclic ring substituent that can serve as a site for oxygenation. The reaction is referred to as a monooxygenation and the enzyme as a **monooxygenase** because only one of the two oxygen atoms is incorporated into the substrate. In mammalian cells, cytochromes P450

TABLE 23.1 Human Cytochrome P450 Forms

Cytochrome P450 Subfamilies							
CYP1	***CYP2***	***CYP3***	***CYP4***	***CYP11***	***CYP17***	***CYP19***	***CYP21***
Individual Forms							
1A1 1A2	2A6 2A7	3A3 3A4	4A9 4A11	11A1 11B1			21A2
	2B6 2C8	3A5 3A7	4B1 4F2	11B2			
	2C9 2C10		4F3				
	2C18 2C19						
	2D6 2E1						

serve as terminal electron acceptors in **electron transport systems,** which are present either in the endoplasmic reticulum or **inner mitochondrial membrane.** The cytochrome P450 proteins contain a single iron **protoporphyrin IX** prosthetic group (see p. 1009), which binds oxygen, and the resulting heme protein contains binding sites for the substrate. Heme iron of all known cytochromes P450 is bound to the four pyrrole nitrogen atoms of the porphyrin ring and two axial ligands, one of which is a sulfhydryl group from a cysteine residue located toward the carboxyl end of the molecule (Figure 23.2). Heme iron may exist in two different spin states: (1) a hexa-coordinated low-spin

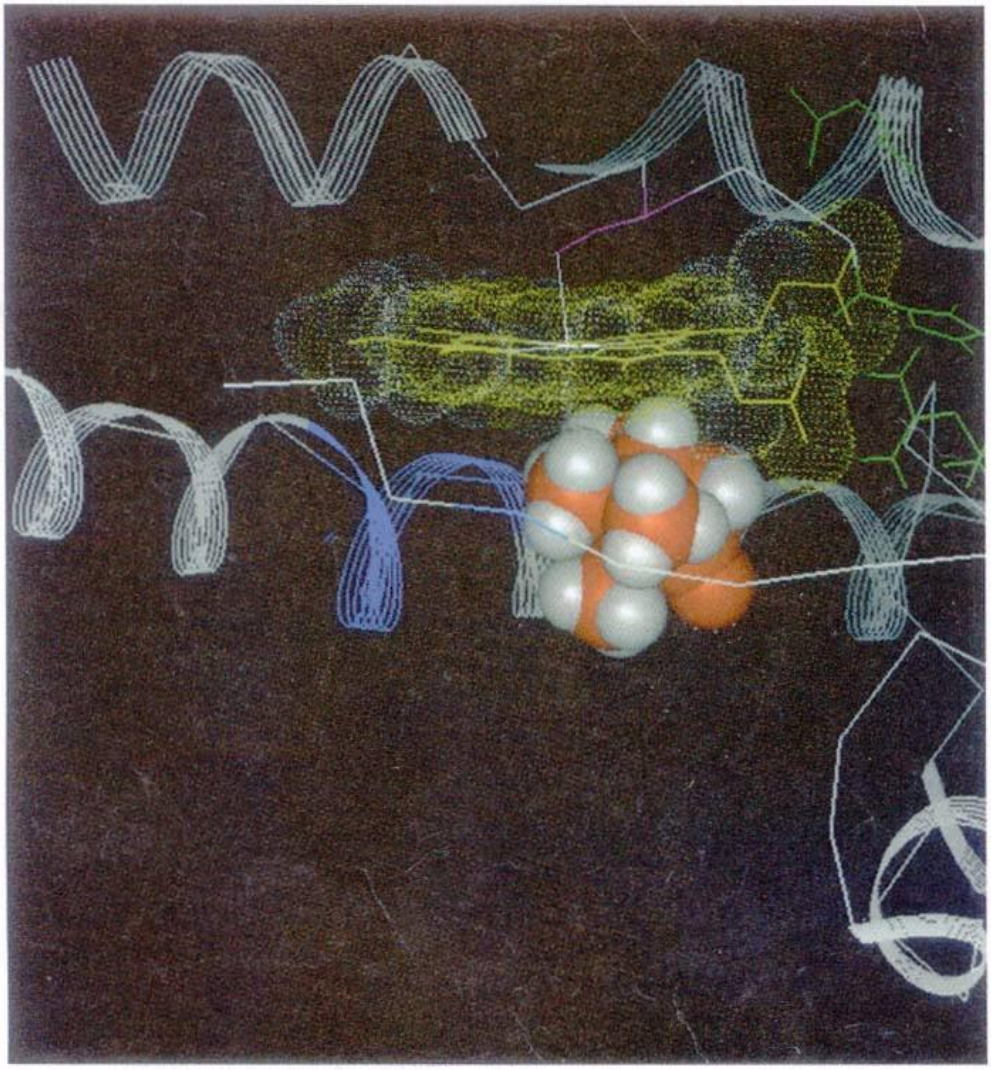

Figure 23.2
Binding of protoporphyrin IX prosthetic group of cytochromes P450.
The cysteine thiolate ligand (Cys 357) liganded to the heme iron is shown in the top of the figure and the space-filling model shows the camphor in the active site of the cytochrome $P450_{cam}$. Generated by Dr. John Salerno from Dr. Tom Poulos' $P450_{cam}$ structure using Biosym's Insight program run on a Silicon Graphics Indigo Extreme platform.

iron or (2) a penta-coordinated high-spin state. Low- and high-spin states are descriptions of the electronic shells within the iron atom. When a cytochrome P450 molecule binds a substrate, there is a perturbation of the structure surrounding heme iron such that a more positive reduction potential (–170 mV) results than in the absence of substrate (–270 mV). This accelerates the rate at which cytochrome P450 may be reduced by electrons donated from NADPH through the flavoprotein enzyme **NADPH–cytochrome P450 reductase** (Figure 23.3). In order for **hydroxylation** (monooxygenation) to occur, heme iron must be reduced from the ferric (Fe^{3+}) to its ferrous (Fe^{2+}) state so that oxygen may bind to the heme iron. A total of two electrons is required for the **mono-oxygenation reaction.** Electrons are transferred to the cytochrome P450 molecule individually, the first to allow oxygen binding and the second to cleave the oxygen molecule to generate the active oxygen species for insertion into the reaction site of the substrate.

23.3— Cytochromes P450: Multiple Forms

Since the mid-1950s it has been known that one atom of molecular O_2 is inserted into a substrate being metabolized. This process of monooxygenation is also performed by other specialized proteins such as flavoprotein monooxygenases (hydroxylases). None of the other proteins classified as **oxygenases,** however, displays the versatility of the members of the cytochrome P450 family. In the past decade, information on the sequence and structure of cytochromes P450 has led to a further understanding of their evolution and regulation.

Multiplicity of Genes Produces Many Forms of Cytochrome P450

Many cytochrome P450 forms have emerged due to **gene duplication** events occurring in the last 5–50 million years. The different forms of cytochrome P450 among various animal species have likely arisen from the selective pressure of environmental influences, such as dietary habits or exposure to environmental agents. It is logical that the primordial genes gave rise to those cytochromes P450 that metabolized endogenous substrates. Examination of the phylogenetic tree, generated by comparing amino acid sequences and assuming a constant evolutionary change rate, leads to the conclusion that the earliest cytochromes P450 evolved to metabolize cholesterol and fatty acids. Therefore they may have played a role in the maintenance of membrane integrity in early eukaryotes.

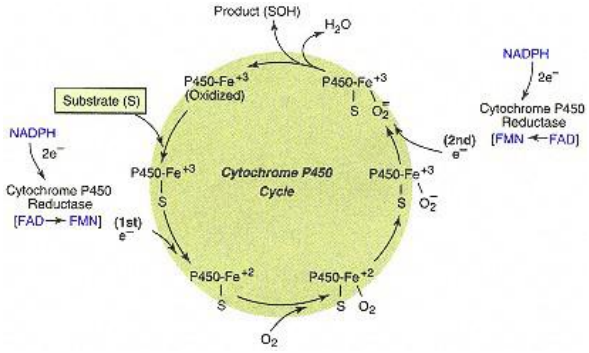

Figure 23.3
Sequence of reactions at cytochrome P450.
Diagram demonstrates the binding of substrate, transfer of the first and second electrons from NADPH–cytochrome P450 reductase, and binding of molecular oxygen.

Substrate Specificity

By the mid-1990s, nucleotide sequences for over 300 cytochrome P450 genes, coding for different proteins catalyzing the oxygenation of a variety of endogenous and exogenous substrates, had been characterized. There remain other members of this **gene superfamily** for which sequences have not yet been determined. One of the ways of characterizing these enzymes is the determination of **substrate specificity.** While this has been possible with many of the members of this family, the similarity of molecular weights and other molecular properties has made purification of individual cytochromes P450 from the same organ or even the same subcellular organelle very difficult, if not impossible. One way of determining the substrate specificity of a cytochrome P450 has been to express the cDNA for the particular protein via an expression vector in an appropriate cellular **expression system** in which that specific cytochrome P450 form is not expressed constitutively. This has been achieved in bacterial, insect, yeast, and mammalian cell systems and permits the unequivocal determination of substrate specificity uncomplicated by impurities of protein purification. The assumption is that knowing the nucleotide sequences of the expressed genes leaves little doubt as to the source of enzyme activity expressed in those cells.

Induction of Cytochromes P450

Induction of various cytochromes P450 by both endogenous and exogenous compounds has been known since the mid-1960s. The mechanisms of induction of cytochromes P450 have been demonstrated to be at either the **transcriptional** or **posttranscriptional** level and it is not possible to predict the mode of induction based on the inducing compound. For example, a single cytochrome P450 can be induced by different mechanisms. In one case, induction occurs at the transcriptional level and, in the other, it involves posttranscriptional events, that is, stabilization of mRNA. An example of the complexity of the induction process occurs with rat CYP2E1 as a result of treatment with small organic molecules, such as ethanol, acetone, or pyrazole, or during fasting or diabetic conditions. Administration of these small organic compounds produces larger amounts of the CYP2E1 protein without affecting the levels of mRNA. While the mechanism is not completely understood, pyrazole may stabilize this specific cytochrome P450 from proteolytic degradation. However, in diabetic rats the sixfold induction of CYP2E1 protein is accompanied by a tenfold increase in mRNA in the absence of an increase in gene transcription, suggesting stabilization of the mRNA.

The role of specific cytosolic receptor proteins has been indicated in the case of some of the known inducing agents. One of the most extensively studied is the interaction of **2,3,7,8-tetrachlorodibenzo-*p*-dioxin** (TCDD) with its cytosolic receptor, called the **aryl hydrocarbon (or Ah) receptor,** which functions in the induction of CYP1A1 and CYP1A2 forms. **Polycyclic aromatic hydrocarbons** serve as ligands which bind to the Ah receptor, producing a complex that is translocated to the nucleus and is involved in binding to the upstream regulatory regions (specific **response elements**) of cytochrome P450 genes. A second protein called the **Ah receptor nuclear translocator** or **Arnt protein** was found to interact with the ligand bound Ah receptor. The Arnt protein was essential for enabling this ligand–Ah receptor complex to recognize and bind to its specific DNA response element. Utilizing cytochrome P450 gene transfection and expression vector technology, it has been possible to express those portions of the cytochrome P450 genomic DNA representing the RNA polymerase II promoter region and the upstream DNA sequences in conjunction with another gene coding for an enzyme that is not expressed constitutively in eukaryotes. In an assay of the prokaryotic enzyme activity, for example, chloramphenicol acetyltransferase (CAT) in the expression system, it is possible to determine which specific nucleotide sequences of DNA are involved in

CLINICAL CORRELATION 23.1

Consequences of Induction of Drug-Metabolizing Enzymes

Induction of the cytochrome P450 system may result in altered efficacy of therapeutic drugs, as the accelerated rate of hydroxylation will increase the inactivation and/or enhance the excretion rate of drugs. Induction of this protein system may also produce unexpected and unwanted side effects of therapeutic agents due to increased formation of toxic metabolites that may cause cell injury if produced in large enough concentrations. The induction of different cytochrome P450 forms by a drug may stimulate the metabolism of itself or other drugs that are substrates for the cytochrome P450 system. Clinical problems may develop as a consequence of cytochrome P450 induction.

The increase in clearance of oral contraceptives by rifampicin, an antituberculosis drug and CYP3A4 inducer, has been shown to decrease the effectiveness of the contraceptive agent and increase the incidence of pregnancy in women who are prescribed both drugs. Fatalities have been reported in patients who are simultaneously treated with phenobarbital, a long-acting sedative and potent cytochrome P450 inducer, and warfarin, an anticoagulant, which is prescribed to patients with clotting disorders. Higher doses of warfarin are required in these patients to maintain the same effective concentration of the drug to delay coagulation because warfarin is a substrate for the cytochrome P450 induced by phenobarbital. Consequently, the drug is metabolized and cleared at a faster rate, which reduces its therapeutic efficacy. Clinical problems are created when phenobarbital is removed from the treatment regimen with no corresponding decrease in warfarin levels. With time, cytochrome P450 levels decrease to the noninduced state but the high concentrations of warfarin, proper under conditions of accelerated metabolism and clearance, are in excess and produce unwanted hemorrhaging.

Induction of CYP2E1 by chronic alcohol use has led to a warning for consumers of acetaminophen, a common over-the-counter analgesic agent, because this cytochrome P450 will metabolize acetaminophen to a toxic metabolite that may lead to liver cell damage. These represent classic examples of cytochrome P450–drug interactions that can lead to unwanted and unexpected clinical problems.

regulating these genes. These nucleotide sequences are referred to as **xenobiotic regulatory elements** or XREs.

Another much studied inducer of cytochrome P450 genes is **phenobarbital,** which increases the transcription rate of certain cytochrome P450 forms. A receptor that binds phenobarbital has not been described, but a specific DNA response element that is essential for phenobarbital-mediated induction has been identified in the upstream regulatory region of *CYP2B2* and *CYP3A1* genes. Although the mechanism by which phenobarbital increases transcription is unknown, the intracellular messenger, adenosine 2 ,3 -cyclic monophosphate (cAMP), is a negative modifier, suppressing phenobarbital-mediated cytochrome P450 gene expression. An increase in cAMP levels in rat hepatocytes was found to prevent the phenobarbital-directed induction of CYP2B2 and CYP3A1 by activating protein kinase A activity. Some clinical consequences of induction of drug-metabolizing enzymes are presented in Clin. Corr. 23.1.

Polymorphisms

In addition to exposure to different inducing agents, individuals may differ in their rates of metabolism of a particular drug because of differences in the cytochrome P450 genes they possess. Different forms of a cytochrome P450 gene may exist in a given population, which will alter the functional activity of the complement of cytochromes P450. These **genetic polymorphisms** may be present in a small percentage of the population and cause an individual to be unable to metabolize a drug at a sufficient rate, thereby producing significantly elevated drug levels. These **"poor metabolizers"** may be at risk for a dose-dependent toxicity if the unmetabolized form of the drug is pharmacologically active. Examples of genetic polymorphisms in drug metabolism are described in Clin. Corr. 23.2.

23.4— Inhibitors of Cytochromes P450

Due to the many forms of cytochrome P450, it is of interest to examine the metabolic roles of these various enzymes in the organs in which they function. Several inhibitors have been utilized to demonstrate that cytochrome(s) P450 may be involved in a metabolic pathway, for example, the metabolism of steroids in the adrenal or specific reproductive organs. As has been discussed, the detection of cytochrome P450 in most tissues can be ascertained by the reduced-**carbon monoxide difference spectrum.** Carbon monoxide (CO) binds to the heme iron, in lieu of oxygen, with a much higher binding affinity and thereby is a potent inhibitor of its function. The identity of a cytochrome P450 in the catalysis of a putative substrate in a metabolic pathway rested on the reversal of CO inhibition by light at 450 nm, corresponding to the reduced-CO absorption maximum. This was first demonstrated for steroids as substrates for adrenal mitochondrial cytochromes P450 and later for drugs metabolized by liver microsomal cytochromes P450. However, this is a nonspecific inhibition characteristic of most cytochromes P450 and does not differentiate among the various forms.

More specific inhibitors are needed that can determine the role of a specific cytochrome P450 in a particular metabolic pathway. Although monospecific polyclonal and monoclonal antibodies have been developed to a number of cytochromes P450, it is not always possible to determine that a single form is responsible because of inhibition of a given reaction. The strong structural homology among the various forms may allow cross-reactivity among cytochromes P450. This is particularly true of members of the same gene family that exhibit immune cross-reactivity.

Recently, efforts have been directed to develop **mechanism-based inhibitors,** so-called **suicide substrates,** which bear strong resemblance to the sub-

CLINICAL CORRELATION 23.2

Genetic Polymorphisms of Drug-Metabolizing Enzymes

Genetic polymorphisms of cytochromes P450 result in the expression of cytochromes P450 that are nonfunctional or exhibit lower enzymatic activities. This may result in unwanted side effects because of the inability to eliminate the active form of the drug, causing elevated concentrations in the body. It may also result in the absence of a therapeutic effect because the active form of a drug is not formed.

The discovery of an individual who suffered exaggerated hypotensive effects when administered the antihypertensive drug, debrisoquine, led to the characterization of individuals who metabolized substrates catalyzed by the CYP2D6 form inefficiently. Approximately 5–10% of the Caucasian population, 2% of the Asian, and 1% of the Arabic populations were deficient for the catalytically active CYP2D6 form. In addition to debrisoquine, other drugs that are metabolized by CYP2D6 are sparteine, amitriptyline, dextromethorphan, and codeine. In the case of codeine, CYP2D6 catalyzes the *O*-demethylation of codeine to morphine. Approximately 10% of the dose of codeine is metabolized to morphine in individuals who have a normal CYP2D6 and this metabolism is responsible for the analgesic effects of this drug. Individuals who lack the normal gene for CYP2D6 are unable to catalyze this reaction and are unable to achieve the analgesic effects associated with codeine.

Another genetic polymorphism was demonstrated in individuals who were poor metabolizers of the drug mephenytoin. This drug is used in the treatment of epilepsy. Poor metabolizers of this drug suffer greater sedative effects at normal dosages. The 4-hydroxylation of the *S*-enantiomer of mephenytoin is carried out by CYP2C19. Approximately 14–22% of the Asian population are reported to be poor metabolizers of the *S*-isomer of mephenytoin whereas only 3–6% of the Caucasian population are affected. These genetic polymorphisms may explain some of the interindividual or interracial differences in the way individuals respond to therapeutic drugs.

Eichelbaum, M., and Gross, A. S. The genetic polymorphism of debrisoquine/sparteine metabolism—clinical aspects. In: W. Kalow (Ed.), *Pharmacogenetics of Drug Metabolism*. New York: Pergamon Press, 1992, Chap. 21, p. 625; and Meyer, U. A., Skoda, R. D., Zanger, U. M., Heim, M., and Broly, F. The genetic polymorphism of debrisoquine/sparteine metabolism—molecular mechanism. In: W. Kalow (Ed.), *Pharmocogenetics of Drug Metabolism*. New York: Pergamon Press, 1992, Chap. 20, p. 609.

strate(s) of the specific cytochrome P450, but which during catalytic turnover form an irreversible inhibition product with the enzyme prosthetic group or protein. Because of their structural resemblance to the substrate(s), these inhibitors become highly specific for that particular form of cytochrome P450. These inhibitors contain functional groups that are metabolized to intermediates that result in their covalent binding to the enzymes, thereby accounting for their irreversibility. This represents a possible tactical approach to drug design.

23.5— Cytochrome P450 Electron Transport Systems

Although cytochrome P450-catalyzed reactions require two electrons to accomplish the tasks of heme iron reduction, oxygen binding, and oxygen cleavage, a basic mechanistic problem is the direct and simultaneous transfer of electrons from NADPH to the cytochrome P450. Pyridine nucleotides are two electron donors (see p. 250), but cytochrome P450, with its single heme prosthetic group, may only accept one electron at a time. Thus a protein that serves to transfer electrons from NADPH to the cytochrome P450 molecule must have the capacity to accept two electrons but serve as a one-electron donor. This problem is solved by the presence of a NADPH-dependent flavoprotein reductase, which accepts two electrons from NADPH simultaneously but transfers the electrons individually either to an intermediate **iron–sulfur protein** (mitochondria) or directly to cytochrome P450 (endoplasmic reticulum). The active redox group of the flavin moiety is the isoalloxazine ring (see p. 251). The isoalloxazine nucleus is uniquely suited to perform this chemical task since it can exist in oxidized and one- and two-electron reduced states (Figure 23.4). The transfer of electrons from NADPH to cytochrome P450 is accomplished by two distinct electron transport systems that reside almost exclusively in either mitochondria or endoplasmic reticulum.

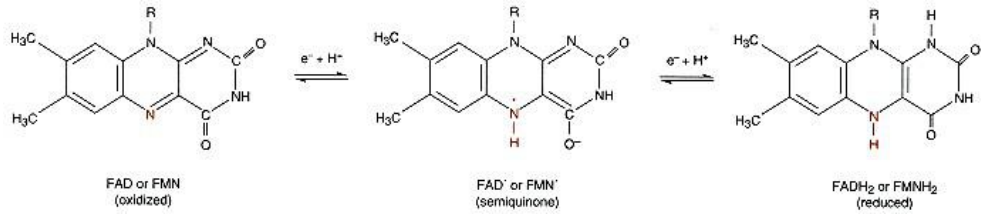

Figure 23.4
Isoalloxazine ring of FMN or FAD in its oxidized, semiquinone ($1e^-$ reduced), or fully reduced ($2e^-$ reduced) states.

NADPH–Cytochrome P450 Reductase Is the Flavoprotein Donor in the Endoplasmic Reticulum

In the endoplasmic reticulum, NADPH donates electrons to a flavoprotein called NADPH–cytochrome P450 reductase. The rat enzyme has a mass of 76,962 Da and contains both flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) as prosthetic groups. Until the recent characterization of nitric oxide synthases, it was the only mammalian flavoprotein known to contain both FAD and FMN. A significant number of residues at the amino end of the molecule are hydrophobic, and this segment of the protein is embedded in the endoplasmic reticulum (Figure 23.5). FAD serves as the entry point for electrons from NADPH, and FMN serves as the exit point, transferring electrons individually to cytochrome P450. Because the flavin molecule may exist as one- or two-electron-reduced forms and two flavin molecules are bound per reductase molecule, the enzyme may receive electrons from NADPH and store them between the two flavin molecules before transferring them individually to the cytochrome P450.

In certain reactions catalyzed by the microsomal cytochrome P450, the transfer of the second electron may not be directly from **NADPH–cytochrome P450 reductase** but may occur from **cytochrome b_5,** a small heme protein of molecular mass 15,330 Da. Cytochrome b_5, is reduced either by NADPH–cytochrome P450 reductase or another microsome-bound flavoprotein, **NADH–cytochrome b_5 reductase.** It is not known why reactions catalyzed by specific cytochromes P450 apparently require cytochrome b_5 for optimal enzymatic activity. In addition, NADH–cytochrome b_5 reductase and cytochrome b_5 constitute the electron transfer system for NADH to the iron–sulfur protein, **fatty acid desaturase,** which catalyzes the formation of double bonds in fatty acids (see p. 372).

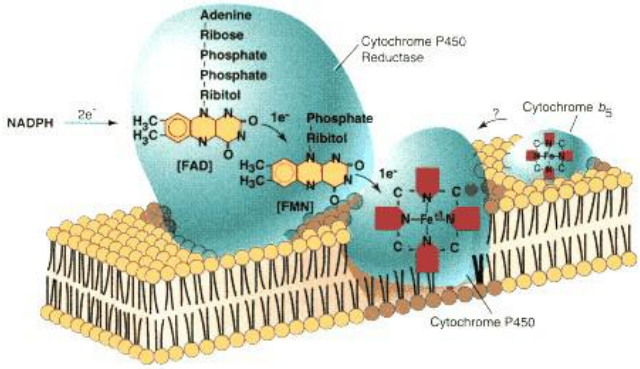

Figure 23.5
Components of the endoplasmic reticulum (microsomal) cytochrome P450 system.
NADPH–cytochrome P450 reductase is bound by its hydrophobic tail to the membrane, whereas cytochrome P450 is deeply embedded in the membrane. Also shown is cytochrome b_5, which may participate in selected cytochrome P450-mediated reactions.

NADPH–Adrenodoxin Reductase Is the Flavoprotein Donor in Mitochondria

In mitochondria, a flavoprotein reductase also acts as the electron acceptor from NADPH. This protein is referred to as **NADPH–adrenodoxin reductase** because its characteristics were described for the flavoprotein first isolated from the adrenal gland. This protein contains only FAD and the bovine NADPH–adrenodoxin reductase has a mass of 50,709 Da. Adrenodoxin reductase is only weakly associated with its membrane milieu, unlike NADPH–cytochrome P450 reductase of endoplasmic reticulum. Adrenodoxin reductase cannot directly transfer either the first or second electron to heme iron of cytochrome P450 (Figure 23.6). A small molecular weight protein, called **adrenodoxin** (12,500 Da), serves as an intermediate between the adrenodoxin reductase and **mitochondrial cytochrome P450.** The adrenodoxin molecule is also weakly associated with the inner mitochondrial membrane through interaction with the membrane-bound cytochrome P450. Adrenodoxin contains two iron–sulfur clusters, which serve as redox centers for this molecule and function as an electron shuttle between the adrenodoxin reductase and the mitochondrial cytochromes P450. One adrenodoxin molecule receives an electron from its mitochondrial flavoprotein reductase and interacts with a second adrenodoxin, which then transfers its electron to the cytochrome P450 (Figure 23.6). Components of the mitochondrial cytochrome P450 system are synthesized in the cytosol as larger molecular weight precursors, transported into mitochondria, and processed by proteases into smaller molecular weight, mature proteins.

23.6—
Physiological Functions of Cytochromes P450

Cytochromes P450 metabolize a variety of lipophilic compounds of endogenous or exogenous origin. These enzymes may catalyze simple hydroxylations of the carbon atom of a methyl group, insertion of a hydroxyl group into a methylene carbon of an alkane, hydroxylation of an aromatic ring to form a phenol, or addition of an oxygen atom across a double bond to form an **epoxide.** In dealkylation reactions, the oxygen is inserted into the carbon–hydrogen bond, but the resulting product is unstable and rearranges to the primary alcohol, amine, or sulfhydryl compound. Oxidation of nitrogen, sulfur, and phosphorus atoms and dehalogenation reactions are also catalyzed by cytochromes P450. Reactions catalyzed by cytochrome P450 forms are shown in Figure 23.7.

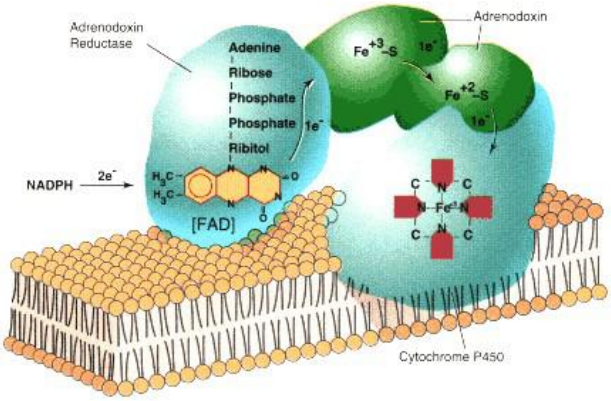

Figure 23.6
Components of mitochondrial cytochrome P450 system.
Cytochrome P450 is an integral protein of the inner mitochondrial membrane. NADPH–adrenodoxin reductase and adrenodoxin (ADR) are peripheral proteins and are `not embedded in the membrane.

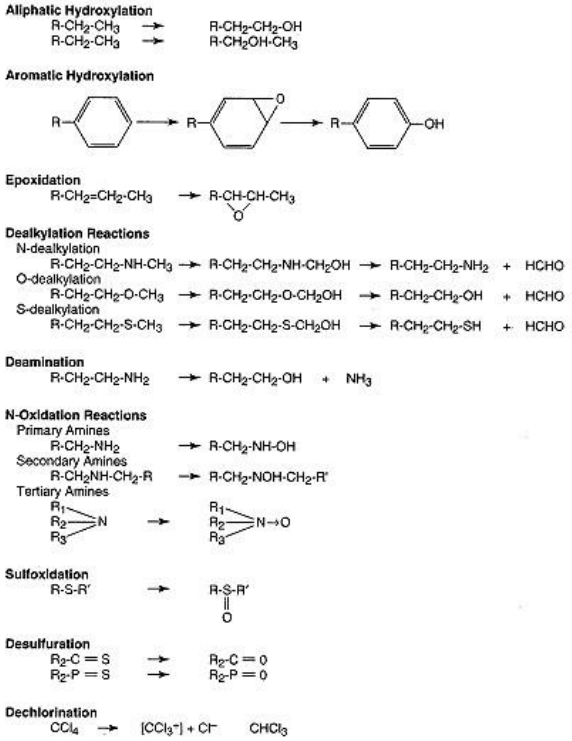

Figure 23.7
Reaction types catalyzed by cytochromes P450.

Cytochromes P450 Participate in Synthesis of Steroid Hormones and Oxygenation of Eicosanoids

The importance of cytochrome P450-catalyzed reactions is illustrated by the synthesis of **steroid hormones** from cholesterol in the adrenal cortex and sex organs. Mitochondrial and endoplasmic reticulum cytochrome P450 systems are required to metabolize cholesterol stepwise into **aldosterone** and **cortisol** in adrenal cortex, **testosterone** in testes, and **estradiol** in ovaries.

Cytochromes P450 are responsible for several steps in the adrenal synthesis of aldosterone, the mineralocorticoid responsible for regulating salt and water balance, and cortisol, the glucocorticoid that governs protein, carbohydrate, and lipid metabolism. In addition, adrenal cytochromes P450 catalyze the synthesis of small quantities of the androgen, **androstenedione,** a precursor of both estrogens and testosterone (see p. 900). Production of androstenedione regulates secondary sex characteristics. Figure 23.8 presents a summary of these pathways.

In adrenal mitochondria, a cytochrome P450 (CYP11A1) catalyzes the **side chain cleavage** converting cholesterol to pregnenolone, a committed step in steroid synthesis. The removal of isocaproic aldehyde results from a cytochrome P450-catalyzed reaction involving sequential hydroxylation at C-22 and C-20 to produce 22-hydroxycholesterol and then 20,22-dihydroxycholesterol (Figure 23.9). An additional P450-catalyzed step is necessary to cleave the bond between C-20 and C-22 to produce pregnenolone. This reaction sequence, which requires

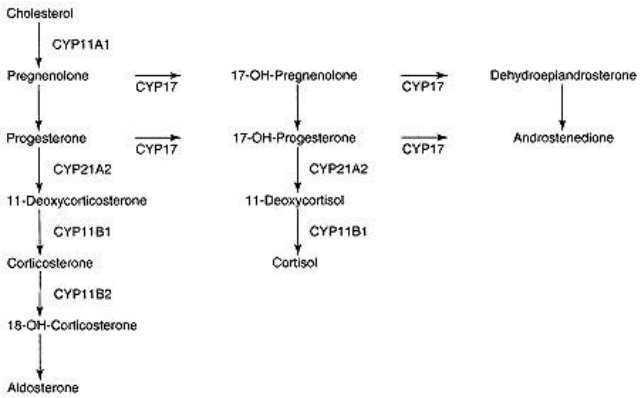

Figure 23.8
Steroid hormone synthesis in the adrenal gland.
The reactions catalyzed by cytochromes P450 (CYP) are indicated.

3 NADPH and 3 O_2 molecules, results in the breakage of a carbon–carbon bond and is catalyzed by a single cytochrome P450 enzyme, CYP11A1. After pregnenolone is produced in mitochondria, it is transported into the cytosol where it is oxidized by 3β-hydroxysteroid dehydrogenase/ ^{4,5}-isomerase to progesterone. Progesterone is metabolized to **11-deoxycorticosterone(DOC)** by an endoplasmic reticulum cytochrome P450 **(CYP21),** which catalyzes the 21-hydroxylation reaction. DOC is hydroxylated by an additional mitochondrial

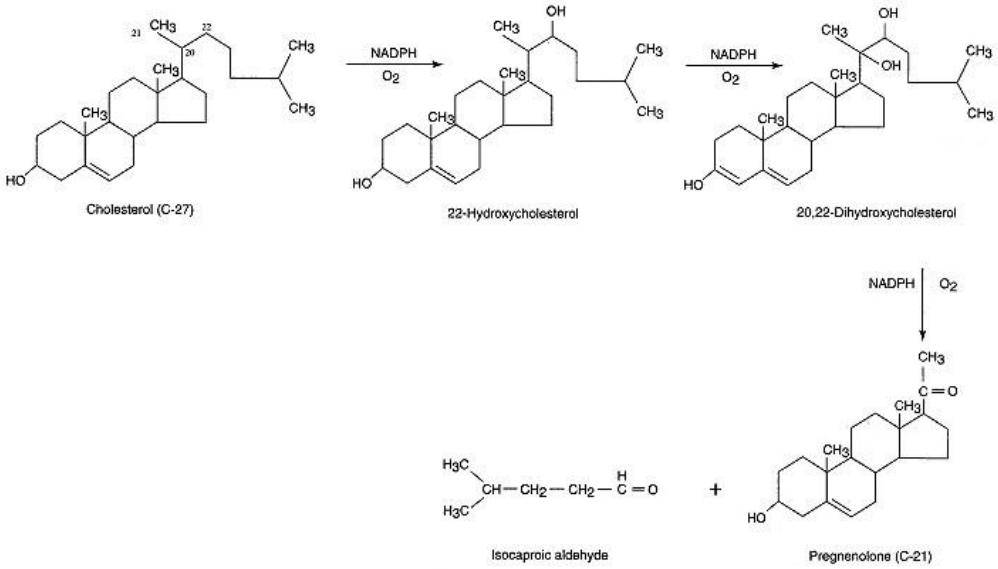

Figure 23.9
Side chain cleavage reaction of cholesterol.
Three sequential reactions are catalyzed by cytochrome P450 to produce pregnenolone and isocaproic aldehyde.

CLINICAL CORRELATION 23.3

Deficiency of Cytochrome P450 Steroid 21-Hydroxylase (CYP21A2)

The adrenal cortex is a major site of steroid hormone production during fetal and adult life. The adrenal gland is metabolically more active in fetal life and may produce 100–200 mg of steroids per day in comparison to the 20–30 mg produced per day in the non-stressed adult adrenal gland. A number of enzymes are required for the production of cortisol, and enzyme deficiencies have been reported at all steps of cortisol production. Diseases associated with insufficient cortisol production are referred to as congenital adrenal hyperplasias (CAHs). The enzyme deficiency that is most common in CAH is the cytochrome P450-dependent 21-hydroxylase or CYP21A2. A deficiency in a functional 21-hydroxylase enzyme prevents the metabolism of 17α-hydroxyprogesterone to 11-deoxycortisol and subsequently to cortisol. This causes an increase in ACTH secretion, the pituitary hormone that regulates adrenal cortex production of cortisol. Prolonged periods of elevated ACTH levels causes adrenal hyperplasia and an increased production of the androgenic hormones, DHEA and androstenedione. Clinical problems arise because the additional production of androgenic steroids causes virilization in females, precocious sex organ development in prepubertal males, or diseases related to salt imbalance because of decreased levels of aldosterone. Clinical consequences of severe 21-hydroxylase deficiency may be recognizable at birth, particularly in females, because the excessive buildup of androgenic steroids may cause obvious irregular development of their genitalia. In male newborns, a deficiency in 21-hydroxylase activity may be overlooked, because male genitalia will appear normal, but there will be precocious masculinization and physical development. In late onset CAH, individuals are born without obvious signs of prenatal exposure to excessive androgen levels, and clinical symptoms may vary considerably from early development of pubic hair, early fusion of epiphyseal growth plates causing premature cessation of growth, or male baldness patterns in females.

Donohoue, P. A., Parker, K., and Migeon, C. J. Congenital adrenal hyperplasia. In: C. S. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed., Vol. II. New York; McGraw-Hill, 1995, Chap. 94, p. 2929.

cytochrome P450 **(CYP11B2),** which catalyzes both the 11β-hydroxylase and 18-hydroxylase activities to form the mineralocorticoid, aldosterone, in the zona glomerulosa (Chapter 21, p. 899).

Synthesis of cortisol proceeds from either pregnenolone or progesterone and involves a cytochrome P450 **(CYP17),** an endoplasmic reticulum cytochrome P450, which catalyzes the 17α-hydroxylation reaction. Hydroxylation of the C-21 of 17α-hydroxyprogesterone by CYP21 produces 11-deoxycortisol, which is transported into mitochondria where it is hydroxylated at carbon atom 11 by **CYP11B1** to form cortisol. These reactions occur primarily in the zona fasciculata of the adrenal cortex. The consequences of a genetic polymorphism in CYP21 is presented in Clin. Corr. 23.3.

Synthesis of steroids containing 19 carbon atoms from 17α-hydroxypregnenolone or 17α-hydroxyprogesterone is the result of the loss of the acetyl group at C-17. This reaction is catalyzed by CYP17, identified as the same cytochrome P450 that hydroxylates C-17. Thus cleavage of the bond between C-17 and C-20 with loss of the acetyl group is also catalyzed by a cytochrome P450 molecule. The factors that determine whether this cytochrome P450 performs only a single hydroxylation step to produce the 17-OH product or proceeds further to cleave the C-17–C-20 bond has not been determined. The products are **dehydroepiandrosterone** (DHEA) from 17α-hydroxypregnenolone or androstenedione from 17α-hydroxyprogesterone. DHEA in the sex organs may be metabolized by dehydrogenation of the 3-OH group to androstenedione, a potent androgenic steroid that serves as the immediate precursor of testosterone.

Another physiologically important reaction catalyzed by cytochromes P450 is synthesis of estrogens from androgens, collectively called **aromatization** because an aromatic ring is introduced into the product. This is a complex reaction not unlike the side chain cleavage of cholesterol in which multiple hydroxylation reactions are carried out by a single cytochrome P450 enzyme to form the aromatic ring and remove the methyl group at C-19. Figure 23.10 outlines the aromatization reaction of ring A. Two cytochrome P450-mediated hydroxylation reactions at the methyl carbon atom at position 19 introduce an aldehyde group. It has been proposed that the final step involves a peroxidative attack at C-19 with loss of the methyl group and elimination of the hydrogen atom to produce the aromatic ring. The reaction steps of this sequence are catalyzed by the same cytochrome P450 and the enzyme is called aromatase or $P450_{arom}$. $P450_{arom}$ is a member of the CYP19 subfamily. The complexity of steroid hormone production and the role of cytochromes P450 are illustrated in Clin. Corr. 234.

Other cytochromes P450 metabolize **vitamin D_3** to produce the **1,25-dihydroxy vitamin D_3,** which is the active form of this important hormone (see p. 907), leukotriene B_4 to produce 20-hydroxy-leukotriene B_4, which is the less active form of this chemotactic agent (see p. 438), and arachidonic acid to produce epoxides, hydroxy and dihydroxy derivatives of arachidonic acid, which may have important regulatory functions (see p. 433).

Cytochromes P450 Oxidize Exogenous Lipophilic Substrates

Exogenous substrates are often referred to as **xenobiotics,** meaning "foreign to life." They include therapeutic drugs, chemicals used in the workplace, industrial by-products that become environmental contaminants, and food additives. Cytochromes P450 oxidize a variety of xenobiotics, particularly lipophilic compounds. The addition of a hydroxyl group makes the compound more polar and thus more soluble in the aqueous environment of the cell. Many exogenous compounds are highly lipophilic and accumulate within cells, interfering with cellular function over a period of time. Examples of xenobiotics that are oxidized by cytochromes P450 are presented in Tables 23.2 and 23.3 (p. 994). In many cases the action of the cytochromes P450 leads to a compound

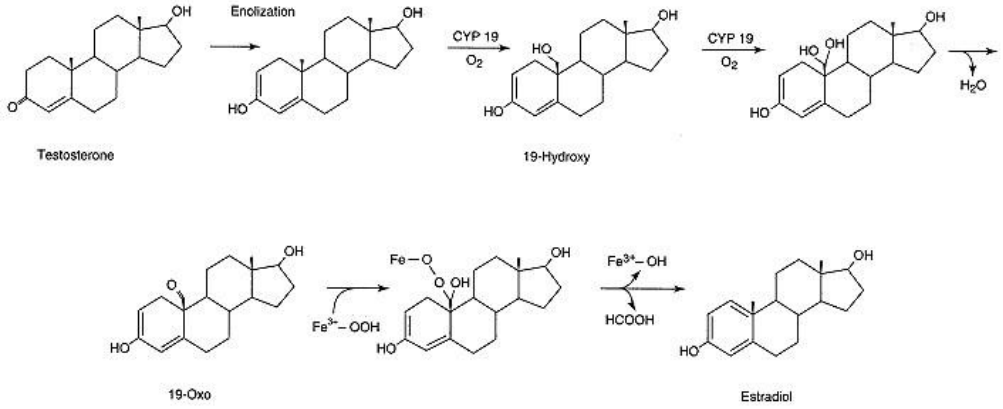

Figure 23.10
Sequence of reactions leading to aromatization of androgens to estrogens.
Adapted from Graham-Lorence, S., Amarneh, B., White, R. E., Peterson, J. A., and Simpson E. R. *Protein Sci.* 4:1065, 1995.

with reduced pharmacological activity or toxicity, which can readily be excreted in the urine or bile. Modified and unmodified xenobiotics can be altered chemically by a variety of conjugating enzyme systems forming products that are even less toxic and that can readily be eliminated from the body. A list of enzymes that metabolize xenobiotics is presented in Table 23.3; many occur primarily in the liver.

One xenobiotic that has received considerable attention is **benzol[*a*]pyrene,** a common environmental contaminant produced from the burning of

CLINICAL CORRELATION 23.4

Steroid Hormone Production during Pregnancy

Steroid hormone production increases dramatically during pregnancy and, at term, the pregnant woman produces 15–20 mg of estradiol, 50–100 mg of estriol, and approximately 250 mg of progesterone per 24-h period. The amount of estrogens synthesized during pregnancy far exceeds the amount synthesized by nonpregnant women. For example, the pregnant woman at the end of gestation produces 1000 times more estrogen than premenopausal women per day.

Production of progesterone and estrogens in pregnant women is decidedly different from that in the nonpregnant woman. The corpus luteum of the ovary is the major site for estrogen production in the first few weeks of pregnancy, but at approximately 4 weeks of gestation, the placenta begins synthesizing and secreting progesterone and estrogens. After 8 weeks of gestation, the placenta becomes the dominant source for the synthesis of progesterone. An interesting difference between the steroid hydroxylating systems in the placenta and the ovary is that the human placenta lacks the cytochrome P450 (CYP11A1) that catalyzes the 17β-hydroxylation reaction and the cleavage of the 17,20 carbon–carbon bond (see Chapter 21, p. 898, for details of synthesis of steroid hormones). Thus the placenta cannot, by itself, synthesize estrogens from cholesterol. The placenta catalyzes the side chain cleavage reaction to form pregnenolone from cholesterol and oxidizes pregnenolone to progesterone but releases this hormone into the maternal circulation. How then does the placenta produce estrogens if it cannot synthesize DHEA or androstenedione from progesterone? This is accomplished in the fetal adrenal gland, which represents a significant proportion of the total fetal weight compared to its adult state. The fetal adrenal gland catalyzes the synthesis of DHEA from cholesterol and releases it into the fetal circulation. A large proportion of the fetal DHEA is metabolized by the fetal liver to 16α-hydroxy-DHEA, and this product is aromatized in the placenta to the estrogen, estriol. This is an elegant demonstration of the cooperativity of the cytochrome P450-mediated hydroxylating systems in the fetal and maternal organ systems leading to the progressive formation of estrogens during the gestational development of the human fetus.

Cunningham, F. G., MacDonald, P. C., Gant, N. F., Leveno, K. J., and Gilstrap, L. C. The placental hormones. In: *Williams Obstetrics*, 19th ed. East Norwalk, CT: Appleton & Lange, 1993, Chap. 6, p. 139.

TABLE 23.2 Xenobiotics Metabolized by Cytochromes P450

Reaction	*Examples*
Aliphatic hydroxylation	Valproic acid, pentobarbital
Aromatic hydroxylation	Debrisoquine, acetanilide
Epoxidation	Benzene, benzo[*a*]pyrene
Dealkylation	Aminopyrine, phenacetin, 6-methyl-thiopurine
Oxidative deamination	Amphetamine
Nitrogen or sulfur oxidation	2-Acetylaminofluorene, chlorpromazine
Dehalogenation	Halothane
Alcohol oxidation	Ethanol

coal, from the combustion of plant materials in tobacco, from food barbecued on charcoal, and as an industrial by-product. Benzo[*a*]pyrene binds to the **aryl hydrocarbon receptor** and induces cytochromes P450 in the 1A subfamily, thus increasing its own metabolism. Several sites of the molecule may be hydroxylated by different forms of cytochrome P450. Benzo[*a*]pyrene is metabolized to a **carcinogen** in animals and a mutagen in bacteria, prompting considerable work in identifying the enzymes involved in this process. The product found to represent the ultimate carcinogen is **benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide,** the formation of which is illustrated in Figure 23.11. The initial step involves a cytochrome P450-catalyzed epoxidation at the 7,8 position, hydrolysis by **epoxide hydrolase** to the vicinal hydroxylated compound, benzo[*a*]pyrene-7,8-dihydrodiol, and then another epoxidation reaction to form benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide. The parent compound, benzo[*a*]pyrene, is a weak carcinogen and, like most carcinogens that have been characterized, requires metabolic activation to its more potent carcinogenic form.

In a number of cases, the cytochrome P450 system is responsible for generation of the ultimate carcinogen Formation of toxic compounds by the cytochrome P450 system, however, does not mean that cell damage or cancer will occur, because many other factors will determine whether or not the toxic metabolite will cause cell injury. These include the involvement of detoxification enzyme systems, the status of the immune system, nutritional state, genetic predisposition, and environmental factors. One may ask why the body should possess an enzyme system that would create highly toxic compounds? As indi-

TABLE 23.3 Xenobiotic-Metabolizing Enzymes

Type of Reaction	*Enzyme*	*Representative Substrate*
Oxidation	Cytochrome P450	Toluene
	Alcohol dehydrogenase	Ethyl alcohol
	Flavin-containing monooxygenase	Dimethylaniline
Reduction	Ketone reductase	Metyrapone
Hydration	Epoxide hydrolase	Benzo[*a*]pyrene- 7,8-epoxide
Hydrolysis	Esterase	Procaine
Conjugation	UDP-glucuronyltransferase	Acetaminophen
	Sulfotransferase	β-Naphthol
	N-acetyltransferase	Sulfanilamide
	Methyltransferase	Thiouracil
	Glutathionetransferase	Acetaminophen

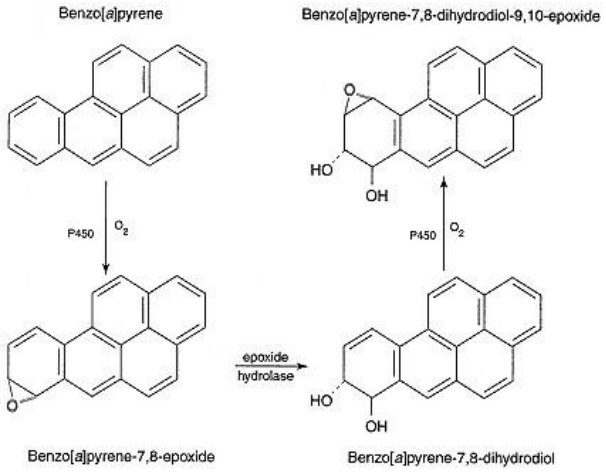

Figure 23.11
Metabolism of benzo[*a*]pyrene by cytochrome P450 and epoxide hydrolase to form benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide.

cated, the purpose of the cytochrome P450 system is to add or expose functional groups making the molecule more polar and/or more susceptible to attack by additional detoxification enzyme systems. In addition, many of these compounds resemble hormones that are our natural communication signals and would interfere with cell–cell or organ–organ communication.

Thus the cytochrome P450 system plays a significant role in the health and disease of humans. Different cytochromes P450 are responsible for generation of essential steroid hormones, the regulation of blood levels of therapeutic agents, the removal of unwanted chemicals that would accumulate because of their lipophilicity, and the generation of potentially toxic metabolites that may cause acute cell injury or damage to genetic material and lead to production of tumors.

23.7— Other Hemoprotein- and Flavoprotein-Mediated Oxygenations: The Nitric Oxide Synthases

Three Distinct Nitric Oxide Synthase Gene Products Display Diverse Physiological Functions

Release of nitric oxide from therapeutic drugs has been used as a treatment for angina pectoris since 1867, when Sir Thomas Lauder Brunton reported the use of nitroglycerin and amyl nitrate in his patients. However, it was not known until the 1980s that **nitric oxide,** or $NO^{\cdot}$, was the active agent in the dilation of blood vessels. The demonstration that this free radical diatomic gas was the primary endogenous vasodilator released by the vascular endothelium led to the search for an enzymatic source of $NO^{\cdot}$. The source of $NO^{\cdot}$ is the guanidino group of the naturally occurring amino acid, L-arginine. The reaction catalyzed by the enzymes responsible for the conversion of L-arginine to L-citrulline and $NO^{\cdot}$ is shown below:

L-Arginine + NADPH → L-citrulline + $NO^{\cdot}$ + $NADP^{+}$

Nitric oxide synthases have been examined in whole animals, tissues, and cells for functional properties and recently three genes have been identified for

CLINICAL CORRELATION 23.5

Clinical Aspects of Nitric Oxide Production

Although the role of $NO^{\cdot}$ in the tumoricidal and bactericidal functions of macrophages is essential in these cells, the overproduction of $NO^{\cdot}$ (from the inducible isoform of nitric oxide synthase, iNOS or NOS-II) has been implicated in septic/cytokine-induced circulatory shock in humans through the activation of guanylate cyclase. This mechanism is responsible for profound hypotension in patients after abdominal surgery or abdominal trauma complicated by bacterial infections that produce endotoxins, as well as in patients with neoplasias treated by IL-2 chemotherapy. Hypotension in these patients is often refractory to treatment with conventional vasoconstrictor drugs. Therapeutic interventions using NOS inhibitors are being examined in gastrointestinal inflammatory diseases, such as pancreatitis and ulcerative colitis, and in arthritis. Administration of NOS inhibitors (e.g., specific to iNOS) might be a treatment of choice in such patients.

The endothelial isoform of nitric oxide synthase, eNOS or NOS-III, is thought to play a critical role in maintaining a basic vasotonus in hemodynamic regulation such that an imbalance in the production of $NO^{\cdot}$ could result in hypertension, thrombosis, or atherosclerosis. Direct application of $NO^{\cdot}$ gas may also be beneficial in the treatment of pulmonary hypertension. In addition, recent experiments with mice in which the gene for the neuronal isoform of nitric oxide synthase, nNOS or NOS-I, has been deleted have resulted in animals with distended stomachs due to constriction of the pyloric sphincter. This work has unexpectedly produced a model for the clinical disease, infantile hypertrophic pyloric stenosis. It has also been shown that these nNOS-deficient mice are resistant to brain damage as a result of ischemic injury usually resulting in vascular strokes. While the direct connection to human disease has not yet been made, in this instance, it presents a paradigm that can now be examined in clinical and pathological settings.

The development of potent, specific inhibitors of the isoforms of nitric oxide synthase is an active area of research being pursued collaboratively by investigators in academia and the pharmaceutical industry.

the isoforms responsible for the activities in various tissues. Accordingly, the respective enzymes have been designated as **neuronal (NOS-I), macrophage** or **induced (NOS-II),** or **endothelial (NOS-III).** Any tissue or cell may contain more than one isoform of nitric oxide synthase, thus contributing to the production of $NO^{\cdot}$ under various physiological circumstances. Studies of the macrophage type of nitric oxide synthase led to the conclusion that, upon treatment of animals with cytokines or lipopolysaccharide, the increase in production of $NO^{\cdot}$ was due to this isoform, since it is quantitatively the major source of $NO^{\cdot}$. Subsequently, L-arginine was shown to be the precursor of $NO^{\cdot}$ in both endothelial and neuronal cells. Production of $NO^{\cdot}$ is necessary for maintenance of vascular tone, platelet aggregation, neural transmission, and bacterial and/ or tumor cytotoxicity (see Clin. Corr. 23.5).

As further evidence of the importance of heme enzymes, signaling events require binding to the heme prosthetic group of guanylate cyclase of $NO^{\cdot}$ produced in neuronal and endothelial cells for activation of signaling events. The formation of cGMP leads to the subsequent downregulation of intracellular $\mathbf{Ca^{2+}}$ concentrations and to a cellular response appropriate to the specific cell involved. For example, the production of cGMP in vascular smooth muscle cells resulting from $NO^{\cdot}$ production leads to the lowering of Ca^{2+} concentrations, resulting in vasodilatation due to smooth muscle relaxation.

Structural Aspects of Nitric Oxide Synthases

Although the written reaction does not reveal the overall stoichiometry, it is representative of a monooxygenation reaction and the mechanism is similar to that catalyzed by cytochromes P450. The oxygen atoms incorporated into both L-citrulline and $NO^{\cdot}$ are derived from atmospheric oxygen. It was originally assumed that oxygenation was occurring through mediation of **tetrahydrobiopterin (BH_4),** a required cofactor for the overall reaction, analogous to the phenylalanine hydroxylase reaction (see p. 464). The discovery that **heme** (iron protoporphyrin IX) is a functional prosthetic group associated with all three isoforms of nitric oxide synthase has directed subsequent studies to include interactions between the flavoprotein and hemoprotein domains of these enzymes. These complex proteins must now be understood from the standpoint of the roles of the flavins, heme, and BH_4, under the control of Ca^{2+}/calmodulin in the case of the neuronal (NOS-I) and endothelial (NOS-II) isoforms. Figure 23.12 shows the overall structural organization of the neuronal NOS isoform. In addition to the various protein modules or domains of NOS-I which are involved in electron transfer, substrate binding, oxygen activation, and calcium binding, a four-amino acid motif (glycine–leucine–glycine–phenylalanine, GLGF) has been identified in the amino terminal region of NOS-I. Although the function of this amino acid motif in NOS-I has not been established, studies on other proteins containing this motif indicate that it may serve to target proteins to specific sites in the cell.

The flow of electrons is assumed to occur in an analogous fashion to that of cytochrome P450-mediated electron systems. The electron donor is NADPH, which donates two electrons to the enzyme-bound entry FAD, which, in turn, reduces the exit FMN. It is the latter flavin that reduces the heme iron prosthetic group to Fe^{2+} to which oxygen can now bind for the oxygenation of the substrate, L-arginine. The overall reaction is inhibited by carbon monoxide and enzyme activity is totally dependent on bound calmodulin, which requires high concentrations of Ca^{2+} for the neuronal and endothelial isoforms. **Calmodulin** is involved in the control of electron flow between the flavin prosthetic groups and between the exit flavin, FMN, and the heme prosthetic group in the oxygenase module. While the precise residues constituting the binding site of BH_4 have not been identified, its location has been narrowed to the

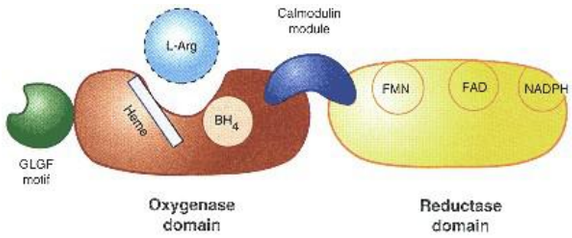

Figure 23.12
Modular structure of neuronal nitric oxide synthase showing approximate locations of prosthetic groups and cofactors.
Adapted from Masters, B.S.S., McMillan, K., Sheta, E. A., Nishimura, J. S. et al. *FASEB J.,*10:552, 1996.

oxygenase module in the vicinity of the heme-binding site. The analogy between the systems synthesizing nitric oxide and the cytochrome P450-mediated systems is remarkable, but the differences are significant and the oxygenase module probably represents an example of convergent evolution with the cytochromes P450. The three-dimensional structures of mammalian representatives of either the cytochromes P450 or the nitric oxide synthases are yet to be determined.

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Questions

C. N. Angstadt and J. Baggott

1. All of the following are correct about a molecule designated as a cytochrome P450 EXCEPT:

   A. it contains a heme as a prosthetic group.

   B. it catalyzes the hydroxylation of a hydrophobic substrate.

   C. it may accept electrons from a substance such as NADPH.

   D. it undergoes a change in the heme iron upon binding a substrate.

   E. it comes from the same gene family as all other molecules designated as cytochromes P450.

2. Known roles for cytochromes P450 include all of the following EXCEPT:

   A. synthesis of steroid hormones.

   B. conversion of some chemicals to mutagens.

   C. hydroxylation of an amino acid.

   D. inactivation of some hydrophobic drugs.

   E. metabolism of fatty acid derivatives.

3. The induction of cytochromes P450:

   A. occurs only by endogenous compounds.

   B. occurs only at the transcriptional level.

   C. necessarily results from increased transcription of the appropriate mRNA.

   D. necessitates the formation of an inducer–receptor protein complex.

   E. may occur by posttranscriptional processes.

4. Flavoproteins are usually intermediates in the transfer of electrons from NADPH to cytochrome P450 because:

   A. NADPH cannot enter the membrane.

   B. flavoproteins can accept two electrons from NADPH and donate them one at a time to cytochrome P450.

   C. they have a more negative reduction potential than NADPH and so accept electrons more readily.

   D. as redox proteins, they can directly reduce cytochromes P450 while the nonprotein NADPH cannot.

   E. they contain iron–sulfur centers.

5. NADPH–cytochrome P450 reductase:

   A. uses both FAD and FMN as prosthetic groups.

   B. is found in mitochondria.

   C. requires an iron–sulfur center for activity.

   D. always passes its electrons to cytochrome b_5.

   E. can use NADH as readily as NADPH.

6. The system necessary for the formation of double bonds in fatty acids:

   A. is the cytochrome P450 electron transport system in the endoplasmic reticulum.

   B. is the cytochrome P450 electron transport system in the mitochondria.

   C. contains NADH–cytochrome b_5 reductase.

   D. uses NADPH–adrenodoxin reductase to reduce cytochrome b_5.

   E. uses both FAD and FMN as prosthetic groups.

7. NADPH–adrenodoxin reductase:

   A. is located in the endoplasmic reticulum

   B. passes its electrons to a protein with iron–sulfur centers.

   C. has a stretch of hydrophobic amino acid residues at the N-terminal end.

   D. reacts directly with cytochrome P450.

   E. reacts directly with cytochrome b_5.

8. Cytochrome P450 systems are able to oxidize:

   A. —CH_2— groups.

   B. benzene rings.

   C. nitrogen atoms in an organic compound.

   D. sulfur atoms in an organic compound.

   E. all of the above.

9. In the conversion of cholesterol to steroid hormones in the adrenal gland:

   A. all of the cytochrome P450 oxidations occur in the endoplasmic reticulum.

   B. all of the cytochrome P450 oxidations occur in the mitochondria.

   C. side chain cleavage of cholesterol to pregnenolone is one of the cytochrome P450 systems that uses adrenodoxin reductase.

   D. cytochrome P450 is necessary for the formation of aldosterone and cortisol but not for the formation of the androgens and estrogens.

   E. aromatization of the first ring of the steroid does not use cytochrome P450 because it involves removal of a methyl group, not a hydroxylation.

10. Many xenobiotics (exogenous substrates) are oxidized by cytochromes P450 in order to:

    A. make them carcinogenic.

    B. increase their solubility in an aqueous environment.

    C. enhance their deposition in adipose tissue.

    D. increase their pharmacological activity.

    E. all of the above.

11. Benzo[a]pyrene, a xenobiotic produced by combustion of a variety of substances:

    A. induces the synthesis of cytochrome P450.

    B. undergoes epoxidation by a cytochrome P450.

    C. is converted to a potent carcinogen in animals by cytochrome P450.

    D. would be rendered more water-soluble after the action of cytochrome P450.

    E. all of the above.

12. Phenobarbital is a potent inducer of cytochrome P450. Warfarin, an anticoagulant, is a substrate for cytochrome P450 with the result that the drug is metabolized and cleared from the body more rapidly than normal. If phenobarbital is added to the therapeutic regimen of a patient, with no change in the dosage of warfarin, the expected consequence would be:

A. no change in the clinical results.

B. an increased possibility of clot formation.

C. an increased possibility of hemorrhaging.

13. Nitric oxide:

A. is formed spontaneously by a reduction of NO_2.

B. is synthesized only in macrophages.

C. is synthesized from arginine.

D. acts as a potent vasoconstrictor.

E. has three isoforms.

14. Nitric oxide synthase:

A. catalyzes a dioxygenase reaction.

B. is similar mechanistically to phenylalanine hydroxylase since it requires tetrahydrobiopterin.

C. accepts electrons from NADH.

D. uses a flow of electrons from NADPH to FAD to FMN to heme iron.

E. is inhibited by Ca^{2+}.

Answers

1. E Several gene families are known. The number after CYP designates the family. B: The types of substrates are hydrophobic. It is classified as a monooxygenase. C: See Figure 23.3. D: The change from hexa to penta coordinated gives the compound a more positive reduction potential (pp. 982–984).

2. C Cytochromes P450 are not the only hydroxylases and other types are active with amino acids (pp. 982, 989–995).

3. E There may be a stabilization of mRNA (as seen in diabetic rats) or decrease in the degradation of the protein, which may be a mechanism for pyrazole (p. 985). A: One of the roles of cytochromes P450 is in the metabolism of exogenous substances. B and C: Transcriptional modification is only one of the mechanisms of induction (see E). D: This has been shown with induction by some compounds, but with others, like phenobarbital, this is not so (p. 985).

4. B Heme can accept only one electron at a time while NADPH always donates two at a time. A: NADPH passes only electrons; it does not have to enter the membrane. C: If this were true, the flow of electrons would not occur in the way it does. D: Protein–protein binding is not known to play a role here. E: Iron–sulfur centers play a role in some, but not all, systems. Flavoproteins are not the only system with iron–sulfur centers (p. 987).

5. A This enzyme is one of two mammalian proteins known to do so. B: This is in the endoplasmic reticulum. C: Some reductases do so but not this one. D: Only certain reactions catalyzed by the enzyme do. E: There are NADH-dependent reductases but they are different enzymes (p. 988).

6. C This enzyme reduces desaturase. A: Desaturase does not react with cytochrome P450. B: Desaturase is in the endoplasmic reticulum. D: This is a mitochondrial system. E: This is not one of the two enzymes that use both flavins (p. 988).

7. B The iron–sulfur protein is adrenodoxin, which passes the electron to cytochrome P450. A and C: This is a mitochondrial enzyme. D and E: See B (p. 989).

8. E See Figure 23.7, p. 989.

9. C This is a mitochondrial process (Figure 23.8, p. 990). A and B: Hormone synthesis involves a series of reactions that move back and forth between mitochondria and endoplasmic reticulum (p. 991). D and E: Removal of side chains frequently begins with oxidation reactions (p. 992).

10. B The types of xenobiotics oxidized by cytochrome P450 are usually highly lipophilic but must be excreted in the aqueous urine or bile. A: This may happen but is certainly not the purpose. C: They do that prior to oxidation. D: Oxidation tends to reduce pharmacological activity (p. 992).

11. E A: It is not uncommon for xenobiotics to induce synthesis of something that will enhance their own metabolism. B and C: Epoxidation is the first step in the conversion of this compound to one that is carcinogenic—again, a common occurrence (p. 994). D: Benzo[a]pyrene, with its four fused benzene rings, is highly hydrophobic; introducing oxygens increases water solubility (p. 993).

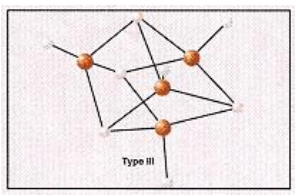
12. B If warfarin is metabolized and cleared more rapidly by cytochrome P450, its therapeutic efficiency is decreased. Therefore, at the same dosage, it will be less effective as an anticoagulant. Think what would happen if the warfarin dosage were adjusted for a proper response, and then phenobarbital were withdrawn without adjusting the warfarin dose (see Clin. Corr. 23.1).

13. C The other product is citrulline. A and E: Three isoforms of nitric oxide synthase have been identified. B: One of the isoforms of NO synthase has been found in macrophages but neuronal and endothelial isoforms also exist. D: Nitric oxide is a vasodilator, which is the basis for the use of nitroglycerin in angina pectoris (p. 995).

14. D This is the second mammalian enzyme known to use both FAD and FMN. A: The reaction is a monooxygenation. B: BH_4 is required but the action of the enzyme is similar to a cytochrome P450-mediated system. C: The donor is NADPH. E: The system requires Ca^{2+}–calmodulin, at least the neuronal and endothelial isoforms (p. 996).

Chapter 24—
Iron and Heme Metabolism

William M. Awad, Jr.



24.1— Iron Metabolism: Overview

Iron is closely involved in the metabolism of oxygen, permitting the transportation and participation of oxygen in a variety of biochemical processes. The common **oxidation states** are either **ferrous** (Fe^{2+}) or **ferric** (Fe^{3+}); higher oxidation levels occur as short-lived intermediates in certain redox processes. Iron has an affinity for electronegative atoms such as oxygen, nitrogen, and sulfur, which provide the electrons that form the bonds with iron. These can be of very high affinity when favorably oriented on macromolecules. In forming complexes, no bonding electrons are derived from iron. There is an added complexity to the structure of iron: the nonbonding electrons in the outer shell of the metal (the incompletely filled 3*d* orbitals) can exist in two states. Where bonding interactions with iron are weak, the outer nonbonding electrons will avoid pairing and distribute throughout the 3*d* orbitals. Where bonding electrons interact strongly with iron, however, there will be pairing of the outer nonbonding electrons, favoring lower-energy 3*d* orbitals. These two different distributions for each oxidation state of iron can be determined by electron spin resonance measurements. Dispersion of 3*d* electrons to all orbitals leads to the high-spin state, whereas restriction of 3*d* electrons to lower energy orbitals, because of electron pairing, leads to a low-spin state. Some iron–protein complexes reveal changes in spin state without changes in oxidation during chemical events (e.g., binding and release of oxygen by hemoglobin).

At neutral and alkaline pH ranges, the redox potential for iron in aqueous solutions favors the Fe^{3+} state; at acid pH values, the equilibrium favors the Fe^{2+} state. In the Fe^{3+} state iron slowly forms large polynuclear complexes with hydroxide ion, water, and other anions that may be present. These complexes can become so large as to exceed their solubility products, leading to their aggregation and precipitation with pathological consequences.

Iron can bind to and influence the structure and function of various macromolecules, with deleterious results to the organism. To protect against such reactions, several iron-binding proteins function specifically to store and transport iron. These proteins have both a very high affinity for the metal and, in the normal physiological state, also have incompletely filled iron-binding sites. The interaction of iron with its ligands has been well characterized in some proteins (e.g., hemoglobin and myoglobin), whereas for others (e.g., transferrin) it is presently in the process of being defined. The major area of ignorance in the biochemistry of iron lies in the *in vivo* transfer processes of iron from one macromolecule to another. Several proposed mechanisms may explain the process of iron transfer. Two are supported by excellent model studies but have varying degrees of relevance to the physiological state. The proposed processes are the following. First, the redox change of iron has been an attractive mechanism because it is supported by selective *in vitro* studies and because in some cases macromolecules have a very selective affinity for Fe^{3+}, binding Fe^{2+} poorly. Thus reduction of iron would permit ferrous ions to dissociate, and reoxidation would allow the iron to redistribute to appropriate macromolecules. Redox mechanisms have only been defined in a very few settings, some of which will be described below. An alternative hypothesis involves chelation of ferric ions by specific small molecules with high affinities for iron; this mechanism has been supported also by selective *in vitro* studies. The chelation mechanism suffers from the lack of a demonstrably specific *in vivo* chelator. Because the redox potential strongly favors ferric ion at almost all tissue sites and because Fe^{3+} binds so strongly to liganding groups, the probability is that there are cooperating mechanisms regulating the intermolecular transfer of iron.

CLINICAL CORRELATION 24.1

Iron Overload and Infection

If an individual is overloaded with iron by any of several causes, the serum transferrin value can be close to saturation, making small amounts of free serum iron available. Microorganisms that are usually nonpathogenic, because they are iron dependent and cannot compete against partially saturated transferrin in the normal individual, can now become pathogenic under these circumstances. For example, *Vibrio vulnificus,* a marine halophile, is found in a small percentage of oysters and commercial shellfish. Individuals who are iron overloaded can develop a rapidly progressive infection, with death ensuing within 24 h after ingestion of the offending meal, whereas normal individuals consuming the same food are entirely free of symptoms.

Muench, K. H. Hemochromatosis and infection: alcohol and iron, oysters and sepsis. *Am. J. Med.* 87:3, 1989.

24.2— Iron-Containing Proteins

Iron binds to proteins either by incorporation into a **protoporphyrin IX** ring (see below) or by interaction with other protein ligands. Ferrous- and ferric-protoporphyrin IX complexes are designated **heme** and **hematin,** respectively. Heme-containing proteins include those that transport (e.g., hemoglobin) and store (e.g., myoglobin) oxygen, and certain enzymes that contain heme as part of their prosthetic groups (e.g., catalase, peroxidases, tryptophan pyrrolase, prostaglandin synthase, guanylate cyclase, NO synthase, and the microsomal and mitochondrial cytochromes.). Discussions on structure–function relationships of heme proteins are presented in Chapters 6 and 25.

Nonheme proteins include transferrin, ferritin, a variety of redox enzymes that contain iron at the active site, and iron–sulfur proteins. A significant body of information has been acquired that relates to the structure–function relationships of some of these molecules.

Transferrin Transports Iron in Serum

The protein in serum involved in the transport of iron is **transferrin,** a β1-glycoprotein synthesized in the liver, consisting of a single polypeptide chain of 78,000 Da with two noncooperative iron-binding sites. The protein is a product of gene duplication derived from a putative ancestral gene coding for a protein binding only one atom of iron. Several metals bind to transferrin; the highest affinity is for Fe^{3+}; Fe^{2+} ion is not bound. The binding of each Fe^{3+} ion is absolutely dependent on the coordinate binding of an anion, which in the physiological state is carbonate as indicated below:

$$\text{Transferrin} + Fe^{3+} + Co_3^{2-} \rightarrow \text{transferrin} \cdot Fe^{3} \cdot CO_3^{2-}$$
$$\text{Transferrin} \cdot Fe^{3+} \cdot CO_3^{2-} \rightarrow \text{transferrin} \cdot 2\,(Fe^{3+}CO_3^{2-})$$

Estimates of the association constants for the binding of Fe^{3+} to transferrins from different species range from 10^{19} to 10^{31} M^{-1}, indicating for practical purposes that wherever there is excess transferrin free ferric ions will not be found. In the normal physiological state, approximately one-ninth of all transferrin molecules are saturated with iron at both sites; four-ninths of transferrin molecules have iron at either site; and four-ninths of circulating transferrin are free of iron. Unsaturated transferrin protects against infections (see Clin. Corr. 24.1). The two iron-binding sites show differences in sequences and in affinities for other metals. Transferrin binds to specific cell surface receptors that mediate the internalization of the protein.

The **transferrin receptor** is a transmembrane protein consisting of two subunits of 90,000 Da each, joined by a disulfide bond. Each subunit contains one transmembrane segment and about 670 residues that are extracellular and bind a transferrin molecule, favoring the diferric form. Internalization of the receptor-transferrin complex is dependent on receptor phosphorylation by a Ca^{2+}–calmodulin–protein kinase C complex. Release of the iron atoms occurs within the acidic milieu of the lysosome after which the receptor–apotransferrin complex returns to the cell surface where the apotransferrin is released to be reutilized in the plasma.

Lactoferrin Binds Iron in Milk

Milk contains iron that is bound almost exclusively to a glycoprotein, **lactoferrin,** closely homologous to transferrin, with two sites binding the metal. The iron content of the protein varies, but it is never saturated. Studies on the function of lactoferrin have been directed toward its antimicrobial effect, protecting the newborn from gastrointestinal infections. Microorganisms require iron for

replication and function. Presence of incompletely saturated lactoferrin results in the rapid binding of any free iron, leading to the inhibition of microbial growth by preventing a sufficient amount of iron from entering these microorganisms. Other microbes, such as *Escherichia coli,* which release competitive iron chelators, are able to proliferate despite the presence of lactoferrin, since the chelators transfer the iron specifically to the microorganism. Lactoferrin is present in granulocytes being released during bacterial infections. It is also present in mucous secretions. Besides its bacteriostatic function it is believed to facilitate iron transport and storage in milk. Lactoferrin has been found in urine of premature infants fed human milk.

Ferritin Is a Protein Involved in Storage of Iron

Ferritin is the major protein involved in the storage of iron. The protein consists of an outer polypeptide shell 130 Å in diameter with a central ferric-hydroxide-phosphate core 60 Å across. The apoprotein, **apoferritin,** consists of 24 subunits of a varying mixture of H subunits (178 amino acids) and L subunits (171 amino acids) that provide various isoprotein forms. H subunits predominate in nucleated blood cells and heart, L subunits in liver and spleen. Synthesis of the subunits is regulated mainly by the concentration of free intracellular iron. The bulk of iron storage occurs in hepatocytes, reticuloendothelial cells, and skeletal muscle. The ratio of iron to polypeptide is not constant, since the protein has the ability to gain and release iron according to physiological needs. With a capacity of 4500 iron atoms, the molecule contains usually less than 3000. Channels from the surface permit the accumulation and release of iron. When iron is in excess, the storage capacity of newly synthesized apoferritin may be exceeded. This leads to iron deposition adjacent to ferritin spheres. Histologically, such amorphous iron deposition is called **hemosiderin.** The H chains of ferritin oxidize ferrous ions to the ferric state. Ferritins derived from different tissues of the same species differ in electrophoretic mobility in a fashion analogous to the differences noted with isoenzymes. In some tissues ferritin spheres form lattice-like arrays, which are identifiable by electron microscopy. Plasma ferritin (low in iron, rich in L subunits) has a half-life of 50 h and is cleared by reticuloendothelial cells and hepatocytes, and its concentration, although very low, correlates closely to the size of the body iron stores.

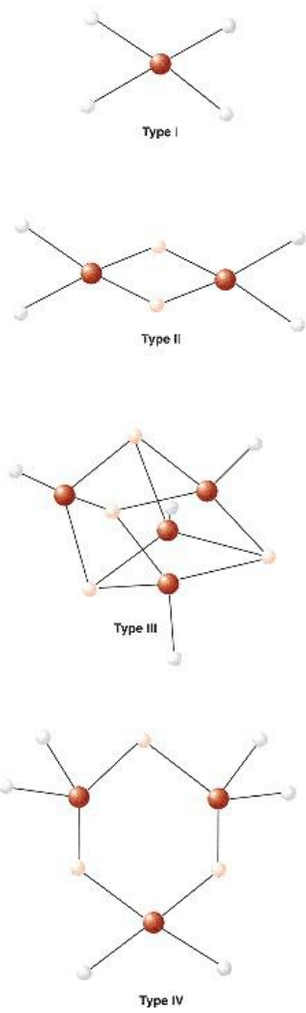


Figure 24.1
Structure of ferredoxins.
Dark red circles represent iron atoms; light red circles represent the inorganic sulfur atoms; and small gray circles represent the cysteinyl sulfur atoms derived from the polypeptide chain. Variation in type IV ferredoxins can occur where one of the cysteinyl residues can be substituted by a solvent oxygen atom of an OH group.

Other Nonheme Iron-Containing Proteins Are Involved in Enzymatic Processes

Many iron-containing proteins are involved in enzymatic processes, most of which are related to oxidation mechanisms. The structural features of the ligands binding the iron are not well known, except for a few components involved in mitochondrial electron transport. These latter proteins, termed **ferredoxins,** are characterized by iron being bonded, with one exception, only to sulfur atoms. Four major types of such proteins are known (see Figure 24.1). The smallest, type I (e.g., nebredoxin), found only in microorganisms, consists of a small polypeptide chain with a mass of about 6000 and contains one iron atom bound to four cysteine residues. Type II consists of ferredoxins found in both plants and animal tissues where two iron atoms are found, each liganding to two separate cysteine residues and sharing two sulfide anions. The most complicated of the iron–sulfur proteins are the bacterial ferredoxins, type III, which contain four atoms of iron, each of which is linked to single separate cysteine residues but also shares three sulfide anions with neighboring iron molecules to form a cube-like structure. In some anaerobic bacteria, a family of ferredoxins may contain two type III iron–sulfur groups per macromolecule. Type IV ferredoxins contain structures with three atoms of iron, each linked to two separate cysteine residues and each sharing two sulfide anions, forming a

CLINICAL CORRELATION 24.2

Duodenal Iron Absorption

Mucin in the duodenal lumen helps to solubilize ferric ions with presentation of the metal to an integrin, a transmembrane protein consisting of a heterodimer of 230 kDa. The cytosolic surface of the integrin interacts with a 56-kDa protein known as mobilferrin. The integrin transfers the iron from the luminal to cytoplasmic surface of the cell, where it is bound by mobilferrin. Mobilferrin acts as a cytosolic shuttle, transferring iron either to cytosolic ferritin or to the opposite pole of the duodenal cell where the iron is transported by an as yet undefined mechanism to capillaries to be picked up by transferrin.

Conrad, M. D., and Umbreit, J. N. Iron absorption—the mucin–mobilferrin–integrin pathway. A competitive pathway for metal absorption. *Am. J. Hematol.* 42:67, 1993.

planar ring. In one example of this ferredoxin type, an exception of iron atoms being liganded only to sulfur atoms was found where the sulfur of a cysteinyl residue was substituted by a solvent oxygen atom. The redox potential afforded by these different ferredoxins varies widely and is in part dependent on the environment of the surrounding polypeptide chain that envelops these iron–sulfur groups. In nebredoxin the iron undergoes ferric–ferrous conversion during electron transport. With the plant and animal ferredoxins (type II iron–sulfur proteins) both irons are in the Fe^{3+} form in the oxidized state; upon reduction only one iron goes to Fe^{2+}. In the bacterial ferredoxin (type III iron–sulfur protein) the oxidized state can be either 2 Fe^{3+} · 2 Fe^{2+} or 3 Fe^{3+} · Fe^{2+}, with corresponding reduced forms of Fe^{3+} · 3 Fe^{2+} or 2 Fe^{3+} · 2 Fe^{2+}.

24.3— Intestinal Absorption of Iron

The high affinity of iron for both specific and nonspecific macromolecules leads to the absence of significant formation of free iron salts, and thus this metal is not lost via usual excretory routes. Rather, excretion of iron occurs only through the normal sloughing of tissues that are not reutilized (e.g., epidermis and gastrointestinal mucosal cells). In the healthy adult male the loss is about 1 mg day^{-1}. In premenopausal women, the normal physiological events of menses and parturition substantially augment iron loss. A wide variation of such loss exists, depending on the amounts of menstrual flow and the multiplicity of births. In the extremes of the latter settings, a premenopausal woman may require an amount of iron that is four to five times that needed in an adult male for prolonged periods of time. The postmenopausal woman who is not iron-deficient has an iron requirement similar to that of the adult male. Children and patients with blood loss naturally have increased iron requirements.

Cooking of food facilitates the breakdown of ligands attached to iron, increasing the availability of the metal in the gut. The low pH of stomach contents permits the reduction of Fe^{3+} to Fe^{2+}, facilitating dissociation from ligands. The latter requires the presence of an accompanying **reductant,** which is usually achieved by adding **ascorbate** to the diet. The absence of a normally functioning stomach reduces substantially the amount of iron that is absorbed. Some iron-containing compounds bind the metal so tightly that it is not available for assimilation. Contrary to popular belief, spinach is a poor source of iron because of an earlier erroneous record of the iron content and because some of the iron is bound to phytate (inositol hexaphosphate), which is resistant to the chemical actions of the gastrointestinal tract. Specific protein cofactors derived from the stomach or pancreas have been suggested as being facilitators of iron absorption in the small intestine.

The major site of **absorption of iron** is in the small intestine, with the largest amount being absorbed in the duodenum and a gradient of lesser absorption occurring in the more distal portions of the small intestine. The metal enters the mucosal cell either as the free ion or as heme; in the latter case the metal is split off from the porphyrin ring in the mucosal cytoplasm. The large amount of bicarbonate secreted by the pancreas neutralizes the acidic material delivered by the stomach and thus favors the oxidation of Fe^{2+} to Fe^{3+}. The major barrier to the absorption of iron is not at the luminal surface of the duodenal mucosal cell. Whatever the requirements of the host are, in the face of an adequate delivery of iron to the lumen, a substantial amount of iron will enter the mucosal cell. Regulation of iron transfer occurs between the mucosal cell and the capillary bed (see Figure 24.2 and Clin. Corr. 24.2). In the normal state, certain processes define the amount of iron that will be transferred. Where there is **iron deficiency,** the amount of transfer increases; where there is **iron overload** in the host, the amount transferred is curtailed substantially. One mechanism that has been demonstrated to regulate this transfer of iron across the mucosal–capillary

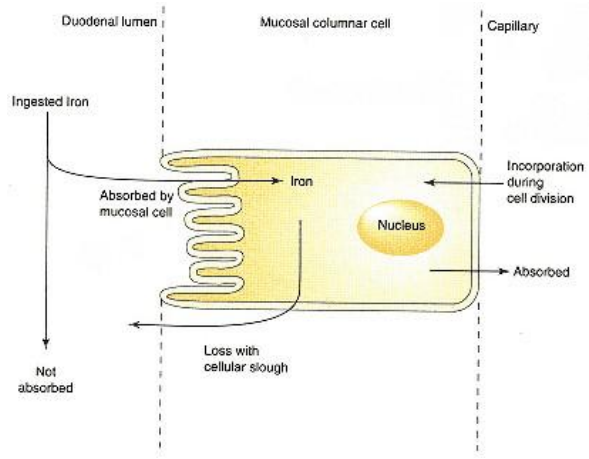


Figure 24.2
Intestinal mucosal regulation of iron absorption.
The flux of iron in the duodenal mucosal cell is indicated. A fraction of the iron that is potentially acceptable is transferred from the intestinal lumen into the epithelial cell. A large portion of ingested iron is not absorbed, in part because it is not presented in a readily acceptable form. Some iron is retained within the cell, bound by apoferritin to form ferritin. This iron is sloughed into the intestinal lumen with the normal turnover of the cell. A portion of the iron within the mucosal cell is absorbed and transferred to the capillary bed to be incorporated into transferrin. During cell division, which occurs at the bases of the intestinal crypts, iron is incorporated for cellular requirements. These fluxes change dramatically in iron-depleted or iron-excess states.

interface is the synthesis of apoferritin by the mucosal cell. In situations in which little iron is required by the host, a large amount of apoferritin is synthesized to trap the iron within the mucosal cell and prevent transfer to the capillary bed. As the cells turn over (within a week), their contents are extruded into the intestinal lumen without absorption occurring. In situations in which there is iron deficiency, virtually no apoferritin is synthesized so as not to compete against the transfer of iron to the deficient host. There are other as yet undefined positive mechanisms that increase the rate of iron absorption in the iron-deficient state. Iron transferred to the capillaries is trapped exclusively by transferrin.

24.4— Molecular Regulation of Iron Utilization

Cytosol contains at least two proteins that respond to changes in iron concentration. They act as effector molecules controlling the translation of mRNAs, which are important in iron metabolism. These **iron regulatory proteins (IRPs)** bind to specific **stem–loop structures** on certain mRNAs. IRP-1 is the best defined of these proteins. It contains an Fe_4S_4 cubane group when the cellular concentration of iron is high. This prosthetic group activates IRP-1 so that it possesses an **aconitase** activity. However, since neither citrate nor isocitrate is present in significant amounts in the cytosol, the activity is only a potential

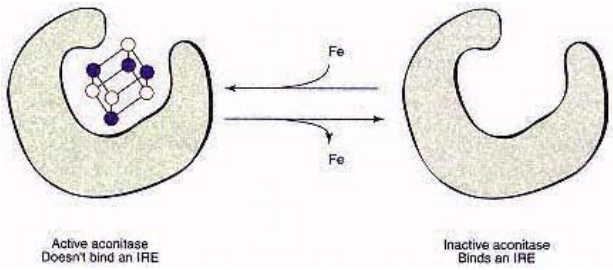


Figure 24.3
Iron-responsive protein-1.
Dark blue circles represent iron atoms and open circles inorganic sulfur atoms.

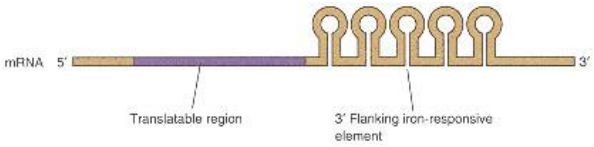


Figure 24.4
Structure of transferrin receptor mRNA.

CLINICAL CORRELATION 24.3

Mutant Iron-Responsive Element

Single mutations have been described of two adjacent bases in the loop segment of the iron-responsive element of ferritin light chain mRNA with an increased amount of apoferritin being synthesized but without an increase in total body iron. This mutation leads to a 28-fold lower affinity for IRP-1 in one case and perhaps an even lower affinity in the other. The reason why these patients have cataracts is unknown. The gene for MP-19, an abundant protein in the lens, which is very close to the light chain gene on chrosomome 19, might possibly be affected by the regulatory process on the mRNA. However, it is more probable that a greatly increased synthesis of ferritin in the lens leads to an increased amount of iron-catalyzed reactions with well-described oxidative lenticular damage.

Girelli, D., Corrocher, R., Bisceglia, L., et al. Molecular basis for the recently described hereditary hyperferritinemia–cataract syndrome: a mutation in the iron-responsive elements of ferritin L-subunit gene (the "Verona mutation"). *Blood* 86:4050, 1995; and Beaumont, C., Leneuve, P., Devaux, I., Scoazec, J. Y., et al. Mutation in the iron responsive element of the L ferritin mRNA in a family with dominant hyperferritinaemia and cataract. *Nature Genet.* 11:444, 1995.

one. At low iron concentrations, the cubane structure collapses, dissociating from the protein and leaving an apoenzyme without catalytic activity. However, it can now bind to specific mRNA stem–loop structures, known as **iron-responsive elements (IREs)** (Figure 24.3). Five mRNAs are known to contain IREs: those for the light and heavy chains of ferritin, the erythrocytic form of amino-levulinic acid synthase, the mitochondrial form of aconitase, and transferrin receptor. (Mitochondrial aconitase, the physiologically active isozyme, has no IRP function.) The first four mRNAs have single IREs in the 5′ flanking region, which bind a single IRP. In contrast, the transferrin receptor has five tandem IREs that bind IRPs in the 3′ flanking region. The binding of the 5′ and 3′ flanking IREs leads to different translational effects. In the iron-deprived state, binding to the 3′ IRE of transferrin receptor (Figure 24.4) leads to stabilization of the mRNA with reduced turnover and, therefore, an increased number of receptor-specific RNA molecules, thereby leading to the increased synthesis of receptor protein. The single 5′ stem–loop of ferritin mRNA (Figure 24.5) is homologous to the 3′ stem–loops of the transferrin receptor mRNA. However, in the former case, binding of the IRP leads to a decreased rate of translation of the mRNA and, thereby, to a decreased concentration of ferritin molecules. Note that the molecular events that are controlled are different in the syntheses of transferrin receptor and apoferritin (see Clin. Corr. 24.3).

In summary, low iron concentrations lead to activation of an IRP that binds to the mRNAs for transferrin receptor and ferritin. In the former case, more receptor is synthesized, while in the latter case less apoferritin is synthesized. The net effect is utilization of iron by proliferating cells. In contrast, high iron concentrations lead to loss of binding by the IRPs to IREs, with a shift of iron from uptake by proliferating cells to storage in the liver.

IRP-1 is regulated by its change from active to inactive states in mRNA-binding properties as noted above. **IRP-2,** a second regulatory protein, also responds to varying concentrations of iron, but in this case, the protein is regulated by increased synthesis at low iron concentrations and increased degradation by a proteasome at high iron concentrations. In addition to the effects of changed iron concentration, increased production of NO (see p. 995) also acts to regulate IRPs.

24.5— Iron Distribution and Kinetics

A normal 70-kg male has 3–4 g of iron, of which only 0.1% (3.5 mg) is in the plasma. Approximately 2.5 g are in hemoglobin. Table 24.1 lists the distribution

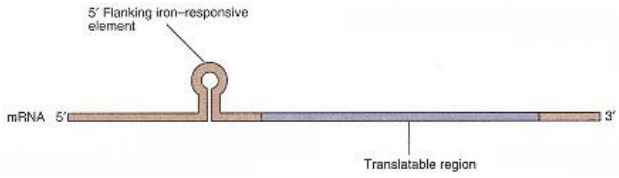


Figure 24.5
Structure of apoferritin H-subunit mRNA.

CLINICAL CORRELATION 24.4

Ceruloplasmin Deficiency

A deficiency of ceruloplasmin, a copper-containing protein, but not its absence, is associated with Wilson's disease in which there is progressive hepatic failure and degeneration of the basal ganglia, associated with a characteristic copper deposition in the cornea (Kayser–Fleischer rings). Because there was no evidence for significant impairment of mobilization of iron in Wilson's disease, it was originally thought that the ferroxidase activity of ceruloplasmin was not physiologically important. However, a recently discovered very rare genetic defect in ceruloplasmin biosynthesis, where the protein was virtually absent in serum, leads to a marked elevation of liver-iron content and serum ferritin levels. These patients develop diabetes, retinal degeneration, and central nervous system findings. The diabetes and central nervous system findings are associated with increased iron in the pancreas and brain, respectively. Thus, in contrast to earlier considerations, it appears that ceruloplasmin has a significant role in iron metabolism.

Harris, E. D. The iron–copper connection: the link to ceruloplasmin grows stronger. *Nutr. Rev.* 53:226, 1995.

of iron in humans. Normally about 33% of the sites on transferrin contain iron. Iron picked up from the intestine is delivered primarily to the marrow for incorporation into the hemoglobin of red blood cells. The mobilization of iron from the mucosa and from storage sites involves in part the reduction of iron to the ferrous state and its reoxidation to the ferric form. The reduction mechanisms have not been well described. On the other hand, conversion of the Fe^{2+} back to Fe^{3+} state is regulated by serum enzymes called ferroxidases as indicated below:

$$Fe^{2+} + \text{ferroxidase} \rightarrow Fe^{3+} + \text{reduced ferroxidase}$$

TABLE 24.1 Approximate Iron Distribution: 70–kg Man

	g	*%*
Hemoglobin	2.5	68
Myoglobin	0.15	4
Transferrin	0.003	0.1
Ferritin, tissue	1.0	27
Ferritin, serum	0.0001	0.004
Enzymes	0.02	0.6
Total	3.7	100

Ferroxidase I is also known as **ceruloplasmin** (see Clin. Corr. 24.4). Another serum protein, **ferroxidase II**, appears to be the major serum component that oxidizes ferrous ions. In any disease process in which iron loss exceeds iron repletion, a sequence of physiological responses occurs. The initial events are without symptoms to the subject and involve depletion of iron stores without compromise of any physiological function. This depletion will be manifested by a reduction or absence of iron stores in the liver and in the bone marrow and also by a decrease in the content of the very small amount of ferritin that is normally present in plasma. Serum ferritin levels reflect slow release from storage sites during the normal cellular turnover that occurs in the liver; measurements are made by radioimmunoassays. Serum ferritin is mostly apoferritin in form, containing very little iron. During this early phase, the level and percentage saturation of serum transferrin are not distinctly abnormal. As the iron deficiency progresses, the level of hemoglobin begins to fall and morphological changes appear in the red blood cells. Concurrently, the serum iron falls with a rise in the level of total serum transferrin, the latter reflecting a physiological adaptation in an attempt to absorb more iron from the gastrointestinal tract. At this state of iron depletion a very sensitive index is the percentage saturation of serum transferrin with iron (normal range, 21–50%). At this point the patient usually comes to medical attention, and the diagnosis of iron deficiency is made. In countries in which iron deficiency is severe without available corrective medical measures, a third and severe stage of iron deficiency can occur, where a depletion of iron-containing enzymes leads to very pronounced metabolic effects (see Clin. Corr. 24.5).

Iron overload can occur in patients so that the iron content of the body can be elevated to values as high as 100 g. This may happen for a variety of reasons. Some patients have a recessive heritable disorder associated with a marked inappropriate increase in iron absorption. In such cases the serum transferrin can be almost completely saturated with iron. This state, which is known as **idiopathic hemochromatosis,** is more commonly seen in men because women with the abnormal gene are protected somewhat by menstrual and childbearing events. The accumulation of iron in the liver, pancreas, and heart can lead to cirrhosis and liver tumors, diabetes mellitus, and cardiac failure, respectively. Treatment for these patients is periodic withdrawals of large amounts of blood, where the iron is contained in the hemoglobin. Another group of patients has severe anemias, among the most common of which are the thalassemias, a group of hereditary **hemolytic anemias.** In these cases the subjects require transfusions throughout their lives, leading to the accumulation of large amounts of iron derived from the transfused blood. Clearly bleeding would be an inappropriate measure in these cases; rather, the patients are treated by the administration of iron chelators, such as desferrioxamine, which leads to the excretion of large amounts of complexed iron in the urine. Rarely, a third group of patients will acquire excess iron because they ingest large amounts of both iron and ethanol, the latter promoting iron absorption. In these cases excess stored iron can be removed by bleeding (see Clin. Corr. 24.6).

CLINICAL CORRELATION 24.5

Iron-Deficiency Anemia

Microscopic examination of a blood smear in patients with iron-deficiency anemia usually reveals the characteristic findings of microcytic (small in size) and hypochromic (underpigmented) red blood cells. These changes in the red cell result from decreased rates of globin synthesis when heme is not available. A bone marrow aspiration will reveal no storage iron to be present and serum ferritin values are virtually zero. The serum transferrin value (expressed as the total iron-binding capacity) will be elevated (upper limits of normal: 410 g dL^{-1}) with a serum iron saturation of less than 16%. Common causes for iron deficiency include excessive menstrual flow, multiple births, and gastrointestinal bleeding that may be occult. The common causes of gastrointestinal bleeding include medications that can cause ulcers or erosion of the gastric mucosa (especially aspirin or cortisone-like drugs), hiatal hernia, peptic ulcer disease, gastritis associated with chronic alcoholism, and gastrointestinal tumor. The management of such patients must include both a careful examination for the cause and source of bleeding and supplementation with iron. The latter is usually provided in the form of oral ferrous sulfate tablets; occasionally, intravenous iron therapy may be required. Where the iron deficiency is severe, transfusion with packed red blood cells may also be indicated.

Finch, C. A., and Huebers, H. Perspectives in iron metabolism. *N. Engl. J. Med.* 306:1520, 1982.

24.6— Heme Biosynthesis

Heme is produced in virtually all mammalian tissues. Its synthesis is most pronounced in the bone marrow and liver because of the requirements for incorporation into hemoglobin and the cytochromes, respectively. As depicted in Figure 24.6, heme is largely a planar molecule. It consists of one ferrous ion and a tetrapyrrole ring, **protoporphyrin IX.** The diameter of the iron atom is a little too large to be accommodated within the plane of the porphyrin ring, and thus the metal puckers out to one side as it coordinates with the apical nitrogen atoms of the four pyrrole groups. Heme is one of the most stable compounds, reflecting its strong resonance features.

Figure 24.7 depicts the pathway for heme biosynthesis. The following are the important aspects to be noted. First, the initial and last three enzymatic steps are catalyzed by enzymes that are in the mitochondrion, whereas the intermediate steps take place in the cytoplasm. This is important in considering the regulation by heme of the first biosynthetic step; this aspect is discussed below. Second, the organic portion of heme is derived totally from eight residues each of glycine and succinyl CoA. Third, the reactions occurring on the side groups attached to the tetrapyrrole ring involve the colorless intermediates known as **porphyrinogens.** The latter compounds, though exhibiting reso-

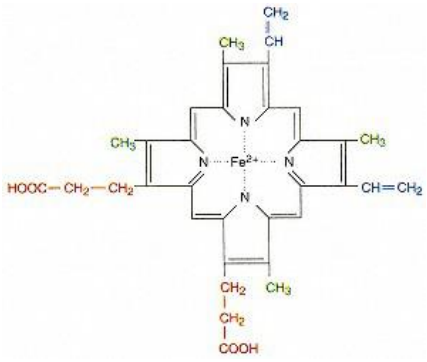


Figure 24.6
Structure of heme.

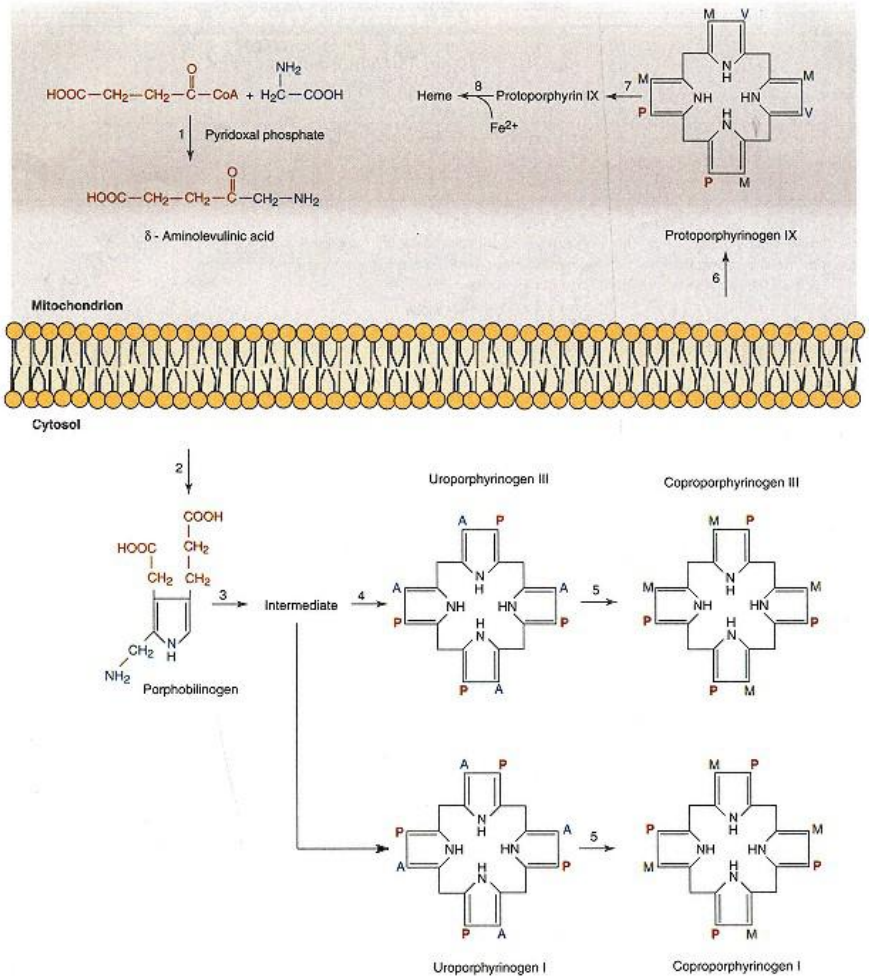


Figure 24.7
Pathway for heme biosynthesis.
Numbers indicate enzymes involved in each step as follows: 1, ALA synthase; 2, ALA dehydratase; 3, porphobilinogen deaminase; 4, uroporphyrinogen III cosynthase; 5, uroporphyrinogen decarboxylase; 6, coproporphyrinogen III oxidase; 7, protoporphyrinogen IX oxidase; 8, ferrochelatase. Pyrrole ligands are indicated as follows: P, propionic (*β*-carboxyethyl); A, acetic (carboxymethyl); M, methyl; V, = vinyl.

nance features within each pyrrole ring, do not demonstrate resonance between the pyrrole groups. As a consequence, the porphyrinogens are unstable and can readily be oxidized, especially in the presence of light, by nonenzymatic means to their stable **porphyrin** products. In the latter cases resonance between pyrrole groups is established by oxidation of the four methylene bridges. Figure 24.8 depicts the enzymatic conversion of protoporphyrinogen to protoporphyrin

CLINICAL CORRELATION 24.6

Hemochromatosis: Molecular Genetics and the Issue of Iron-Fortified Diets

The hemochromatosis gene is heterozygous in about 9% of the population. The disease is expressed primarily in the homozygous state; about 0.25% of all individuals are at risk. Normal individuals have a major histocompatibility complex class-1 gene (HLA-H) with unknown function that encodes for the α-chain, containing three immunoglobulin-like domains. The normal gene product has a structure that cannot present an antigen. Most individuals with hemochromatosis are homozygous for a Cys_{282}-Tyr mutation which prevents the normal conformation of an immunoglobulin domain.

A controversy has developed as to whether food should be fortified with iron because of the prevalence of iron-deficiency anemia, especially among premenopausal women. It was suggested that dietary iron deficiency would be reduced if at least 50 mg of iron was incorporated per pound of enriched flour. Others suggested that toxicity from excess iron absorption through iron fortification was too great. Sweden has mandated iron fortification for 45 years and about 42% of the average daily intake of iron is derived from these sources. However, 5% of males had elevation of serum iron values, with 2% having iron stores consonant with the distribution found in early stages of hemochromatosis, pointing out the danger of iron-fortified diets. In countries where iron deficiency is widespread, however, fortification may still be the most appropriate measure.

McLaren, C. E., Gorddeuk, V. R., Looker, A. C., et al. Prevalence of heterozygotes for hemochromatosis in the white population of the United States. *Blood* 86:2021, 1995; Feder, J. N., Gnirki, A., Thomas, W., et al. A novel MHC class 1-like gene is mutated in patients with hereditary haemochromatosis. *Nature Genetics* 13:399, 1996; Olsson, K. S., Heedman, P. A., and Staugard, F. Preclinical hemochromatosis in a population on a high-iron-fortified diet. *J. Am. Med. Assoc*. 239:1999, 1978; Olsson, K. S., Marsell, R., Ritter, B., Olander, B., et al. Iron deficiency and iron overload in Swedish male adolescents. *J. Intern. Med.* 237:187, 1995.

by this oxidation mechanism. This is the only known porphyrinogen oxidation that is enzyme regulated in humans; all other porphyrinogen porphyrin conversions are nonenzymatic and catalyzed by light rather than catalyzed by specific enzymes. Fourth, once the tetrapyrrole ring is formed, the order of the R groups as one goes clockwise around the tetrapyrrole ring defines which of the four possible types of **uro-** or **coproporphyrinogens** are being synthesized. These latter compounds have two different substituents, one each for every pyrrole group. Going clockwise around the ring, the substituents can be arranged as ABABABAB (where A is one substituent and B the other), forming a type I porphyrinogen, or the arrangement can be ABABABBA, forming a type III porphyrinogen. In principle, two other arrangements can occur to form porphyrinogens II and IV, and these can be synthesized chemically; however, they do not occur naturally. In protoporphyrinogen and protoporphyrin there are three types of substituents, and the classification becomes more complicated; type IX is the only form that is synthesized naturally.

Derangements of porphyrin metabolism are known clinically as the **porphyrias**. This family of diseases is of great interest because it has revealed that the regulation of heme biosynthesis is complicated. The clinical presentations of the different porphyrias provide a fascinating exposition of biochemical regulatory abnormalities and their relationship to pathophysiological processes. Table 24.2 lists the details of the different porphyrias (see Clin. Corr. 24.7).

Enzymes in Heme Biosynthesis Occur in Both Mitochondria and Cytosol

Aminolevulinic Acid Synthase

Aminolevulinic acid (ALA) synthase controls the rate-limiting step of heme synthesis in all tissues studied. Synthesis of the enzyme is not directed by mitochondrial DNA but occurs rather in the cytosol, being directed by mRNA derived from the nucleus. The enzyme is incorporated into the matrix of the mitochondrion. Succinyl CoA is one of the substrates and is found only in the mitochondrion. This protein has been purified to homogeneity from rat liver mitochondria. The cytosolic protein is a dimer of a 71,000-Da subunit, containing a basic N-terminal signaling sequence that directs the enzyme into the mitochondrion. An ATP-dependent 70,000-Da cytostolic component, known as a chaperone protein, maintains ALA synthase in the unfolded extended state, the only form that can pass through the mitochondrial membrane. Thereafter, the N-

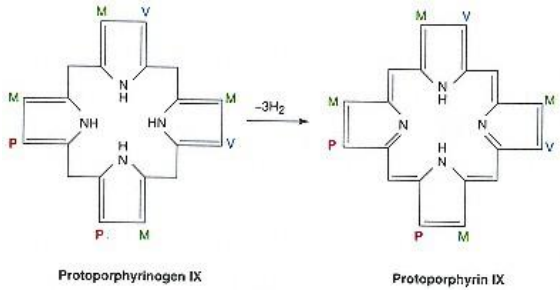


Figure 24.8
Action of protoporphyrinogen IX oxidase, an example of the conversion of a porphyrinogen to a porphyrin.

terminal signaling sequence is cleaved by a metal-dependent protease in the mitochondrial matrix, yielding an ALA synthase with subunits of 65,000 Da each. Within the matrix another oligomeric chaperone protein, of 14 subunits of 60,000 Da each, catalyzes the correct folding of the protein in a second ATP-dependent process (Figure 24.9, p. 1014). The ALA synthase has a short biological half-life (~60 min). Both the synthesis and activity of the enzyme are subject to regulation by a variety of substances; 50% inhibition of activity occurs in the presence of 5 mM of hemin, and virtually complete inhibition is noted at a 20-mM concentration. The enzymatic reaction involves the condensation of a **glycine** residue with a residue of **succinyl CoA.** The reaction has an absolute requirement for **pyridoxal phosphate.** Two isoenzymes exist for ALA synthase; only the erythrocytic form contains an IRE.

ALA Dehydratase

Aminolevulinic acid dehydratase (280 Da) (or porphobilinogen synthase) is a cytosol component consisting of eight subunits, of which only four interact with the substrate. This protein also interacts with the substrate to form a Schiff base, but in this case the -amino group of a lysine residue binds to the ketonic carbon of the substrate molecule (Figure 24.10, p. 1015). Two molecules of

TABLE 24.2 Derangements in Porphyrin Metabolism

Disease State	*Genetics*	*Tissue*	*Enzyme*	*Activity*	*Organ Pathology*
Acute intermittent porphyria	Dominant	Liver	1. ALA synthase	Increase	Nervous system
			2. Porphobilinogen deaminase	Decrease	
			3. ^{4}-5α-Reductase	Decrease	
Hereditary coproporphyria	Dominant	Liver	1. ALA synthase	Increase	Nervous system; skin
			2. Coproporphyrinogen oxidase	Decrease	
Variegate porphyria	Dominant	Liver	1. ALA synthase	Increase	Nervous system; skin
			2. Protoporphyrinogen oxidase	Decrease	
Porphyria cutanea tarda	Dominant	Liver	1. Uroporphyrinogen decarboxylase	Decrease	Skin, induced by liver disease
Hereditary protoporphyria	Dominant	Marrow	1. Ferrochelatase	Decrease	Gallstones, liver disease, skin
Erythropoietic porphyria	Recessive	Marrow	1. Uroporphyrinogen III cosynthase	Decrease	Skin and appendages; reticuloengothelial system
Lead poisoning	None	All tissues	1. ALA dehydrase	Decrease	Nervous system; blood; others
			2. Ferrochelatase	Decrease	

CLINICAL CORRELATION 24.7

Acute Intermittent Porphyria

A 40-year-old woman appears in the emergency room in an agitated state, weeping and complaining of severe abdominal pain. She has been constipated for several days and has noted marked weakness in the arms and legs and that "things do not appear to be quite right." Physical examination reveals a slightly rapid heart rate (100/min) and moderate hypertension (blood pressure of 160/110 mmHg). There have been earlier episodes of severe abdominal pain; operations undertaken on two occasions revealed no abnormalities. The usual laboratory tests are normal. The neurological complaints are not localized to an anatomical focus. The decision is made that the present symptoms are largely psychiatric in origin and have a functional rather than an organic basis. The patient is sedated with 60 mg of phenobarbital; a consultant psychiatrist agrees by telephone to see the patient in about 4 h. The staff notices a marked deterioration; generalized weakness rapidly appears, progressing to a compromise of respiratory function. This ominous development leads to immediate incorporation of a ventilatory assistance regimen, with transfer to intensive care for physiological monitoring. Her condition deteriorates and she dies 48 h later. A urine sample of the patient is reported later to have a markedly elevated level of porphobilinogen. This patient had acute intermittent porphyria, a disease of incompletely understood derangement of heme biosynthesis. There is a dominant pattern of inheritance associated with an overproduction of the porphyrin precursors, ALA and porphobilinogen. Three enzyme abnormalities are noted in the cases that have been studied carefully. These include (1) a marked increase in ALA synthase, (2) a reduction by one-half of activity of porphobilinogen deaminase, and (3) a reduction of one-half of the activity of steroid ^{4}-5α-reductase. The change in content of the second enzyme is consonant with a dominant expression. The change in content of the third enzyme is acquired and not apparently a heritable expression of the disease. It is believed that a decrease in porphobilinogen deaminase leads to a minor decrease in content of heme in liver. The lower concentration of heme leads to a failure both to repress the synthesis and to inhibit the activity of ALA synthase. Almost never manifested before puberty, the disease is thought to appear only with the induction of ^{4}-5β-reductase at adolescence. Without a sufficient amount of ^{4}-5α-reductase, the observed increase in the 5β steroids is due to a shunting of ^{4} steroids into the 5β-reductase pathway. The importance of abnormalities of this last metabolic pathway in the pathogenesis of porphyria is controversial. Pathophysiologically, the disease poses a great riddle: the derangement of porphyrin metabolism is confined to the liver, which anatomically appears normal, whereas the pathological findings are restricted to the nervous system. In the present case, involvement of (1) the brain led to the agitated and confused state and the respiratory collapse, (2) the autonomic system led to the hypertension, increased heart rate, constipation, and abdominal pain, and (3) the peripheral nervous system and spinal cord led to the weakness and sensory disturbances. Experimentally, no known metabolic intermediate of heme biosynthesis can cause the pathology noted in acute intermittent porphyria. There should have been a greater suspicion of the possibility of porphyria early in the patient's presentation. The analysis of porphobilinogen in the urine is a relatively simple test. The treatment would have been glucose infusion, the exclusion of any drugs that could cause elevation of ALA synthase (e.g., barbiturates), and, if her disease failed to respond satisfactorily despite these measures, the administration of intravenous hematin to inhibit the synthesis and activity of ALA synthase. Acute hepatic porphyria is of historic political interest. The disease has been diagnosed in two descendants of King George III, suggesting that the latter's deranged personality preceding and during the American Revolution could possibly be ascribed to porphyria.

Meyer, U. A., Strand, L. J., Doss, M., et al. Intermittent acute porphyria: demonstration of a genetic defect in porphobilinogen metabolism. *N. Engl. J. Med.* 286:1277, 1972; and Stein, J. A., and Tschudy, D. D. Acute intermittent porphyria: a clinical and biochemical study of 46 patients. *Medicine (Baltimore)* 49:1, 1970.

ALA condense asymmetrically to form **porphobilinogen.** The ALA dehydratase is a sulfhydryl enzyme and is very sensitive to inhibition by heavy metals. A characteristic finding of **lead poisoning** is the elevation of ALA in the absence of an elevation of porphobilinogen.

Porphobilinogen Deaminase

Synthesis of the porphyrin ring is a complicated process. A sulfhydryl group on porphobilinogen deaminase forms a thioether bond with a porphobilinogen residue through a deamination reaction. Thereafter, five additional porphobilinogen residues are deaminated successively to form a linear hexapyrrole adduct with the enzyme. The adduct is cleaved hydrolytically to form both an enzyme–dipyrromethane complex and the linear tetrapyrrole, hydroxymethylbilane. The enzyme–dipyrromethane complex is then ready for another cycle of addition of four porphobilinogen residues to generate another tetrapyrrole. Thus dipyrromethane is the covalently attached novel cofactor for the enzyme. Porphobilinogen deaminase has no ring-closing function; hydroxymethylbilane closes in an enzyme-independent step to form uroporphyrinogen I if no additional factors are present. However, the deaminase is closely associated with a second protein,

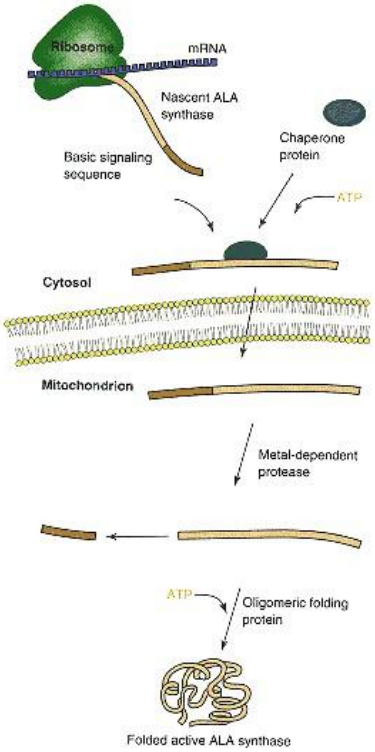


Figure 24.9
Synthesis of -aminolevulinic acid synthase.

uroporphyrinogen III cosynthase, which directs the synthesis of the III isomer. The formation of the latter involves a spiro intermediate generated from hydroxymethylbilane; this allows inversion of one of the pyrrole groups (Figure 24.11, p. 1016). In the absence of the cosynthase, uroporphyrinogen I is synthesized slowly; in its presence, the III isomer is synthesized rapidly. A rare recessively inherited disease, **erythropoietic porphyria,** associated with marked cutaneous light sensitization, is due to an abnormality of red blood cell cosynthase. Here, large amounts of the type I isomers of uroporphyrinogen and coproporphyrinogen are synthesized in the bone marrow. Two isoenzymes exist for porphobilinogen deaminase due to alternative splicing of exon 1 or exon 2 to the rest of the mRNA.

Uroporphyrinogen Decarboxylase

This enzyme acts on the side chains of the uroporphyrinogens to form the coproporphyrinogens. The protein catalyzes the conversion of both I and III isomers of uroporphyrinogen to the respective coproporphyrinogen isomers. Uroporphyrinogen decarboxylase is inhibited by iron salts. Clinically, the most common cause of porphyrin derangement is associated with patients who have a single gene abnormality for this enzyme, leading to 50% depression of the enzyme's activity. This disease, which shows cutaneous manifestations primarily with sensitivity to light, is known as **porphyria cutanea tarda.** The condition

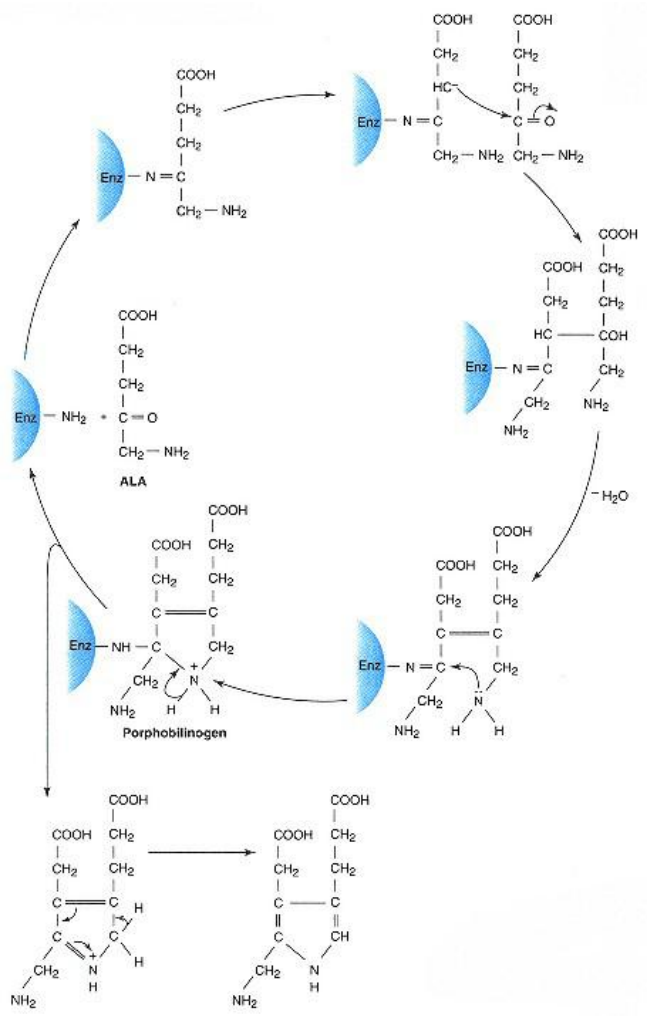


Figure 24.10
Synthesis of porphobilinogen.

is not expressed unless patients either take drugs that cause an increase in porphyrin synthesis or drink large amounts of alcohol, leading to the accumulation of iron, which then acts to inhibit further the activity of uroporphyrinogen decarboxylase.

Coproporphyrinogen Oxidase

This mitochondrial enzyme is specific for the type III isomer of coproporphyrinogen, not acting on the type I isomer. Coproporphyrinogen III enters the mitochondrion and is converted to protoporphyrinogen IX. The mechanism of action is not understood. A dominant disease associated with a deficiency of this

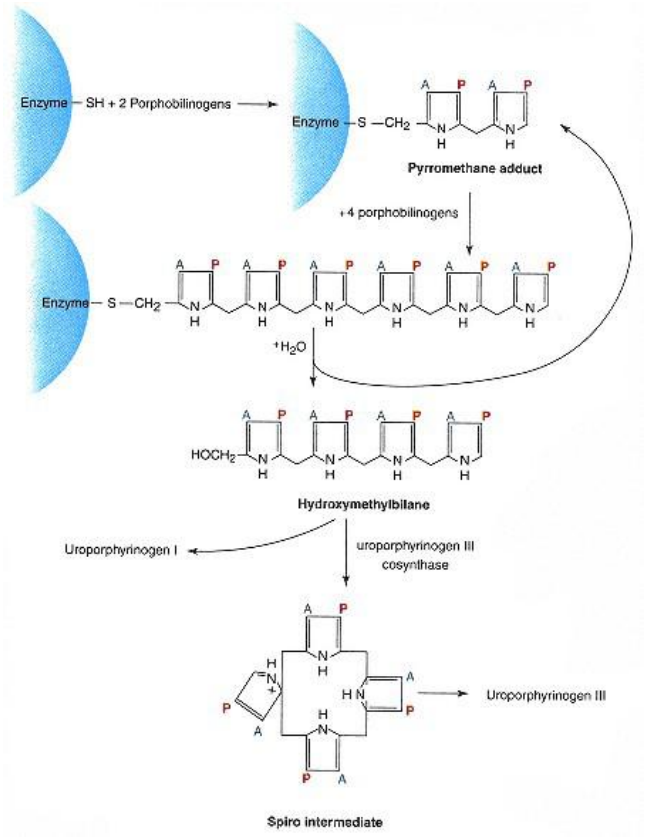


Figure 24.11
Synthesis of uroporphyrinogens I and III.
Enzyme in blue is uroporphyrinogen I synthase.

enzyme leads to a form of hereditary hepatic porphyria, known as **hereditary coproporphyria.**

Protoporphyrinogen Oxidase

This mitochondrial enzyme generates a product, protoporphyrin IX, which, in contrast to the other heme precursors, is very water-insoluble. Excess amounts of protoporphyrin IX that are not converted to heme are excreted by the biliary system into the intestinal tract. A dominant disease, **variegate porphyria,** is due to a deficiency of protoporphyrinogen oxidase.

Ferrochelatase

Ferrochelatase inserts ferrous iron into protoporphyrin IX in the final step of the synthesis of heme. The protein is sensitive to the effects of heavy metals (especially lead) and, of course, to iron deprivation. In these latter instances, zinc instead of iron is incorporated to form a zinc–protoporphyrin IX complex. In contrast to heme, the zinc–protoporphyrin IX complex is brilliantly fluorescent and easily detectable in small amounts. The enzyme contains an Fe_2S_2 group and has been proposed as an IRP-3 that controls translation of the erythrocytic ALA synthase mRNA.

ALA Synthase Catalyzes Rate-Limiting Step of Heme Biosynthesis

ALA synthase controls the rate-limiting step of heme synthesis in all tissues. Succinyl CoA and glycine are substrates for a variety of reactions. The modulation of the activity of ALA synthase determines the quantity of the substrates that will be shunted into heme biosynthesis. Heme (and also hematin) acts both as a repressor of the synthesis of ALA synthase and as an inhibitor of its activity. Since heme resembles neither the substrates nor the product of the enzyme's action, it is probable that the latter inhibition occurs at an allosteric site. Almost 100 different drugs and metabolites can cause induction of ALA synthase; for example, a 40-fold increase is noted in the rat after treatment with 3,5-dicarbethoxy-1,4-dihydrocollidine. The effect of pharmacological agents has led to the important clinical feature where some patients with certain kinds of porphyria have had exacerbations of their condition following the inappropriate administration of certain drugs (e.g., barbiturates). ALA dehydratase is also inhibited by heme; but this is of little physiological consequence, since the activity of ALA dehydrase is about 80-fold greater than that of ALA synthase, and thus heme-inhibitory effects are reflected first in the activity of ALA synthase.

Glucose or a proximal metabolite serves to inhibit heme biosynthesis in a mechanism that is not yet defined. This is of clinical relevance, since some patients manifest their porphyric state for the first time when placed on a very low caloric (and therefore glucose) intake. Other regulators of porphyrin metabolism include certain steroids. Steroid hormones (e.g., oral contraceptive pills) with a double bond in ring A between C-4 and C-5 atoms can be reduced by two different reductases. The product of 5α-reduction has little effect on heme biosynthesis; however, the product of 5α-reduction serves as a stimulus for the synthesis of ALA synthase.

24.7— Heme Catabolism

Catabolism of heme-containing proteins presents two requirements to the mammalian host: (1) development of a means of processing the hydrophobic products of porphyrin ring cleavage and (2) retention and mobilization of the contained iron so that it may be reutilized. Red blood cells have a life span of approximately 120 days. Senescent cells are recognized by their membrane changes and removed and engulfed by the reticuloendothelial system at extravascular sites. The globin chains denature, releasing heme into the cytoplasm. The globin is degraded to its constituent amino acids, which are reutilized for general metabolic needs.

Figure 24.12 depicts the events of heme catabolism. Heme is degraded primarily by a microsomal enzyme system in reticuloendothelial cells that requires molecular oxygen and NADPH. **Heme oxygenase** is substrate inducible and catalyzes the cleavage of the α-methene bridge, which joins the two pyrrole residues containing the vinyl substituents. The α-methene carbon is converted quantitatively to carbon monoxide. The only endogenous source of **carbon monoxide** in humans is the α-methene carbon. A fraction of the carbon monoxide is released via the respiratory tract. Thus the measurement of carbon monoxide in an exhaled breath provides an index to the quantity of heme that is degraded in an individual. The oxygen present in the carbon monoxide and in the newly derivatized lactam rings are generated entirely from molecular oxygen. The stoichiometry of the reaction requires 3 mol of oxygen for each ring cleavage. Heme oxygenase will only use heme as a substrate, with the iron possibly participating in the cleavage mechanism. Thus free protoporphyrin IX is not a substrate. The linear tetrapyrrole **biliverdin IX** is the product formed by the action of heme oxygenase. Biliverdin IX is reduced by **biliverdin reductase** to bilirubin IX.

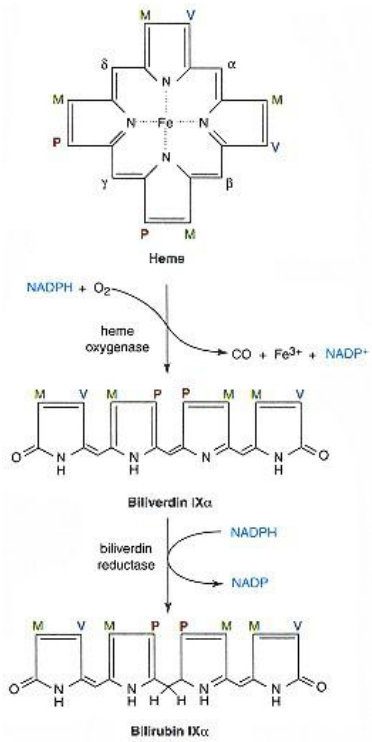


Figure 24.12
Formation of bilirubin from heme.
Greek letters indicate the labeling of the methene carbon atoms in heme.

Bilirubin Is Conjugated to Form Bilirubin Diglucuronide in Liver

Bilirubin is derived not only from senescent red cells but also from the turnover of other heme-containing proteins, such as the cytochromes. Studies with labeled glycine as a precursor have revealed that an early-labeled bilirubin, with a peak within 1–3 h, appears a very short time after a pulsed administration of the labeled precursor. A larger amount of bilirubin appears much later at about 120 days, reflecting the turnover of heme in red blood cells. Early-labeled bilirubin can be divided into two parts: an early–early part, which reflects the turnover of heme proteins in the liver, and a late–early part, which consists of both the turnover of heme-containing hepatic proteins and the turnover of bone marrow heme, which is either poorly incorporated or easily released from red blood cells. The latter is a measurement of ineffective erythropoiesis and can be very pronounced in disease states such as pernicious anemia (see Chapter 28) and the thalassemias.

Bilirubin is poorly soluble in aqueous solutions at physiological pH values. When transported in plasma, it is bound to serum albumin with an association constant greater than 10^6 M^{-1}. Albumin contains one such high-affinity site and another with a lesser affinity. At the normal albumin concentration of 4 g dL^{-1}, about 70 mg of bilirubin per deciliter of plasma can be bound on the two sites. However, bilirubin toxicity **(kernicterus)**, which is manifested by the transfer of bilirubin to membrane lipids, commonly occurs at concentrations greater than 25 mg dL^{-1}. This suggests that the weak affinity of the second site does not allow it to serve effectively in the transport of bilirubin. Bilirubin on serum albumin is rapidly cleared by the liver, where there is a free bidirectional flux of the tetrapyrrole across the sinusoidal–hepatocyte interface. Once in the hepatocyte, bilirubin is bound to several cytostolic proteins, of which only one has been well characterized. The latter component, **ligandin**, is a small basic component making up to 6% of the total cytosolic protein of rat liver. Ligandin has been purified to homogeneity from rat liver and characterized as having two subunits with molecular masses of 22 kDa and 27 kDa. Each subunit contains glutathione *S*-epoxidetransferase activity, a function important in detoxification mechanisms of aryl groups. The stoichiometry of binding is one bilirubin molecule per complete ligandin molecule. The functional role of ligandin and other hepatic bilirubin-binding proteins remains to be defined.

Once in the hepatocyte the propionyl side chains of bilirubin are conjugated to form a diglucuronide (Figure 24.13). The reaction utilizes uridine diphosphoglucuronate derived from the oxidation of uridine diphosphoglucose. The former serves as a glucuronate donor to bilirubin. In normal bile, the diglucuronide is the major form of excreted bilirubin, with only small amounts of the monoglucuronide or other glycosidic adducts present. **Bilirubin diglucuronide** is much more water-soluble than free bilirubin, and thus the transferase facilitates excretion of the bilirubin into bile. Bilirubin diglucuronide is poorly absorbed by the intestinal mucosa. The glucuronide residues are released in the terminal ileum and large intestine by bacterial hydrolases; the released free bilirubin is reduced to the colorless linear tetrapyrroles known as **urobilinogens**. Urobilinogens can be oxidized to colored products known as **urobilins**, which are excreted in the feces. A small fraction of urobilinogen can be reabsorbed by the terminal ileum and large intestine to be removed by hepatic cells and resecreted in bile. When urobilinogen is reabsorbed in large amounts in certain disease states, the kidney serves as a major excretory site.

In the normal state, plasma bilirubin concentrations are 0.3–1 mg dL^{-1}, and this is almost all in the unconjugated state. In the clinical setting, conjugated bilirubin is expressed as **direct bilirubin** because it can be coupled readily with diazonium salts to yield azo dyes; this is the direct **van den Bergh reaction**. Unconjugated bilirubin is bound noncovalently to albumin and will not react until it is released by the addition of an organic solvent such as

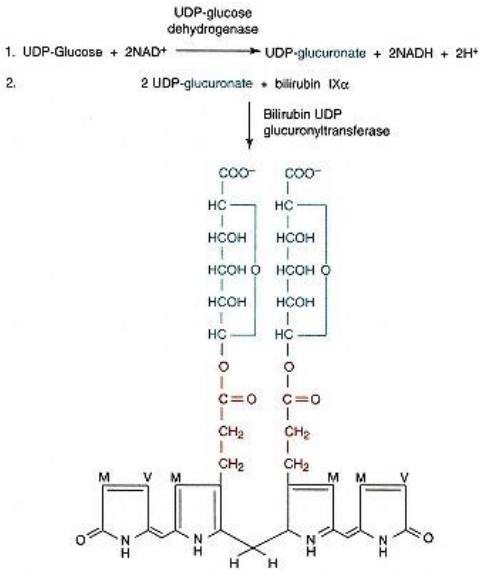


Figure 24.13
Biosynthesis of bilirubin diglucuronide.

ethanol. The reaction with diazonium salts yielding the azo dye after the addition of ethanol is the indirect van den Bergh reaction, and this measures the **indirect bilirubin** or the unconjugated bilirubin. Unconjugated bilirubin binds so tightly to serum albumin and lipid that it does not diffuse freely in plasma and therefore does not lead to an elevation of bilirubin in the urine. Unconjugated bilirubin has a high affinity for membrane lipids, which leads to the impairment of cell membrane function, especially in the nervous system. In contrast, conjugated bilirubin is relatively water-soluble, and elevations of this bilirubin form lead to high urinary concentrations with the characteristic deep yellow-brown color. The deposition of conjugated and unconjugated bilirubin in skin and the sclera gives the yellow to yellow-green color seen in patients with jaundice.

A third form of plasma bilirubin occurs only with hepatocellular disease in which a fraction of the bilirubin binds so tightly that it is not released from serum albumin by the usual techniques and is linked covalently to the protein. In some cases up to 90% of total bilirubin can be in this covalently bound form.

The normal liver has a very large capacity to conjugate and mobilize the bilirubin that is delivered. As a consequence, **hyperbilirubinemia** due to excess heme destruction, as in hemolytic diseases, rarely leads to bilirubin levels that exceed 5 mg dL^{-1}, except in situations in which functional derangement of the liver is present (see Clin. Corr. 24.8). Thus marked elevation of unconjugated bilirubin reflects primarily a variety of hepatic diseases, including those that are heritable and those that are acquired (see Clin. Corr. 24.9).

Elevations of conjugated bilirubin level in plasma are attributable to liver and/or biliary tract disease. In simple uncomplicated biliary tract obstruction, the major component of the elevated serum bilirubin is the diglucuronide form, which is released by the liver into the vascular compartment. Biliary tract disease may be extrahepatic or intrahepatic, the latter involving the canaliculi and biliary ductules (see Clin. Corr. 24.10).

CLINICAL CORRELATION 24.8

Neonatal Isoimmune Hemolysis

Rh-negative women pregnant with Rh-positive fetuses will develop antibodies to Rh factors. These antibodies will cross the placenta to hemolyze fetal red blood cells. Usually this is not of clinical relevance until about the third Rh-positive pregnancy, in which the mother has had antigenic challenges with earlier babies. Antenatal studies will reveal rising maternal levels of IgG antibodies against Rh-positive red blood cells, indicating that the fetus is Rh-positive. Before birth, placental transfer of fetal bilirubin occurs with excretion through the maternal liver. Because hepatic enzymes of bilirubin metabolism are poorly expressed in the newborn, infants may not be able to excrete the large amounts of bilirubin that can be generated from red cell breakdown. At birth these infants usually appear normal; however, the unconjugated bilirubin in the umbilical cord blood is elevated up to 4 mg dL^{-1}; due to the hemolysis initiated by maternal antibodies. During the next 2 days the serum bilirubin rises, reflecting continuing isoimmune hemolysis, leading to jaundice, hepatosplenomegaly, ascites, and edema. If untreated, signs of central nervous system damage can occur, with the appearance of lethargy, hypotonia, spasticity, and respiratory difficulty, constituting the syndrome of kernicterus. Treatment involves exchange transfusion with whole blood, which is serologically compatible with both the infant's blood and maternal serum. The latter requirement is necessary to prevent hemolysis of the transfused cells. Additional treatment includes external phototherapy, which facilitates the breakdown of bilirubin. The entire problem can be prevented by treating Rh-negative mothers with anti-Rh globulin. These antibodies recognize the fetal red cells, block the Rh antigens, and cause them to be destroyed without stimulating an immune response in the mothers.

Mauer, H. M., Shumway, C. N., Draper, D. A., and Hossaini, A. A. Controlled trial comparing agar, intermittent phototherapy, and continuous phototherapy for reducing neonatal hyperbilirubinemia. *J. Pediatr.* 82:73, 1973; and Bowman, J. J. Management of Rh-isoimmunization. *Obstet. Gynecol.* 52:1, 1978.

Intravascular Hemolysis Requires Scavenging of Iron

In certain diseases destruction of red blood cells occurs in the intravascular compartment rather than in the extravascular reticuloendothelial cells. In the former case the appearance of free hemoglobin and heme in the plasma potentially could lead to the excretion of these substances through the kidney with a substantial loss of iron. To prevent this occurrence, specific plasma proteins are involved in scavenging mechanisms. Transferrin binds free iron and thus permits its reutilization. Free hemoglobin, after oxygenation in the pulmonary capillaries, dissociates into α,β dimers, which are bound to a family of circulating

CLINICAL CORRELATION 24.9

Bilirubin UDP-Glucuronosyltransferase Deficiency

Bilirubin UDP-glucuronosyltransferase has two isoenzyme forms, derived from alternative mRNA splicing between variable forms of exon 1 and common exons 2, 3, 4, and 5. The latter exons define the part of the protein that binds the UDP-glucuronate, whereas the various exons 1 have defined specificities for either bilirubin or other acceptors, such as phenol. Two exons have bilirubin specificity leading to two forms of bilirubin UDP-glucuronosyltransferase forms. Two major families of diseases are seen with deficiencies of the enzyme. Crigler–Najjar syndrome is seen in infants and is associated with extraordinarily high serum unconjugated bilirubin due to an autosomal recessive inheritance of mutations on both alleles in exons 2, 3, 4, or 5. Gilbert's syndrome is also associated with a deficiency of the enzyme's activity, but only to about 25% of normal. The patients appear jaundiced but without other clinical symptoms. The major complication is an exhaustive search by the physician looking for some serious liver disease and failing to recognize the benign condition. Two different findings that may be restricted to different populations account for the condition. In Japan a dominant pattern of inheritance is noted with a mutation on only one allele. The 75% reduction of activity is ascribed to the fact that the enzyme exists as an oligomer, where mutant and normal monomers might associate to form heterooligomers. The explanation is that not only is the mutant monomer inactive, but it forces conformational effects on the normal subunit, reducing its activity substantially. In contrast, in the Western world the condition is due largely to a homozygous expansion of the bases in the promoter region with less efficient transcription of the gene.

Aono, S., Adachi, Y., Uyama, S., et al. Analysis of genes for bilirubin UDP-glucuronosyltransferase in Gilbert's syndrome. *Lancet* 345:958, 1995; and Bosma, P. J., Chowdhury, J. R., Bakker, C., et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilberts syndrome. *N. Engl. J. Med.* 333:1171, 1995.

CLINICAL CORRELATION 24.10

Elevation of Serum Conjugated Bilirubin

Elevations of serum conjugated bilirubin are attributable to liver and/or biliary tract disease. In simple uncomplicated biliary tract obstruction, the major component of the elevated serum bilirubin is the diglucuronide form, which is released by the liver into the vascular compartment. Biliary tract disease may be extrahepatic or intrahepatic, the latter involving the canaliculi and biliary ductules. Dubin–Johnson syndrome is an autosomal recessive disease involving a defect in the biliary secretory mechanisms in liver. Excretion through the biliary tract of a variety of (but not all) organic anions is affected. Retention of melanin-like pigment in the liver in this disorder leads to a characteristic gray-black color of this organ. A second heritable disorder associated with elevated levels of serum conjugated bilirubin is Rotor's syndrome. In this poorly defined disease no hepatic pigmentation occurs.

Kitamura, T., Alroy, J., Gatmaitan, Z., et al. Defective biliary excretion of epinephrine metabolites in mutant (TR-) rats: relation to the pathogenesis of black liver in the Dubin–Johnson syndrome and Corriedale sheep with an analogous excretory defect. *Hepatology* 15:1154, 1992.

plasma proteins, the **haptoglobins,** having a high affinity for the oxyhemoglobin dimer. Since deoxyhemoglobin does not dissociate into dimers in physiological settings, it is not bound by haptoglobin. The stoichiometry of binding is two α,β-oxyhemoglobin dimers per haptoglobin molecule. Interesting studies have been made with rabbit antihuman-hemoglobin antibodies on the haptoglobin–hemoglobin interaction. Human haptoglobin interacts with a variety of hemoglobins from different species. The binding of human haptoglobin with human hemoglobin is not affected by the binding of rabbit antihuman-hemoglobin antibody. These studies suggest that haptoglobin binds to sites on hemoglobin that are highly conserved in evolution and therefore are not sufficiently antigenic to generate antibodies. The most likely site for the molecular interaction of hemoglobin and haptoglobin is the interface of the α and β globins of the tetramer that dissociates to yield α,β dimers. Sequence determinations have indicated that these contact regions are highly conserved in evolution.

The haptoglobins are **α_2-globulins.** Synthesized in the liver, they consist of two pairs of polypeptide chains (α being the lighter and β the heavier). The genes for the α and α chains are linked so that a single mRNA is synthesized, generating a single polypeptide chain that is cleaved to form the two different chains. The β chains are glycopeptides of 39 kDa and are invariant in structure; α chains are of several kinds. The haptoglobin peptide chains are joined by disulfide bonds between the α and β chains and between the two α chains.

Interaction of haptoglobin with hemoglobin forms a complex that is too large to be filtered through the renal glomerulus. Free hemoglobin (appearing in renal tubules and in urine) will occur during intravascular hemolysis only when the binding capacity of circulating haptoglobin has been exceeded. Haptoglobin delivers hemoglobin to the reticuloendothelial cells. The heme in free hemoglobin is relatively resistant to the action of heme oxygenase, whereas the heme residues in an α,β dimer of hemoglobin bound to haptoglobin are very susceptible.

The measurement of serum haptoglobin is used clinically as an indication of the degree of intravascular hemolysis. Patients who have significant intravascular hemolysis will have little or no levels of haptoglobin because of the removal of haptoglobin–hemoglobin complexes by the reticuloendothelial system. Haptoglobin levels can also be low in severe extravascular hemolysis, in which the large load of hemoglobin in the reticuloendothelial system leads to the transfer of free hemoglobin into plasma.

Free heme and hematin appearing in plasma are bound by a β-globulin, **hemopexin** (57 kDa). One heme residue binds per hemopexin molecule. Hemopexin transfers heme to liver, where further metabolism by heme oxygenase occurs. Normal plasma hemopexin contains very little bound heme, whereas in intravascular hemolysis, the hemopexin is almost completely saturated by heme and is cleared with a half-life of about 7 h. In the latter instance, excess heme binds to albumin, with newly synthesized hemopexin serving as a mediator for the transfer of the heme from albumin to the liver. Hemopexin also binds free protoporphyrin.

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Questions

C. N. Angstadt and J. Baggott

Refer to the following for Questions 1–3:

A. ferritin

B. ferredoxin

C. hemosiderin

D. lactoferrin

E. transferrin

1. A type of protein in which iron is specifically bound to sulfur.

2. Exhibits an antimicrobial effect in the intestinal tract of newborns because of its ability to bind iron.

3. Delivers iron to tissues by binding to specific cell surface receptors.

4. In the intestinal absorption of iron:

A. the presence of a reductant like ascorbate enhances the availability of iron.

B. regulation of uptake occurs between the lumen and mucosal cells.

C. the amount of apoferritin synthesized in the mucosal cell is directly related to the need for iron by the host.

D. iron bound tightly to a ligand, such as phytate, is more readily absorbed than free iron.

E. low pH in the stomach inhibits absorption by favoring $Fe3^{+}$.

5. Which of the following statements about iron distribution is correct?

A. Iron overload cannot occur because very efficient excretory mechanisms are available.

B. Cells cannot regulate their uptake of iron with changing iron content.

C. Transferrin decreases in iron deficiency to facilitate storage of iron.

D. Iron homeostasis is maintained in part by iron regulatory proteins binding to iron-responsive elements in mRNA.

E. In the early stages of iron depletion, serum ferritin levels rise rapidly as iron is released from storage forms.

6. The biosynthesis of heme requires all of the following EXCEPT:

A. propionic acid.

B. succinyl CoA.

C. glycine.

D. ferrous ion.

7. Uroporphyrin III:

A. is an intermediate in the biosynthesis of heme.

B. does not contain a tetrapyrrole ring.

C. differs from coproporphyrin III in the substituents around the ring.

D. is formed from uroporphyrinogen III by an oxidase.

E. formation is the primary control step in heme synthesis.

8. Aminolevulinic acid synthase:

A. requires NAD for activity.

B. is allosterically activated by heme.

C. synthesis is inhibited by steroids.

D. is synthesized in mitochondria.

E. synthesis can be induced by a variety of drugs.

9. Lead poisoning would be expected to result in an elevated level of:

A. aminolevulinic acid.

B. porphobilinogen.

C. protoporphyrin I.

D. heme.

E. bilirubin.

10. Ferrochelatase:

A. is an iron-chelating compound.

B. releases iron from heme in the degradation of hemoglobin.

C. binds iron to sulfide ions and cysteine residues.

D. is inhibited by heavy metals.

E. is involved in the cytoplasmic portion of heme synthesis.

11. Heme oxygenase:

A. can oxidize the methene bridge between any two pyrrole rings of heme.

B. requires molecular oxygen.

C. produces bilirubin.

D. produces carbon dioxide.

E. can use either heme or protoporphyrin IX as substrate.

12. The substance deposited in skin and sclera in jaundice is:

A. biliverdin.

B. only unconjugated bilirubin.

C. only direct bilirubin.

D. both bilirubin and bilirubin diglucuronide.

E. hematin.

13. Hepatic disease leads to major elevation of the blood level of:

A. heme.

B. biliverdin.

C. bilirubin.

D. bilirubin diglucuronide.

E. direct bilirubin.

14. Biliary obstruction leads to major elevation of the blood level of:

A. only direct bilirubin.

B. only indirect bilirubin.

C. both direct and indirect bilirubin.

D. heme but not bilirubin.

E. biliverdin but not bilirubin.

15. Acute intermittent porphyria is accompanied by an increased urinary level of:

A. biliverdin.

B. direct bilirubin.

C. heme.

D. indirect bilirubin.

E. porphobilinogen.

16. Haptoglobin binds:

A. a globin monomer.

B. an oxyhemoglobin molecule.

C. α,β-oxyhemoglobin dimers.

D. a deoxyhemoglobin molecule.

E. α,β-deoxyhemoglobin dimers.

17. Haptoglobin:

A. helps prevent loss of iron following intravascular red blood cell destruction.

B. levels in serum are elevated in severe intravascular hemolysis.

C. inhibits the action of heme oxygenase.

D. binds heme and hematin as well as hemoglobin.

E. is a β-globulin.

Answers

1. B Animal ferredoxins, also known as nonheme iron-containing proteins, have two irons bound to two cysteine residues and sharing two sulfide ions (p. 1004).

2. D As long as lactoferrin is not saturated, its avid binding of iron diminishes the amount available for growth of microorganisms (p. 1003).

3. E Internalization of the receptor–transferrin complex is mediated by a Ca^{2+}–calmodulin–protein kinase C complex. Internalization is followed by release of the iron and recycling of the apotransferrin to the plasma (p. 1003). Ferritin and hemosiderin (p. 1004) are storage forms of iron.

4. A A and D: Ascorbate facilitates reduction to the ferrous state and, therefore, dissociation from ligands and absorption. B: Substantial iron enters the mucosal cell regardless of need, but the amount transferred to the capillary beds is controlled. C: Iron bound to apoferritin is trapped in mucosal cells and not transferred to the host. E: Oxidation to Fe^{3+} is favored by higher pH (p. 1005).

5. D B and D: In the presence of low iron this mechanism leads to increased synthesis of transferrin receptor and decreased synthesis of apoferritin. A: The high affinity of many macromolecules for iron prevents efficient excretion. C: Transferrin increases in iron deficiency to improve absorption. E: Serum ferritin is normally small and decreases (pp. 1007–1008).

6. A B and C: The organic portion of heme comes totally from glycine and succinyl CoA; the propionic acid side chain comes from the succinate. D: The final step of heme synthesis is the insertion of the ferrous ion (p. 1010, Figure 24.7).

7. C A, B, and D: The tetrapyrrole porphyrins (except for protoporphyrin IX) are not intermediates but end products formed from the porphyrinogens nonenzymatically. E: Synthesis of aminolevulinic acid is the rate-limiting step (p. 1010, Figure 24.7).

8. E The enzyme is induced in response to need (many drug detoxifications are cytochrome P450-dependent). A: The mechanism involves a Schiff base with glycine. B: Heme both allosterically inhibits and suppresses synthesis of the enzyme. C: One reduction product of catabolic steroids stimulates synthesis. D: The gene for this enzyme is on nuclear DNA (pp. 1009–1012).

9. A A–D: Lead inhibits ALA dehydratase so it inhibits synthesis of porphobilinogen and subsequent compounds. Heme certainly would not be elevated, because lead also inhibits ferrochelatase. E: Bilirubin is a breakdown product of heme, not an intermediate in synthesis (p. 1013).

10. D This enzyme, in the mitochondria, catalyzes the last step of heme synthesis, the insertion of Fe^{2+}, and is sensitive to the effects of heavy metals (p. 1016).

11. B Oxygenases usually use O_2. A: The enzyme is specific for the methene between the two rings containing the vinyl groups (α-methene bridge). C and D: The products are biliverdin and CO; the measurement of CO in the breath is an index of heme degradation. E: Iron is necessary for activity (p. 1017).

12. D Both conjugated (direct) and unconjugated (indirect) bilirubin are deposited (p. 1019).

13. C Since the liver is responsible for conjugating bilirubin, hepatic disease leads to the elevation of unconjugated (indirect) bilirubin in blood. A and B: Catabolism of heme to bilirubin occurs in reticuloendothelial cells. D and E: These are the same and require conjugation by the liver (p. 1019).

14. A Conjugated (direct) bilirubin is excreted in the bile. B and C: As long as the liver is functioning, bilirubin (indirect) will be conjugated. D and E: These occur in the reticuloendothelial cells so bilirubin will be formed (pp. 1020–1021).

15. E The disease is characterized by increased ALA synthase and decreased porphobilinogen deaminase activities. A, B, and E: These all represent heme catabolism. D: Heme synthesis is reduced (p. 1013).

16. C Haptoglobin binds dimers, two per haptoglobin molecule, specifically the oxyhemoglobin dimers since deoxyhemoglobin does not dissociate to dimers physiologically (p. 1021).

17. A Haptoglobin is part of the scavenging mechanism to prevent urinary loss of heme and hemoglobin from intravascular degradation of red blood cells. B: Since the scavenged complex is taken up by the reticuloendothelial system, the haptoglobin levels in serum are low. C: Heme residues in the dimers bound to haptoglobin are more susceptible than free heme to oxidation by heme oxygenase. D and E: Heme and hematin are bound by a β-globulin, while haptoglobin is an β-globulin (p. 1021).