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# Metabolism of Toxicants

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## 7.1 INTRODUCTION

One of the most important determinants of xenobiotic persistence in the body and subsequent toxicity to the organism is the extent to which they can be metabolized and excreted. Several families of metabolic enzymes, often with wide arrays of substrate specificity, are involved in xenobiotic metabolism. Some of the more important families of enzymes involved in xenobiotic metabolism include the cytochrome P450 monooxygenases (CYPs), flavin-containing monooxygenases (FMOs), alcohol and aldehyde dehydrogenases, amine oxidases, cyclooxygenases, reductases, hydrolases, and a variety of conjugating enzymes such as glucuronidases, sulfotransferases, methyltransferases, glutathione transferases, and acetyl transferases.

Most xenobiotic metabolism occurs in the liver, an organ devoted to the synthesis of many important biologically functional proteins and thus with the capacity to mediate chemical transformations of xenobiotics. Most xenobiotics that enter the body are lipophilic, a property that enables them to bind to lipid membranes and be transported by lipoproteins in the blood. After entrance into the liver, as well as in other organs, xenobiotics may undergo one or two phases of metabolism. In phase I a polar reactive group is introduced into the molecule rendering it a suitable substrate for phase II enzymes. Enzymes typically involved in phase I metabolism include the CYPs, FMOs, and hydrolases, as will be discussed later. Following the addition of a polar group, conjugating enzymes typically add much more bulky substituents, such as sugars, sulfates, or amino acids that result in a substantially increased water solubility of the xenobiotic, making it easily excreted. Although this process is generally a detoxication sequence, reactive intermediates may be formed that are much more toxic than the parent compound. It is, however, usually a sequence that increases water solubility and hence decreases the biological half life ( $t_{0.5}$ ) of the xenobiotic in vivo.

Phase I monooxygenations are more likely to form reactive intermediates than phase II metabolism because the products are usually potent electrophiles capable of reacting with nucleophilic substituents on macromolecules, unless detoxified by some subsequent reaction. In the following discussion, examples of both detoxication and intoxication reactions are given, although greater emphasis on activation products is provided in Chapter 8.

## 7.2 PHASE I REACTIONS

Phase I reactions include microsomal monooxygenations, cytosolic and mitochondrial oxidations, co-oxidations in the prostaglandin synthetase reaction, reductions, hydrolyses, and epoxide hydration. All of these reactions, with the exception of reductions, introduce polar groups to the molecule that, in most cases, can be conjugated during phase II metabolism. The major phase I reactions are summarized in Table 7.1.

### 7.2.1 The Endoplasmic Reticulum, Microsomal Preparation, and Monooxygenations

Monooxygenation of xenobiotics are catalyzed either by the cytochrome P450 (CYP)-dependent monooxygenase system or by flavin-containing monooxygenases (FMO).

**Table 7.1 Summary of Some Important Oxidative and Reductive Reactions of Xenobiotics**

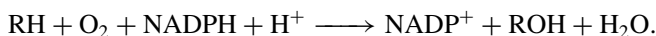
Enzymes and Reactions	Examples
Cytochrome P450	
Epoxidation/hydroxylation	Aldrin, benzo(a)pyrene, aflatoxin, bromobenzene
<i>N</i> -, <i>O</i> -, <i>S</i> -Dealkylation	Ethylmorphine, atrazine, <i>p</i> -nitroanisole, methylmercaptan
<i>N</i> -, <i>S</i> -, <i>P</i> -Oxidation	Thiobenzamide, chlorpromazine, 2-acetylaminofluorene
Desulfuration	Parathion, carbon disulfide
Dehalogenation	Carbon tetrachloride, chloroform
Nitro reduction	Nitrobenzene
Azo reduction	<i>O</i> -Aminoazotoluene
Flavin-containing monooxygenase	
<i>N</i> -, <i>S</i> -, <i>P</i> -Oxidation	Nicotine, imiprimine, thiourea, methimazole
Desulfuration	Fonofos
Prostaglandin synthetase cooxidation	
Dehydrogenation	Acetaminophen, benzidine, epinephrine
<i>N</i> -Dealkylation	Benzphetamine, dimethylaniline
Epoxidation/hydroxylation	Benzo(a)pyrene, 2-aminofluorene, phenylbutazone
Oxidation	FANFT, ANFT, bilirubin
Molybdenum hydroxylases	
Oxidation	Purines, pteridine, methotrexate, 6-deoxycyclovir
Reductions	Aromatic nitrocompounds, azo dyes, nitrosoamines
Alcohol dehydrogenase	
Oxidation	Methanol, ethanol, glycols, glycol ethers
Reduction	Aldehydes and ketones
Aldehyde dehydrogenase	
Oxidation	Aldehydes resulting from alcohol and glycol oxidations
Esterases and amidases	
Hydrolysis	Parathion, paraoxon, dimethoate
Epoxide hydrolase	
Hydrolysis	Benzo(a)pyrene epoxide, styrene oxide

Both are located in the endoplasmic reticulum of the cell and have been studied in many tissues and organisms. This is particularly true of CYPs, probably the most studied of all enzymes.

Microsomes are derived from the endoplasmic reticulum as a result of tissue homogenization and are isolated by centrifugation of the postmitochondrial supernatant fraction, described below. The endoplasmic reticulum is an anastomosing network of lipoprotein membranes extending from the plasma membrane to the nucleus and mitochondria, whereas the microsomal fraction derived from it consists of membranous vesicles contaminated with free ribosomes, glycogen granules, and fragments of other subcellular structures such as mitochondria and Golgi apparatus. The endoplasmic reticulum, and consequently the microsomes derived from it, consists of two types, rough and smooth, the former having an outer membrane studded with ribosomes, which the latter characteristically lack. Although both rough and smooth microsomes have all of the components of the CYP-dependent monooxygenase system, the specific activity of the smooth type is usually higher.

The preparation of microsomal fractions, S9, and cytosolic fractions from tissue homogenates involves the use of two to three centrifugation steps. Following tissue extraction, careful mincing, and rinses of tissue for blood removal, the tissues are typically homogenized in buffer and centrifuged at  $10,000 \times g$  for 20 minutes. The resulting supernatant, often referred to as the S9 fraction, can be used in studies where both microsomal and cytosolic enzymes are desired. More often, however, the S9 fraction is centrifuged at  $100,000 \times g$  for 60 minutes to yield a microsomal pellet and a cytosolic supernatant. The pellet is typically resuspended in a volume of buffer, which will give 20 to 50 mg protein/ml and stored at  $-20$  to  $-70^{\circ}\text{C}$ . Often, the microsomal pellet is resuspended a second time and resedimented at  $100,000 \times g$  for 60 minutes to further remove contaminating hemoglobin and other proteins. As described above, enzymes within the microsomal fraction (or microsomes) include CYPs, FMOs, cyclooxygenases, and other membrane-bound enzymes, including necessary coenzymes such as NADPH cytochrome P450 reductase for CYP. Enzymes found in the cytosolic fraction (derived from the supernatant of the first  $100,000 \times g$  spin) include hydrolases and most of the conjugating enzymes such as glutathione transferases, glucuronidases, sulfotransferases, methyl transferases, and acetylases. It is important to note that some cytosolic enzymes can also be found in microsomal fractions, although the opposite is not generally the case.

Monooxygenations, previously known as mixed-function oxidations, are those oxidations in which one atom of a molecule of oxygen is incorporated into the substrate while the other is reduced to water. Because the electrons involved in the reduction of CYPs or FMOs are derived from NADPH, the overall reaction can be written as follows (where RH is the substrate):



## 7.2.2 The Cytochrome P450-Dependent Monooxygenase System

The CYPs, the carbon monoxide-binding pigments of microsomes, are heme proteins of the b cytochrome type. Originally described as a single protein, there are now known to be more than 2000 CYPs widely distributed throughout animals, plants, and microorganisms. A system of nomenclature utilizing the prefix CYP has been devised

for the genes and cDNAs corresponding to the different forms (as discussed later in this section), although P450 is still appropriate as a prefix for the protein products. Unlike most cytochromes, the name CYP is derived not from the absorption maximum of the reduced form in the visible region but from the unique wavelength of the absorption maximum of the carbon monoxide derivative of the reduced form, namely 450 nm.

The role of CYP as the terminal oxidase in monooxygenase reactions is supported by considerable evidence. The initial proof was derived from the demonstration of the concomitant light reversibility of the CO complex of CYP and the inhibition, by CO, of the C-21 hydroxylation of 17  $\alpha$ -hydroxy-progesterone by adrenal gland microsomes. This was followed by a number of indirect, but nevertheless convincing, proofs involving the effects on both CYP and monooxygenase activity of CO, inducing agents, and spectra resulting from ligand binding and the loss of activity on degradation of CYP to cytochrome P420. Direct proof was subsequently provided by the demonstration that monooxygenase systems, reconstituted from apparently homogeneous purified CYP, NADPH-CYP reductase, and phosphatidylcholine, can catalyze many monooxygenase reactions.

CYPs, like other hemoproteins, have characteristic absorptions in the visible region. The addition of many organic, and some inorganic, ligands results in perturbations of this spectrum. Although the detection and measurement of these spectra requires a high-resolution spectrophotometer, these perturbations, measured as optical difference spectra, have been of tremendous use in the characterization of CYPs, particularly in the decades preceding the molecular cloning and expression of specific CYP isoforms.

The most important difference spectra of oxidized CYP are type I, with an absorption maximum at 385 to 390 nm. Type I ligands are found in many different chemical classes and include drugs, environmental contaminants, pesticides, and so on. They appear to be generally unsuitable, on chemical grounds, as ligands for the heme iron and are believed to bind to a hydrophobic site in the protein that is close enough to the heme to allow both spectral perturbation and interaction with the activated oxygen. Although most type I ligands are substrates, it has not been possible to demonstrate a quantitative relationship between  $K_S$  (concentration required for half-maximal spectral development) and  $K_M$  (Michaelis constant). Type II ligands, however, interact directly with the heme iron of CYP, and are associated with organic compounds having nitrogen atoms with  $sp^2$  or  $sp^3$  nonbonded electrons that are sterically accessible. Such ligands are frequently inhibitors of CYP activity.

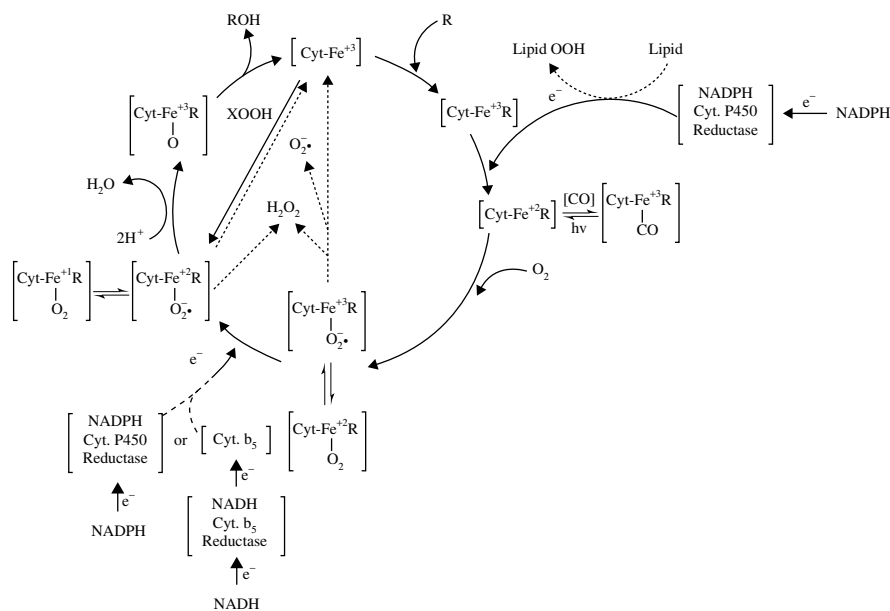
The two most important difference spectra of reduced CYP are the well-known CO spectrum, with its maximum at or about 450 nm, and the type III spectrum, with two pH-dependent peaks at approximately 430 and 455 nm. The CO spectrum forms the basis for the quantitative estimation of CYP. The best-known type III ligands for CYP are ethyl isocyanide and compounds such as the methylenedioxyphenyl synergists and SKF 525A, the last two forming stable type III complexes that appear to be related to the mechanism by which they inhibit monooxygenations.

In the catalytic cycle of CYP, reducing equivalents are transferred from NADPH to CYP by a flavoprotein enzyme known as NADPH-cytochrome P450 reductase. The evidence that this enzyme is involved in CYP monooxygenations was originally derived from the observation that cytochrome c, which can function as an artificial electron acceptor for the enzyme, is an inhibitor of such oxidations. This reductase is an essential component in CYP-catalyzed enzyme systems reconstituted from purified components. Moreover antibodies prepared from purified reductase are inhibitors of microsomal

monooxygenase reactions. The reductase is a flavoprotein of approximately 80,000 daltons that contain 2 mole each of flavin mononucleotide (FMN) and flavinadenine dinucleotide (FAD) per mole of enzyme. The only other component necessary for activity in the reconstituted system is a phospholipid, phosphatidylcholine. This is not involved directly in electron transfer but appears to be involved in the coupling of the reductase to the cytochrome and in the binding of the substrate to the cytochrome.

The mechanism of CYP function has not been established unequivocally; however, the generally recognized steps are shown in Figure 7.1. The initial step consists of the binding of substrate to oxidize CYP followed by a one electron reduction catalyzed by NADPH-cytochrome P450 reductase to form a reduced cytochrome-substrate complex. This complex can interact with CO to form the CO-complex, which gives rise to the well-known difference spectrum with a peak at 450 nm and also inhibits monooxygenase activity. The next several steps are less well understood. They involve an initial interaction with molecular oxygen to form a ternary oxygenated complex. This ternary complex accepts a second electron, resulting in the further formation of one or more less understood complexes. One of these, however, is probably the equivalent of the peroxide anion derivative of the substrate-bound hemoprotein. Under some conditions this complex may break down to yield hydrogen peroxide and the oxidized cytochrome substrate complex. Normally, however, one atom of molecular oxygen is transferred to the substrate and the other is reduced to water, followed by dismutation reactions leading to the formation of the oxygenated product, water, and the oxidized cytochrome.

The possibility that the second electron is derived from NADH through cytochrome  $b_5$  has been the subject of argument for some time and has yet to be completely resolved. Cytochrome  $b_5$  is a widely distributed microsomal heme protein that is involved in metabolic reactions such as fatty acid desaturation that involve endogenous substrates. It is clear, however, that cytochrome  $b_5$  is not essential for all



**Figure 7.1** Generalized scheme showing the sequence of events for P450 monooxygenations.

CYP-dependent monooxygenations because many occur in systems reconstituted from NADPH, O<sub>2</sub>, phosphatidylcholine, and highly purified CYP and NADPH-cytochrome P450 reductase. Nevertheless, there is good evidence that many catalytic activities by isoforms including CYP3A4, CYP3A5, and CYP2E1 are stimulated by cytochrome b<sub>5</sub>. In some cases apocytochrome b<sub>5</sub> (devoid of heme) has also been found to be stimulatory, suggesting that an alternate role of cytochrome b<sub>5</sub> may be the result of conformational changes in the CYP/NADPH cytochrome P450 reductase systems. Thus cytochrome b<sub>5</sub> may facilitate oxidative activity in the intact endoplasmic reticulum. The isolation of forms of CYP that bind avidly to cytochrome b<sub>5</sub> also tends to support this idea.

***Distribution of Cytochrome P450.*** In vertebrates the liver is the richest source of CYP and is most active in the monooxygenation of xenobiotics. CYP and other components of the CYP-dependent monooxygenase system are also in the skin, nasal mucosa, lung, and gastrointestinal tract, presumably reflecting the evolution of defense mechanisms at portals of entry. In addition to these organs, CYP has been demonstrated in the kidney, adrenal cortex and medulla, placenta, testes, ovaries, fetal and embryonic liver, corpus luteum, aorta, blood platelets, and the nervous system. In humans, CYP has been demonstrated in the fetal and adult liver, the placenta, kidney, testes, fetal and adult adrenal gland, skin, blood platelets, and lymphocytes.

Although CYPs are found in many tissues, the function of the particular subset of isoforms in organ, tissue, or cell type does not appear to be the same in all cases. In the liver, CYPs oxidize a large number of xenobiotics as well as some endogenous steroids and bile pigments. The CYPs of the lung also appear to be concerned primarily with xenobiotic oxidations, although the range of substrates is more limited than that of the liver. The skin and small intestine also carry out xenobiotic oxidations, but their activities have been less well characterized. In normal pregnant females, the placental microsomes display little or no ability to oxidize foreign compounds, appearing to function as a steroid hormone metabolizing system. On induction of the CYP enzymes, such as occurs in pregnant women who smoke, CYP-catalyzed aryl hydrocarbon hydroxylase activity is readily apparent. The CYPs of the kidney are active in the  $\omega$ -oxidation of fatty acids, such as lauric acid, but are relatively inactive in xenobiotic oxidation. Mitochondrial CYPs, such as those of the placenta and adrenal cortex, are active in the oxidation of steroid hormones rather than xenobiotics.

Distribution of CYPs within the cell has been studied primarily in the mammalian liver, where it is present in greatest quantity in the smooth endoplasmic reticulum and in smaller but appreciable amounts in the rough endoplasmic reticulum. The nuclear membrane has also been reported to contain CYP and to have detectable aryl hydrocarbon hydroxylase activity, an observation that may be of considerable importance in studies of the metabolic activation of carcinogens.

***Multiplicity of Cytochrome P450, Purification, and Reconstitution of Cytochrome P450 Activity.*** Even before appreciable purification of CYP had been accomplished, it was apparent from indirect evidence that mammalian liver cells contained more than one CYP enzyme. Subsequent direct evidence on the multiplicity of CYPs included the separation and purification of CYP isozymes, distinguished from each other by chromatographic behavior, immunologic specificity, and/or substrate specificity after reconstitution and separation of distinct polypeptides by sodium

dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), which could then be related to distinct CYPs present in the original microsomes.

Purification of CYP and its usual constituent isoforms was, for many years, an elusive goal; one, however, that has been largely resolved. The problem of instability on solubilization was resolved by the use of glycerol and dithiothreitol as protectants, and the problem of reaggregation by maintaining a low concentration of a suitable detergent, such as Emulgen 911 (Kao-Atlas, Tokyo), throughout the procedure. Multiple CYP isoforms, as discussed previously, may be separated from each other and purified as separate entities, although individual isoforms are now routinely cloned and expressed as single entities. The lengthy processes of column purification of CYPs have now been largely superseded by the cloning and expression of transgenic isoforms in a variety of expression systems.

Systems reconstituted from purified CYP, NADPH-cytochrome P450 reductase and phosphatidylcholine will, in the presence of NADPH and O<sub>2</sub>, oxidize xenobiotics such as benzphetamine, often at rates comparable to microsomes. Although systems reconstituted from this minimal number of components are enzymatically active, other microsomal components, such as cytochrome b<sub>5</sub>, may facilitate activity either in vivo or in vitro or may even be essential for the oxidation of certain substrates.

One important finding from purification studies as well as cloning and expressing of individual isoforms is that the lack of substrate specificity of microsomes for monooxygenase activity is not an artifact caused by the presence of several specific cytochromes. Rather, it appears that many of the cytochromes isolated are still relatively nonspecific. The relative activity toward different substrates does nevertheless vary greatly from one CYP isoform to another even when both are relatively nonspecific. This lack of specificity is illustrated in Table 7.2, using human isoforms as examples.

**Classification and Evolution of Cytochrome P450.** The techniques of molecular biology have been applied extensively to the study of CYP. More than 1925 genes have been characterized as of 2002, and the nucleotide and derived amino acid sequences compared. In some cases the location of the gene on a particular chromosome has been determined and the mechanism of gene expression investigated.

A system of nomenclature proposed in 1987 has since been updated several times, most recently in 1996. The accepted guidelines from nomenclature designate cytochrome P450 genes as CYP (or *cyp* in the case of mouse genes). The CYP designation is followed by an Arabic numeral to denote the gene family, followed by a letter designating the subfamily. The individual isoform is then identified using a second Arabic numeral following the subfamily designation. Polymorphic isoforms of genes are indicated by an asterisk followed by an arabic numeral. If there are no subfamilies or if there is only a single gene within the family or subfamily, the letter and/or the second numeral may be omitted (e.g., CYP17). The name of the gene is italicized, whereas the protein (enzyme) is not.

In general, enzymes within a gene family share more than 40% amino acid sequence identity. Protein sequences within subfamilies have greater than 55% similarity in the case of mammalian genes, or 46% in the case of nonmammalian genes. So far, genes in the same subfamily have been found to lie on the same chromosome within the same gene cluster and are nonsegregating, suggesting a common origin through gene duplication events. Sequences showing less than 3% divergence are arbitrarily designated allelic variants unless other evidence exists to the contrary. Known sequences fit

**Table 7.2 Some Important Human Cytochrome P450 Isozymes and Selected Substrates**

P450	Drugs	Carcinogens/Toxicants/ Endogenous Substrates	Diagnostic Substrates In vivo [In vitro]
1A1	Verlukast (very few drugs)	Benzo(a)pyrene, dimethylbenz(a)anthracene	[Ethoxyresorufin, benzo(a)pyrene]
1A2	Phenacetin, theophylline, acetaminophen, warfarin, caffeine, cimetidine	Aromatic amines, arylhydrocarbons, NNK, <sup>3</sup> aflatoxin, estradiol	Caffeine, [acetanilide, methoxyresorufin, ethoxyresorufin]
2A6	Coumarin, nicotine	Aflatoxin, diethylnitrosamine, NNK <sup>3</sup>	Coumarin
2B6	Cyclophosphamide, ifosphamide, nicotine	6 Aminochrysene, aflatoxin, NNK <sup>3</sup>	[7-ethoxy-4-trifluoro- methyl coumarin]
2C8	Taxol, tolbutamide, carbamazepine	—	[Chloromethyl fluorescein diethyl ether]
2C9	Tienilic acid, tolbutamide, warfarin, phenytoin, THC, hexobarbital, diclofenac	—	[Diclofenac (4' -OH)]
2C19	S-Mephenytoin, diazepam, phenytoin, omeprazole, indomethacin, impramine, propranolol, proguanil	—	[S-Mephentoin (4' -OH)]
2D6	Debrisoquine, sparteine, bufuralol, propranolol, thioridazine, quinidine, phenytoin, fluoxetine	NNK <sup>3</sup>	Dextromethorphan, [bufuralol (4' -OH)]
2E1	Chlorzoxazone, isoniazid, acetaminophen, halothane, enflurane, methoxyflurane	Dimethylnitrosamine, ben- zene, halogenated alkanes (eg, CCl <sub>4</sub> ) acylonitrile, alcohols, aniline, styrene, vinyl chloride	Chlorzoxazone (6-OH), [p-nitrophenol]
3A4	Nifedipine, ethylmorphine, warfarin, quinidine, taxol, ketoconazole, verapamil, erythromycin, diazepam	Aflatoxin, 1-nitropyrene, benzo(a)pyrene 7,8-diol, 6 aminochrysene, estradiol, progesterone, testosterone, other steroids, bile acids	Erythromycin, nifedipine [testosterone (6-β)]
4A9/11 (Very few drugs)		Fatty acids, prostaglandins, thromboxane, prostacyclin	[Lauric acid]

Note: NNK<sup>3</sup> = 4(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a nitrosamine specific to tobacco smoke.

the classification scheme surprisingly well, with few exceptions found at the family, subfamily, or allelic variant levels, and in each case additional information is available to justify the departure from the rules set out.

In some cases a homologue of a particular CYP enzyme is found across species (e.g., CYP1A1). In other cases the genes diverged subsequent to the divergence of the species and no exact analogue is found in various species (e.g., the CYP2C subfamily). In this case the genes are numbered in the order of discovery, and the gene products



from a particular subfamily may even have differing substrate specificity in different species (e.g., rodent vs. human). Relationships between different CYP families and subfamilies are related to the rate and extent of CYP evolution.

Figure 7.2 demonstrates some of the evolutionary relationships between CYP genes between some of the earliest vertebrates and humans. This dendrogram compares CYP genes from the puffer fish (fugu) and 8 other fish species with human CYPs (including 3 pseudogenes). The unweighted pair group method arithmetic averaging (UPGMA) phylogenetic tree demonstrates the presence of five CYP clans (clusters of CYPs that are consistently grouped together) and delineates the 18 known human CYPs. This data set demonstrates that the defining characteristics of vertebrate CYPs have not changed much in 420 million years. Of these 18 human CYPs, only 1 family was missing in fugu (CYP39), indicating that the mammalian diversity of CYPs likely predates the tetrapod-ray finned fish divergence. The fish genome also has new CYP1C, 3B, and 7C subfamilies that are not seen in mammals.

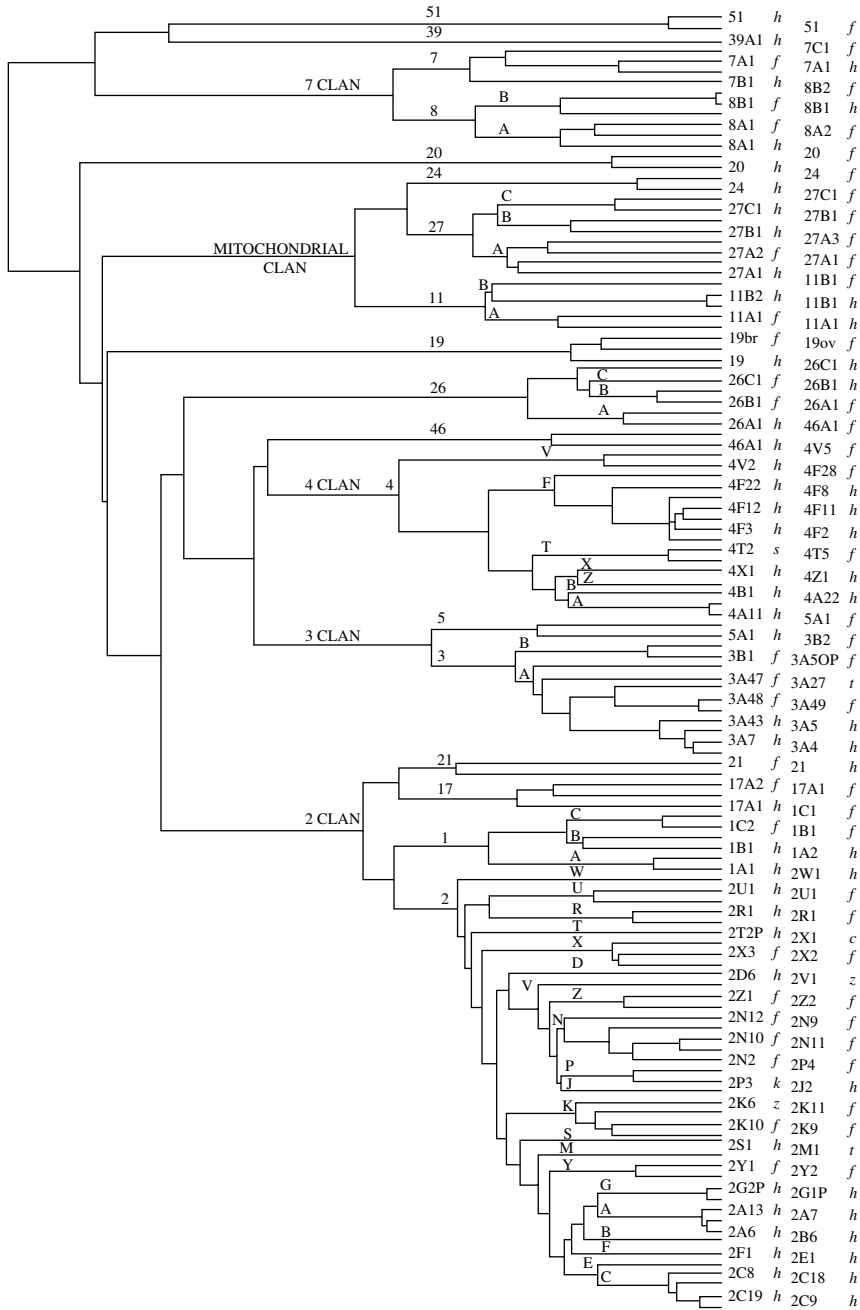
The gene products, the CYP isoforms, may still be designated P450 followed by the same numbering system used for the genes, or the CYP designation may be used, for example, P4501A1 or CYP1A1.

As of May 16, 2002, a total of 1925 CYP sequences have been “named” with several others still awaiting classification. Of these, 977 are animal sequences, 607 from plants, 190 from lower eukaryotes and 151 are bacterial sequences. These sequences fall into more than 265 CYP families, 18 of which belong to mammals. Humans have 40 sequenced CYP genes. As the list of CYPs is continually expanding, progress in this area can be readily accessed via the internet at the Web site of the P450 Gene Superfamily Nomenclature Committee (<http://drnelson.utmem.edu/nelsonhomepage.html>) or at another excellent Web site (<http://www.icgeb.trieste.it/p450>).

**Cytochrome P450 Families with Xenobiotic Metabolizing Potential.** Although mammals are known to have 18 CYP families, only three families are primarily responsible for most xenobiotic metabolism. These families (families 1–3) are considered to be more recently derived from the “ancestral” CYP families. The remaining families are less promiscuous in their metabolizing abilities and are often responsible for specific metabolic steps. For example, members of the CYP4 family are responsible for the end-chain hydroxylation of long-chain fatty acids. The remaining mammalian CYP families are involved in biosynthesis of steroid hormones. In fact some of the nomenclature for some of these families is actually derived from the various positions in the steroid nucleus where the metabolism takes place. For example, CYP7 mediates hydroxylation of cholesterol at the 7 $\alpha$ -position, while CYP17 and 21 catalyze the 17 $\alpha$  and 21-hydroxylations of progesterone, respectively. CYP19 is responsible for the aromatization of androgens to estrogen by the initial step of hydroxylation at the 19-position. Many of the CYPs responsible for steroidogenesis are found in the adrenal cortex, while those involved in xenobiotic metabolism are found predominantly in tissues that are more likely to be involved in exposure such as liver, kidneys, lungs, and olfactory tissues.

To simplify discussion of important CYP family members, the following discussion concentrates upon human CYP family members. However, since there is a great deal of homology among family members, many of the points of discussion are generally applicable to CYP families belonging to several species.

The CYP1 family contains three known human members, CYP1A1, CYP1A2, and CYP1B1. CYP1A1 and CYP1A2 are found in all classes of the animal kingdom.

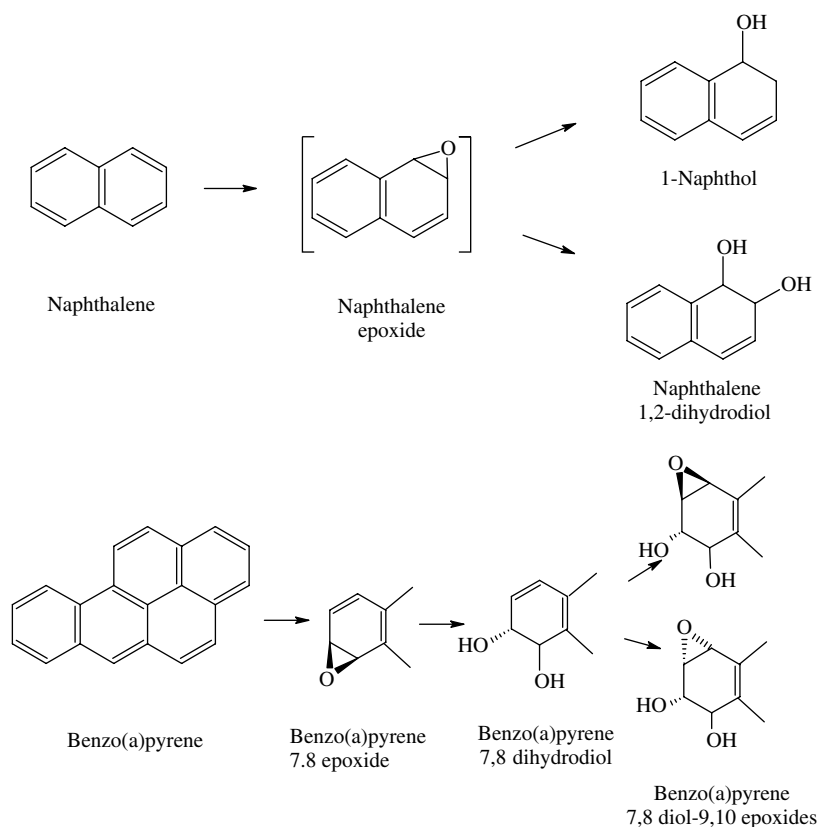


**Figure 7.2** UPGMA tree of 54 puffer fish (fugu), 60 human, and 8 other fish P450s. Species are indicated by *f*, *h*, *z*, *c*, *k*, *s*, and *t* for fugu, human, zebrafish, catfish, killifish, seabass, and trout, respectively. (Reprinted from D. R. Nelson, *Archives of Biochemistry and Biophysics* 409, pp. 18–24. 2003, with permission from Academic Press.)

Because these two highly homologous forms are so highly conserved among species, it is thought that both may possess important endogenous functions that have yet to be elucidated. CYP2E1 is the only other CYP that retains the same gene designation in many different species.

CYP1A1 and CYP1A2 possess distinct but overlapping substrate specificities: CYP1A1 preferring neutral polycyclic aromatic hydrocarbons (PAHs), and the latter preferring polyaromatic and heterocyclic amines and amides. Because of the preference of this family for molecules with highly planar molecular structures, CYP1 family members are closely associated with metabolic activation of many procarcinogens and mutagens including benzo(a)pyrene, aflatoxin B1, dimethylbenzanthracene,  $\beta$ -naphthylamine, 4-aminobiphenyl, 2-acetylaminofluorene, and benzidine. Figure 7.3 illustrates a typical reaction sequence leading to the formation of epoxide and the epoxide diols that are often implicated in the formation of carcinogenic metabolites formed by these enzymes.

Many of the planar PAH compounds induce their own metabolism by inducing transcription of the aryl hydrocarbon receptor (Ah receptor). Although expression of CYP1A1 and 1A2 is often coordinately induced, there are clear differences in regulation, not only with respect to substrate specificity but also in their biological expression. For example, CYP1A1 does not appear to be expressed in human liver unless induced,



**Figure 7.3** Examples of epoxidation reactions.

whereas CYP1A2 is endogenously expressed in the liver. CYP1A1, however, is present in many extrahepatic tissues including the lung, where there is a possible association between CYP-mediated activation of benzo(a)pyrene and other related chemicals present in cigarette smoke and lung cancer in humans.

The CYP2 family consists of 10 subfamilies, five of which are present in mammalian liver. Some of the more important isoforms found in humans within this family are CYP2A6, -2B6, -2C8, -2C9, -2C19, -2D6, and -2E1. The enzyme CYP2A6 is expressed primarily in liver tissue, where it represents 1–10% of total CYP content. CYP2A6 is responsible for the 7-hydroxylation of the naturally occurring plant compound coumarin and its activity is often phenotyped by monitoring this particular metabolic pathway. Other drugs metabolized by CYP2A6 include nicotine, 2-acetylaminofluorene, methoxyflurane, halothane, valproic acid, and disulfiram. Precarcinogens likely activated by 2A6 include aflatoxin B1, 1,3 butadiene, 2,6-dichlorobenzonitrile, and a number of nitrosamines. Because CYP2A6 is responsible for up to 80% of the human metabolism of nicotine, a number of studies have been conducted to determine whether individuals with 2A6 polymorphisms have reduced risk of lung cancers. Although theoretically individuals lacking 2A6 would be expected to smoke less and be less likely to activate carcinogens found in tobacco smoke, studies have not conclusively demonstrated any clear associations between 2A6 polymorphisms and risk of lung cancer.

Like CYP2A6, the human isoform CYP2B6 has recently gained greater recognition for its role in metabolism of many clinical drugs. Some common pharmaceutical substrates for CYP2B6 include cyclophosphamide, nevirapine, *S*-mephobarbitol, artemisinin, bupropion, propofol, ifosfamide, ketamine, selegiline, and methadone. CYP2B6 has also been demonstrated to have a role in the activation of the organophosphate, chlorpyrifos, and in the degradation of the commonly used insecticide repellent, diethyl toluamide (DEET). Historically it was thought that CYP2B6 is found in a small proportion of livers (<25%), but more recent data using antibodies prepared from human proteins have demonstrated that most liver samples have detectable levels of 2B6, though greater than 20-fold differences in levels of protein have been observed.

In contrast with CYP2A6 and CYP2B6, members of the CYP2C family constitute a fairly large percentage of CYP in human liver (ca. 20%) and are responsible for the metabolism of several drugs. All four members of the subfamily in humans exhibit genetic polymorphisms, many of which have important clinical consequences in affected individuals. Genetic polymorphisms in CYP2C19 were shown to be responsible for one of the first described polymorphic effects, that involving mephenytoin metabolism. This polymorphism significantly reduces the metabolism of mephenytoin, resulting in the classification of those individuals possessing this trait as poor metabolizers (PM). Among Caucasians, PMs represent only 3–5% of the populations, while in Asian and Polynesian populations 12–23% and 38–79% of the populations are represented, respectively. At least seven different mutations in this allele have been described, some of which negatively affect catalytic activity while others prevent expression of the protein. Other important drugs affected by these CYP2C19 polymorphisms include the anti-ulcer drug omeprazole, other important proton pump inhibitors, barbiturates, certain tricyclic antidepressants such as imipramine, and the antimalarial drug proguanil. Other important members of the CYP2C family in humans include CYP2C8, -2C9, and -2C18. Substrates metabolized exclusively by CYP2C8 include retinol, retinoic acid, taxol, and arachidonic acid. CYP2C9, the principal CYP2C in

human liver, metabolizes several important drugs including the diabetic agent tolbutamide, the anticonvulsant phenytoin, the anticoagulant warfarin and a number of anti-inflammatory drugs including ibuprofen, diclofenac, and others. Both CYP2C9 and -2C8, which are responsible for metabolism of the anticancer drug paclitaxel, have been demonstrated to be polymorphic.

CYP2E1 is the only member of the CYP2E family in most mammals with the exception of rabbits. Substrates for this family tend to be of small molecular weight and include ethanol, carbon tetrachloride, benzene, and acetaminophen. In contrast to many other inducible CYP families, CYP2E1 is regulated by a combination of increased transcription levels and increased message and protein stabilization.

Undoubtedly the largest amount of CYP in human liver is that of the CYP3 family. CYP3A4 is the most abundant CYP in the human liver, accounting for nearly 30% of the total amount, and is known to metabolize many important drugs including cyclosporine A, nifedipine, rapamycin, ethinyl estradiol, quinidine, digitoxin, lidocaine, erythromycin, midazolam, triazolam, lovastatin, and tamoxifen. Other important oxidations ascribed to the CYP3 family include many steroid hormones, macrolide antibiotics, alkaloids, benzodiazepines, dihydropyridines, warfarin, polycyclic hydrocarbon-derived dihydrodiols, and aflatoxin B<sub>1</sub>. Many chemicals are also capable of inducing this family including phenobarbital, rifampicin, and dexamethasone. Because of potential difficulties arising from CYP induction, drugs metabolized by this family must be closely examined for the possibility of harmful drug-drug interactions.

**Cytochrome P450 Reactions.** Although microsomal monooxygenase reactions are basically similar in the role played by molecular oxygen and in the supply of electrons, the many CYP isoforms can attack a large variety of xenobiotic substrates, with both substrates and products falling into many different chemical classes. In the following sections enzyme activities are therefore classified on the basis of the overall chemical reaction catalyzed; one should bear in mind, however, that not only do these classes often overlap, but often a substrate may also undergo more than one reaction. See Table 7.1 for a listing of important oxidation and reduction reactions of CYPs.

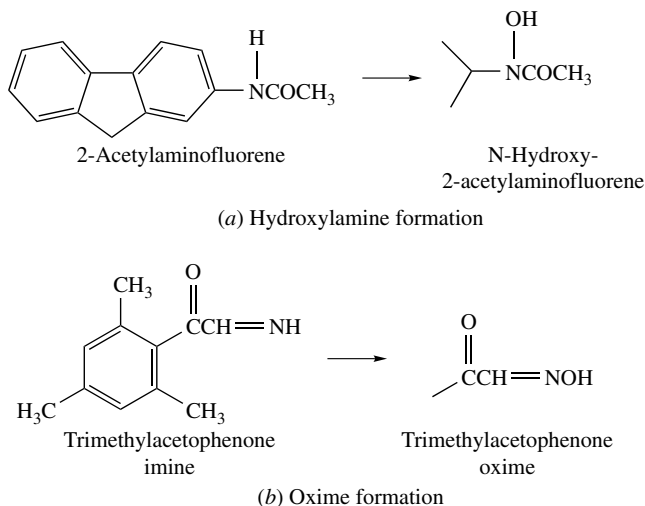
*Epoxidation and Aromatic Hydroxylation.* Epoxidation is an extremely important microsomal reaction because not only can stable and environmentally persistent epoxides be formed (see aliphatic epoxidations, below), but highly reactive intermediates of aromatic hydroxylations, such as arene oxides, can also be produced. These highly reactive intermediates are known to be involved in chemical carcinogenesis as well as chemically induced cellular and tissue necrosis.

The oxidation of naphthalene was one of the earliest examples of an epoxide as an intermediate in aromatic hydroxylation. As shown in Figure 7.3, the epoxide can rearrange nonenzymatically to yield predominantly 1-naphthol, or interact with the enzyme epoxide hydrolase to yield the dihydrodiol, or interact with glutathione S-transferase to yield the glutathione conjugate, which is ultimately metabolized to a mercapturic acid. These reactions are also of importance in the metabolism of other xenobiotics that contain an aromatic nucleus, such as the insecticide carbaryl and the carcinogen benzo(a)pyrene.

The ultimate carcinogens arising from the metabolic activation of benzo(a)pyrene are stereoisomers of benzo(a)pyrene 7,8-diol-9,10-epoxide (Figure 7.3). These metabolites arise by prior formation of the 7,8 epoxide, which gives rise to the 7,8-dihydrodiol





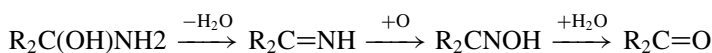


**Figure 7.6** Examples of *N*-oxidation.

**Oxidative Deamination.** Oxidative deamination of amphetamine occurs in the rabbit liver but not to any extent in the liver of either the dog or the rat, which tend to hydroxylate the aromatic ring. A close examination of the reaction indicates that it is probably not an attack on the nitrogen but rather on the adjacent carbon atom, giving rise to a carbinol amine, which eliminates ammonia, producing a ketone:



The carbinol, by another reaction sequence, can also give rise to an oxime, which can be hydrolyzed to yield the ketone. The carbinol is thus formed by two different routes:



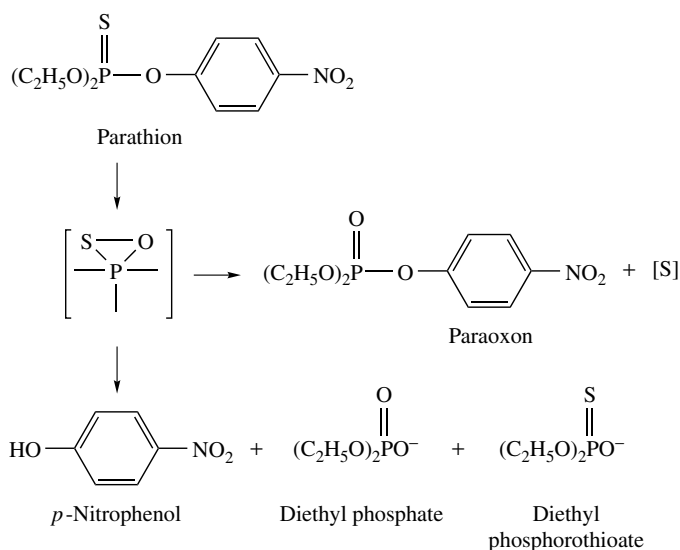
**S-Oxidation.** Thioethers in general are oxidized by microsomal monooxygenases to sulfoxides, some of which are further oxidized to sulfones. This reaction is very common among insecticides of several different chemical classes, including carbamates, organophosphates, and chlorinated hydrocarbons. Recent work suggests that members of the CYP2C family are highly involved in sulfoxidation of several organophosphate compounds including phorate, coumaphos, demeton, and others. The carbamate methiocarb is oxidized to a series of sulfoxides and sulfones, and among the chlorinated hydrocarbons endosulfan is oxidized to endosulfan sulfate and methiochlor to a series of sulfoxides and sulfones, eventually yielding the bis-sulfone. Drugs, including chlorpromazine and solvents such as dimethyl sulfoxide, are also subject to S-oxidation. The fact that FMOs are versatile sulfur oxidation enzymes capable of carrying out many of the previously mentioned reactions raises important questions as to the relative role of this enzyme versus that of CYP. Thus, a reexamination of earlier work in which many of these reactions were ascribed to CYP is required.



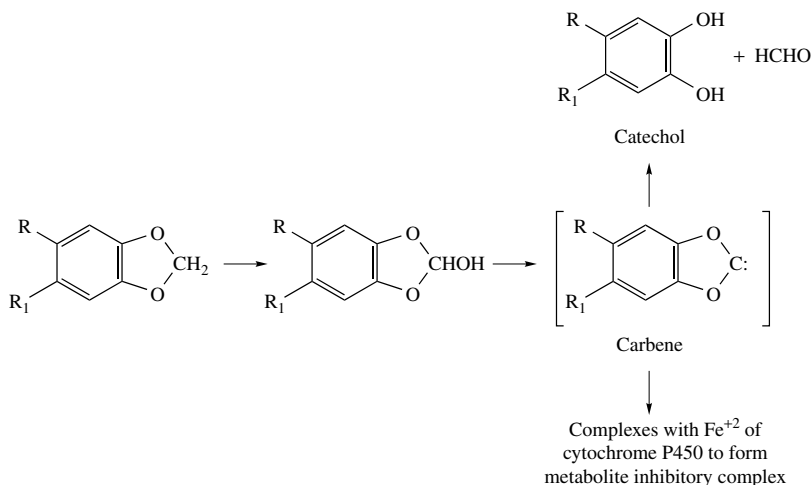
**P-Oxidation.** P-oxidation, a little known reaction, involves the conversion of trisubstituted phosphines to phosphine oxides, for example, diphenylmethylphosphine to diphenylmethylphosphine oxide. Although this reaction is described as a typical CYP-dependent monooxygenation, it too is now known to be catalyzed by the FMO also.

**Desulfuration and Ester Cleavage.** The phosphorothionates  $[(R^1O)_2P(S)OR^2]$  and phosphorodithioate  $[(R^1O)_2P(S)SR^2]$  owe their insecticidal activity and their mammalian toxicity to an oxidative reaction in which the P=S group is converted to P=O, thereby converting the compounds from chemicals relatively inactive toward cholinesterase into potent inhibitors (see Chapter 11 for a discussion of the mechanism of cholinesterase inhibition). This reaction has been described for many organophosphorus compounds but has been studied most intensively in the case of parathion. Much of the splitting of the phosphorus ester bonds in organophosphorus insecticides, formerly believed to be due to hydrolysis, is now known to be due to oxidative dearylation. This is a typical CYP-dependent monooxygenation, requiring NADPH and  $O_2$  and being inhibited by CO. Current evidence supports the hypothesis that this reaction and oxidative desulfuration involve a common intermediate of the "phosphooxithirane" type (Figure 7.7). Some organophosphorus insecticides, all phosphonates, are activated by the FMO as well as the CYP.

**Methylenedioxy (Benzodioxole) Ring Cleavage.** Methylenedioxy-phenyl compounds, such as safrole or the insecticide synergist, piperonyl butoxide, many of which are effective inhibitors of CYP monooxygenations, are themselves metabolized to catechols. The most probable mechanism appears to be an attack on the methylene carbon, followed by elimination of water to yield a carbene. The highly reactive carbene either reacts with the heme iron to form a CYP-inhibitory complex or breaks down to yield the catechol (Figure 7.8).



**Figure 7.7** Desulfuration and oxidative dearylation.



**Figure 7.8** Monooxygenation of methylenedioxyphenyl compounds.

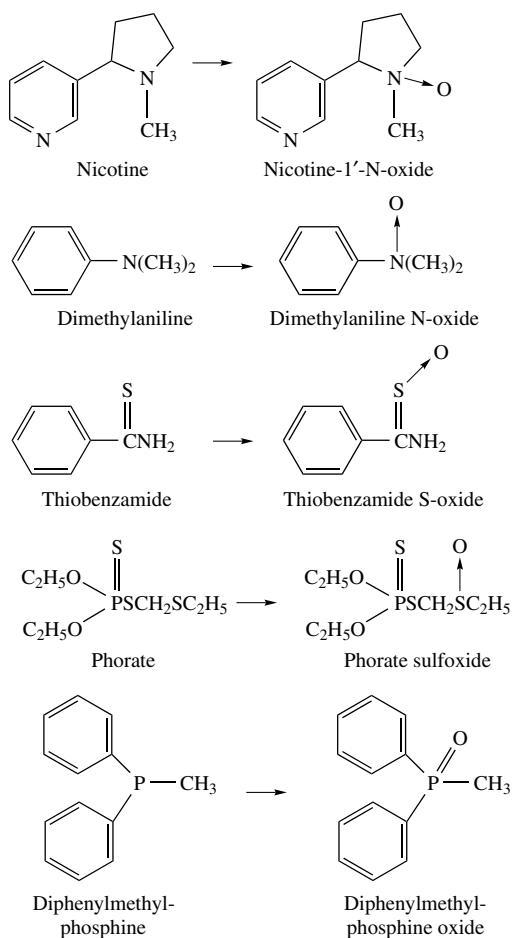
### 7.2.3 The Flavin-Containing Monooxygenase (FMO)

Tertiary amines such as trimethylamine and dimethylamine had long been known to be metabolized to *N*-oxides by a microsomal amine oxidase that was not dependent on CYP. This enzyme, now known as the microsomal flavin-containing monooxygenase (FMO), is also dependent on NADPH and O<sub>2</sub>, and has been purified to homogeneity from a number of species. Isolation and characterization of the enzyme from liver and lung samples provided evidence of clearly distinct physicochemical properties and substrate specificities suggesting the presence of at least two different isoforms. Subsequent studies have verified the presence of multiple forms of the enzyme.

At least six different isoforms have been described by amino acid or cDNA sequencing, and are classified as FMO1 to FMO6. These isoforms share approximately 50–60% amino acid identity across species lines. The identity of orthologues is greater than 82%. Although each isoform has been characterized in humans, several are essentially nonfunctional in adults. For example, FMO1, expressed in the embryo, disappears relatively quickly after birth. FMO2 in most Caucasians and Asians contains a premature stop codon, preventing the expression of functional protein. Functional FMO2 is found in 26% of the African-American population and perhaps also in the Hispanic population. FMO3, the predominant human FMO, is poorly expressed in neonatal humans but is expressed in most individuals by one year of age. Gender independent expression of FMO3 (contrasting with what is observed in other mammals) continues to increase through childhood, reaching maximal levels of expression at adulthood. Several polymorphic forms of FMO3 are responsible for the disease, trimethylamineuria, also known as “fish odor syndrome,” characterized by the inability of some individuals to convert the malodorous trimethylamine, either from the diet or from metabolism, to its odorless *N*-oxide. Although the FMO4 transcript is found in several species, the protein has yet to be successfully expressed in any species. Although FMO5 is expressed in humans at low levels, the poor catalytic activity of FMO5 for most classical FMO substrates suggests that it has minimal participation in xenobiotic oxidation. No data are yet available on the role and abundance of the most recently discovered FMO, FMO6.

Substrates containing soft nucleophiles (e.g., nitrogen, sulfur, phosphorus, and selenium) are good candidates for FMO oxidation (Figure 7.9). A short list of known substrates include drugs such as dimethylaniline, imipramine, thiobenzamide, chlorpromazine, promethazine, cimetidine, and tamoxifen; pesticides such as phorate, fonofos, and methiocarb; environmental agents including the carcinogen 2-aminofluorene, and the neurotoxicants nicotine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Although there is no known physiologically relevant substrate for FMO a few dietary and/or endogenous substrates have been identified, including trimethylamine, cysteamine, methionine and several cysteine-*s*-conjugates. In most cases metabolism by FMO results in detoxication products, although there are several examples of substrates that are bioactivated by FMO oxidation; particularly in the case of substrates involving sulfur oxidation.

Most FMO substrates are also substrates for CYP. Since both enzymes are microsomal and require NADPH and oxygen, it is difficult to distinguish which enzyme is responsible for oxidation without the use of techniques involving specific inactivation or inhibition or one or the other of these enzymes while simultaneously examining the



**Figure 7.9** Examples of oxidations catalyzed by the flavin-containing monooxygenase (FMO).

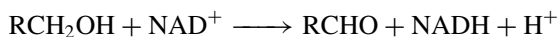
metabolic contribution of the other. Since FMOs are generally heat labile, heating the microsomal preparation to 50°C for one minute inactivates the FMOs while having minimal effects of CYPs. Alternatively, the contribution of FMO can be assessed by use of a general CYP inhibitor such as *N*-benzylimidazole or by an inhibitory antibody to NADPH cytochrome P450 reductase, a necessary CYP coenzyme. Typically results of these tests are sought in combination so that the best estimates of CYP and FMO contribution can be obtained.

Toxicologically it is of interest that the FMO enzyme is responsible for the oxidation of nicotine to nicotine 1'-N-oxide, whereas the oxidation of nicotine to cotinine is catalyzed by two enzymes acting in sequence: CYP followed by a soluble aldehyde dehydrogenase. Thus nicotine is metabolized by two different routes, the relative contributions of which may vary with both the extrinsic and intrinsic factors outlined in Chapter 9.

#### 7.2.4 Nonmicrosomal Oxidations

In addition to the microsomal monooxygenases, other enzymes are involved in the oxidation of xenobiotics. These enzymes are located in the mitochondria or in the soluble cytoplasm of the cell.

**Alcohol Dehydrogenase.** Alcohol dehydrogenases catalyze the conversion of alcohols to aldehydes or ketones:



This reaction should not be confused with the monooxygenation of ethanol by CYP that occurs in the microsomes. The alcohol dehydrogenase reaction is reversible, with the carbonyl compounds being reduced to alcohols.

This enzyme is found in the soluble fraction of the liver, kidney, and lung and is probably the most important enzyme involved in the metabolism of foreign alcohols. Alcohol dehydrogenase is a dimer whose subunits can occur in several forms under genetic control, thus giving rise to a large number of variants of the enzyme. In mammals, six classes of enzymes have been described. Alcohol dehydrogenase can use either NAD or NADP as a coenzyme, but the reaction proceeds at a much slower rate with NADP. In the intact organism the reaction proceeds in the direction of alcohol consumption, because aldehydes are further oxidized to acids. Because aldehydes are toxic and are not readily excreted because of their lipophilicity, alcohol oxidation may be considered an activation reaction, the further oxidation to an acid being a detoxication step.

Primary alcohols are oxidized to aldehydes, *n*-butanol being the substrate oxidized at the highest rate. Although secondary alcohols are oxidized to ketones, the rate is less than for primary alcohols, and tertiary alcohols are not readily oxidized. Alcohol dehydrogenase is inhibited by a number of heterocyclic compounds such as pyrazole, imidazole, and their derivatives.

**Aldehyde Dehydrogenase.** Aldehydes are generated from a variety of endogenous and exogenous substrates. Endogenous aldehydes may be formed during metabolism of amino acids, carbohydrates, lipids, biogenic amines, vitamins, and steroids. Metabolism

of many drugs and environmental agents produces aldehydes. Aldehydes are highly reactive electrophilic compounds; they may react with thiol and amino groups to produce a variety of effects. Some aldehydes produce therapeutic effects, but more often the effects are cytotoxic, genotoxic, mutagenic, and carcinogenic. Aldehyde dehydrogenases are important in helping to alleviate some of the toxic effects of aldehyde generation. This enzyme catalyzes the formation of acids from aliphatic and aromatic aldehydes; the acids are then available as substrates for conjugating enzymes:



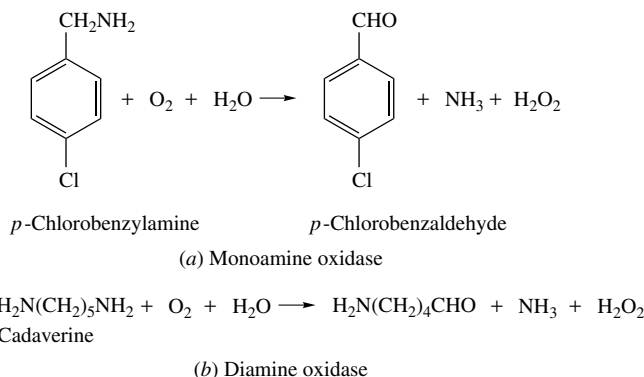
The aldehyde gene superfamily is large with more than 330 aldehyde dehydrogenase genes in prokaryote and eukaryotic species. The eukaryotic aldehyde dehydrogenase gene superfamily consists of 20 gene families, 9 of which contain 16 human genes and 3 pseudogenes. The importance of some of these genes in detoxication pathways is underscored by the fact that identified polymorphisms are associated with several metabolic diseases.

One especially interesting polymorphism is that which occurs at the aldehyde dehydrogenase 2 locus. When inherited as the homozygous trait, this aldehyde dehydrogenase polymorphism results in a 20-fold greater generation of acetaldehyde from ethanol, resulting in the flushing syndrome characteristic of many Asian individuals after ethanol consumption. Alcoholics are not likely to be found among individuals expressing this particular polymorphism.

Other enzymes in the soluble fraction of liver that oxidize aldehydes are aldehyde oxidase and xanthine oxidase, both flavoproteins that contain molybdenum; however, their primary role seems to be the oxidation of endogenous aldehydes formed as a result of deamination reactions.

**Amine Oxidases.** The most important function of amine oxidases appears to be the oxidation of amines formed during normal processes. Two types of amine oxidases are concerned with oxidative deamination of both endogenous and exogenous amines. Typical substrates are shown in Figure 7.10.

**Monoamine Oxidases.** The monoamine oxidases are a family of flavoproteins found in the mitochondria of a wide variety of tissues: liver, kidney, brain, intestine, and



**Figure 7.10** Examples of oxidations catalyzed by amine oxidases.

blood platelets. They are a group of similar enzymes with overlapping specificities and inhibition. Although the enzyme in the central nervous system is concerned primarily with neurotransmitter turnover, that in the liver will deaminate primary, secondary, and tertiary aliphatic amines, reaction rates with the primary amines being faster. Electron-withdrawing substitutions on an aromatic ring increase the reaction rate, whereas compounds with a methyl group on the  $\alpha$ -carbon such as amphetamine and ephedrine are not metabolized.

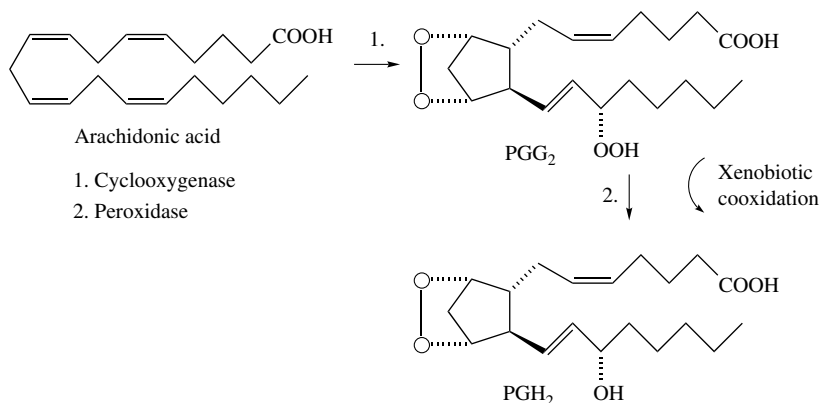
*Diamine Oxidases.* Diamine oxidases are enzymes that also oxidize amines to aldehydes. The preferred substrates are aliphatic diamines in which the chain length is four (putrescine) or five (cadaverine) carbon atoms. Diamines with carbon chains longer than nine will not serve as substrates but can be oxidized by monoamine oxidases. Secondary and tertiary amines are not metabolized. Diamine oxidases are typically soluble pyridoxal phosphate-containing proteins that also contain copper. They have been found in a number of tissues, including liver, intestine, kidney, and placenta.

### 7.2.5 Cooxidation by Cyclooxygenases

During the biosynthesis of prostaglandins, a polyunsaturated fatty acid, such as arachidonic acid, is first oxygenated to yield a hydroperoxy endoperoxide, prostaglandin G<sub>2</sub>. This is then further metabolized to prostaglandin H<sub>2</sub>, both reactions being catalyzed by the same enzyme, cyclooxygenase (COX), also known as prostaglandin synthase (Figure 7.11). This enzyme is located in the microsomal membrane and is found in greatest levels in respiratory tissues such as the lung. It is also common in the kidney and seminal vesicle. It is a glycoprotein with a subunit molecular mass of about 70,000 daltons, containing one heme per subunit. During the second step of the previous sequence (peroxidase), many xenobiotics can be cooxidized, and investigations of the mechanism have shown that the reactions are hydroperoxide-dependent reactions catalyzed by a peroxidase that uses prostaglandin G as a substrate. In at least some of these cases, the identity of this peroxidase has been established as a prostaglandin synthase. Many of the reactions are similar or identical to those catalyzed by other peroxidases and also by microsomal monooxygenases; they include both detoxication and activation reactions. This mechanism is important in xenobiotic metabolism, particularly in tissues that are low in CYP and/or the FMO but high in prostaglandin synthase.

The cyclooxygenase (COX) enzyme is known to exist as two distinct isoforms. COX-1 is a constitutively expressed housekeeping enzyme found in nearly all tissues and mediates physiological responses. COX-2 is an inducible form expressed primarily by cells involved in the inflammatory response. Several tissues low in CYP expression are rich in COX, which is believed to have significance in the carcinogenic effects of aromatic amines in these organs.

During cooxidation, some substrates are activated to become more toxic than they were originally. In some cases substrate oxidation results in the production of free radicals, which may initiate lipid peroxidation or bind to cellular proteins or DNA. Another activation pathway involves the formation of a peroxy radical from subsequent metabolism of prostaglandin G<sub>2</sub>. This reactive molecule can epoxidize many substrates including polycyclic aromatic hydrocarbons, generally resulting in increasing toxicity of the respective substrates.



**Figure 7.11** Cooxidation during prostaglandin biosynthesis.

To differentiate between xenobiotic oxidations by COX and CYP, *in vitro* microsomal incubations of the xenobiotic may be performed either in the presence of arachidonic acid (COX catalyzed) or in the presence of NADPH (CYP catalyzed). In the presence of arachidonic acid while in the absence of NADPH, substrates co-oxidized by COX will be formed while those requiring CYP will not. Specific inhibitors of PG synthase (indomethacin) and CYP (Metyrapone or SKF 525A) have also been used.

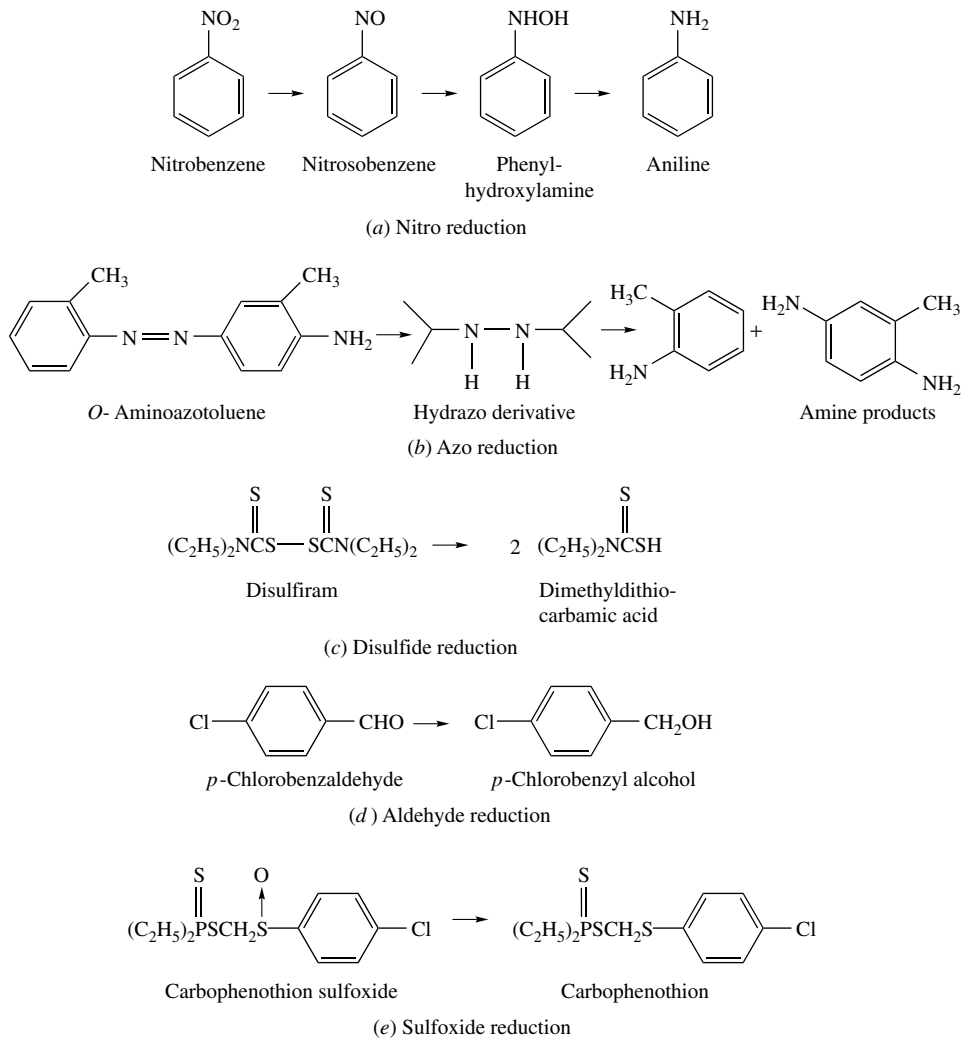
### 7.2.6 Reduction Reactions

A number of functional groups, such as nitro, diazo, carbonyl, disulfide sulfoxide, alkene, and pentavalent arsenic, are susceptible to reduction, although in many cases it is difficult to tell whether the reaction proceeds enzymatically or nonenzymatically by the action of such biologic reducing agents as reduced flavins or reduced pyridine nucleotides. In some cases, such as the reduction of the double bond in cinnamic acid ( $C_6H_5CH=CHCOOH$ ), the reaction has been attributed to the intestinal microflora. Examples of reduction reactions are shown in Figure 7.12.

**Nitro Reduction.** Aromatic amines are susceptible to reduction by both bacterial and mammalian nitroreductase systems. Convincing evidence has been presented that this reaction sequence is catalyzed by CYP. It is inhibited by oxygen, although NADPH is still consumed. Earlier workers had suggested a flavoprotein reductase was involved, and it is not clear if this is incorrect or if both mechanisms occur. It is true, however, that high concentration of FAD or FMN will catalyze the nonenzymatic reduction of nitro groups.

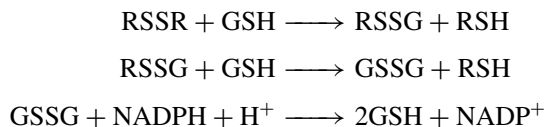
**Azo Reduction.** Requirements for azo reduction are similar to those for nitroreduction, namely anaerobic conditions and NADPH. They are also inhibited by CO, and presumably they involve CYP. The ability of mammalian cells to reduce azo bonds is rather poor, and intestinal microflora may play a role.

**Disulfide Reduction.** Some disulfides, such as the drug disulfiram (Antabuse), are reduced to their sulfhydryl constituents. Many of these reactions are three-step



**Figure 7.12** Examples of metabolic reduction reactions.

sequences, the last reaction of which is catalyzed by glutathione reductase, using glutathione (GSH) as a cofactor:



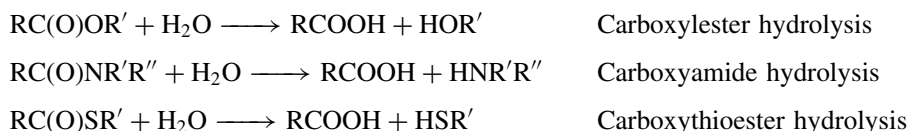
**Ketone and Aldehyde Reduction.** In addition to the reduction of aldehyde and ketones through the reverse reaction of alcohol dehydrogenase, a family of aldehyde reductases also reduces these compounds. These reductases are NADPH-dependent, cytoplasmic enzymes of low molecular weight and have been found in liver, brain, kidney, and other tissues.



**Sulfoxide Reduction.** The reduction of sulfoxides has been reported to occur in mammalian tissues. Soluble thioredoxin-dependent enzymes in the liver are responsible in some cases. It has been suggested that oxidation in the endoplasmic reticulum followed by reduction in the cytoplasm may be a form of recycling that could extend the *in vivo* half-life of certain toxicants.

### 7.2.7 Hydrolysis

Enzymes with carboxylesterase and amidases activity are widely distributed in the body, occurring in many tissues and in both microsomal and soluble fractions. They catalyze the following general reactions:



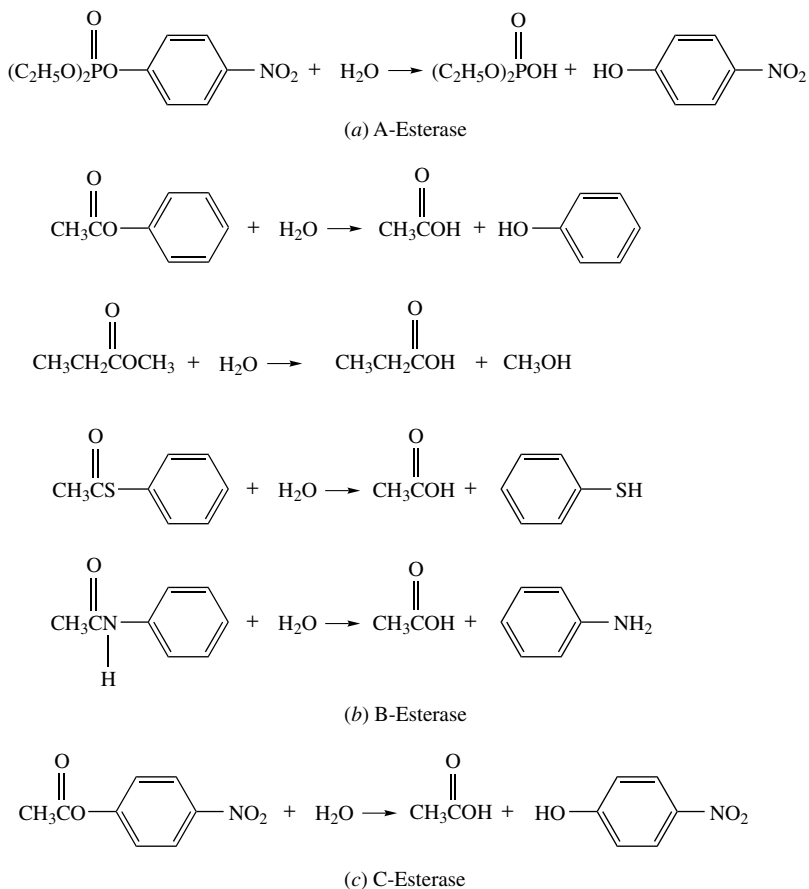
Although carboxylesterases and amidases were thought to be different, no purified carboxylesterase has been found that does not have amidase activity toward the corresponding amide. Similarly enzymes purified on the basis of their amidase activity have been found to have esterase activity. Thus these two activities are now regarded as different manifestations of the same activity, specificity depending on the nature of R, R', and R'' groups and, to a lesser extent, on the atom (O, S, or N) adjacent to the carboxyl group.

In view of the large number of esterases in many tissues and subcellular fractions, as well as the large number of substrates hydrolyzed by them, it is difficult to derive a meaningful classification scheme. The division into A-, B-, and C- esterases on the basis of their behavior toward such phosphate triesters as paraoxon, first devised by Aldridge, is still of some value, although not entirely satisfactory.

A-esterases, also referred to as arylesterases, are distinguished by their ability to hydrolyze esters derived from aromatic compounds. Organophosphates, such as the insecticide paraoxon are often used to characterize this group. B-esterases, the largest and most important group, are inhibited by organophosphates. All the B-esterases have a serine residue in their active site that is phosphorylated by this inhibitor. This group includes a number of different enzymes and their isozymes, many of which have quite different substrate specificities. For example, the group contains carboxylesterase, amidases, cholinesterases, monoacylglycerol lipases, and arylamidases. Many of these enzymes hydrolyze physiological (endogenous) substrates as well as xenobiotics. Several examples of their activity toward xenobiotic substrates are shown in Figure 7.13. C-esterases, or acetyl esterases, are defined as those esterases that prefer acetyl esters as substrates, and for which paraoxon serves as neither substrate nor inhibitor.

### 7.2.8 Epoxide Hydration

Epoxide rings of alkene and arene compounds are hydrated by enzymes known as epoxide hydrolases, the animal enzyme forming the corresponding *trans*-diols, although bacterial hydrolases are known that form *cis*-diols. Although, in general, the hydration

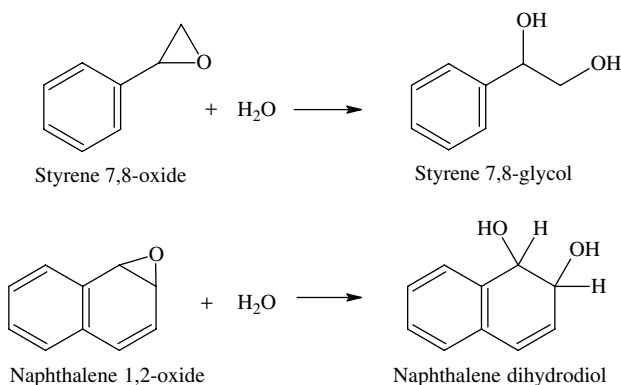


**Figure 7.13** Examples of esterase/amidase reactions involving xenobiotics.

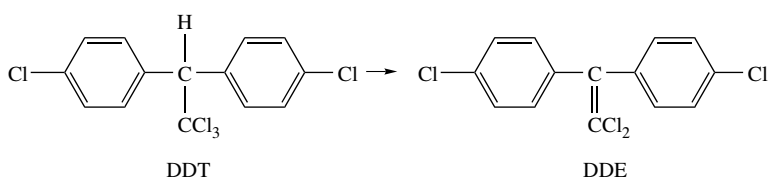
of the oxirane ring results in detoxication of the very reactive epoxide, in some cases, such as benzo(a)pyrene, the hydration of an epoxide is the first step in an activation sequence that ultimately yields highly toxic *trans*-dihydrodiol intermediates. In others, reactive epoxides are detoxified by both glutathione transferase and epoxide hydrolase. The reaction probably involves a nucleophilic attack by  $-\text{OH}$  on the oxirane carbon. The most studied epoxide hydrolase is microsomal, and the enzyme has been purified from hepatic microsomes of several species. Although less well known, soluble epoxide hydrolases with different substrate specificities have also been described. Examples of epoxide hydrolase reactions are shown in Figure 7.14.

### 7.2.9 DDT Dehydrochlorinase

DDT-dehydrochlorinase is an enzyme that occurs in both mammals and insects and has been studied most intensively in DDT-resistant houseflies. It catalyzes the dehydrochlorination of DDT to DDE and occurs in the soluble fraction of tissue homogenates. Although the reaction requires glutathione, it apparently serves in a catalytic role



**Figure 7.14** Examples of epoxide hydrolase reactions.



**Figure 7.15** DDT-dehydrochlorinase.

because it does not appear to be consumed during the reaction. The  $K_m$  for DDT is  $5 \times 10^{-7}$  mol/L with optimum activity at pH 7.4. The monomeric form of the enzyme has a molecular mass of about 36,000 daltons, but the enzyme normally exists as a tetramer. In addition to catalyzing the dehydrochlorination of DDT to DDE and DDD (2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane) to TDE (2,2-bis(*p*-chlorophenyl)-1-chloroethene), DDT dehydrochlorinase also catalyzes the dehydrohalogenation of a number of other DDT analogues. In all cases the *p,p* configuration is required, *o,p*, and other analogues are not utilized as substrates. The reaction is illustrated in Figure 7.15.

### 7.3 PHASE II REACTIONS

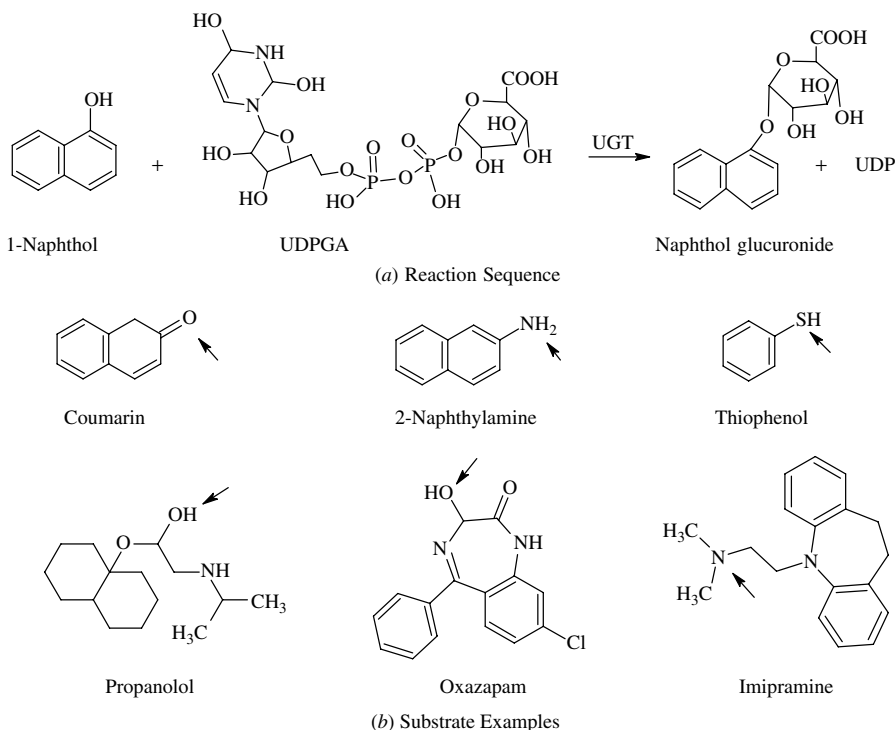
Products of phase I metabolism and other xenobiotics containing functional groups such as hydroxyl, amino, carboxyl, epoxide, or halogen can undergo conjugation reactions with endogenous metabolites, these conjugations being collectively termed phase II reactions. The endogenous metabolites in question include sugars, amino acids, glutathione, sulfate, and so on. Conjugation products, with only rare exceptions, are more polar, less toxic, and more readily excreted than are their parent compounds.

Conjugation reactions usually involve metabolite activation by some high-energy intermediate and have been classified into two general types: type I, in which an activated conjugating agent combines with the substrate to yield the conjugated product, and type II, in which the substrate is activated and then combines with an amino acid

to yield a conjugated product. The formation of sulfates and glycosides are examples of type I, whereas type II consists primarily of amino acid conjugation.

### 7.3.1 Glucuronide Conjugation

The glucuronidation reaction is one of the major pathways for elimination of many lipophilic xenobiotics and endobiotics from the body. The mechanism for this conjugation involves the reaction of one of many possible functional groups (R-OH, Ar-OH, R-NH<sub>2</sub>, Ar-NH<sub>2</sub>, R-COOH, Ar-COOH) with the sugar derivative, uridine 5'-diphosphoglucuronic acid (UDPGA). Homogeneous glucuronosyl transferase has been isolated as a single polypeptide chain of about 59,000 daltons, apparently containing carbohydrate, whose activity appears to be dependent on reconstitution with microsomal lipid. There appears to be an absolute requirement for UDPGA: related UDP-sugars will not suffice. This enzyme, as it exists in the microsomal membrane, does not exhibit its maximal capacity for conjugation; activation by some means (e.g., detergents), is required. The reaction involves a nucleophilic displacement (S<sub>N</sub><sup>2</sup> reaction) of the functional group of the substrate with Walden inversion. UDPGA is in the  $\alpha$ -configuration whereas, due to the inversion, the glucuronide formed is in the  $\beta$ -configuration. The enzyme involved, the UDP glucuronosyl transferase (UGT), is found in the microsomal fraction of liver, kidney, intestine, and other tissues. Examples of various types of glucuronides are shown in Figure 7.16.



**Figure 7.16** Reaction sequences of uridine diphospho glucuronosyl transferase and chemical structures of compounds that form glucuronides. Arrows indicate the position on each molecule where glucuronidation occurs.

Glucuronide conjugation generally results in the formation of products that are less biologically and chemically reactive. This, combined with their greater polarity and greater susceptibility to excretion, contributes greatly to the detoxication of most xenobiotics. However, there are now many examples where glucuronide conjugation results in greater toxicity. Perhaps the best-known example involves the bioactivation of *N*-hydroxy-2-acetylaminofluorine. This substrate, unlike 2-acetylaminofluorine, is unable to bind to DNA in the absence of metabolism. However, following glucuronide conjugation by linkage of the oxygen through the *N*-hydroxy group, this substrate becomes equipotent as a hepatocarcinogen with 2-acetylaminofluorine based on its ability to bind to DNA. Another relatively large class of xenobiotics that are often activated by glucuronide conjugation are the acyl glucuronides of carboxylic acids. Useful therapeutic drugs within this class include nonsteroidal anti-inflammatory drugs (NSAIDs), hypolipidemic drugs (clofibrate), and anticonvulsants (valproic acid). The various syndromes associated with the clinical use of some of these drugs (including cytotoxic, carcinogenic, and various immunologic effects) are thought to be the result of the ability of the glucuronide conjugates to react with nucleophilic macromolecules (protein and DNA).

A wide variety of reactions are mediated by glucuronosyltransferases, O-glucuronides, N-glucuronides, and S-glucuronides have all been identified. At this time over 35 different UGT gene products have been described from several different species. These are responsible for the biotransformation of greater than 350 different substrates. Evidence from molecular cloning suggests that the UGTs belong to one of two large superfamilies, sharing less than 50% amino acid identity. Nomenclature of these genes is similar to that of the CYP superfamily. The UGT1 gene family consists of a number of UGTs that arise from alternate splicing of multiple first exons and share common exons 2–5. Members of the UGT2 family catalyze the glucuronidation of a wide variety of substrates including steroids, bile acids, and opioids.

There are nine known human isozymes within the UGT1 family and six within the UGT2 family. Polymorphic forms of some of these enzymes are associated with diseases and significant adverse effects to some drugs.

Jaundice, a condition resulting from the failure of either transport or conjugation of bilirubin, becomes clinically evident when serum bilirubin levels exceed 35  $\mu\text{M}$ . Although the human UGT1A locus encompasses nine functional transferase genes, only one isoform, UGT1A1, is involved in inherited diseases of bilirubin metabolism. All three inheritable hyperbilirubineamias are the result of either mutant UGT1A1 alleles or UGT1A1 promoter polymorphisms. To date, 33 mutant UGT1A1 alleles have been identified. For the disease to be clinically manifest, one must either be homozygous for the mutant allele or have multiple heterozygous mutant alleles.

### 7.3.2 Glucoside Conjugation

Although rare in vertebrates, glucosides formed from xenobiotics are common in insects and plants. Formed from UDP-glucose, they appear to fall into the same classes as the glucuronides.

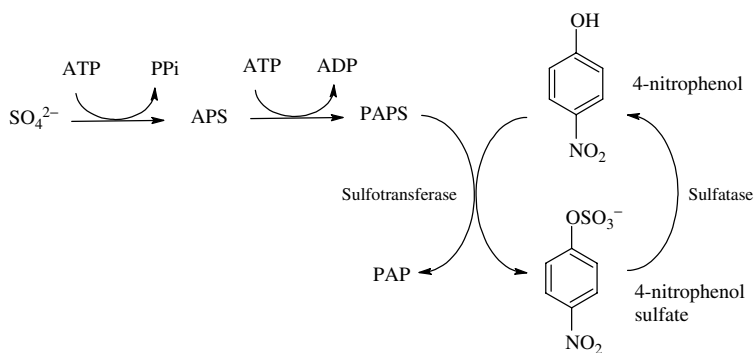
### 7.3.3 Sulfate Conjugation

Sulfation and sulfate conjugate hydrolysis, catalyzed by various members of the sulfotransferases (SULT) and sulfatase enzyme superfamilies, play important roles in the

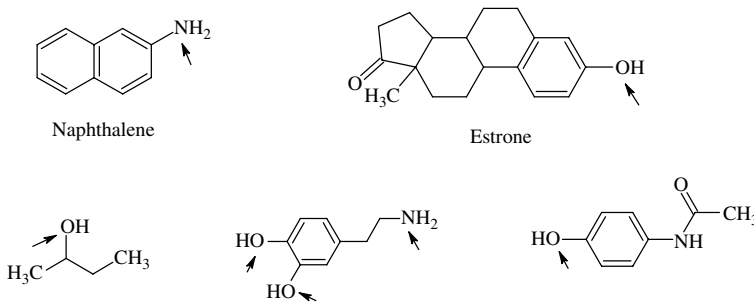
metabolism and disposition of many xenobiotics and endogenous substrates. Reactions of the sulfotransferase enzyme with various xenobiotics, including alcohols, arylamines, and phenols, result in the production of water soluble sulfate esters that often are readily eliminated from the organism. Although generally these reactions are important in detoxication, they have also been shown to be involved in carcinogen activation, prodrug processing, cellular signaling pathways, and the regulation of several potent endogenous chemicals including thyroid hormones, steroids, and catechols. The overall sulfation pathway shown in Figure 7.17, consists of two enzyme systems: the SULTs, which catalyze the sulfation reaction, and the sulfatases, which catalyze the hydrolysis of sulfate esters formed by the action of the SULTs.

Sulfation is expensive in energy terms for the cell, since two molecules of ATP are necessary for the synthesis of 3'-phosphoadenosine 5'-phosphosulfate (PAPS). Both enzymes involved in the synthesis of PAPS, ATP sulfurylase, and APS kinase, reside within a single bifunctional cytosolic protein of approximately 56 kDa, where substrate channeling of APS from ATP sulfurylase to APS kinase occurs. Several group VI anions other than sulfate can also serve as substrates, although the resultant anhydrides are unstable. Because this instability would lead to the overall consumption of ATP, these other anions can exert a toxic effect by depleting the cell of ATP.

In humans, there are five well-characterized *SULT* genes, each possessing widely different amino acid sequences and with widely different substrate specificities. Based



(a) Reaction Sequence



(b) Substrate Examples

**Figure 7.17** Reaction sequence of sulfotransferases and chemical structures of compounds that form sulfates. Arrows indicate positions on each molecule where sulfotransferases may attack.

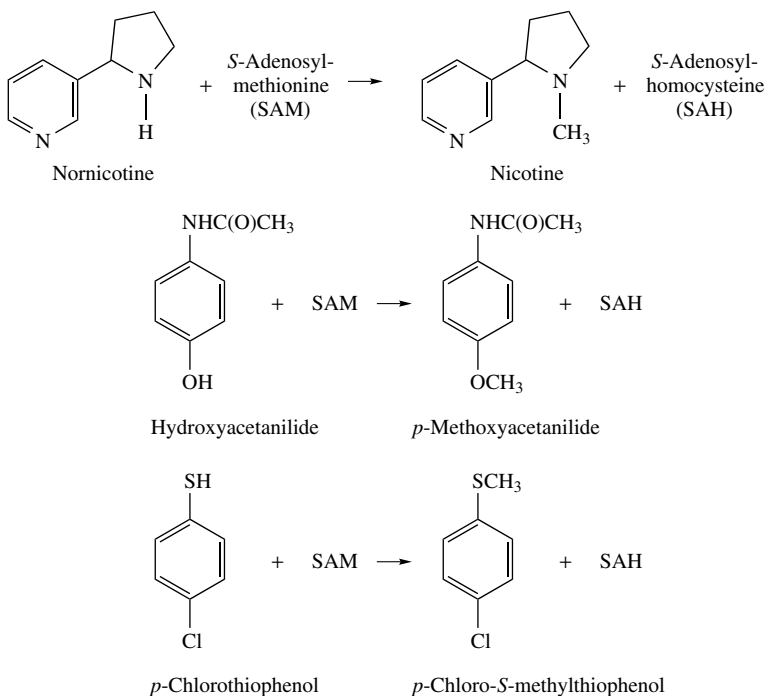
on amino acid sequence identity as well as substrate preference, these can be separated into two families, phenol SULTs (P-PST, SULT1A2, M-PST and EST) and hydroxysteroid SULT (HST). Phenol SULTs from rat liver have been separated into four distinct forms, each of which catalyzes the sulfation of various phenols and catecholamines. They differ, however, in pH optimum, relative substrate specificity, and immunologic properties. The molecules of all of them are in the range of 61,000 to 64,000 daltons.

Hydroxysteroid sulfotransferase also appears to exist in several forms. This reaction is now known to be important, not only as a detoxication mechanism but also in the synthesis and possibly the transport of steroids. Hydroxysteroid sulfotransferase will react with hydroxysterols and primary and secondary alcohols but not with hydroxyl groups in the aromatic rings of steroids.

### 7.3.4 Methyltransferases

A large number of both endogenous and exogenous compounds can be methylated by several *N*-, *O*-, and *S*-methyl transferases. The most common methyl donor is *S*-adenosyl methionine (SAM), which is formed from methionine and ATP. Even though these reactions may involve a decrease in water solubility, they are generally detoxication reactions. Examples of biologic methylation reactions are seen in Figure 7.18.

*N*-Methylation. Several enzymes are known that catalyze *N*-methylation reactions. They include histamine *N*-methyltransferase, a highly specific enzyme that occurs in



**Figure 7.18** Examples of methyl transferase reactions.

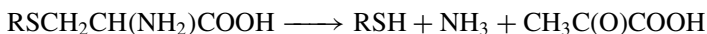
the soluble fraction of the cell, phenylethanolamine *N*-methyltransferase, which catalyzes the methylation of noradrenaline to adrenaline as well as the methylation of other phenylethanolamine derivatives. A third *N*-methyltransferase is the indoethylamine *N*-methyltransferase, or nonspecific *N*-methyltransferase. This enzyme occurs in various tissues. It methylates endogenous compounds such as serotonin and tryptamine and exogenous compounds such as nornicotine and norcodeine. The relationship between this enzyme and phenylethanolamine *N*-methyltransferase is not yet clear.

*O*-Methylation. Catechol *O*-methyltransferase occurs in the soluble fraction of several tissues and has been purified from rat liver. The purified form has a molecular weight 23,000 daltons, requires *S*-adenosylmethionine and  $Mg^{+}$ , and catalyzes the methylation of epinephrine, norepinephrine, and other catechol derivatives. There is evidence that this enzyme exists in multiple forms.

A microsomal *O*-methyltransferase that methylates a number of alkyl-, methoxy-, and halophenols has been described from rabbit liver and lungs. These methylations are inhibited by SKF-525, *N*-ethyl-maleimide and *p*-chloromercuribenzoate. A hydroxyindole *O*-methyltransferase, which methylates *N*-acetyl-serotonin to melatonin and, to a lesser extent, other 5-hydroxyindoles and 5,6-dihydroxyindoles, has been described from the pineal gland of mammals, birds, reptiles, amphibians, and fish.

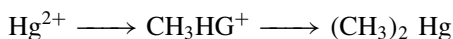
*S*-Methylation. Thiol groups of some foreign compounds are also methylated, the reaction being catalyzed by the enzyme, thiol *S*-methyltransferase. This enzyme is microsomal and, as with most methyl transferases, utilizes *S*-adenosylmethionine. It has been purified from rat liver and is a monomer of about 28,000 daltons. A wide variety of substrates are methylated, including thiacetanilide, mercaptoethanol, and diphenylsulfide. This enzyme may also be important in the detoxication of hydrogen sulfide, which is methylated in two steps, first to the highly toxic methanethiol and then to dimethylsulfide.

Methylthiolation, or the transfer of a methylthio ( $CH_3S-$ ) group to a foreign compound may occur through the action of another recently discovered enzyme, cysteine conjugate  $\beta$ -lyase. This enzyme acts on cysteine conjugates of foreign compounds as follows:



The thiol group can then be methylated to yield the methylthio derivative of the original xenobiotic.

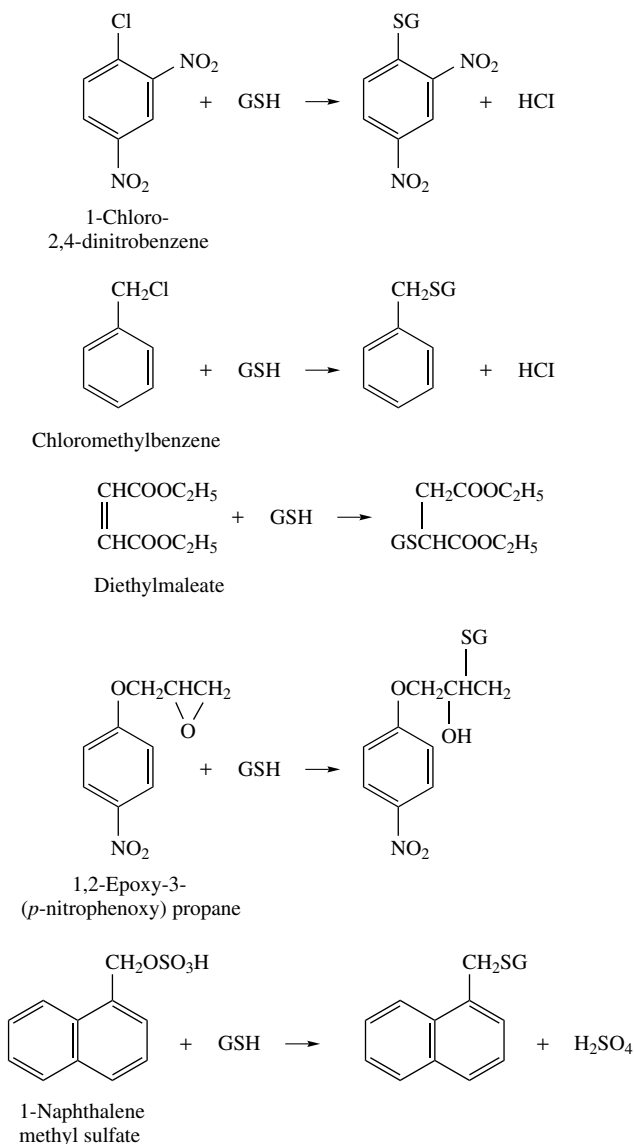
*Biomethylation of Elements.* The biomethylation of elements is carried out principally by microorganisms and is important in environmental toxicology, particularly in the case of heavy metals, because the methylated compounds are absorbed through the membranes of the gut, the blood-brain barrier, and the placenta more readily than are the inorganic forms. For example, inorganic mercury can be methylated first to monomethylmercury and subsequently, to dimethylmercury:



The enzymes involved are reported to use either *S*-adenosylmethionine or vitamin  $B_{12}$  derivatives as methyl donors, and in addition to mercury, the metals, lead, tin,







**Figure 7.20** Examples of glutathione transferases reactions.

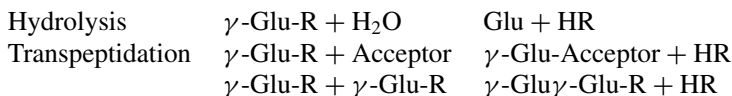
aryltransferase, aralkyltransferase, alkenyltransferase, and epoxidettransferase. Examples are shown in Figure 7.20.

Multiple forms of GST have been demonstrated in the liver of many mammalian species; multiple forms also occur in insects. Most GSTs are soluble dimeric proteins with molecular weights ranging between 45,000 and 50,000 daltons. All forms appear to be nonspecific with respect to the reaction types described, although the kinetic constants for particular substrates vary from one form to another. They are usually named from their chromatographic behavior. At least two are membrane-bound glutathione transferases, one of which is involved in metabolism of xenobiotics and is designated

the microsomal GST. The cytosolic GSTs are divided into six families (historically called classes): the  $\alpha$  (alpha),  $\kappa$  (kappa),  $\mu$  (mu),  $\pi$  (pi),  $\sigma$  (sigma), and  $\theta$  (theta) families. A new system of nomenclature proposes the term GST for the enzyme, preceded by the use of a small roman letter for the species (m for mouse, h for humans, etc.) followed by a capital roman letter for the family (A for  $\alpha$ , K for  $\kappa$ , etc.). Subunits are to be designated by arabic numbers, with the two subunits represented with a hyphen between them. For example, hGSTM1-2 designates a heterodimer of the human family mu, which possesses subunits one and two.

Glutathione conjugation dramatically increases the water solubility of the metabolites compared to the parent compounds. The metabolites are released from the cell by an active transport system belonging to the multi-drug resistance (mdr) protein. Prior to excretion, the metabolites are usually processed by multiple enzymes to release the substrate conjugated to a mercapturic acid (Figure 7.19). The enzymes involved in this process are  $\gamma$ -glutamyltranspeptidase, cysteinyl glycinase, and *N*-acetyl transferase.

$\gamma$ -Glutamyltranspeptidase is a membrane-bound glycoprotein that has been purified from both the liver and kidney of several species. Molecular weights for the kidney enzyme are in the range of 68,000 to 90,000 daltons, and the enzyme appears to consist of two unequal subunits; the different forms appear to differ in the degree of sialylation. This enzyme, which exhibits wide specificity toward  $\gamma$ -glutamyl peptides and has a number of acceptor amino acids, catalyzes two types of reactions:



Aminopeptidases that catalyze the hydrolysis of cysteinyl peptides are known. The membrane-bound aminopeptidases are glycoproteins, usually with molecular weights of about 100,000 daltons. They appear to be metalloproteins, one of the better known being a zinc-containing enzyme. Other enzymes, such as the leucine aminopeptidase, are cytosolic but, at least in this case, are also zinc-containing. The substrate specificity of these enzymes varies but most are relatively nonspecific.

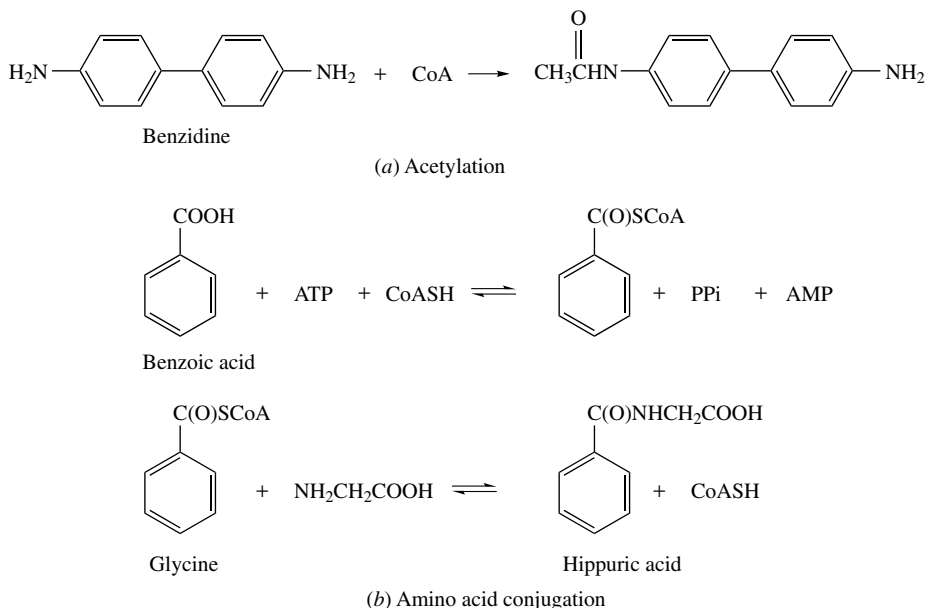
Little is known of the *N*-acetyltransferase(s) responsible for the acetylation of the S-substituted cysteine. It is found in the microsomes of the kidney and the liver, however, and is specific for acetyl CoA as the acetyl donor. It is distinguished from other *N*-acetyltransferases by its substrate specificity and subcellular location.

### 7.3.6 Cysteine Conjugate $\beta$ -Lyase

This enzyme uses cysteine conjugates as substrates, releasing the thiol of the xenobiotic, pyruvic acid, and ammonia, with subsequent methylation giving rise to the methylthio derivative. The enzyme from the cytosolic fraction of rat liver is pyridoxal phosphate requiring protein of about 175,000 daltons. Cysteine conjugates of aromatic compounds are the best substrates, and it is necessary for the cysteine amino and carboxyl groups to be unsubstituted for enzyme activity.

### 7.3.7 Acylation

Acylation reactions are of two general types, the first involving an activated conjugation agent, coenzyme A (CoA), and the second involving activation of the foreign



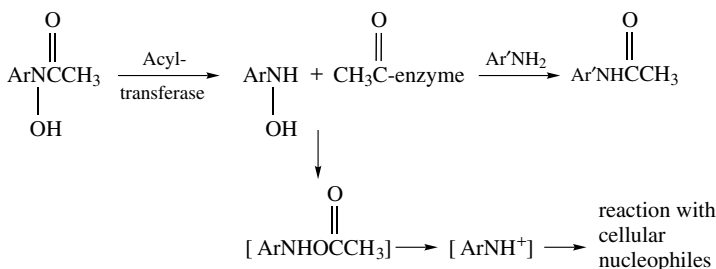
**Figure 7.21** Examples of acylation reactions.

compounds and subsequent acylation of an amino acid. This type of conjugation is commonly undergone by exogenous carboxylic acids and amides, and although the products are often less water soluble than the parent compound, they are usually less toxic. Examples of acylation reactions are shown in Figure 7.21.

**Acetylation.** Acetylated derivatives of foreign exogenous amines are acetylated by *N*-acetyl transferase, the acetyl donor being CoA. This enzyme is cytosolic, has been purified from rat liver, and is known to occur in several other organs. Evidence exists for the existence of multiple forms of this enzyme. Although endogenous amino, hydroxy, and thiol compounds are acetylated *in vivo*, the acetylation of exogenous hydroxy and thiol groups is presently unknown.

Acetylation of foreign compounds is influenced by both development and genetics. Newborn mammals generally have a low level of the transferase, whereas due to the different genes involved, fast and slow acetylators have been identified in both rabbit and human populations. Slow acetylators are more susceptible to the effects of compounds detoxified by acetylation.

***N,O*-Acyltransferase.** The *N*-acyltransferase enzyme is believed to be involved in the carcinogenicity of arylamines. These compounds are first *N*-oxidized, and then, in species capable of their *N*-acetylation, acetylated to arylhydroxamic acids. The effect of *N,O*-transacetylation is shown in Figure 7.22. The *N*-acyl group of the hydroxamic acid is first removed and is then transferred, either to an amine to yield a stable amide or to the oxygen of the hydroxylamine to yield a reactive *N*-acyloxyarylamine. These compounds are highly reactive in the formation of adducts with both proteins and nucleic acids, and *N,O*-acyltransferase, added to the medium in the Ames test, increases the mutagenicity of compounds such as *N*-hydroxy-2-acetylaminofluorene.



**Figure 7.22** *N*-, *O*-Acyltransferase reactions of arylhydroxamic acid. Ar = aryl group.

Despite its great instability this enzyme has been purified from the cytosolic fraction of the rat liver.

**Amino Acid Conjugation.** In the second type of acylation reaction, exogenous carboxylic acids are activated to form S-CoA derivative in a reaction involving ATP and CoA. These CoA derivatives then acylate the amino group of a variety of amino acids. Glycine and glutamate appear to be the most common acceptor of amino acids in mammals; in other organisms, other amino acids are involved. These include ornithine in reptiles and birds and taurine in fish.

The activating enzyme occurs in the mitochondria and belongs to a class of enzymes known as the ATP-dependent acid: CoA ligases (AMP) but has also been known as acyl CoA synthetase and acid-activating enzyme. It appears to be identical to the intermediate chain length fatty acyl-CoA-synthetase.

Two acyl-CoA: amino acid *N*-acyltransferases have been purified from liver mitochondria of cattle, Rhesus monkeys, and humans. One is a benzoyltransferase CoA that utilizes benzyl-CoA, isovaleryl-CoA, and tiglyl-CoA, but not phenylacetyl CoA, malonyl-CoA, or indolacetyl-CoA. The other is a phenylacetyl transferase that utilizes phenylacetyl-CoA and indolacetyl-CoA but is inactive toward benzoyl-CoA. Neither is specific for glycine, as had been supposed from studies using less defined systems; both also utilize asparagine and glutamine, although at lesser rates than glycine.

Bile acids are also conjugated by a similar sequence of reactions involving a microsomal bile acid: CoA ligase and a soluble bile acid *N*-acyl-transferase. The latter has been extensively purified, and differences in acceptor amino acids, of which taurine is the most common, have been related to the evolutionary history of the species.

**Deacetylation.** Deacetylation occurs in a number of species, but there is a large difference between species, strains, and individuals in the extent to which the reaction occurs. Because acetylation and deacetylation are catalyzed by different enzymes, the levels of which vary independently in different species, the importance of deacetylation as a xenobiotic metabolizing mechanism also varies between species. This can be seen in a comparison of the rabbit and the dog. The rabbit, which has high acetyltransferase activity and low deacetylase, excretes significant amounts of acetylated amines. The dog, in which the opposite situation obtains, does not.

A typical substrate for the aromatic deacetylases of the liver and kidney is acetanilide, which is deacetylated to yield aniline.

### 7.3.8 Phosphate Conjugation

Phosphorylation of xenobiotics is not a widely distributed conjugation reaction, insects being the only major group of animals in which it is found. The enzyme from the gut of cockroaches utilizes ATP, requires  $Mg^{+}$ , and is active in the phosphorylation of 1-naphthol and *p*-nitrophenol.

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