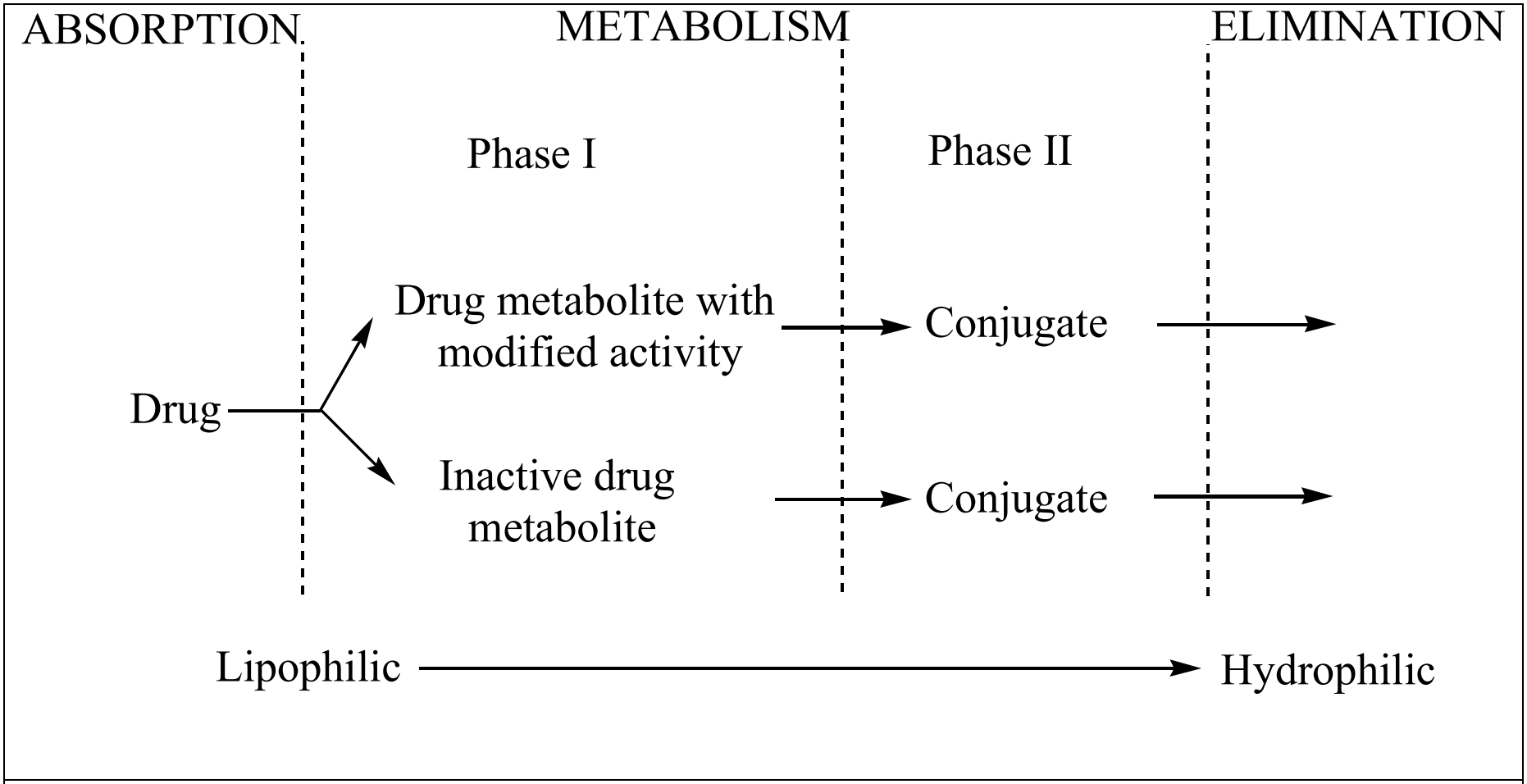
Humans are exposed daily to a wide variety of foreign compounds called **xenobiotics—**substances absorbed across the lungs or skin or, more commonly, ingested either unintentionally as compounds present in food and drink or deliberately as drugs for therapeutic or "recreational" purposes. Exposure to environmental xenobiotics may be inadvertent and accidental and may even be inescapable. Some xenobiotics are innocuous, but many can provoke biologic responses both pharmaco-logic and toxic in nature that are discussed in Chapters 58-60. These biologic responses often depend on conversion of the absorbed substance into an active metabolite. The discussion that follows is applicable to xenobiotics in general as well as to drugs and to some extent to endogenous compounds.

Phenobarbital would have extremely long half-lives if it were not for their metabolic conversion to more water-soluble compounds.
Metabolic products are often less active than the parent drug and may even be inactive. However, some biotransformation products have enhanced activity or toxic properties, including mutagenic-ity, teratogenicity, and carcinogenicity. It is noteworthy that the synthesis of endogenous substrates such as steroid hormones, cholesterol, and bile acids involves many enzyme-catalyzed pathways associated with the metabolism of xenobiotics. The same is true of the formation and excretion of endogenous metabolic products such as bilirubin, the end catabolite ofheme. Finally, drug-metabolizing enzymes have been exploited through the design of pharmacologically inactive pro-drugs that are converted in vivo to pharmacologically active molecules.

WHY IS DRUG BIOTRANSFORMATION NECESSARY?

Renal excretion plays a pivotal role in terminating the biologic activity of a few drugs, particularly those that have small molecular volumes or possess polar characteristics such as functional groups fully ionized at physiologic pH. Most drugs do not possess such physicochemical properties. Pharmacologically active organic molecules tend to be li-pophilic and remain un-ionized or only partially ionized at physiologic pH. They are often strongly bound to plasma proteins. Such substances are not readily filtered at the glomerulus. The lipophilic nature of renal tubular membranes also facilitates the reabsorption of hydrophobic compounds following their glomerular filtration. Consequently, most drugs would have a prolonged duration of action if termination of their action depended solely on renal excretion. An alternative process that may lead to the termination or alteration of biologic activity is metabolism. In general, lipophilic xenobiotics are transformed to more polar and hence more readily excretable products. The role metabolism may play in the inactivation of lipid-soluble drugs can be quite dramatic. For example, lipophilic barbiturates such as thiopental and Phenobarbital.



Phase I and phase II reactions in drug biodisposition. Phase II reactions may also precede phase I reactions.

MICROSOMAL MIXED FUNCTION OXIDASE SYSTEM

Localization

Many **xenobiotics**-metabolizing enzymes are located in the lipophilic membranes of the endoplasmic reticulum of the liver and other tissues. When these lamellar membranes are isolated by homogenization and fractionation of the cell, they reform into vesicles called **microsomes.**

Microsomes

Microsomes retain most of the morphologic and functional characteristics of the intact membranes, including the rough and smooth surface features of the rough (ribosome-studded) and smooth (no ribosomes) endoplasmic reticulum. Whereas the rough microsomes tend to be dedicated to protein synthesis, the smooth microsomes are relatively rich in enzymes responsible for oxidative xenobiotics metabolism.

Definition

In particular, they contain the important class of enzymes known as the mixed function oxidases (MFO), or monooxygenases. The activity of these enzymes requires both a reducing agent (NADPH) and molecular oxygen; in a typical reaction, one molecule of oxygen is consumed (reduced) per substrate molecule, with one oxygen atom appearing in the product and the other in the form of water.

Origin of mixed function oxidases

In this oxidation-reduction process, two microsomal enzymes play a key role.

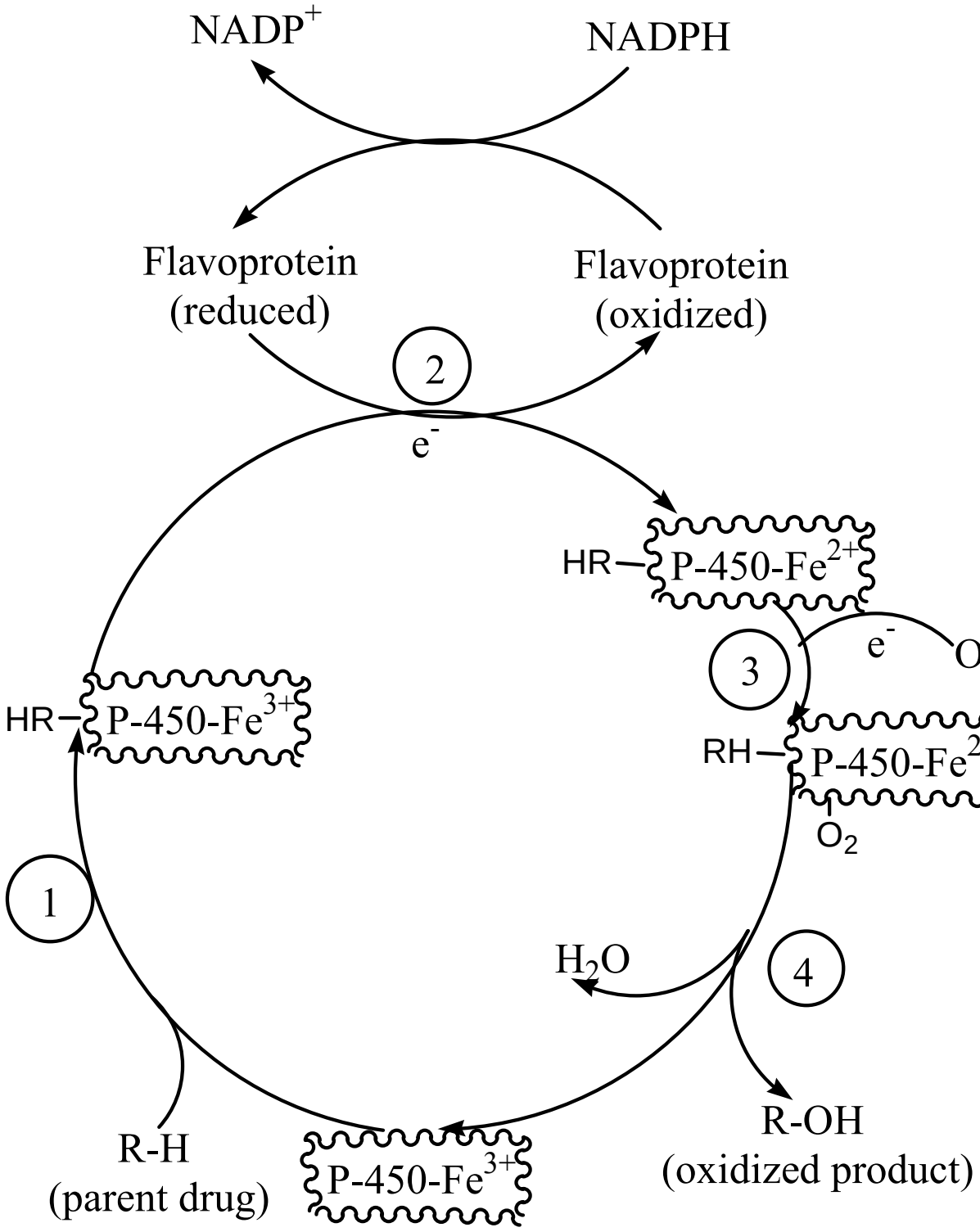
1. The first of these is a flavoprotein, **NADPH-cytochrome P-450 reductase**. One mole of this enzyme contains 1 mol each of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Because cytochrome c can serve as an electron acceptor, the enzyme is often referred to as NADPH-cytochrome c reductase.
2. The second microsomal enzyme is a hemoprotein called **cytochrome P-450** that serves as the terminal oxidase. In fact, the microsomal membrane harbors multiple forms of this hemoprotein, and this multiplicity is increased by repeated administration of exogenous chemicals. The name cytochrome P-450 is derived from the spectral properties of this hemoprotein. In its reduced (ferrous) form, it binds carbon monoxide to give a complex that absorbs light maximally at 450 nm. The relative abundance of cytochrome P-450, as compared to that of the reductase in the liver, contributes to making cytochrome P-450 heme reduction a rate-limiting step in hepatic xenobiotic oxidations.

Mechanism of monooxygenese reaction

Microsomal drug oxidations require cytochrome P-450, cytochrome P-450 reductase, NADPH, and molecular oxygen.

1. Briefly, oxidized (Fe^{3+}) cytochrome P-450 combines with a drug substrate to form a binary complex.
2. NADPH donates an electron to the flavoprotein reductase, which in turn reduces the oxidized cytochrome P-450-drug complex.
3. A second electron is introduced from NADPH via the same flavoprotein reductase, which serves to reduce molecular oxygen and to form an "activated oxygen"-cytochrome P-450-substrate complex.
4. This complex in turn transfers "activated" oxygen to the drug substrate to form the oxidized product.

The potent oxidizing properties of this activated oxygen permit oxidation of a large number of substrates. Substrate specificity is very low for this enzyme complex. High solubility in lipids is the only common structural feature of the wide variety of structurally unrelated drugs and chemicals that serve as substrates in this system.



Cytochrome P-450 cycle in drug oxidations. (RH = parent drug; ROH = oxidized metabolite; Fp = flavoprotein; e^- = electron.

Monooxygenases (Mixed-Function Oxidases, Hydroxylases) Incorporate Only One Atom of Molecular Oxygen Into the Substrate

The other oxygen atom is reduced to water, an additional electron donor or cosubstrate being necessary for this purpose.

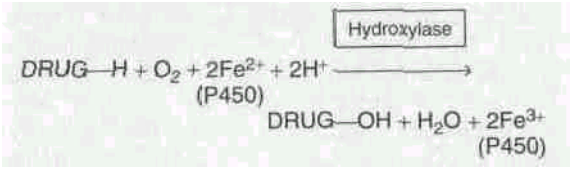
$$A—H + O_2 + ZH_2 \rightarrow A—OH + H_2O + Z$$

OXYGENASES CATALYZE THE DIRECT TRANSFER AND INCORPORATION OF OXYGEN INTO A SUBSTRATE MOLECULE

Oxygenases are concerned with the synthesis or degradation of many different types of metabolites rather than taking part in reactions that have as their purpose the provision of energy to the cell. Enzymes in this group catalyze the incorporation of oxygen into a substrate molecule. This takes place in two steps: (1) oxygen binding to the enzyme at the active site and (2) the reaction in which the bound oxygen is reduced or transferred to the substrate.

Microsomal Cytochrome P450 Monooxygenase Systems Are Important for the Hydroxylation of Many Drugs

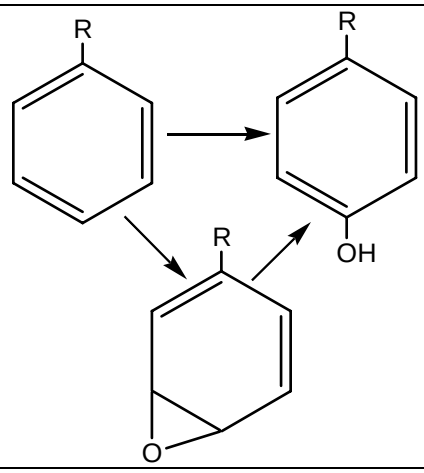
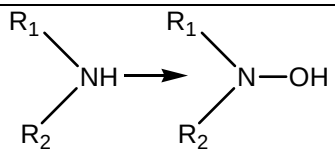
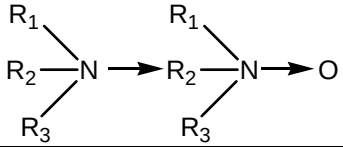
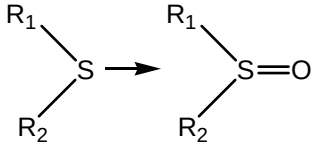
These monooxygenases are found in the micro-somes of the liver together with cytochrome P450 and **cytochrome** *b5*. Both NADH and NADPH donate reducing equivalents for the reduction of these cytochromes (Figure 13-7), which in turn are oxidized by substrates in a series of enzymatic reactions collectively known as the **hydroxylase cycle** (Figure 13-8).

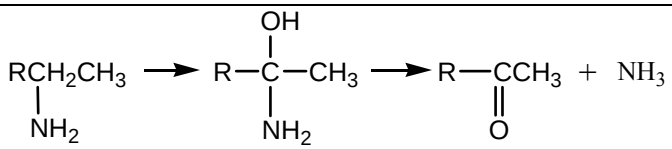
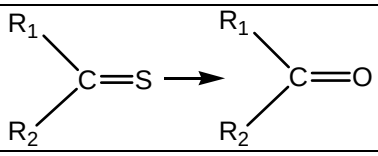
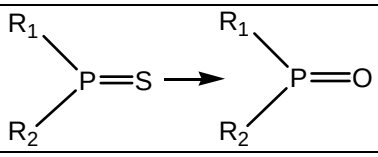
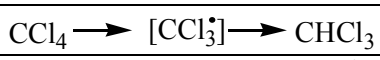
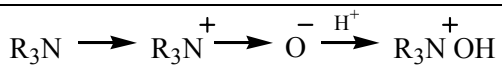
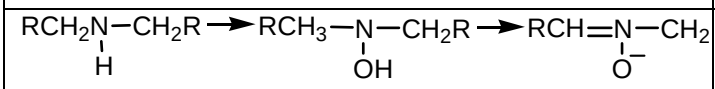
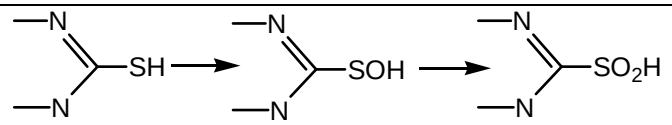
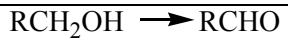
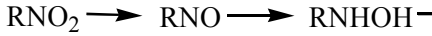
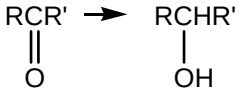
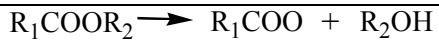


Among the drugs metabolized by this system are benzpyrene, aminopyrine, aniline, morphine, and benzphetamine. Many drugs such as phenobarbital have the ability to induce the formation of microsomal enzymes and of cytochrome P450.

Mitochondrial Cytochrome P450 Monooxygenase Systems Catalyze Steroidal Hydroxylations

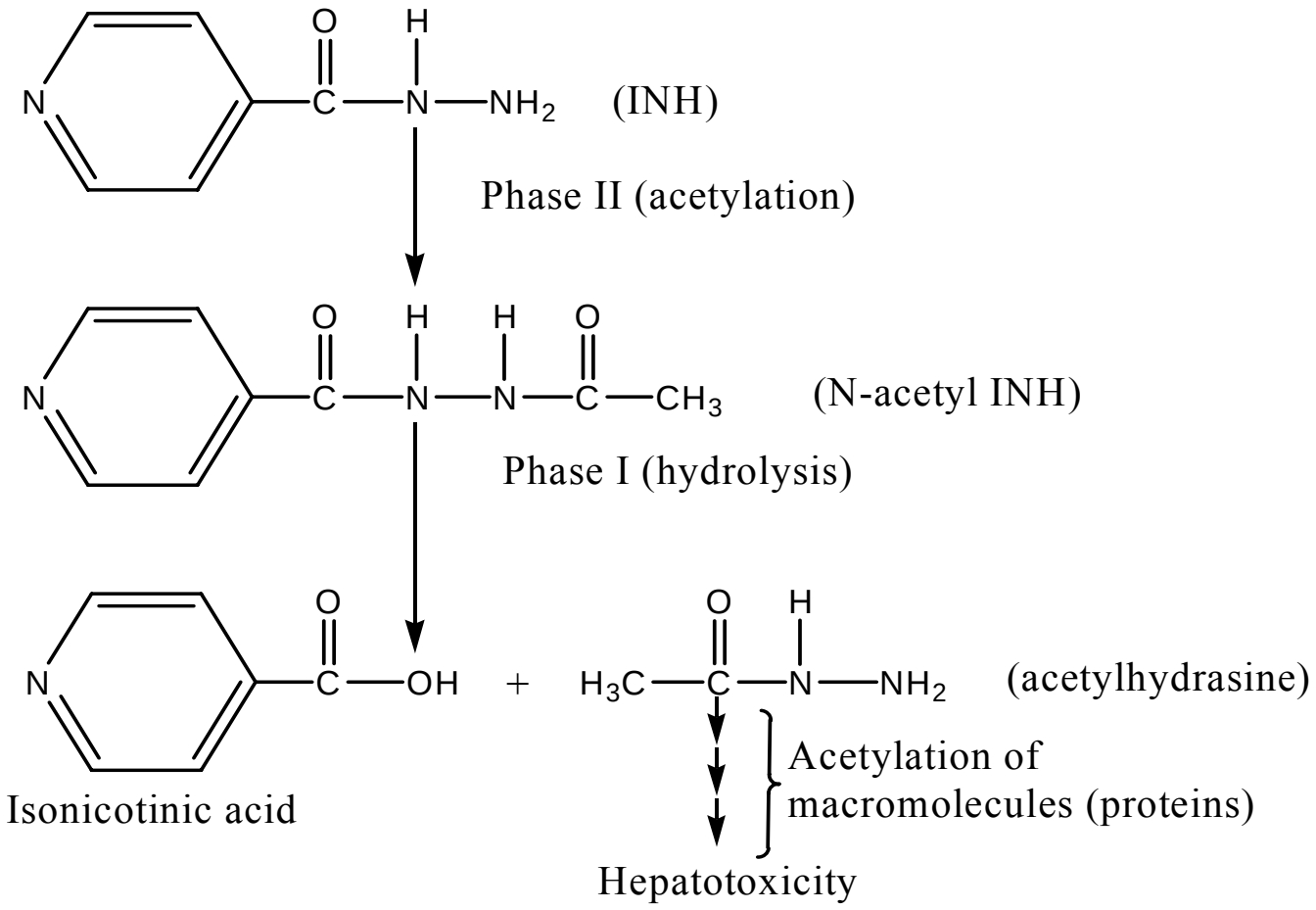
These systems are found in steroidogenic tissues such as adrenal cortex, testis, ovary, and placenta and are concerned with the biosynthesis of steroid hormones from cholesterol (hydroxylation in side-chain cleavage and at the 1113 and 18 positions). Renal systems catalyze la- and 24-hydroxylations of 25-hydroxycholecalciferol, and the liver catalyzes 26-hydroxylation in bile acid biosynthesis. In the adrenal cortex, mitochrondrial cytochrome P450 is six times more abundant than cytochromes of the respiratory chain.

Reaction Class		Drug Substrates
Oxidations Cytochrome P-450-depende Aromatic hydroxylations		Acetanilid, propranolol, phenobarbital, phenytoin, phenylbutazone, amphetamine, warfarin, 17a-ethinyl estradiol, naphthalene, benzpyrene.
Aliphatic hydroxylations	$RCH_2CH_3 \longrightarrow RCH_2CH_2OH$ $RCH_2CH_3 \longrightarrow RCHCH_3$ (OH)	Amobarbital, pentobarbital, secobarbital, chlorpropamide, ibuprofen, meprobamate, glutethimide, phenylbutazone, digitoxin.
Epoxidation	$RHC{=}CHR \longrightarrow R{-}C(H)(O){-}C(H){-}R$	Aldrin.
Oxidative dealkylation N-Dealkylation	$RNHCH_3 \longrightarrow RNH_2 + CH_2O$	Morphine, ethylmorphine, benzphetamine, aminopyrine, caffeine, theophylline.
0-Dealkylation	$ROCH_3 \longrightarrow ROH + CH_2O$	Codeine, p-nitroanisole.
S-Dealkylation	$RSCH_3 \longrightarrow RSH + CH_2O$	6-Methylthiopurine, methitural.
N-Oxidation Primary amines	$RNH_2 \longrightarrow RNHOH$	Aniline, chlorphentermine.
Secondary amines		2-Acetylaminofluorene, acetaminophen.
Tertiary amines		Nicotine, methaqualone.
S-Oxidation		Thioridazine, cimetidine, chlorpromazine.

Deamination		Amphetamine, diazepam.
Desulfuration		Thiopental.
		Parathion.
Dechlorination		Carbon tetrachloride.
Cytochrome P-450-indepen Flavin monooxygenase (Ziegler's enzyme)		Chlorpromazine, amitriptyline, benzphetamine
		Desipramine, nortriptyline
Cytochrome P-450-independe Flavin monooxygenase		Methimazole, propylthiouracil
Amine oxidases		Phenylethylamine, epinephrine.
Dehydrogenations		Ethanol, chloral hydrate.
Reductions Azo reductions		Prontosil, tartrazine.
Nitro reductions		Nitrobenzene, chloramphenicol, cloraze-pam, dantrolene.
Carbonyl reductions		Metyrapone, methadone, naloxone.
Hydrolyses Esters		Procaine, succinylcholine, aspirin, clofi-brate, methylphenidate.
Amides		Procainamide, lidocaine, indometnacin.

Phase II reactions.

Type of Conjugation	Endogenous Reactant	Transferase (Location)	Types of Substrates	Examples
Glucuronidation	**UDP glucuronic acid.**	**UDP-glucuronyl transferase (microsomes).**	**Phenols, alcohols, carboxylic acids, hydroxylamines, sulfonamides.**	**Nitrophenol, morphine, acetaminophen, diazepam, meprobamate, digitoxin, digoxin.**
Acetylation	**Acetyl-CoA.**	**N-Acetyl transferase (cytosol).**	**Amines.**	**Sulfonamides, isoniazid, clonazepam, dapsone, mescaline.**
Glutathione conjugation	**Glutathione.**	**GSH-S-transferase (cytosol, microsomes).**	**Epoxides, arene oxides, nitro groups, hydroxylamines.**	**Ethacrynic acid, bromobenzene.**
Glycine conjugation	**Glycine.**	**Acyl-CoA glycine transferase (mitochondria).**	**Acyl-CoA derivatives of carboxylic acids.**	**Salicylic acid, benzoic acid, nicotinic acid, cinnamic acid, cholic acid, deoxychollc acid.**
Sulfate conjugation	**Phosphoadenosyl phosphosulfate**	**Sulfotransferase (cytosol).**	**Phenols, alcohols, aromatic amines.**	**Estrone, phenol, 3-hydroxycoumarin, indol, acetaminophen, methyldopa.**
Methylation	**S-Adenosylmethionine.**	**Transmethylases (cytosol).**	**Catecholamines, phenols, amines, histamine.**	**Dopamine, epinephrine, histamine, thiouracil.**



Phase II-mediated activation of isoniazid (INH) to a hepatotoxic metabolite.