

SITES OF DRUG BIOTRANSFORMATION

Although biotransformation reactions may occur in many tissues, the liver is the most important organ in drug metabolism and detoxification of endogenous and exogenous compounds. Another important site, especially for orally administered drugs, is the intestinal mucosa which contains the CYP3A4 isozyme and P-glycoprotein (Permeability glycoprotein) that can capture the drug and secrete it back into the intestinal tract. In contrast, the liver is particularly rich in almost all of the drug-metabolizing enzymes. Orally administered drugs that are absorbed into the bloodstream through the GI tract must pass through the liver before being further distributed into body compartments. Therefore, they are susceptible to hepatic metabolism known as the *first-pass effect* before reaching the systemic circulation. Depending on the drug, this metabolism can sometimes be quite significant and results in decreased oral bioavailability. For example, in humans, several drugs are metabolized extensively by the first-pass effect. The following list includes some of those drugs:

Isoproterenol	Morphine	Propoxyphene
Lidocaine	Nitroglycerin	Propranolol
Meperidine	Pentazocine	Salicylamide

Some drugs (e.g., lidocaine) are removed so effectively by first-pass metabolism that they are ineffective when given orally. Nitroglycerin is administered buccally to bypass the liver.

Because most drugs are administered orally, the **intestine** appears to play an important role in the extrahepatic metabolism of xenobiotics.

- ❖ For example, in humans, orally administered isoproterenol undergoes considerable sulfate conjugation in the intestinal wall. Several other drugs (e.g., levodopa, chlorpromazine, and diethylstilbestrol) are also reportedly metabolized in the GI tract.
- ❖ **Esterases and lipases** present in the intestine may be particularly important in carrying out hydrolysis of many ester prodrugs.
- ❖ **Bacterial flora** present in the intestine and colon appear to play an important role in the reduction of many aromatic azo and nitro drugs (e.g., sulfasalazine).
- ❖ **Intestinal glucuronidase enzymes** can hydrolyze glucuronide conjugates excreted in the bile, thereby liberating the free drug or its metabolite for possible reabsorption (enterohepatic circulation or recycling).

Although other tissues, such as kidney, lungs, adrenal glands, placenta, brain, and skin, have some drug-metabolizing capability, the biotransformations that they carry out are often more substrate selective and more limited to particular types of reaction (e.g., oxidation, glucuronidation).

ROLE OF CYTOCHROME P450 MONOOXYGENASES IN OXIDATIVE BIOTRANSFORMATIONS

The oxidative biotransformation processes are the most common and important in phase I reactions of drug metabolism. The general stoichiometry that describes the oxidation of many xenobiotics (R-H) to their corresponding oxidized metabolites (R-OH) is given by the following equation:



The enzyme systems carrying out this biotransformation are referred to as **mixed-function oxidases or monooxygenases**. There is a large family that carry out the same basic chemical reactions. Their nomenclature is based on amino acid homology. There are four components to the name. CYP refers to the cytochrome system. This is followed by the Arabic number that specifies the cytochrome family (CYP1, CYP2, CYP3, etc.). Next is a capital letter that represents the subfamily (CYP1A, CYP1B, CYP2A, CYP2B, CYP3A, CYP3B, etc.). Finally, the cytochrome name ends with another Arabic number that specifies the specific enzyme responsible for a particular reaction (CYP1A2, CYP2C9, CYP2C19, CYP3A4, etc.).

Cytochrome P450 Enzymes Nomenclature

CYP-Arabic Number-Capital Letter-Arabic Number

1. CYP: Cytochrome P450 enzymes

2. Arabic number: Family (CYP1, CYP2, CYP3, etc.)

Must have more than 40% identical amino acid sequence

3. Capital letter: Subfamily (CYP1A, CYP2C, CYP3A, etc.)

Must have more than 55% identical amino acid sequence

4. Arabic number: Individual enzyme in a subfamily (CYP1A2, CYP2C9, CYP2D6, CYP2E1, CYP3A4, etc.)

Identity of amino acid sequences can exceed 90%

The reaction requires both molecular oxygen and the reducing agent NADPH (reduced form of nicotinamide adenosine dinucleotide phosphate). During this oxidative process, one atom of molecular oxygen (O_2) is introduced into the substrate R-H to form R-OH and the other oxygen atom is incorporated into water. The mixed-function oxidase system is actually made up of several components, the most important being the superfamily of CYP enzymes which are responsible for transferring an oxygen atom to the substrate R-H. Other important components of this system include the NADPH-dependent CYP reductase and the NADH-linked cytochrome b_5 . The latter two components, along with the cofactors NADPH and NADH, supply the reducing equivalents (electrons) needed in the overall metabolic oxidation of foreign compounds.

- ❖ The CYP enzymes are heme proteins. The heme portion is an iron-containing porphyrin called **protoporphyrin IX**, and the protein portion is called the **apoprotein**.

- ❖ CYP is found in high concentrations in the liver, the major organ involved in the metabolism of xenobiotics.
- ❖ The presence of this enzyme in many other tissues (e.g., lung, kidney, intestine, skin, placenta, adrenal cortex) shows that these tissues have drug-oxidizing capability too.
- ❖ The name cytochrome P450 is derived from the fact that the reduced (Fe^{2+}) form of this enzyme binds with carbon monoxide to form a complex that has a distinguishing spectroscopic absorption maximum at 450 nm.

One important feature of the hepatic CYP mixed-function oxidase system is its ability to metabolize an almost unlimited number of diverse substrates by various oxidative transformations. This is due to be

1. The substrate non-specificity of CYP
2. The presence of multiple forms of the enzyme.

Some of these P450 enzymes are selectively inducible by various chemicals (e.g., phenobarbital, benzo[α]pyrene, 3-methyl cholanthrene).

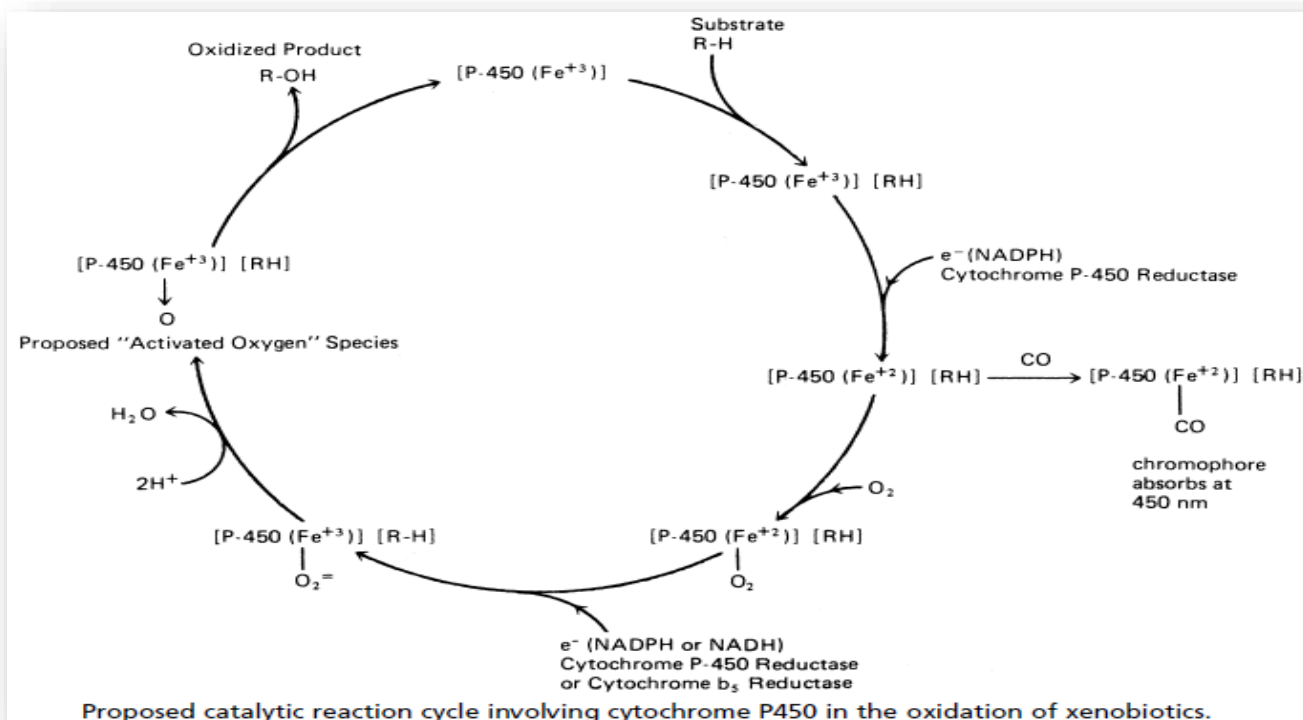
The CYP monooxygenases are located in the endoplasmic reticulum, a highly organized and complex network of intracellular membranes that is particularly abundant in tissues such as the liver. When these tissues are disrupted by homogenization, the endoplasmic reticulum loses its structure and is converted into small vesicular bodies known as *microsomes*. Mitochondria house many of the cytochrome enzymes that are responsible for the biosynthesis of steroidal hormones and metabolism of certain vitamins.

Microsomes isolated from hepatic tissue appear to retain all of the mixed-function oxidase capabilities of intact hepatocytes; because of this, microsomal preparations (with the necessary cofactors, e.g., NADPH, Mg^{2+}) are used frequently for in vitro drug metabolism studies. Because of its membrane-bound nature, the CYP monooxygenase system appears to be housed in a lipoidal environment. This may explain, in part, why lipophilic xenobiotics are generally good substrates for the monooxygenase system.

The catalytic role that the CYP monooxygenase system plays in the oxidation of xenobiotics is summarized in the cycle

- ❖ The initial step of this catalytic reaction cycle starts with the binding of the substrate to the oxidized (Fe^{3+}) resting state of CYP to form a P450-substrate complex.
- ❖ The next step involves the transfer of one electron from NADPH-dependent CYP reductase to the P450-substrate complex. This one-electron transfer reduces Fe^{3+} to Fe^{2+} . It is this reduced (Fe^{2+}) P450-substrate complex that is capable of binding dioxygen (O_2).
- ❖ The dioxygen–P450-substrate complex that is formed then undergoes another one-electron reduction (by CYP reductase-NADPH and/or cytochrome b5 reductase-NADH) to yield what is believed to be a peroxide dianion–P450 (Fe^{3+})-substrate complex.

- ❖ Water (containing one of the oxygen atoms from the original dioxygen molecule) is released from the latter intermediate to form an activated oxygen–P450-substrate complex.
- ❖ The activated oxygen $[\text{FeO}]^{3+}$ in this complex is highly electron deficient and a potent oxidizing agent. The activated oxygen is transferred to the substrate (R-H), and the oxidized substrate product (R-OH) is released from the enzyme complex to regenerate the oxidized form of CYP.



The potent oxidizing nature of the activated oxygen being transferred that makes CYP can oxidize numerous substrates. The many types of oxidative reaction carried out by CYP are enumerated in the sections below. Many of these oxidative pathways are summarized schematically as follow

