METABOLISM

Drug metabolism in liver, intestine, kidney, lung, brain. Individual factors altering drug effects (eg, age, gender, disease, tolerance and physical dependence, compliance, body weight)
Metabolism of drugs and Drug Biotransformation

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 or—when they are present as components of air, water, and food—inescapable.

Some xenobiotics are innocuous, but many can provoke biologic responses. Some of the toxic effects of these substances and the biologic responses to them often depend on conversion of the absorbed substance into an active metabolite.

The discussion that follows is applicable to xenobiotics in general (including drugs) and to some extent to endogenous compounds.
• In pharmacology, the word "metabolism" often refers to the process of making a drug more polar and water soluble.

• Biotransformation or metabolism refers to the chemical alteration of the drug in the body.

• Although this often results in drug inactivation and excretion, it is INCORRECT to assume that a metabolite will be less active or more easily excreted than the parent drug.

• Some drugs actually become activated when metabolized.

• Metabolic reactions can transform...
  • an active drug into less-active or inactive forms or
  • from inactive to active e.g PRODRUG (inactive or less-active drug) into a more active drug.

• Generally drugs are made more hydrophilic by the process.
• IT IS IMPORTANT TO REALIZE THAT
• a) metabolites are sometime pharmacologically active (e.g. mushrooms, nicotine)
• b) neonates have few drug-metabolic enzymes and that their kidneys not fully functioning
• c) drug action (depressants administered to mother) persists longer in neonates, and so infants may be depressed for long time after delivery.
The purpose of drug metabolism is to

- activate drug activity in the case of drugs administered as prodrugs, (some agents are initially administered as inactive compounds (pro-drugs) and must be metabolized to their active forms)
- terminate drug activity,
- render drugs water soluble, so they can be excreted by the kidney.

Drugs, chemicals, and toxins are all foreign to our bodies. Our body attempts to rid itself of foreign chemicals, regardless of whether they are therapeutic or harmful.

Most drugs must be biotransformed, or metabolized, before they can be excreted.

The liver is the main organ of drug metabolism.
The Kinetics of metabolism is:
- first-order kinetics
- zero-order kinetics
A. Kinetics of metabolism
1. First-order kinetics: The metabolic transformation of drugs is catalyzed by enzymes, and most of the reactions obey Michaelis-Menten kinetics:

\[ v = \text{rate of drug metabolism} = \frac{V_{\text{max}} [C]}{K_m + [C]} \]

- In most clinical situations, the concentration of the drug, \([C]\), is much less than the Michaelis constant, \(K_m\), and the Michaelis-Menten equation reduces to

\[ v = \text{rate of drug metabolism} = \frac{V_{\text{max}} [C]}{K_m} \]

- That is, the rate of drug metabolism is directly proportional to the concentration of free drug, and first-order kinetics are observed (Figure 1.14). This means that a constant fraction of drug is metabolized per unit of time.
2. Zero-order kinetics: With a few drugs, such as aspirin, ethanol and phenytoin, the doses are very large. Therefore the \([C]\) is much greater than \(K_m\), and the velocity equation becomes:

\[
v = \text{rate of drug metabolism} = \frac{V_{\text{max}} [C]}{[C]} = V_{\text{max}}
\]

- The enzyme is saturated by a high free-drug concentration, and the rate of metabolism remains constant over time. This is called zero-order kinetics (or sometimes is referred to clinically as nonlinear kinetics). A constant amount of drug is metabolized per unit of time.
Figure 1.14
Effect of drug dose on the rate of metabolism.

At high doses, drug metabolism is zero order—that is, constant and independent of the drug dose.

At low doses, drug metabolism is first order—that is, proportional to the drug dose.
• Sites of drug metabolism

• Drugs are most often eliminated by biotransformation and/or excretion into the urine or bile.

• The liver is the major site for drug metabolism. Most drugs are metabolized by the liver.

• The kidneys are another important site of metabolism.

• Specific drugs may undergo biotransformation in other tissues.

• The enzymes of degradation for a small number of drugs are spread widely throughout other tissues, including the blood and the wall of the GI tract.
Biochemical reactions in drug metabolism:
- oxidation,
- reduction,
- hydrolysis,
- conjugation

The kidney cannot efficiently eliminate lipophilic drugs that readily cross cell membranes and are reabsorbed in the distal tubules.

Therefore, lipid-soluble agents must first be metabolized in the liver using two general sets of reactions, called Phase I and Phase II (Figure 1.15).
Figure 1.15
The biotransformation of drugs.

Following Phase I, the drug may be activated, unchanged, or most often, inactivated.

Some drugs directly enter Phase II metabolism.

Conjugated drug is usually inactive.

Drug → Phase I → Oxidation, reduction, and/or hydrolysis → Phase II → Conjugation products.
Phase I:
Phase 1 reactions function to convert lipophilic drug molecules into more polar molecules by introducing or unmasking a polar functional group, such as -OH or -NH2.
Phase I metabolism may increase, decrease, or leave unaltered the drug's pharmacologic activity.
Phase I reactions include: oxidation, reduction, deamination, and hydrolysis

- Phase I reactions utilizing the P450 system:
- The Phase I reactions most frequently involved in drug metabolism are catalyzed by the cytochrome P450 system (also called microsomal mixed function oxidase):
  - Drug + O2 + NADPH + H+ > Drug modified + H2O + NADP+
- The oxidation proceeds by the drug binding to the oxidized form of cytochrome P450, and then oxygen is introduced through a reductive step coupled to NADPH cytochrome P450 oxidoreductase
- Phase I reactions not involving the P450 system include amine oxidation (for example, oxidation of catecholamines or histamine), alcohol dehydrogenation (for example, ethanol oxidation), and hydrolysis (for example, of procaine).
Phase II:

- Phase 2 reactions are conjugation reactions that link a polar group to the drug molecule to increase its water solubility. Polar moieties include glucuronate, acetate, gluathione, glycine, sulfate, and methyl.

If the metabolite from Phase I metabolism is sufficiently polar, it can be excreted by the kidneys. However, many metabolites are too lipophilic to be retained in the kidney tubules. A subsequent conjugation reaction with an endogenous substrate, such as glucuronic acid, sulfuric acid, acetic acid, or an amino acid, results in polar, usually more water-soluble compounds that are most often therapeutically inactive.

- A notable exception is morphine-6-glucuronide, which is twice as potent as morphine in many models of analgesia.

- Glucuronidation is the most common and the most important conjugation reaction. Neonates are deficient in this conjugating system, making them particularly vulnerable to drugs such as chloramphenicol.

- Drugs already possessing an -OH, -HN2, or -COOH group may enter Phase II directly, and become conjugated without prior Phase I metabolism. The highly polar drug conjugates may then be excreted by the kidney or bile.

- It is important to grasp that not all drugs undergo Phase I and II reactions in that order. They can be a reversal of the order of the phases. For example, isoniazid is first acetylated (a Phase II reaction) and then hydrolyzed to isonicotinic acid (a Phase I reaction).
• Determinants of metabolism

• Genetics

• Chemical properties of drugs

• Route of administration

• Diet

• Dosage

• Age differences:

• Gender:

• Disease

• Species differences

• Circadian rhythm
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 that are fully ionized at physiologic pH.

However, most drugs do not possess such physicochemical properties.

Pharmacologically active organic molecules tend to be lipophilic 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 pentobarbital would have extremely long half-lives if it were not for their metabolic conversion to more water-soluble compounds.

On the other hand, lipophilic substances such as DDT that are stored in fat and protected from the major organs of drug metabolism may persist in body fat years after exposure has ceased.

Metabolic products are often less pharmacodynamically active than the parent drug and may even be inactive. However, some biotransformation products have enhanced activity or toxic properties, including cytotoxicity, mutagenicity, 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 of heme.

• Finally, drug-metabolizing enzymes have been exploited through the design of pharmacologically inactive prodrugs that are converted in vivo to pharmacologically active molecules.
Most metabolic biotransformations occur at some point between absorption of the drug into the general circulation and its renal elimination. A few transformations occur in the intestinal lumen or intestinal wall.

In general, all of these reactions can be assigned to one of two major categories called phase I and phase II reactions as shown below.
Drug and toxin metabolism is divided into "Phase I" and "Phase II" reactions.

**Phase I reactions (nonsynthetic),** usually convert the parent drug to a more polar metabolite by oxidation, reduction, hydrolysis reactions.

Such Phase I – functionalization reactions exposes (unmasks) or introduces a functional group (—OH, —NH —SH).

Often these metabolites are inactive, though in some instances activity is only modified.

If phase I metabolites are sufficiently polar, they may be readily excreted.

However, many phase I products are not eliminated rapidly and undergo a subsequent reaction called phase II metabolism.
• **PHASE I: Mixed-Function Oxidases**, formed by microsomes made out of Smooth-ER folded over on itself.
  
  – Cytochrome-P450 Enzyme Complex:
  
  – Has four required components in order to work.
    
    • Cytochrome-P450 Enzyme
    • Cytochrome-P450 Reductase
    • O₂
    • **NADPH**: NADPH is the only energy source. No ATP is required!
  
  – Phase I enzymes perform multiple types of reactions:
    
    • **OXIDATIVE REACTIONS**: on drugs, such as: Aromatic hydroxylation, aliphatic hydroxylation, N-dealkylation, O-dealkylation, S-dealkylation, N-Oxidation, S-Oxidation, Desulfuration.
    
    • **REDUCTIVE REACTIONS**: Azo, Nitrile, Carbamyl
    
    • **HYDROLYTIC REACTIONS**: Ester hydrolysis, Amide hydrolysis.

• **Note that phase phase I reactions may actually occur AFTER phase II reactions.**
Phase I (nonsynthetic) Reactions

- **Microsomal (P450) oxidation reactions**
  1. Hydroxylation
     \[
     \text{NADPH, } O_2 \rightarrow \text{phenol}
     \]
     \[
     \text{Microsomes}
     \]
  2. Dealkylation
     \[
     \text{NADPH, } O_2 \rightarrow \text{phenol}
     \]
     \[
     \text{Microsomes}
     \]
  3. Oxidation
     \[
     \text{NADPH, } O_2 \rightarrow \text{SO}_{2}\text{O}
     \]
     \[
     \text{Microsomes}
     \]
  4. Polarizing atom exchange
     \[
     \text{NADPH, } O_2 \rightarrow \text{PO}_{4}\text{O}
     \]
     \[
     \text{Microsomes}
     \]

- **Microsomal (P450) reduction reactions**
  1. Azo-reduction
     \[
     \text{NADPH} \rightarrow \text{phenylamine}
     \]
     \[
     \text{Microsomes}
     \]
  2. Nitro-reduction
     \[
     \text{NO} \rightarrow \text{nitrosono} \rightarrow \text{hydroxynitro} \rightarrow \text{amino}
     \]
     \[
     \text{Microsomes}
     \]

- **Nonmicrosomal oxidation and reduction reactions**
  1. Alcohol oxidation
     \[
     \text{CH}_3\text{CH}_2\text{OH} \rightarrow \text{CH}_3\text{CH}_2\text{O} \rightarrow \text{CH}_3\text{CH}_2\text{O}
     \]
     \[
     \text{NAD}^+ \rightarrow \text{NAD}^+ \rightarrow \text{NAD}^+
     \]
  2. Alcohol reduction
     \[
     \text{R-C-OH} + \text{NAD} + \text{H}^+ \rightarrow \text{R-C-OH} + \text{NAD} + \text{H}_2\text{O}
     \]
     \[
     \text{OH}
     \]

Figure 1.2A Examples of Phase I metabolic reactions.
Table 4-1. Phase I reactions.

<table>
<thead>
<tr>
<th>Reaction Class</th>
<th>Structural Change</th>
<th>Drug Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytochrome P450-dependent oxidations: Aromatic hydroxylations</td>
<td>![Chemical Structure]</td>
<td>Acetaminolide, propranolol, phenobarbital, phenytoin, phenylbutazone, amphetamine, warfarin, 17α-ethyl estradiol, naphthalene, benzpyrene</td>
</tr>
<tr>
<td>Aliphatic hydroxylations</td>
<td>RCH₂CH₃ → RCH₂CH₂OH</td>
<td>Amobarbital, pentobarbital, secobarbital, chlorpropamide, ibuprofen, meprobamate, glutethimide, phenylbutazone, digitoxin</td>
</tr>
<tr>
<td>Epoxidation</td>
<td>RCH = CHₓ → R – C – C – R</td>
<td>Aldrin</td>
</tr>
<tr>
<td><strong>Oxidative dealkylation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Dealkylation</td>
<td>RNH₂CH₃ → RNH₂ + CH₂O</td>
<td>Morphine, ethylmorphine, benzphetamine, aminopyrine, caffeine, theophylline</td>
</tr>
<tr>
<td>O-Dealkylation</td>
<td>ROCH₃ → ROH + CH₂O</td>
<td>Codeine, p-nitroanisole</td>
</tr>
<tr>
<td>S-Dealkylation</td>
<td>RSCH₃ → RSH + CH₂O</td>
<td>6-Methylthiopurine, methiturial</td>
</tr>
<tr>
<td><strong>N-Oxidation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary amines</td>
<td>RNH₂ → RNH₂OH</td>
<td>Aniline, chlorphentermine</td>
</tr>
<tr>
<td>Secondary amines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertiary amines</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>S-Oxidation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deamination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deepulfuration</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4–1. Phase I reactions (cont’d).

<table>
<thead>
<tr>
<th>Reaction Class</th>
<th>Structural Change</th>
<th>Drug Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P450-dependent oxidations: (cont’d)</td>
<td></td>
<td>Parathion</td>
</tr>
</tbody>
</table>
| | \[
P = S \rightarrow P = O
\] | |
| Dechlorination | \[CCl_4 \rightarrow [CCl_3^-] \rightarrow CHCl_3\] | Carbon tetrachloride |
| Cytochrome P450-independent oxidations: | | |
| Flavin monooxygenase (Ziegler’s enzyme) | \[-N\] | Methimazole, propylthiouracil |
| | \[R_2N \rightarrow R_3N^+ \rightarrow O^- \rightarrow R_3N^+OH\] | Chlorpromazine, amitriptyline, benzphetamine |
| | \[RCH_2N \rightarrow CH_2R \rightarrow RCH_2 \rightarrow N \rightarrow CH_2R \rightarrow OH\] | Desipramine, nortriptyline |
| | \[RCH=NR \leftarrow \] | |
| | \[\] | |
| Amine oxidases | \[RCH_2NH_2 \rightarrow RCHO + NH_3\] | Phenylethylamine, epinephrine |
| Dehydrogenations | \[RCH_2OH \rightarrow RCHO\] | Ethanol |
| Reductions | | |
| Azo reductions | \[RN \rightarrow NR \rightarrow RNH \rightarrow NHR \rightarrow RNH_2 + R_1NH_2\] | Prontosil, tartrazine |
| Nitro reductions | \[RNO_2 \rightarrow RNO \rightarrow RNHOH \rightarrow RNH_2\] | Nitrobenzene, chloramphenicol, clonazepam, dantrolene |
| Carbonyl reductions | \[RCR' \rightarrow RCHR'\] | Metyrapone, methadone, naloxone |
| Hydrolyses | | |
| Esters | \[R_1COOR_2 \rightarrow R_1COOH + R_2OH\] | Procaine, succinylcholine, aspirin, clofibrate, methylphenidate |
| Amides | \[RCONHR_1 \rightarrow RCOOH + R_1NH_2\] | Procainamide, lidocaine, indomethacin |
As noted, many phase I products are not eliminated rapidly and undergo a subsequent reaction in which an endogenous substrate such as glucuronic acid, sulfuric acid, acetic acid, or an amino acid combines with the newly established functional group to form a highly polar conjugate.

Such conjugation or synthetic reactions are the hallmarks of phase II metabolism.

In PHASE II there is drug conjugation usually to glucuronides, making the drug more soluble. In Phase II Reactions (synthetic), a polar group, such as glutathione, is conjugated to the drug. This substantially increases the polarity of the drug.

Drugs undergoing Phase II conjugation reactions may have already undergone Phase I transformation.

Phase II reactions are biosynthetic (conjugation) reactions via the cytochrome P450 system – involving covalent linkage between a functional group with endogenously derived glucuronic acid, sulfate, glutathione, amino acids or acetate (generally inactive) except: 6-glucuronide of morphine is more active.
In phase II reactions, parent drugs or their phase I metabolites that contain suitable chemical groups often undergo coupling or conjugation reactions with an endogenous substance to yield drug conjugates (Table 4—3).

In general, conjugates are polar molecules that are readily excreted and often inactive.

Conjugate formation involves high-energy intermediates and specific transfer enzymes. Such enzymes (transferases) may be located in microsomes or in the cytosol.

They catalyze the coupling of an activated endogenous substance (such as the uridine 5’-diphosphate [derivative of glucuronic acid) with a drug (or endogenous compound), or of an activated drug (such as the S-CoA derivative of benzoic acid) with an endogenous substrate.
Because the endogenous substrates originate in the diet, nutrition plays a critical role in the regulation of drug conjugations.

Drug conjugations were once believed to represent terminal inactivation events and as such have been viewed as “true detoxification” reactions.

However, this concept must be modified, since it is now known that certain conjugation reactions (acyl glucuronidation of nonsteroidal anti-inflammatory drugs, 0-sulfation of N-hydroxyacetylaminofluorene, and N-acetylation of isoniazid) may lead to the formation of reactive species responsible for the hepatotoxicity of the drug.
<table>
<thead>
<tr>
<th>Type of Conjugation</th>
<th>Endogenous Reactant</th>
<th>Transferase (Location)</th>
<th>Types of Substrates</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucuronidation</td>
<td>UDP glucuronic acid</td>
<td>UDP glucuronosyltransferase (microsomes)</td>
<td>Phenols, alcohols, carboxylic acids, hydroxylamines, sulfonamides</td>
<td>Nitrophenol, morphine, acetaminophen, diazepam, N-hydroxydapsone, sulfathiazole, meprobamate, digoxin, digoxin</td>
</tr>
<tr>
<td>Acetylation</td>
<td>Acetyl-CoA</td>
<td>N-Acetyltransferase (cytosol)</td>
<td>Amines</td>
<td>Sulfonamides, isoniazid, clonazepam, dapsone, mesacaline</td>
</tr>
<tr>
<td>Glutathione</td>
<td>Glutathione</td>
<td>GSH-S-transferase (cytosol) and (microsomes)</td>
<td>Epoxides, arene oxides, nitro groups, hydroxylamines</td>
<td>Ethacrynic acid, bromobenzene</td>
</tr>
<tr>
<td>Glycine conjugation</td>
<td>Glycine</td>
<td>Acyl-CoA glycinetransferase (mitochondria)</td>
<td>Acyl-CoA derivatives of carboxylic acids</td>
<td>Salicylic acid, benzoic acid, nicotinic acid, cinnamic acid, cholic acid, deoxycholic acid</td>
</tr>
<tr>
<td>Sulfate conjugation</td>
<td>Phosphoadenosyl phosphosulfate</td>
<td>Sulfotransferase (cytosol)</td>
<td>Phenols, alcohols, aromatic amines</td>
<td>Estrone, aniline, phenol, 3-hydroxycoumarin, acetaminophen, methyl-dopa</td>
</tr>
<tr>
<td>Methylation</td>
<td>S-Adenosylmethionine</td>
<td>Transmethylases (cytosol)</td>
<td>Catecholamines, phenols, amines</td>
<td>Dopamine, epinephrine, pyridine, histamine, thiouacil</td>
</tr>
<tr>
<td>Water conjugation</td>
<td>Water</td>
<td>Epoxide hydrolase (microsomes)</td>
<td>Arene oxides, cis-disubstituted and monosubstituted oxiranes</td>
<td>Benzopyrene 7,8-epoxide, styrene 1,2-oxide, carbamazepine epoxide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(cytosol)</td>
<td>Alkene oxides, fatty acid epoxides</td>
<td>Leukotriene A₄</td>
</tr>
</tbody>
</table>
Phase II (Synthetic) Reactions
Catalyzed by specific enzymes rather than \( P_{450} \)

1. Glucuronide conjugation
\[
\text{drug} - \text{OH} + \text{UDPGA} \xrightarrow{\text{glucuronyl transferase}} \text{drug} - \text{OC}_6\text{H}_5\text{O}_6 + \text{UDP}
\]

2. Ethereal sulfate conjugation
\[
\text{drug} - \text{OH} + \text{PAPS} \xrightarrow{\text{Sulfokinase}} \text{drug} - \text{OSO}_3^- + \text{PAP}
\]

3. Acetylation
\[
\text{drug} - \text{NH}_2 + \text{CH}_3\text{CO-CoA} \xrightarrow{\text{Acetyl transferase}} \text{drug} - \text{N} - \text{CCH}_3 + \text{CoA}
\]

4. Transulfuration (occurs in mitochondria)
\[
\text{CN}^- + \text{S}_2\text{O}_3^- \rightarrow \text{CNS}^- + \text{SO}_3^-
\]

5. Glutathione conjugation
\[
\text{drug} - \text{CH}_2\text{Cl} + \text{GSH} \xrightarrow{\text{glutathionase}} \text{drug} - \text{SH} - \text{SH} - \text{CHCOOH}
\]

Abbreviations of donors:
- UDPGA = uridine diphosphoglucuronic acid
- PAPS = 3-phosphoadenosine 5' phosphosulfate
- GSH = glutathione (g-glutamyl-cysteinyl-glycine)

Figure 1.2B Examples of Phase II metabolic reactions.
• Note that a great variety of drugs undergo these sequential biotransformation reactions, although in some instances the parent drug may already possess a functional group that may form a conjugate directly.

• For example, the hydrazide moiety of isoniazid is known to form an N-acetyl conjugate in a phase II reaction.

• This conjugate is then a substrate for a phase I type reaction, namely, hydrolysis to isonicotinic acid (Figure 4—2). Thus, phase II reactions may actually precede phase I reactions.
WHERE DO DRUG BIOTRANSFORMATIONS (METABOLISM) OCCUR?

Although every tissue has some ability to metabolize drugs, the liver is the principal organ of drug metabolism. Other tissues that display considerable activity include the gastrointestinal tract, the lungs, the skin, and the kidneys.

Following oral administration, many drugs (eg, isoproterenol, meperidine, pentazocine, morphine) are absorbed intact from the small intestine and transported first via the portal system to the liver, where they undergo extensive metabolism. This process has been called a first-pass effect.

Some orally administered drugs (eg, clonazepam, chlorpromazine) are more extensively metabolized in the intestine than in the liver.

Thus, intestinal metabolism may contribute to the overall first-pass effect.

First-pass effects may so greatly limit the bioavailability of orally administered drugs that alternative routes of administration must be employed to achieve therapeutically effective blood levels.
The lower gut harbors intestinal microorganisms that are capable of many biotransformation reactions. In addition, drugs may be metabolized by gastric acid (eg, penicillin), by digestive enzymes (eg, polypeptides such as insulin), or by enzymes in the wall of the intestine (eg, sympathomimetic catecholamines).

Although drug biotransformation in vivo can occur by spontaneous, noncatalyzed chemical reactions, the vast majority of transformations are catalyzed by specific cellular enzymes.

At the subcellular level, these enzymes may be located in the endoplasmic reticulum, mitochondria, cytosol, lysosomes, or even the nuclear envelope or plasma membrane.
When cells are homogenized and subjected to differential centrifugation we can separate a fraction of microvesicles derived from the ER. **This is known as microsomes and it contains phase I enzymes.**

- The most important phase I enzymes are the Cytochrome P450 monooxygenases. This is a superfamily of heme thiolate proteins which functions as a terminal oxidase to introduce a single atom of molecular oxygen into the substrate.
- Electrons for this reaction are supplied from NADPH via Cyt. P450 reductase.
- 1000 currently known cyt. P450s, 50 active in human beings.
- Categorized into 17 families and subfamilies designate as CYP1, CYP2, CYP3 families. Enzymes within a family have amino acid sequences with greater than 40% identity.
The liver microsomal drug oxidation/reduction system (The P450 system) is responsible for the metabolism of many drugs. Cytochrome P450 (so named because it maximally absorbs light at 450 nm) is an enzyme located in the endoplasmic reticulum (microsomal fraction) of hepatocytes.

Through an electron transport chain which uses NADPH as a proton carrier, a drug bound to cytochrome P450 can be oxidized or reduced (phase I reaction).
MICROSOMAL MIXED FUNCTION OXIDASE SYSTEM & PHASE REACTIONS

Many drug-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 re-form into vesicles called 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 drug metabolism.

In particular, they contain the important class of enzymes known as the mixed function oxidases (MFOs), 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.

• In this oxidation-reduction process, two microsomal enzymes play a key role. The first of these is a flavoprotein, NADPH-cytochrome P450 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.
• The second microsomal enzyme is a hemoprotein called cytochrome P450 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 P450 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 P450, as compared with that of the reductase in the liver, contributes to making cytochrome P450 heme reduction a rate-limiting step in hepatic drug oxidations.
• Microsomal drug oxidations require cytochrome P450, cytochrome P450 reductase, NADPH, and molecular oxygen. A simplified scheme of the oxidative cycle is presented in Figure 4—3.
Figure 4–3. Cytochrome P450 cycle in drug oxidations. (R-H, parent drug; R-OH, oxidized metabolite; e\(^-\), electron.)
• Briefly, oxidized (Fe cytochrome P450 combines with a drug substrate to form a binary complex (step 1).

• NADPH donates an electron to the flavoprotein reductase, which in turn reduces the oxidized cytochrome P450-drug complex (step 2).

• 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 P450-substrate complex (step 3).

• This complex in turn transfers “activated” oxygen to the drug substrate to form the oxidized product (step 4).

• The potent oxidizing properties of this activated oxygen permitS 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 (Table 4—1).
• **CYTOCHROME-P450 COMPLEX:**

• There are multiple isotypes.
  - **CYT-P450-2** and **CYT-P450-3A** are responsible for the metabolism of most drugs.
  - **CYT-P450-3A4** metabolizes many drugs in the GI-Tract, where it decreases the bioavailability of many orally absorbed drugs.

• **INDUCERS of CYT-P450 COMPLEX:** Drugs that increase the production of Cytochrome-P450 enzymes.
  - **ANTI-CONVULSANTS:** Phenobarbitol, Phenytoin, Carbamazepine induce CYT-P450-3A4
  - Phenobarbitol, Phenytoin also induce CYT-P450-2B1
  - Polycyclic Aromatics (PAH): Induce CYT-P450-1A1
  - Glucocorticoids induce CYT-P450-3A4
  - Chronic Alcohol, Isoniazid induce CYT-P450-2E1. This is important as this drug activates some carcinogens such as Nitrosamines.
    • Chronic alcoholics have up-regulated many of their CYT-P450 enzymes.
Factors Impacting Drug Metabolism

Enzyme Induction
- Induction of both phase I (P450) and phase II enzymes
  - Generally increased transcription
  - Change in spectrum of expressed drug metabolizing enzymes
- Polycyclic aromatic hydrocarbons (dioxin)
  - CYP1A and CYP1B and UGT
  - Ah receptor (AhR) and AhR nuclear translocator (Arnt)
- CYP1A↑↑: Charcoal Broiled foods. Cruciferous vegetables
- CYP3A↓↓: Grapefruit juices

Drug –Drug Interactions During Metabolism:
- Drug –Drug Interactions may involve induction or inhibition
- Lipophilicity: at active site; Competitive inhibition.
  ∧ ↑↑ metabolism: own and others.
- Genetic variability (Pharmacogenetics)
- Age differences
  - Differential susceptibility due to differences in expression with age
• **Gender differences**
  – Minimal impact in human

• **Drug/Drug Interactions**

• Infants: Sulfation well developed but not glucuronidation


• Phenytoin; by 2 weeks surpasses adults.

• **Disease states resulting in loss of metabolic capacity**
  – Fatty liver disease, hepatitis, cirrhosis
  – Cancer

• High Extraction Ratio CL affected by hepatic blood flow:

  • **Cirrhosis CHF**: ↓↓ CL (Liver extraction ratio)

• Low extraction Ratio drugs: and low affinity for plasma protein. (e.g. Theophylline, Acetaminophen, chlooramph)

• Metabolism affected by hepatocellular function or plasma protein binding.

• Viral hepatitis ↓↓ Cl by 45% in children.

• Polmonary diseases:

• **Thyroid dysfunction**:
• **Inducers:**
  - The cytochrome P450-dependent enzymes are an important target for pharmacokinetic drug interactions.
  - One such interaction is the induction of selected CYP isozymes.
  - Certain drugs, most notably phenobarbital, rifampin, and carbamazepine, are capable of increasing the synthesis of one or more CYP isozymes.
  - The increased biotransformation rates can lead to significant decreases in plasma concentrations of drugs as measured by AUC, with concurrent loss of pharmacologic effect.
  - For example, rifampin, an antituberculosis drug significantly decreases the plasma concentrations of HIV protease inhibitors, diminishing their ability to suppress HIV viron maturation.
  - Figure 1.16 lists some of the more important inducers for representative CYP isozymes.
### Isozyme: CYP2C9/10

<table>
<thead>
<tr>
<th>COMMON SUBSTRATES</th>
<th>INDUCERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin</td>
<td>Phenobarbital</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Rifampin</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td></td>
</tr>
<tr>
<td>Tolbutamide</td>
<td></td>
</tr>
</tbody>
</table>

### Isozyme: CYP2D6

<table>
<thead>
<tr>
<th>COMMON SUBSTRATES</th>
<th>INDUCERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desipramine</td>
<td></td>
</tr>
<tr>
<td>Imipramine</td>
<td></td>
</tr>
<tr>
<td>Haloperidol</td>
<td></td>
</tr>
<tr>
<td>Propanolol</td>
<td></td>
</tr>
</tbody>
</table>

### Isozyme: CYP3A4/5

<table>
<thead>
<tr>
<th>COMMON SUBSTRATES</th>
<th>INDUCERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Dexamethasone</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Phenobarbital</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>Verapamil</td>
<td>Rifampin</td>
</tr>
</tbody>
</table>

Figure 1.16 Some representative P450 isozymes.
Enzyme Induction

Some drugs have the ability to increase the rate of their metabolism.

The enzyme induction process is one mechanism for producing tolerance (where increasing dose needed to maintain same level of drug in plasma and produce same behavioral effect).

An interesting feature of some of these chemically dissimilar drug substrates is their ability, on repeated administration, to “induce” cytochrome P450 by enhancing the rate of its synthesis or reducing its rate of degradation.

Induction results in an acceleration of metabolism and usually in a decrease in the pharmacologic action of the inducer and also of coadministered drugs.

However, in the case of drugs metabolically transformed to reactive metabolites, enzyme induction may exacerbate metabolite-mediated tissue toxicity.
Various substrates appear to induce isoforms of cytochrome P450 having different molecular masses and exhibiting different substrate specificities and immunochemical and spectral characteristics.

The two isoforms that have been most extensively studied are CYP2B1 (cytochrome P450 2B 1, which is induced by treatment with phenobarbital; and CYPIA1 which is induced by polycyclic aromatic hydrocarbons (PAHs), of which benzo[A] pyrene and 3-methylcholanthrene are prototypes.

In addition, glucocorticoids, macrolide antibiotics, anticonvulsants, and some steroids induce specific isoforms called CYP3A, the most abundant isoforms in the human liver.
• Isoniazid or chronic ethanol administration induces a different isoform, CYP2E1, that oxidizes ethanol and activates carcinogenic nitrosamines.

• The VLDL-lowering drug clofibrate induces other distinct enzymes of the CYP4A class that are responsible for ω-hydroxylation of several fatty acids, leukotrienes, and prostaglandins.

• Environmental pollutants are capable of inducing cytochrome P450 enzymes. For example, exposure to benzo[a]pyrene and other polycyclic aromatic hydrocarbons, which are present in tobacco smoke, charcoal-broiled meat, and other organic pyrolysis products, is known to induce CYP1A enzymes and to alter the rates of drug metabolism in both experimental animals and in humans.

• Other environmental chemicals known to induce specific cytochromes P450 include the polychlorinated biphenyls (PCBs), which were used widely in industry as insulating materials and plasticizers, and 2,3,7,8-tetrachlorodibenzo-p-dioxin toxin, TCDD), a trace by-product of the chemical synthesis of the defoliant 2,4,5-T
• Increased P450 synthesis requires enhanced transcription and translation.

• P450 enzymes may also be induced by “stabilization,” ie, decreased degradation, as is the case with troleandomycin-mediated induction of CYP3A enzymes.
• Cytochrome P450 can be induced (increased in activity) by a number of drugs or chemicals.

• Induction occurs in response to the presence of a chemical which is metabolized by P450 (more enzyme is produced to handle the chemical load). Once the enzyme is induced, it will metabolize the "inducing agent" more rapidly. Because cytochrome P450 is not specific for the inducer, however, other drugs metabolized by the induced enzyme will also be biotransformed more rapidly.

• Alcohol tolerance is a common example of P450 (microsomal enzyme) induction. Alcohol is metabolized by P450. If a person hasn't been drinking alcohol regularly, perhaps two drinks will make him tipsy.

• If that same person were to consume two drinks daily for several weeks, it would likely take more than two drinks to achieve the same degree of intoxication. This occurs because the liver enzymes have been induced, causing the alcohol to be metabolized more rapidly to an inactive form.

• This same person would also metabolize any of a multitude of drugs more rapidly once the enzymes were induced, a common mechanism of drug interaction. Therefore, a dose of such a drug that was adequate a few months ago might have little or no effect following several weeks of increased alcohol consumption.
Summary of the P450 system:

- The P450 system is important for the metabolism of many endogenous compounds (steroids, lipids, etc.) and for the biotransformation of exogenous substances.

- Cytochrome P450, designated as CYP, is composed of many families of heme-containing isozymes that are located in most cells, but mainly those in the liver and GI tract.

- The family name is indicated by a number followed by a capital letter for the subfamily (for example, CYP3A). Another number is added to indicate the specific isozyme (CYP3A4).

- Six isozymes are responsible for the vast majority of P450 catalyzed reactions: CYP3A4, CYP2D6, CYP2C9/10, CYP2C19, CYP2E1, and CYP1A2. The percentages of currently available drugs that are substrates for these isozymes is 60, 25, 15, 15, 2, and 2 percent, respectively.

- [Note: An individual drug may be a substrate for more than one isozyme.]
Considerable amounts of CYP3A4 are found in intestinal mucosa, accounting for first-pass metabolism of drugs such as chlorpromazine and clonazepam.

As might be expected, these enzymes exhibit considerable genetic variability, which has implications for individual dosing regimens, and, even more importantly, as determinants of therapeutic responsiveness and the risk of adverse events.

CYP2D6, in particular, has been shown to exhibit genetic polymorphism. Mutations result in very low capacities to metabolize substrates. Some individuals, for example, obtain no benefit from the opioid analgesic codeine, because it must be O-demethylated for activation.

This reaction is CYP2D6-dependent. The frequency of this polymorphism is racially determined, with a prevalence of five to ten percent in European Caucasians as compared to less than two percent of Southeast Asians.

Similar polymorphisms have been characterized for the CYP2C subfamily of isozymes. Although CYP3A4 exhibits a greater than ten-fold interindividual variability, no polymorphisms have been identified for this P450 isozyme.
• INHIBITORS of CYT-P450 COMPLEX: Drugs that inhibit the production of Cytochrome-P450 enzymes.
  
  – Acute Alcohol suppresses many of the CYT-P450 enzymes, explaining some of the drug-interactions of acute alcohol use.

  – Erythromycin, Ketanazole inhibit CYT-P450-3A4.
    • Terfenadine (Seldane) is metabolized by CYT-P450-3A4, so the toxic unmetabolized form builds up in the presence of Erythromycin. The unmetabolized form is toxic and causes lethal arrhythmias. This is why Seldane was taken off the market.

  – Chloramphenicol, Cimetidine, Disulfiram also inhibit CYT-P450's.
• **Inhibitors:** Inhibition of CYP isozyme activity is an important source of drug interactions that leads to serious adverse events. The most common form of inhibition is through competition for the same isozyme. Some drugs, however, are capable of inhibiting reactions for which they are not substrates (for example, ketoconazole).

• Numerous drugs have been shown to inhibit one or more of the CYP-dependent biotransformation pathways of warfarin. For example, omeprazole is a potent inhibitor of three of the CYP isozymes responsible for warfarin metabolism. If the two drugs are taken together, plasma concentrations of warfarin increase, which leads to greater inhibition of coagulation and risk of hemorrhage and serious bleeding reactions.

• [Note: The more important CYP inhibitors are erythromycin, ketoconazole, and ritonavir, because they each inhibit several CYP isozymes.].
• **Enzyme Inhibition**

• Certain drug substrates may inhibit cytochrome P450 enzyme activity.

• Imidazole-containing drugs such as cimetidine and ketoconazole bind tightly to the heme iron of cytochrome P450 and effectively reduce the metabolism of endogenous substrates (testosterone) or other coadministered drugs through competitive inhibition.

• However, *macrolide antibiotics* such as troleandomycin, erythromycin, and other erythromycin derivatives are metabolized, apparently by CYP3A, to metabolites that complex the cytochrome heme-iron and render it catalytically inactive.

• Another compound that acts through this mechanism is the well-known inhibitor proadifen (SKF-525-A), which binds tightly to the heme-iron and quasi irreversibly inactivates the enzyme, thereby inhibiting the metabolism of potential substrates.
• Some substrates irreversibly inhibit cytochrome P450 via covalent interaction of a metabolically generated reactive intermediate that may react with the apoprotein or the heme moiety of the cytochrome or even cause the heme to fragment and irreversibly modify the apoprotein.

• The antibiotic chloramphenicol is metabolized by cytochrome P450 to a species that alkylates its protein and thus also in activates the enzyme.

• A growing list of inactivators that attack the heme or the protein moiety includes the steroids ethinyl estradiol, norethindrone, and spironolactone; the anesthetic agent fluroxene; the barbiturate allobarbital; the analgesic sedatives allylisopropyl acetylurea, diethylpentenamide, and ethchlorvynol; the solvent carbon disulfide; and propylthiouracil.

• On the other hand, the barbiturate secobarbital is found to in activate CYP2B 1 by alkylation of both its heme and protein moieties.
• Human Liver P450 Enzymes

• Immunoblotting analyses—coupled with the use of relatively selective functional markers and selective P450 inhibitors—have identified numerous P450 iso-forms (CYP1A2, 2A6, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5, 4A1 1 and 7) in human liver microsomal preparations.

• Of these, CYP1A2, 2A6, 2C9, 2D6, 2E1, and 3A4 appear to be the major forms, accounting for approximately, 12, 4, 20, 4, 6, and 28 percent, respectively, of the total human liver P450 content.

• Together they are responsible for catalyzing the bulk of the hepatic drug and xenobiotic metabolism (Table 4—2).
<table>
<thead>
<tr>
<th>CYP</th>
<th>Substrates</th>
<th>Inducers</th>
<th>Noninvasive Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>Acetaminophen, antipyrine, caffeine, clomipramine, phenacetin, tamoxifen, theophylline, warfarin</td>
<td>Smoking, charcoal-broiled foods, cruciferous vegetables, omeprazole</td>
<td>Caffeine</td>
</tr>
<tr>
<td>2A6</td>
<td>Coumarin</td>
<td></td>
<td>Coumarin</td>
</tr>
<tr>
<td>2C9</td>
<td>Hexobarbital, ibuprofen, phenytoin, tolbutamide, trimethadione, sulfaphenazole, S-warfarin, ticrynafen</td>
<td>Barbiturates, rifampin</td>
<td>Tolbutamide, warfarin</td>
</tr>
<tr>
<td>2C19</td>
<td>Diazepam, S-mephenytoin, naproxen, nirvanol, omeprazole, propranolol</td>
<td>Barbiturates, rifampin</td>
<td>S-mephenytoin</td>
</tr>
<tr>
<td>2D6</td>
<td>Bufuralol, bupranolol, clomipramine, clozapine, codeine, debrisoquin, dextromethorphan, encainide, flecainide, fluoxetine, guanoxan, haloperidol, hydrocodone, 4-methoxyamphetamine, metoprolol, mexitelinate, oxycodone, paroxetine, phenformin, propafenone, propoxyphene, risperidone, selegiline (deprenil), sparteine, thioridazine, timolol, tricyclic antidepressants</td>
<td>None known</td>
<td>Debrisoquin, dextromethorphan</td>
</tr>
<tr>
<td>2E1</td>
<td>Acetaminophen, chlorzoxazone, enflurane, halothane, ethanol (a minor pathway)</td>
<td>Ethanol, isoniazid</td>
<td>Chlorzoxazone</td>
</tr>
<tr>
<td>3A4</td>
<td>Acetaminophen, alfentanil, amiodarone, astemizole, cocaine, cortisol, cyclosporine, dapsone, diazepam, dihydroergotamine, dihydropyridines, diltiazem, ethinyl estradiol, gestodene, indinavir, lidocaine, lovastatin, macrolides, methadone, miconazole, midazolam, mifepristone (RU 486), paclitaxel, progesterone, quinidine, rapamycin, ritonavir, saquinavir, spironolactone, sulfamethoxazole, sufentanil, tacrolimus, tamoxifen, terfenadine, testosterone, tetrahydrocannabinol, triazolam, troleandomycin, verapamil</td>
<td>Barbiturates, carbamazepine, glucocorticoids, pioglitazone, phenytoin, rifampin</td>
<td>Erythromycin, 6β-hydroxycortisol</td>
</tr>
</tbody>
</table>
• It is noteworthy that CYP3A4 alone is responsible for more than 60% of the clinically prescribed drugs metabolized by the liver.

• The involvement of individual P450s in the metabolism of a given drug may be screened in vitro by means of selective functional markers, selective chemical P450 inhibitors, and anti-P450 antibodies.

• In vivo, such screening may be accomplished by means of relatively selective noninvasive markers, which include breath tests or urinary analyses of specific metabolites after administration of a P450-selective substrate probe.
• **BIOTRANSFORMATION**: Alteration of drugs by the liver. Drugs can be metabolized from active to inactive, or from inactive to active. Generally drugs are made more hydrophilic by the process.

• **PHASE I: Mixed-Function Oxidases**, formed by **microsomes** made out of Smooth-ER folded over on itself.
  
  – Cytochrome-P450 Enzyme Complex: Has four required components in order to work.
    
    • Cytochrome-P450 Enzyme
    • Cytochrome-P450 Reductase
    • O2
    • NADPH: NADPH is the only energy source. No ATP is required!
  
  – Phase I enzymes perform multiple types of reactions:
    
    • **OXIDATIVE REACTIONS**: on drugs, such as: Aromatic hydroxylation, aliphatic hydroxylation, N-dealkylation, O-dealkylation, S-dealkylation, N-Oxidation, S-Oxidation, Desulfuration.
    
    • **REDUCTIVE REACTIONS**: Azo, Nitrile, Carbamyl
    
    • **HYDROLYTIC REACTIONS**: Ester hydrolysis, Amide hydrolysis.

• **PHASE II**: Drug Conjugation. usually to **glucuronides**, making the drug more soluble.
• CYTOCHROME-P450 COMPLEX:

• There are multiple isotypes.
  – CYT-P450-2 and CYT-P450-3A are responsible for the metabolism of most drugs.
  – CYT-P450-3A4 metabolizes many drugs in the GI-Tract, where it decreases the bioavailability of many orally absorbed drugs.

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  – Glucocorticoids induce CYT-P450-3A4
  – Chronic Alcohol, Isoniazid induce CYT-P450-2E1. This is important as this drug activates some carcinogens such as Nitrosamines.
    • Chronic alcoholics have up-regulated many of their CYT-P450 enzymes.
• **INHIBITORS of CYT-P450 COMPLEX:** Drugs that inhibit the production of Cytochrome-P450 enzymes.

- **Acute Alcohol** suppresses many of the CYT-P450 enzymes, explaining some of the drug-interactions of acute alcohol use.

- **Erythromycin, Ketanazole** inhibit CYT-P450-3A4.
  - **Terfenadine** (Seldane) is metabolized by CYT-P450-3A4, so the toxic unmetabolized form builds up in the presence of Erythromycin. The unmetabolized form is toxic and causes lethal arrhythmias. This is why Seldane was taken off the market.

- **Chloramphenicol, Cimetidine, Disulfiram** also inhibit CYT-P450's.
METABOLISM OF DRUGS TO TOXIC PRODUCTS

It is becoming increasingly evident that metabolism of drugs and other foreign chemicals may not always be an innocuous biochemical event leading to detoxification and elimination of the compound.

Indeed, several compounds have been shown to be metabolically transformed to reactive intermediates that are toxic to various organs. Such toxic reactions may not be apparent at low levels of exposure to parent compounds when alternative detoxification mechanisms are not yet overwhelmed or compromised and the availability of endogenous detoxifying cosubstrates (glutathione, glucuronic acid, sulfate) is not limited.

However, when these resources are exhausted, the toxic pathway may prevail, resulting in overt organ toxicity or carcinogenesis. The number of specific examples of such drug-induced toxicity is expanding rapidly.

An example is acetaminophen (paracetamol)-induced hepatotoxicity (Figure 4—4).
• This analgesic antipyretic drug is quite safe in therapeutic doses (1.2 g/d for an adult).
• It normally undergoes glucuronidation and sulfation to the corresponding conjugates, which together comprise 95% of the total excreted metabolites.
• The alternative cytochrome P450-dependent glutathione (GSH) conjugation pathway accounts for the remaining 5%.
• When acetaminophen intake far exceeds therapeutic doses, the glucuronidation and sulfation pathways are saturated, and the cytochrome P450-dependent pathway becomes increasingly important.
• Little or no hepatotoxicity results as long as glutathione is available for conjugation.
• However, with time, hepatic glutathione is depleted faster than it can be regenerated, and accumulation of a reactive and toxic metabolite occurs.
Figure 4–4. Metabolism of acetaminophen (Ac) to hepatotoxic metabolites. (GSH, glutathione; GS, glutathione moiety; Ac*, reactive intermediate.)
• In the absence of **intracellular nucleophiles such as glutathione**, this reactive metabolite (thought to be an N-hydroxylated product or an N-acetylbenzoiminoquinone) reacts with nucleophilic groups present on cellular macromolecules such as protein, resulting in **hepatotoxicity** (Figure 4-4).

• The chemical and toxicologic characterization of the electrophilic nature of the reactive acetaminophen metabolite has led to the development of **effective antidotes** —cysteamine and N-acetylcysteine.

• **Administration of N-acetylcysteine** (the safer of the two) within 8—16 hours following acetaminophen over-dosage has been shown to protect victims from fulminant hepatotoxicity and death.

• Similar mechanistic interpretations can be invoked to explain the nephrotoxicity of phenacetin and the hepatotoxicity of aflatoxin.
• MASTER THE METABOLISM OF ACETAMENOPHEN (PARACETAMOL). It is clinically very important!
• **CLINICAL RELEVANCE OF DRUG METABOLISM**

• The dose and the frequency of administration required to achieve effective therapeutic blood and tissue levels vary in different patients because of **individual differences in drug distribution and rates of drug metabolism and elimination.**

• These differences are determined by genetic factors and nongenetic variables such as age, sex, liver size, liver function, circadian rhythm, body temperature, and nutritional and environmental factors such as concomitant exposure to inducers or inhibitors of drug metabolism.
Let us discuss the most important variables relating to drug metabolism that are of clinical relevance.

**Individual Differences**

Individual differences in metabolic rate depend on the nature of the drug itself.

Thus, within the same population, steady state plasma levels may reflect a 30-fold variation in the metabolism of one drug and only a twofold variation in the metabolism of another.
• Genetic Factors

• Genetic factors that influence enzyme levels account for some of these differences. **Succinylcholine**, for example, is metabolized only half as rapidly in persons with genetically determined defects in pseudocholinesterase as in persons with normally functioning pseudocholinesterase.

• Analogous pharmacogenetic differences are seen in the acetylation of **isoniazid** (Figure 4—5) and the hydroxylation of **warfarin**. The defect in slow acetylators (of isoniazid and similar amines) appears to be caused by the synthesis of less of the enzyme rather than of an abnormal form of it.

• Inherited as an autosomal recessive trait, the slow acetylator phenotype occurs in about 50% of blacks and whites in the USA, more frequently in Europeans living in high northern latitudes, and much less commonly in Asians and Inuits (Eskimos).

• Similarly, genetically determined defects in the oxidative metabolism of debrisoquin, phenacetin, guanoxan, sparteine, phenformin, and others have been reported (Table 4—4).

• The defects are apparently transmitted as autosomal recessive traits and may be expressed at any one of the multiple metabolic transformations that a chemical might undergo in vivo.
Table 4-4. Some examples of genetic polymorphisms in drug metabolism.

<table>
<thead>
<tr>
<th>Defect</th>
<th>Drug and Therapeutic Use</th>
<th>Clinical Consequences¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidation</td>
<td>Bufuralol (β-adrenoceptor blocker)</td>
<td>Exacerbation of β-blockade, nausea</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Debrisoquin (antihypertensive)</td>
<td>Orthostatic hypotension</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Ethanol</td>
<td>Facial flushing, cardiovascular symptoms</td>
</tr>
<tr>
<td>N-Acetylation</td>
<td>Hydralazine (antihypertensive)</td>
<td>Lupus erythematosus-like syndrome</td>
</tr>
<tr>
<td>N-Acetylation</td>
<td>Isoniazid (antitubercular)</td>
<td>Peripheral neuropathy</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Mephenytoin (antiepileptic)</td>
<td>Overdose toxicity</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Sparteine</td>
<td>Oxytotic symptoms</td>
</tr>
<tr>
<td>Ester hydrolysis</td>
<td>Succinylcholine (neuromuscular blocker)</td>
<td>Prolonged apnea</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Tolbutamide (hypoglycemic)</td>
<td>Cardiotoxicity</td>
</tr>
</tbody>
</table>

¹Observed or predictable.
Three genetic variations of these drug metabolism polymorphisms have been particularly well investigated and so afford some insight into possible underlying mechanisms.

First is the debrisoquin-sparteine oxidation type of polymorphism, which apparently occurs in 3—10% of whites and is inherited as an autosomal recessive trait.

In affected individuals, the CYP2D6-dependent oxidations of debrisoquin and other drugs (Table 4—2) are impaired.

These defects in oxidative drug metabolism are probably co-inherited.

The precise molecular basis for the defect appears to be faulty expression of the cytochrome P450 protein, resulting in little or no isoform-catalyzed drug metabolism.
More recently, however, another polymorphic genotype has been reported that results in ultrarapid metabolism of relevant drugs due to the presence of 2D6 allelic variants with up to 13 gene copies in tandem.

This genotype is most common in Ethiopians and Saudi Arabians, populations that display it in up to one-third of individuals.

As a result, these subjects require twofold to threefold higher daily doses of nortriptyline (a 2D6 substrate) to achieve therapeutic plasma levels.

Conversely, in these ultrarapidly metabolizing populations, the pro-drug codeine (another 2D6 substrate) is metabolized much faster to morphine, often resulting in typical undesirable side effects of morphine such as severe abdominal pain.
A second well-studied genetic drug polymorphism involves the stereoselective aromatic (4)-hydroxylation of the anticonvulsant mephenytoin, catalyzed by CYP2C19.

This polymorphism, which is also inherited as an autosomal recessive trait, occurs in 3—5% of Caucasians and 18—23% of Japanese populations.

It is genetically independent of the debrisoquin-sparteine polymorphism.

In normal “extensive metabolizers,” (S)-mephenytoin is extensively hydroxylated by CYP2C19 at the 4 position of the phenyl ring before its glucuronidation and rapid excretion in the urine, whereas (R)-mephenytoin is slowly N-demethylated to nirvanol.
• “Poor metabolizers,” however, appear to totally lack the stereospecific (5)-mephenytoin hydroxylase activity, so both (5)- and (R)-mephenytoin enantiomers are N-demethylated to nirvanol before excretion.

• The molecular basis for this defect is a single base pair mutation (G —> A) in exon 5 of the CYP2C19 gene that creates an aberrant splice site, a correspondingly altered reading frame of the mRNA, and, finally, a truncated nonfunctional protein.

• It is clinically important to recognize that the safety of a drug may be severely reduced in individuals who are poor metabolizers.

• For example, poor metabolizers of mephenytoin show signs of profound sedation and ataxia after doses of the drug that are well tolerated by normal metabolizers.
The third genetic polymorphism recently characterized is that of CYP2C9.

Two variants of this enzyme exist, each with amino acid mutations that result in altered metabolism: CYP2C9*2 allele encodes an Arg144Cys mutation, exhibiting impaired functional interactions with P450 reductase.

The other allelic variant, CYP2C9*3, encodes an enzyme with an Ile359Leu mutation that has lowered affinity for many substrates. Consequently, individuals displaying the CYP2C9*3 phenotype have greatly reduced tolerance for the anticoagulant warfarin.

The warfarin clearance in CYP2C9*3 individuals is only 10% of normal values, and these people can tolerate only much smaller daily doses of the drug than those who are homozygous for the normal wild type allele.

These individuals also have a much higher risk of adverse effects with warfarin (eg, bleeding) and with other CYP2C9 substrates such as phenytoin, losartan, tolbutamide, and some NSAIDs.
Additional genetic polymorphisms in drug metabolism that are inherited independently from those already described are being discovered.

Studies of theophylline metabolism in monozygotic and dizygotic twins that included pedigree analysis of various families have revealed that a distinct polymorphism may exist for this drug and may be inherited as a recessive genetic trait.

Genetic drug metabolism polymorphisms also appear to occur for aminopyrine, and carbocysteine oxidations.

Although genetic polymorphisms in drug oxidations often involve specific cytochrome P450 enzymes, such genetic variations can occur at other sites.

The recent descriptions of a polymorphism in the oxidation of trimethylamine, believed to be metabolized largely by the flavin monooxygenase (Ziegler’s enzyme), suggest that genetic variants of other non-P450-dependent oxidative enzymes may also contribute to such polymorphisms.
• **Diet & Environmental Factors**

• Diet and environmental factors also contribute to individual variations in drug metabolism.

• **Charcoal-broiled foods and cruciferous vegetables** are known to induce CYP1A enzymes, whereas **grapefruit juice** is known to inhibit the CYP3A metabolism of coadministered drug substrates.

• **Cigarette smokers** metabolize some drugs more rapidly than nonsmokers because of enzyme induction.

• Industrial workers exposed to **some pesticides** metabolize certain drugs more rapidly than nonexposed individuals.

• Such differences make it difficult to determine effective and safe doses of drugs that have narrow therapeutic indices.
• **Age & Sex**

- Increased susceptibility to the pharmacologic or toxic activity of drugs has been reported in very young and old patients as compared with young adults.

- Studies in other mammalian species indicating that drugs are metabolized at reduced rates during the prepubertal period and senescence.

- **Slower metabolism could be due to reduced activity of metabolic enzymes or reduced availability of essential endogenous cofactors.** Similar trends have been observed in humans, but incontrovertible evidence is yet to be obtained.

- Young adult male rats metabolize drugs much faster than mature female rats or prepubertal male rats. These **differences in drug metabolism have been clearly associated with androgenic hormones.**

- A few clinical reports suggest that **sex-dependent differences in drug metabolism also exist in humans for ethanol, propranolol, benzodiazepines, estrogens, and salicylates.**
Drug-Drug Interactions During Metabolism

Many substrates, by virtue of their relatively high lipophilicility, are retained not only at the active site of the enzyme but remain nonspecifically bound to the lipid membrane of the endoplasmic reticulum. In this state, they may induce microsomal enzymes; depending on the residual drug levels at the active site, they also may competitively inhibit metabolism of a simultaneously administered drug.

Enzyme-inducing drugs include various sedative-hypnotics, tranquilizers, anticonvulsants, and insecticides (Table 4—5).
<table>
<thead>
<tr>
<th>Inducer</th>
<th>Drug Whose Metabolism Is Enhanced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[a]pyrene</td>
<td>Theophylline</td>
</tr>
<tr>
<td>Chlorcyclizine</td>
<td>Steroid hormones</td>
</tr>
<tr>
<td>Ethchlorvynol</td>
<td>Warfarin</td>
</tr>
<tr>
<td>Glutethimide</td>
<td>Antipyrene, glutethimide, warfarin</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>Warfarin</td>
</tr>
<tr>
<td>Phenobarbital and other barbiturates¹</td>
<td>Barbiturates, chloramphenicol, chlorpromazine, cortisol, coumarin anticoagulants, desmethyldiphenidilpramine, digitoxin, doxorubicin, estradiol, phenylbutazone, phenytoin, quinine, testosterone</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>Aminopyrine, cortisol, digitoxin</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Cortisol, dexamethasone, digitoxin, theophylline</td>
</tr>
<tr>
<td>Rifampin</td>
<td>Coumarin anticoagulants, digitoxin, glucocorticoids, methadone, metoprolol, oral contraceptives, prednisone, propranolol, quinidine</td>
</tr>
</tbody>
</table>

¹Secobarbital is an exception. See Table 4–6 and text.
Patients who routinely ingest barbiturates, other sedative-hypnotics, or tranquilizers may require considerably higher doses of warfarin, when being treated with this oral anticoagulant, to maintain a prolonged prothrombin time.

On the other hand, discontinuance of the sedative may result in reduced metabolism of the anticoagulant and bleeding—a toxic effect of the enhanced plasma levels of the anticoagulant. (a paradox)

Similar interactions have been observed in individuals receiving various combination drug regimens such as antipsychotics or sedatives with contraceptive agents, sedatives with anticonvulsant drugs, and even alcohol with hypoglycemic drugs (tolbutamide).
• It must also be noted that an inducer may enhance not only the metabolism of other drugs but also its own metabolism. Thus, continued use of some drugs may result in a pharmacokinetic type of tolerance—progressively reduced effectiveness due to enhancement of their own metabolism.

• Conversely, simultaneous administration of two or more drugs may result in impaired elimination of the more slowly metabolized drug and prolongation or potentiation of its pharmacologic effects (Table 4—6).
Table 4–6. Partial list of drugs that inhibit drug metabolism in humans.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Drug Whose Metabolism Is Inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allopurinol, chloramphenicol, isoniazid</td>
<td>Antipyrene, dicumarol, probenecid, tolbutamide</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>Chlordiazepoxide, diazepam, warfarin, others</td>
</tr>
<tr>
<td>Dicumarol</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>Diethylpentenamide</td>
<td>Diethylpentenamide</td>
</tr>
<tr>
<td>Disulfiram</td>
<td>Antipyrene, ethanol, phenytoin, warfarin</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Chlordiazepoxide (?), diazepam (?), methanol</td>
</tr>
<tr>
<td>Grapefruit juice&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Alprazolam, atorvastatin, cisapride, cyclosporine, midazolam, triazolam</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>Cyclosporine, astemizole, terfenadine</td>
</tr>
<tr>
<td>Nortriptyline</td>
<td>Antipyrene</td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>Antipyrene</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>Phenytoin, tolbutamide</td>
</tr>
<tr>
<td>Secobarbital</td>
<td>Secobarbital</td>
</tr>
<tr>
<td>Troleandomycin</td>
<td>Theophylline, methylprednisolone</td>
</tr>
</tbody>
</table>

<sup>1</sup>Active components in grapefruit juice include furanocoumarins such as 6', 7'-dihydroxybergamottin (which is known to inactivate both intestinal and liver CYP3A4) as well as other unknown components that inhibit p-glycoprotein-mediated intestinal drug efflux and consequently further enhance the bioavailability of certain drugs such as cyclosporine.
Both competitive substrate inhibition and irreversible substrate-mediated enzyme inactivation may augment plasma drug levels and lead to toxic effects from drugs with narrow therapeutic indices.

For example, it has been shown that erythromycin inhibits the metabolism of the antihistamine terfenadine and leads to the expression of adverse effects such as cardiac arrhythmias. Similarly, allopurinol both prolongs the duration and enhances the chemotherapeutic action of mercaptopurine by competitive inhibition of xanthine oxidase.

Consequently, to avoid bone marrow toxicity, the dose of mercaptopurine is usually reduced in patients receiving allopurinol. Cimetidine, a drug used in the treatment of peptic ulcer, has been shown to potentiate the pharmacologic actions of anticoagulants and sedatives. The metabolism of chlordiazepoxide has been shown to be inhibited by 63% after a single dose of cimetidine; such effects are reversed within 48 hours after withdrawal of cimetidine.

Impairment of metabolism may also result if a simultaneously administered drug irreversibly inactivates a common metabolizing enzyme, as is the case with secobarbital or diethyipentenamide overdoses. These compounds, in the course of their metabolism by cytochrome P450, inactivate the enzyme and result in impairment of their own metabolism and that of other cosubstrates.
• Interactions Between Drugs & Endogenous Compounds

• Various drugs require conjugation with endogenous substrates such as glutathione, glucuronic acid, and sulfate for their inactivation.

• Consequently, different drugs may compete for the same endogenous substrates, and the faster-reacting drug may effectively deplete endogenous substrate levels and impair the metabolism of the slower-reacting drug.

• If the latter has a steep dose-response curve or a narrow margin of safety, potentiation of its pharmacologic and toxic effects may result.
• Diseases Affecting Drug Metabolism
  • Acute or chronic diseases that affect liver architecture or function markedly affect hepatic metabolism of some drugs.
  • Such conditions include fat accumulation, alcoholic hepatitis, active or inactive alcoholic cirrhosis, hemochromatosis, chronic active hepatitis, biliary cirrhosis, and acute viral or drug-induced hepatitis.
  • Depending on their severity, these conditions impair hepatic drug-metabolizing enzymes, particularly microsomal oxidases, and thereby markedly affect drug elimination.
  • For example, the half-lives of chlordiazepoxide and diazepam in patients with liver cirrhosis or acute viral hepatitis are greatly increased, with a corresponding prolongation of their effects.
  • Consequently, these drugs may cause coma in patients with liver disease when given in ordinary doses.
Liver cancer has been reported to impair hepatic drug metabolism in humans.

For example, aminopyrine metabolism is slower in patients with malignant hepatic tumors than in normal controls.

These patients also exhibit markedly diminished aminopyrine clearance.

Studies of liver biopsy specimens from patients with hepatocellular carcinoma also indicate impaired ability to oxidatively metabolize drugs in vitro.

This is associated with a correspondingly reduced cytochrome P450 content.
• Cardiac disease, by limiting blood flow to the liver, may impair disposition of those drugs whose metabolism is flow-limited (Table 4—7).

• These drugs are so readily metabolized by the liver that hepatic clearance is essentially equal to liver blood flow.
Table 4–7. Rapidly metabolized drugs whose hepatic clearance is blood flow-limited.

| Alprenolol | Lidocaine |
| Amiotriptyline | Meperidine |
| Clomethiazole | Morphine |
| Desipramine | Pentazocine |
| Imipramine | Propoxyphene |
| Isoniazid | Propranolol |
| Labetalol | Verapamil |
Pulmonary disease may also affect drug metabolism as indicated by the impaired hydrolysis of procainamide and procaine in patients with chronic respiratory insufficiency and the increased half-life of antipyrine in patients with lung cancer.

Impairment of enzyme activity or defective formation of enzymes associated with heavy metal poisoning or porphyria also results in reduction of hepatic drug metabolism.

For example, lead poisoning has been shown to increase the half-life of antipyrine in humans.
Thyroid dysfunction has been associated with altered metabolism of some drugs and of some endogenous compounds as well.

Hypothyroidism increases the half-life of antipyrine, digoxin, methimazole, and practolol, whereas hyperthyroidism has the opposite effect.

A few clinical studies in diabetic patients indicate no apparent impairment of drug metabolism, as reflected by the half-lives of antipyrine, tolbutamide, and phenylbutazone.
• Disease states resulting in loss of metabolic capacity
  – Fatty liver disease, hepatitis, cirrhosis
  – Cancer
• High Extraction Ratio CL affected by hepatic blood flow:
• **Cirrhosis CHF**: ↓↓ CL (Liver extraction ratio)

• Low extraction Ratio drugs: and low affinity for plasma protein. (e.g. Theophylline, Acetaminophen, chlooramph)
• Metabolism affected by hepatocellular function or plasma protein binding.
• **Viral hepatitis**: ↓↓ Cl by 45% in children.