

# **Drug Biotransformation**

**Yacoub Irshaid MD, PhD, ABCP**

**Department of Pharmacology, FOM, UJ  
2016**

# **Drug Biotransformation**

- **Humans are exposed always to foreign compounds called xenobiotics, through the GIT, skin, lung, etc.**
- **Xenobiotics include drugs, environmental toxins and industrial toxins.**
- **Exposure may be accidental or intentional.**

# Drug Biotransformation

- Renal excretion plays an important role in removing xenobiotics from the body, and thus, terminating their biologic activity.
- Xenobiotics undergoing renal excretion are usually **small molecules**, that **possess polar characteristics**, or that are **ionized at physiologic pH**.

# Drug Biotransformation

- Many drugs are not polar and tend to be lipophilic at physiologic pH, and are readily reabsorbed from the glomerular filtrate in the nephron.
- Some lipophilic drugs bound to plasma proteins are not readily filtered at the glomerulus.
- An alternative process for elimination of such drugs is metabolism.

# Drug Biotransformation

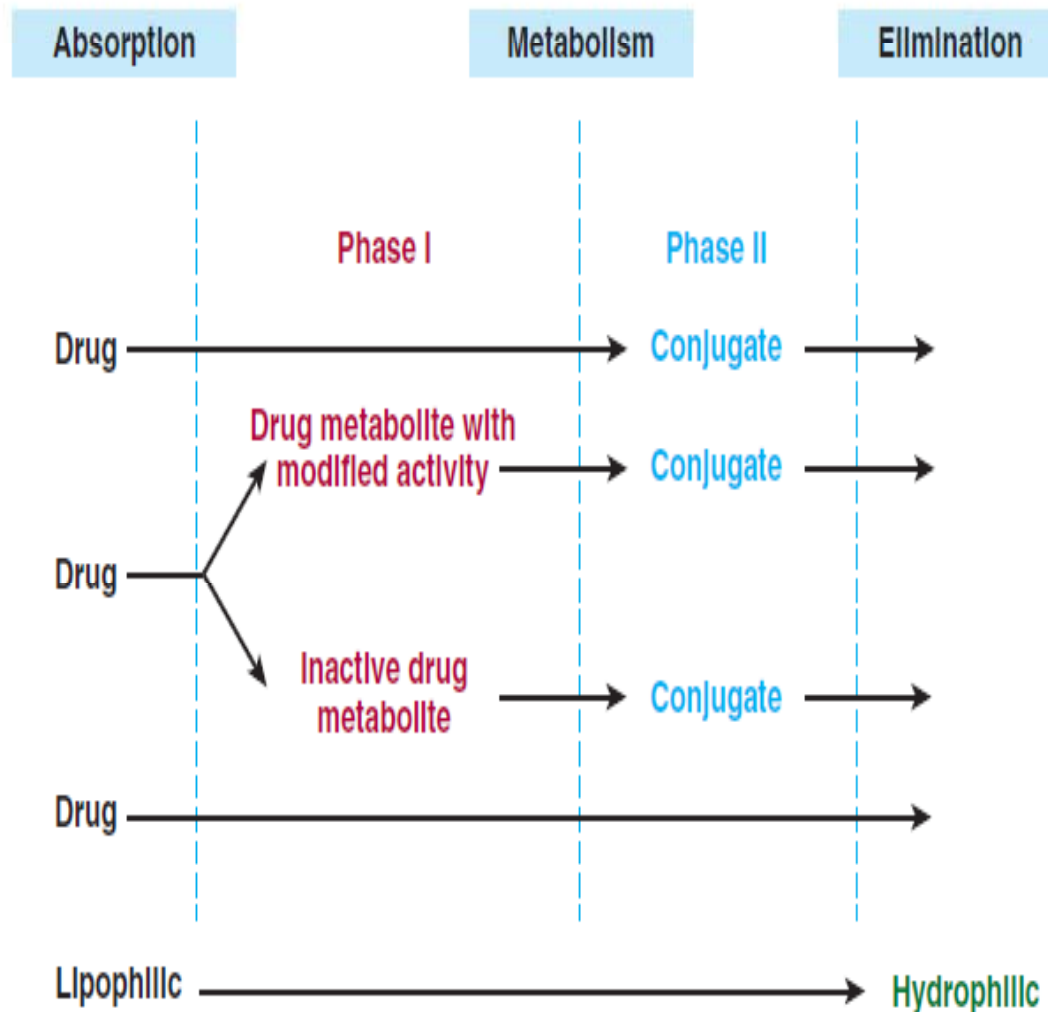
- In general, lipophilic drugs are transformed to more polar and hence more readily excreted products.
- Metabolic products are often less pharmacodynamically active than the parent drug and may be even inactive.
- There are exception to this rule:
  1. Some biotransformation products have enhanced activity or even toxicity.

# **Drug Biotransformation**

- 2. Some drugs are inactive and need activation by metabolism (prodrugs) like levodopa, codeine.**
- 3. Some drugs are metabolized into toxins. Paracetamol may be converted to the hepatotoxin N-acetyl-p-benzoquinone imine. Halothane is metabolized to free radicals that are hepatotoxic.**

# Drug Biotransformation

- Few biotransformation reactions occur in intestinal lumen or intestinal wall.
- Biotransformation reactions can be classified as **phase I** or **phase II** reactions.



**FIGURE 4-1** Phase I and phase II reactions, and direct elimination, in drug biodisposition. Phase II reactions may also precede phase I reactions.



# **Drug Biotransformation**

- **Phase I reactions usually convert the drug to more polar metabolites by introducing (or unmasking) a functional group (- OH, - NH<sub>2</sub>, - SH).**
- **The increase in polarity may facilitate renal excretion.**
- **These metabolites are often inactive. Some times activity may be lower or higher than that of the parent compound.**

# Drug Biotransformation

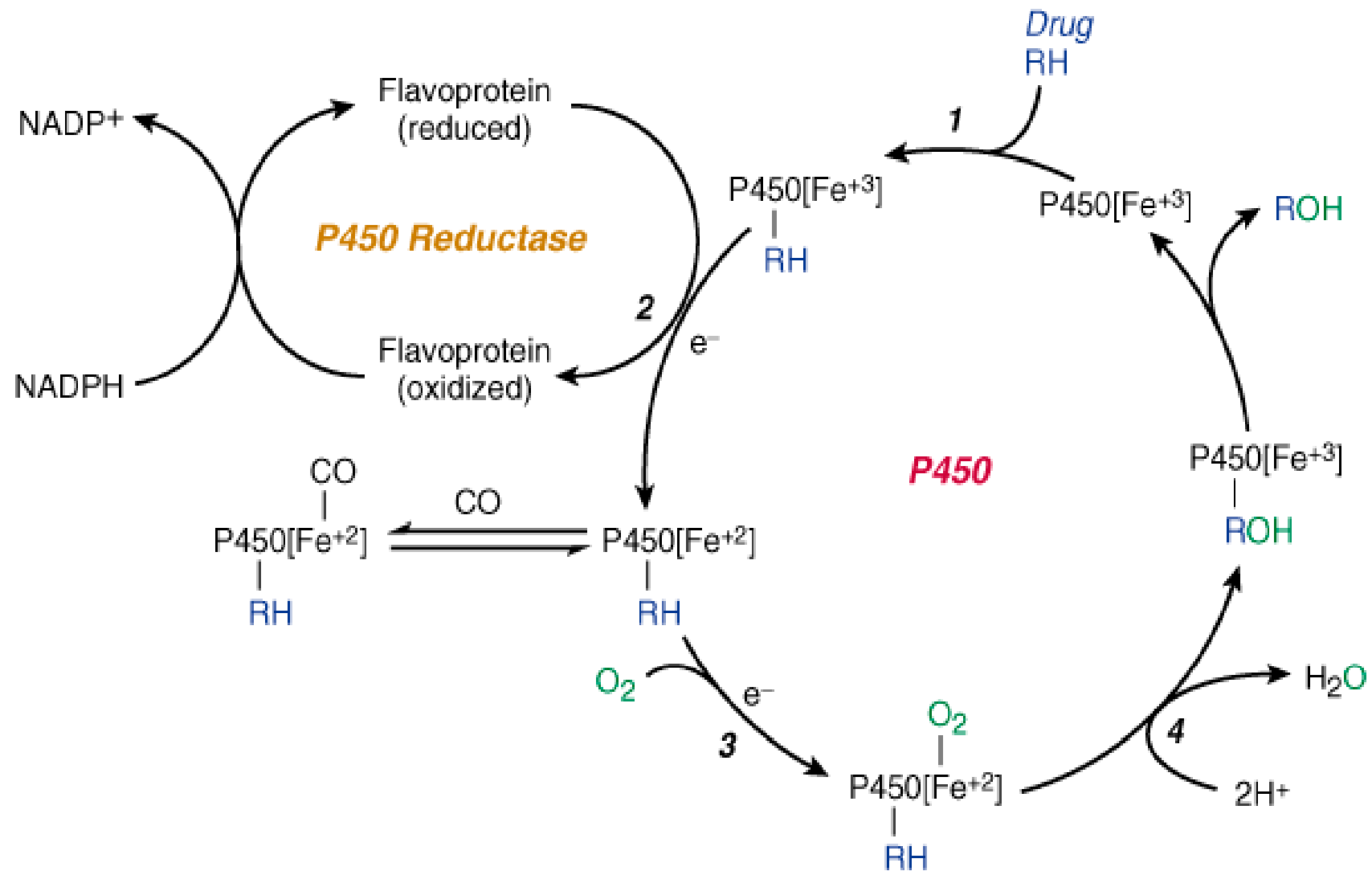
- Many phase I products are not eliminated rapidly and may need a subsequent reaction to become polar enough to be readily excreted.
- Such subsequent reactions involve **conjugation with an endogenous substrate** such as glucuronic acid, sulfuric acid, ..., which are called **phase II reactions**.

# **Phase I Biotransformation reactions**

- 1. Oxidations**
  - 2. Reductions**
  - 3. Hydrolysis**
- Most oxidation-reduction reactions in drug metabolism are carried out by the microsomal mixed function oxidase system or cytochromes P450 enzymes.**

# **Phase I Biotransformation reactions**

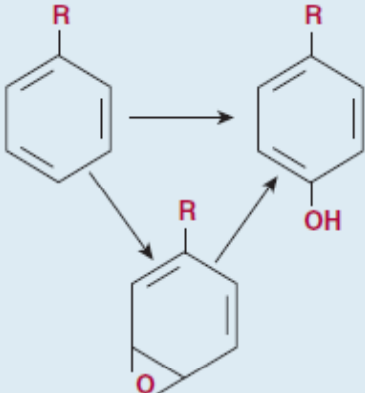
- **Cytochrome P450 enzymes are located in the endoplasmic reticulum.**
- **They have very low substrate specificity, and slow reaction rates.**
- **High lipid solubility is common to the wide variety of structurally unrelated drugs metabolized by this system.**



Source: Katzung BG, Masters SB, Trevor AJ: *Basic & Clinical Pharmacology*, 11th Edition: <http://www.accessmedicine.com>

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**TABLE 4-1** Phase I reactions.

Reaction Class	Structural Change	Drug Substrates
<b>Oxidations</b>		
<i>Cytochrome P450-dependent oxidations:</i>		
Aromatic hydroxylations		Acetanilide, propranolol, phenobarbital, phenytoin, phenylbutazone, amphetamine, warfarin, 17 $\alpha$ -ethinyl estradiol, naphthalene, benzpyrene
Aliphatic hydroxylations	$\begin{array}{l} \text{RCH}_2\text{CH}_3 \longrightarrow \text{RCH}_2\text{CH}_2\text{OH} \\ \text{RCH}_2\text{CH}_3 \longrightarrow \text{RCH}(\text{OH})\text{CH}_3 \end{array}$	Amobarbital, pentobarbital, secobarbital, chlorpropamide, ibuprofen, meprobamate, glutethimide, phenylbutazone, digitoxin
Epoxidation	$\text{RCH}=\text{CHR} \longrightarrow \text{R}-\overset{\text{H}}{\underset{\text{O}}{\text{C}}}-\overset{\text{H}}{\underset{\text{O}}{\text{C}}}-\text{R}$	Aldrin
<b>Oxidative dealkylation</b>		
N-Dealkylation	$\text{RNHCH}_3 \longrightarrow \text{RNH}_2 + \text{CH}_2\text{O}$	Morphine, ethylmorphine, benzphetamine, aminopyrine, caffeine, theophylline
O-Dealkylation	$\text{ROCH}_3 \longrightarrow \text{ROH} + \text{CH}_2\text{O}$	Codeine, <i>p</i> -nitroanisole
S-Dealkylation	$\text{RSCH}_3 \longrightarrow \text{RSH} + \text{CH}_2\text{O}$	6-Methylthiopurine, methitural

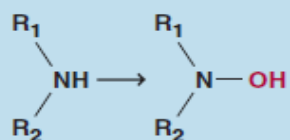
**N-Oxidation**

Primary amines



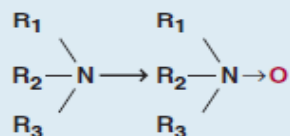
Aniline, chlorphentermine

Secondary amines

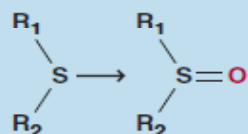


2-Acetylaminofluorene, acetaminophen

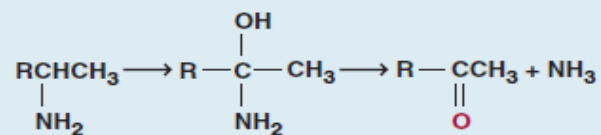
Tertiary amines



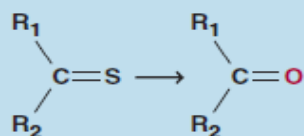
Nicotine, methaqualone

**S-Oxidation**

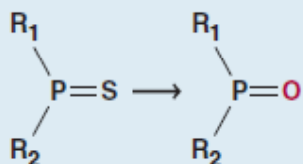
Thioridazine, cimetidine, chlorpromazine

**Deamination**

Amphetamine, diazepam

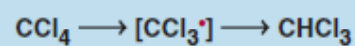
**Desulfuration**

Thiopental



Parathion

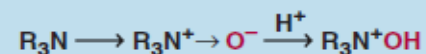
Dechlorination



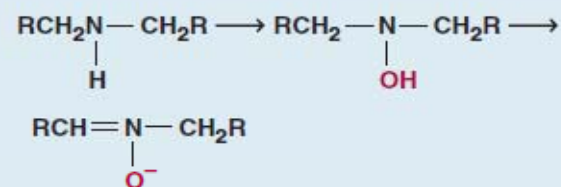
Carbon tetrachloride

### Cytochrome P450-independent oxidations:

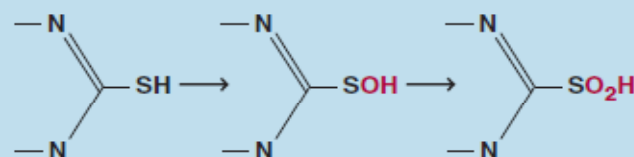
Flavin monooxygenase  
(Ziegler's enzyme)



Chlorpromazine, amitriptyline,  
benzphetamine

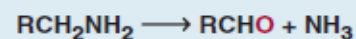


Desipramine, nortriptyline



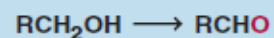
Methimazole, propylthiouracil

Amine oxidases



Phenylethylamine, epinephrine

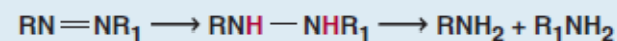
Dehydrogenations



Ethanol

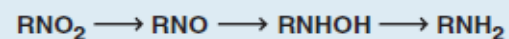
### Reductions

Azo reductions



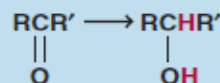
Prontosil, tartrazine

Nitro reductions



Nitrobenzene, chloramphenicol, clonazepam,  
dantrolene

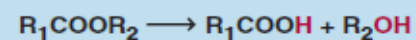
Carbonyl reductions



Metyrapone, methadone, naloxone

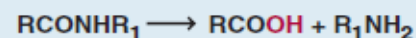
### Hydrolyses

Esters



Procaine, succinylcholine, aspirin, clofibrate,  
methylphenidate

Amides



Procainamide, lidocaine, indomethacin



# Human Liver P450 Enzymes

- There are numerous P450 isoenzymes.
- The most important are **CYP1A2**, **CYP2A6**, **CYP2B6**, **CYP2C8**, **CYP2C9**, **CYP2C18**, **CYP2C19**, **CYP2D6**, **CYP2E1**, and **CYP3A4**.

# Human Liver P450 Enzymes

- **CYP1A2**, **CYP2C9**, and **CYP3A4** account for **15%**, **20%**, and **30%** of the total human liver P450 content, respectively.
- **CYP3A4** alone is responsible for the metabolism of over **50% of prescription drugs** metabolized in the liver.

# Human Liver P450 Enzymes

- *Human liver P450s 3A4, 2C9, 2D6, 2C19, 1A2, and 2B6 are responsible for about 75% of all clinically relevant phase I drug metabolism, and thus for about 60% of all physiologic drug biotransformation and elimination.*



**TABLE 4–2 Human liver P450s (CYPs), and some of the drugs metabolized (substrates), inducers, and selective inhibitors.**

CYP	Substrates	Inducers	Inhibitors
<b>1A2</b>	Acetaminophen, antipyrine, caffeine, clomipramine, phenacetin, tacrine, tamoxifen, theophylline, warfarin	Smoking, charcoal-broiled foods, cruciferous vegetables, omeprazole	Galangin, furafylline, fluvoxamine
<b>2A6</b>	Coumarin, tobacco nitrosamines, nicotine (to cotinine and 2'-hydroxynicotine)	Rifampin, phenobarbital	Tranylcypromine, menthofuran, methoxsalen
<b>2B6</b>	Artemisinin, bupropion, cyclophosphamide, efavirenz, ifosfamide, ketamine, S-mephobarbital, S-mephenytoin (N-demethylation to nirvanol), methadone, nevirapine, propofol, selegiline, sertraline, ticlopidine	Phenobarbital, cyclophosphamide	Ticlopidine, clopidogrel
<b>2C8</b>	Taxol, all- <i>trans</i> -retinoic acid	Rifampin, barbiturates	Trimethoprim
<b>2C9</b>	Celecoxib, flurbiprofen, hexobarbital, ibuprofen, losartan, phenytoin, tolbutamide, trimethadione, sulfaphenazole, S-warfarin, ticrynafen	Barbiturates, rifampin	Tienilic acid, sulfaphenazole
<b>2C18</b>	Tolbutamide, phenytoin	Phenobarbital	
<b>2C19</b>	Diazepam, S-mephenytoin, naproxen, nirvanol, omeprazole, propranolol	Barbiturates, rifampin	N3-benzylnirvanol, N3-benzylphenobarbital, fluconazole
<b>2D6</b>	Bufuralol, bupranolol, clomipramine, clozapine, codeine, debrisoquin, dextromethorphan, encainide, flecainide, fluoxetine, guanoxan, haloperidol, hydrocodone, 4-methoxy-amphetamine, metoprolol, mexiletine, oxycodone, paroxetine, phenformin, propafenone, propoxyphene, risperidone, selegiline (deprenyl), sparteine, tamoxifen, thioridazine, timolol, tricyclic antidepressants	Unknown	Quinidine, paroxetine
<b>2E1</b>	Acetaminophen, chlorzoxazone, enflurane, halothane, ethanol (a minor pathway)	Ethanol, isoniazid	4-Methylpyrazole, disulfiram
<b>3A4<sup>1</sup></b>	Acetaminophen, alfentanil, amiodarone, astemizole, cisapride, cocaine, cortisol, cyclosporine, dapsone, diazepam, dihydroergotamine, dihydropyridines, diltiazem, erythromycin, ethinyl estradiol, gestodene, indinavir, lidocaine, lovastatin, macrolides, methadone, miconazole, midazolam, mifepristone, nifedipine, paclitaxel, progesterone, quinidine, rapamycin, ritonavir, saquinavir, spironolactone, sulfamethoxazole, sufentanil, tacrolimus, tamoxifen, terfenadine, testosterone, tetrahydrocannabinol, triazolam, troleandomycin, verapamil	Barbiturates, carbamazepine, glucocorticoids, pioglitazone, phenytoin, rifampin, St. John's wort	Azamulin, clarithromycin, diltiazem, erythromycin, fluconazole, grapefruit juice (furanocoumarins), itraconazole, ketoconazole, ritonavir, troleandomycin

<sup>1</sup> CYP3A5 has similar substrate and inhibitor profiles, but except for a few drugs is generally less active than CYP3A4.

# Enzyme Induction

- **Enhanced rate of enzyme synthesis, or reduced rate of degradation.**
- **Results in accelerated substrate metabolism, and usually in a decrease in the pharmacological action of the drug.**
- **Toxicity may increase if the drug is metabolized to reactive metabolites.**
- **Induction mostly starts at the gene level.**

# Enzyme Induction

**Inducers include (but are not limited to):**

- 1. Environmental chemicals and pollutants such as polycyclic aromatic hydrocarbons present in tobacco smoke and charcoal-broiled meat, and other pyrolysis products (induce CYP1A).**
- 2. Drugs (see table). + dexamethasone, clofibrate, oral contraceptives, spironolactone.**

# Enzyme Induction

- Environmental chemicals known to induce specific P450s include the polychlorinated biphenyls (PCBs), and 2,3,7,8-tetrachlorodibenzo- *p* -*dioxin* (*dioxin*, *TCDD*), a trace byproduct of the chemical synthesis of the defoliant 2,4,5-T.
- **Cruciferous vegetables.**
- Ethanol (CYP2E1), isosafrole (CYP1A2).



# Enzyme Induction

- Autoinduction refers to a drug that induces its own metabolism, like carbamazepine.
- Autoinduction may lead to **tolerance** to drug action.

**TABLE 4–5 Partial list of drugs that enhance drug metabolism in humans.**

Inducer	Drugs Whose Metabolism Is Enhanced
Benzo[ <i>a</i> ]pyrene	Theophylline
Carbamazepine	Carbamazepine, clonazepam, itraconazole
Chlorcyclizine	Steroid hormones
Ethchlorvynol	Warfarin
Glutethimide	Antipyrine, glutethimide, warfarin
Griseofulvin	Warfarin
Phenobarbital and other barbiturates <sup>1</sup>	Barbiturates, chloramphenicol, chlorpromazine, cortisol, coumarin anticoagulants, desmethylinipramine, digitoxin, doxorubicin, estradiol, itraconazole, phenylbutazone, phenytoin, quinine, testosterone
Phenylbutazone	Aminopyrine, cortisol, digitoxin
Phenytoin	Cortisol, dexamethasone, digitoxin, itraconazole, theophylline
Rifampin	Coumarin anticoagulants, digitoxin, glucocorticoids, itraconazole, methadone, metoprolol, oral contraceptives, prednisone, propranolol, quinidine, saquinavir
Ritonavir <sup>2</sup>	Midazolam
St. John's wort	Alprazolam, cyclosporine, digoxin, indinavir, oral contraceptives, ritonavir, simvastatin, tacrolimus, warfarin

<sup>1</sup>Secobarbital is an exception. See Table 4–6 and text.

<sup>2</sup>With chronic (repeated) administration; acutely, ritonavir is a potent CYP3A4 inhibitor/inactivator.

# Enzyme Inhibition

- **Some drugs inhibit cytochrome P450 by binding to heme iron and inactivating the enzyme:**
  - 1. Imidazole-containing drugs such as cimetidine and ketoconazole bind tightly to the P450 heme iron and effectively reduce the metabolism of drugs through competitive inhibition.**

# Enzyme Inhibition

- 2. Macrolide antibiotics such as erythromycin, and erythromycin metabolites (CYP3A), complex the cytochrome P450 heme iron and inactive it.**
- 3. Some drugs (such as chloramphenicol metabolite) irreversibly inhibit P450s by covalent interaction that destroys P450 apoprotein or heme moiety.**

# Enzyme Inhibition

- 4. Suicide inhibitors (inactivators) include certain steroids (ethinyl estradiol, norethindrone, and spironolactone); allobarbital; grapefruit furanocoumarins; selegiline; phencyclidine; ticlopidine and clopidogrel; ritonavir; and propylthiouracil...**

# **Enzyme Inhibition**

- 5. Substrates compete with each other for the same active site of the enzyme.**
- 6. Deficiency of cofactors impair drug metabolism.**
- 7. Inhibitors of nucleic acid and protein synthesis impair enzyme synthesis and, thus, drug metabolism.**
- 8. Malnutrition.**
- 9. Impairment of hepatic function.**

**TABLE 4–6** Partial list of drugs that inhibit drug metabolism in humans.

Inhibitor <sup>1</sup>	Drug Whose Metabolism Is Inhibited
Allopurinol, chloramphenicol, isoniazid	Antipyrine, dicumarol, probenecid, tolbutamide
Chlorpromazine	Propranolol
Cimetidine	Chlordiazepoxide, diazepam, warfarin, others
Dicumarol	Phenytoin
Diethylpentenamide	Diethylpentenamide
Disulfiram	Antipyrine, ethanol, phenytoin, warfarin
Ethanol	Chlordiazepoxide (?), diazepam (?), methanol
Grapefruit juice <sup>2</sup>	Alprazolam, atorvastatin, cisapride, cyclosporine, midazolam, triazolam
Itraconazole	Alfentanil, alprazolam, astemizole, atorvastatin, buspirone, cisapride, cyclosporine, delavirdine, diazepam, digoxin, felodipine, indinavir, loratadine, lovastatin, midazolam, nisoldipine, phenytoin, quinidine, ritonavir, saquinavir, sildenafil, simvastatin, sirolimus, tacrolimus, triazolam, verapamil, warfarin

Inhibitor <sup>1</sup>	Drug Whose Metabolism Is Inhibited
Ketoconazole	Astemizole, cyclosporine, terfenadine
Nortriptyline	Antipyrine
Oral contraceptives	Antipyrine
Phenylbutazone	Phenytoin, tolbutamide
Ritonavir	Amiodarone, cisapride, itraconazole, midazolam, triazolam
Saquinavir	Cisapride, ergot derivatives, midazolam, triazolam
Secobarbital	Secobarbital
Spironolactone	Digoxin
Troleandomycin	Theophylline, methylprednisolone

<sup>1</sup>While some inhibitors are selective for a given P450 enzyme, others are more general and can inhibit several P450s concurrently.

<sup>2</sup>Active components in grapefruit juice include furanocoumarins such as 6', 7'-dihydroxybergamottin (which inactivates 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.



# Phase II Biotransformation reactions

- Conjugation reactions with endogenous substrates to yield drug conjugates.
- In general, conjugates are polar molecules readily excreted and inactive.
- Conjugations are synthetic reactions, involve high-energy intermediates and specific transfer enzymes called transferases.

# **Phase II Biotransformation reactions**

- 1. Uridine 5'-diphosphate [UDP]-glucuronosyl transferases (UGTs) are the most dominant enzymes. Groups glucuronidated are –OH, -NH, -SH, -COOH, -NHOH.**
- 2. Sulfotransferases (SULTs) use 3'-phosphoadenosine 5'-phosphosulfate (PAPS). Inorganic sulfate is a limiting factor for sulfation. Its sources are food and sulfur-containing amino acids.**

# **Phase II Biotransformation reactions**

- **Almost all chemical groups that are glucuronidated are also sulfated.**
  - **Infants are more capable of sulfation than glucuronidation, but in adults glucuronidation predominates.**
- 3. N-acetyltransferases (NATs) utilize acetyl CoA as the endogenous cofactor.**

# **Phase II Biotransformation reactions**

- 4. Glutathione (GSH) transferases (GSTs).**
  - The donor is glutathione (GSH), which is Glu-Cys-Gly.**
  - GSH is a nucleophile that reacts with and detoxifies electrophiles.**
  - Cause halogen replacement ( $R-Cl \rightarrow R-SG$ ).**
  - Conjugates epoxides.**

# **Phase II Biotransformation reactions**

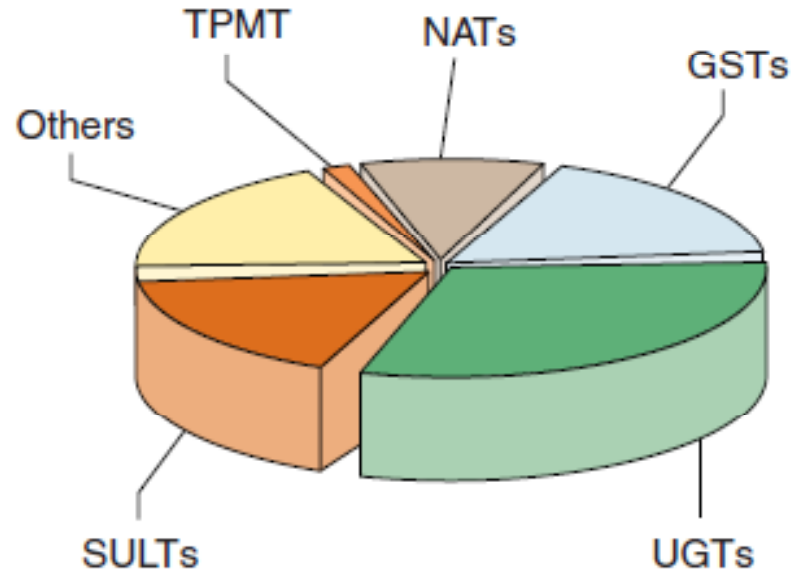
- **Glutathione conjugates do not appear in urine, but may appear in bile.**
- **They are metabolized further to cysteine conjugates and then to mercaptouric acid conjugates (N-acetylated cysteine conjugates), that appear in urine by an active transport process.**

# **Phase II Biotransformation reactions**

- 5. S-Adenosyl-L-methionine (SAM) mediate O-, N-, and S-methylation of drugs and xenobiotics by methyltransferases (MTs).**
- Phase II reactions are relatively faster than Phase I reactions.**

**TABLE 4–3** Phase II reactions.

Type of Conjugation	Endogenous Reactant	Transferase (Location)	Types of Substrates	Examples
<b>Glucuronidation</b>	UDP glucuronic acid	UDP glucuronosyltransferase (microsomes)	Phenols, alcohols, carboxylic acids, hydroxylamines, sulfonamides	Nitrophenol, morphine, acetaminophen, diazepam, <i>N</i> -hydroxydapsone, sulfathiazole, meprobamate, digitoxin, digoxin
<b>Acetylation</b>	Acetyl-CoA	<i>N</i> -Acetyltransferase (cytosol)	Amines	Sulfonamides, isoniazid, clonazepam, dapsone, mescaline
<b>Glutathione conjugation</b>	Glutathione (GSH)	GSH-S-transferase (cytosol, microsomes)	Epoxides, arene oxides, nitro groups, hydroxylamines	Acetaminophen, ethacrynic acid, bromobenzene
<b>Glycine conjugation</b>	Glycine	Acyl-CoA glycinetransferase (mitochondria)	Acyl-CoA derivatives of carboxylic acids	Salicylic acid, benzoic acid, nicotinic acid, cinnamic acid, cholic acid, deoxycholic acid
<b>Sulfation</b>	Phosphoadenosyl phosphosulfate	Sulfotransferase (cytosol)	Phenols, alcohols, aromatic amines	Estrone, aniline, phenol, 3-hydroxycoumarin, acetaminophen, methyl dopa
<b>Methylation</b>	<i>S</i> -Adenosylmethionine	Transmethylases (cytosol)	Catecholamines, phenols, amines	Dopamine, epinephrine, pyridine, histamine, thiouracil
<b>Water conjugation</b>	Water	Epoxide hydrolase (microsomes)  (cytosol)	Arene oxides, <i>cis</i> -disubstituted and monosubstituted oxiranes  Alkene oxides, fatty acid epoxides	Benzopyrene 7,8-epoxide, styrene 1,2-oxide, carbamazepine epoxide  Leukotriene A <sub>4</sub>



**B**

**FIGURE 4-4** Relative contributions of various cytochrome P450 isoforms (**A**) and different phase II pathways (**B**) to metabolism of drugs in clinical use. Many drugs are metabolized by two or more of these pathways. Note that two pathways, CYP3A4/5 and UGT, are involved in the metabolism of more than 75% of drugs in use. DPYD, dihydropyrimidine dehydrogenase; GST, glutathione-S-transferase; NAT, N-acetyltransferase; SULT, sulfotransferase; TPMT, thiopurine methyltransferase; UGT, UDP-glucuronosyltransferase. (Reproduced, with permission, from Brunton LL, Lazo JS, Parker KL: *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 11th ed. McGraw-Hill Medical, 2006.)



# **Phase II Biotransformation reactions**

- **Certain conjugation reactions (acyl glucuronidation of nonsteroidal antiinflammatory drugs, O-sulfation of N-hydroxyacetylaminofluorine, and N-acetylation of isoniazide) may lead to formation of reactive species and drug toxicities.**

# **Phase II Biotransformation reactions**

- **Sulfation leads to activation of the prodrug minoxidil.**
- **Morphine-6-glucuronide is more potent than morphine.**

# **Metabolism of Drugs to Toxic Product**

- **Several drugs may be metabolically transformed to reactive intermediates that are toxic to various organs.**
- **Such toxic reactions may become apparent at high drug doses, especially when alternative detoxification mechanisms are overwhelmed or endogenous detoxifying cosubstrates (GSH, glucuronic acid, sulfate) are depleted.**

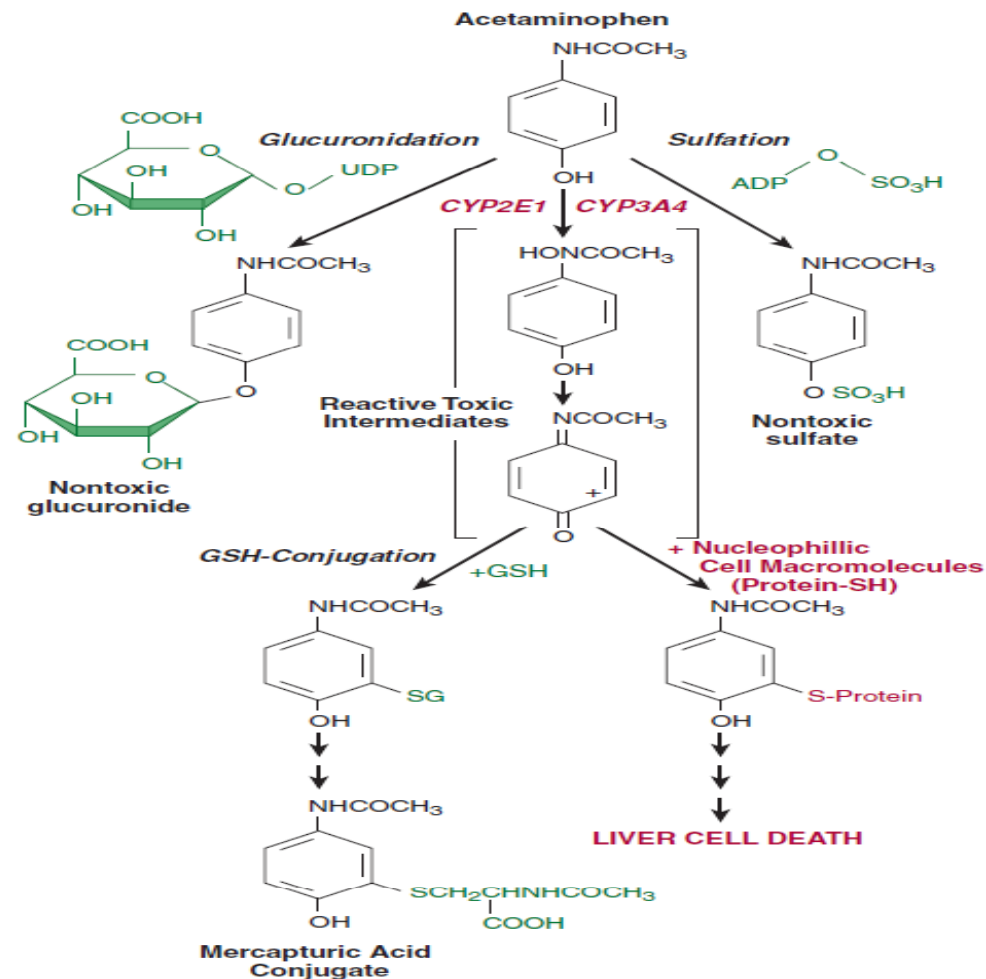
# **Metabolism of Drugs to Toxic Product**

- **An example is acetaminophen (paracetamol)-induced hepatotoxicity.**
- **It normally undergoes glucuronidation and sulfation, which make up 95% of the total excreted metabolites. The alternative P450-dependent GSH conjugation pathway accounts for the remaining 5%.**
- **No hepatotoxicity results as long as hepatic GSH is available for conjugation.**

# **Metabolism of Drugs to Toxic Product**

- **At high paracetamol dose and when GSH is depleted, the toxic metabolite accumulates resulting in hepatotoxicity.**
- **Administration of N -acetylcysteine (antidote) within 8–16 hours after acetaminophen overdose protects victims from fulminant hepatotoxicity and death. Administration of GSH is not effective because it does not cross cell membranes readily.**

# Metabolism of Drugs to Toxic Products



**FIGURE 4-5** Metabolism of acetaminophen (top center) to hepatotoxic metabolites. GSH, glutathione; SG, glutathione moiety.