Drug Metabolism Principles- Phase I and Phase II

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Outline of Presentation:

- Definition
- Consequences
- Types
- Metabolizing Enzymes
- Phase-I Metabolism with examples
- Phase-II Metabolism with examples
- Factors affecting drug metabolism

BIOTRANSFORMATION (Metabolism)

Biotransformation means chemical alteration of the drug in the body. It is needed to render non-polar (lipid-soluble) compounds polar (lipid insoluble/water soluble) so that they are not reabsorbed in the renal tubules and are excreted.

➢Most hydrophilic drugs, e.g. streptomycin, neostigmine, pancuronium, etc. are little biotransformed and are largely excreted unchanged.

➢Mechanisms which metabolize drugs (essentially foreign substances/ xenobiotics) have developed to protect the body from ingested toxins.

➢The primary site for drug metabolism is liver; others are—kidney, intestine, lungs and plasma.

➢Biotransformation of drugs may lead to the following **consequences**:

1. ***Inactivation:*** *Most drugs and their active* metabolites are rendered inactive or less active, e.g. ibuprofen, paracetamol, lidocaine, chloramphenicol, propranolol and its active metabolite 4-hydroxypropranolol.

2. ***Active metabolite from an active drug:*** Many drugs have been found to be partially converted to one or more active metabolite; the effects observed are the sumtotal of that due to the parent drug and its active metabolite(s) (*see* Table 1).

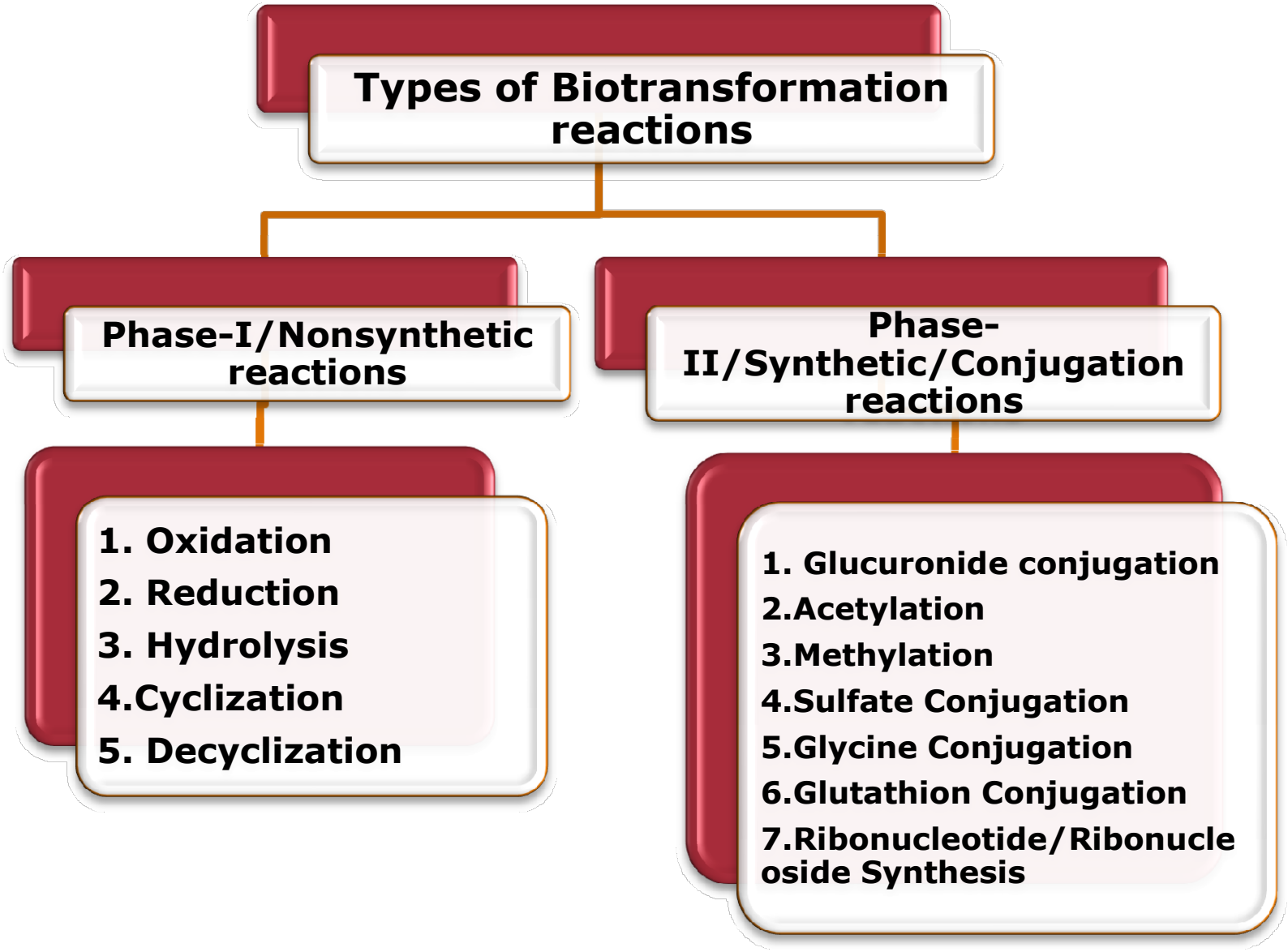
3. ***Activation of inactive drug:*** *Few drugs are* inactive as such and need conversion in the body to one or more active metabolites. Such a drug is called a *prodrug (see Table 2). The prodrug may* offer advantages over the active form in being more stable, having better bioavailability or other desirable pharmacokinetic properties or less side effects and toxicity. Some prodrugs are activated selectively at the site of action.

Table 1: Active metabolites from active drugs

Active drug		*Active metabolite*
Chloral hydrate	—	Trichloroethanol
Morphine	—	Morphine-6-glucuronide
Cefotaxime	—	Desacetyl cefotaxime
Allopurinol	—	Alloxanthine
Procainamide	—	N-acetyl procainamide
Primidone	—	Phenobarbitone, phenylethylmalonamide
Diazepam	—	Desmethyl-diazepam, oxazepam
Digitoxin	—	Digoxin
Imipramine	—	Desipramine
Amitriptyline	—	Nortriptyline
Codeine	—	Morphine
Spironolactone	—	Canrenone
Losartan	—	E 3174

Table 2: Prodrugs and their active froms

Prodrug		*Active form*
Levodopa	—	Dopamine
Enalapril	—	Enalaprilat
a-Methyldopa	—	a-methylnorepinephrine
Dipivefrine	—	Epinephrine
Sulindac	—	Sulfide metabolite
Proguanil	—	Cycloguanil
Prednisone	—	Prednisolone
Bacampicillin	—	Ampicillin
Sulfasalazine	—	5-Aminosalicylic acid
Cyclophos-phamide	—	Aldophosphamide, phosphoramide mustard, acrolein
Fluorouracil	—	Fluorouridine monophosphate
Mercaptopurine	—	Methylmercaptopurine ribonucleotide
Acyclovir	—	Acyclovir triphosphate


Types of Biotransformation reactions
Phase-I/Nonsynthetic reactions
1. Oxidation
2. Reduction
3. Hydrolysis
4.Cyclization
5. Decyclization
Phase-II/Synthetic/Conjugation reactions
1. Glucuronide conjugation
2.Acetylation
3.Methylation
4.Sulfate Conjugation
5.Glycine Conjugation
6.Glutathion Conjugation
7.Ribonucleotide/Ribonucleoside Synthesis

The drug metabolizing enzymes

The drug metabolising enzymes are divided into two types:

A. Microsomal enzymes

B. Non-microsomal enzymes

A. Microsomal enzymes

➢These are located on smooth endoplasmic reticulum (a system of microtubules inside the cell), primarily in liver, also in kidney, intestinal mucosa and lungs.

➢The monooxygenases, cytochrome P450, UGTs, epoxide hydrolases, etc. are microsomal enzymes.

➢They catalyse most of the oxidations, reductions, hydrolysis and glucuronide conjugation.

➢Microsomal enzymes are **inducible** by drugs, diet and other agencies.

B. Non-microsomal enzymes:

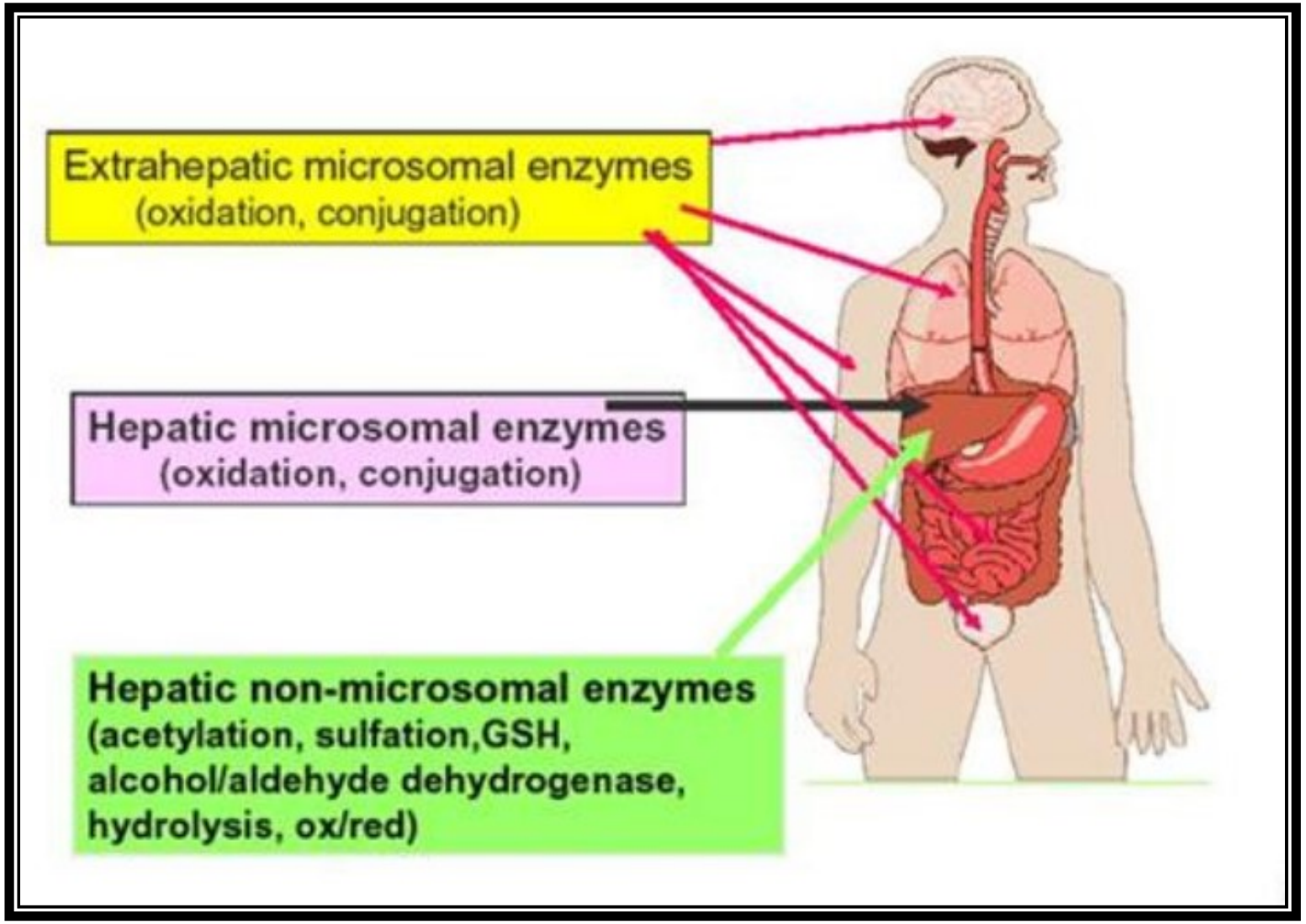
- These are present in the cytoplasm and mitochondria of hepatic cells as well as in other tissues including plasma.

- The esterases, amidases, some flavoprotein oxidases and most conjugases are nonmicrosomal.

- Reactions catalysed are: Some oxidations and reductions, many hydrolytic reactions and all conjugations except glucuronidation.

- The nonmicrosomal enzymes are not inducible but many show genetic polymorphism (acetyl transferase, pseudocholinesterase).

Sites of drug metabolism: Hepatic and Extra-hepatic enzymes



Phase-I Metabolic reactions

1. Oxidation:

- This reaction involves addition of oxygen/negatively charged radical or removal of hydrogen/positively charged radical. Oxidations are the most important drug metabolizing reactions.

- Oxidation is the main process of metabolism.

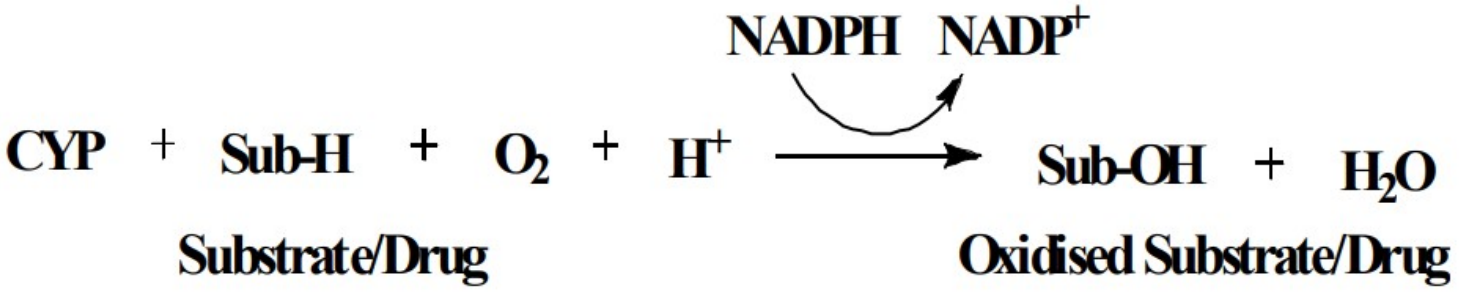
- Produces unstable intermediates - Epoxides, Superoxides, Quinones

The Role of Cytochrome P450 (CYP) in Oxidative reactions

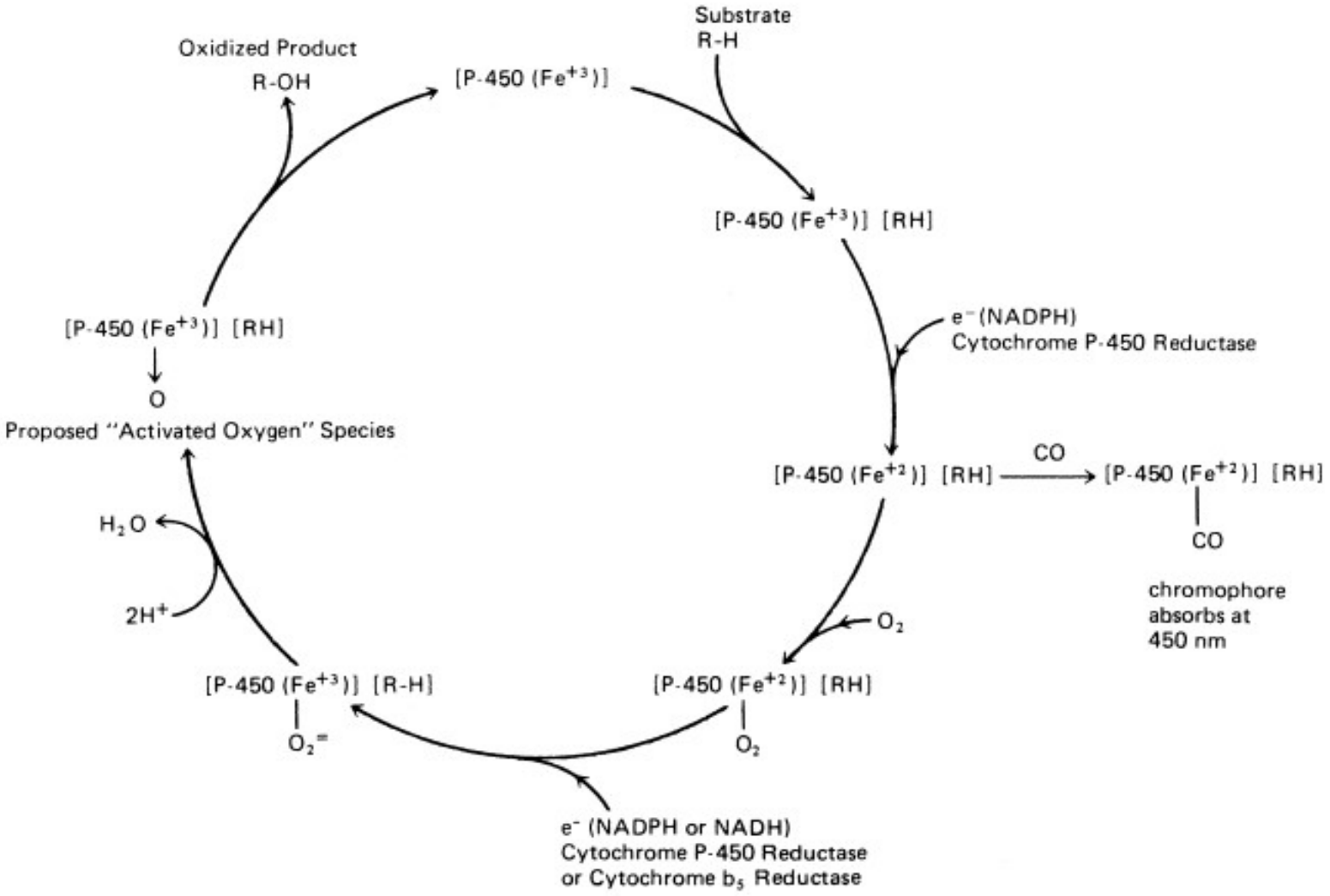
- The cytochrome P450 (CYP) enzymes are also known as microsomal mixed function oxidases.
- The CYP enzymes are membrane-bound proteins, present in the smooth endoplasmic reticulum of liver and other tissues.
- They are the most important enzymes for Phase I biotransformation of drugs.
- These enzymes contain a heme prosthetic group, where heme group is the iron-porphyrin unit.
- The oxidizing site in these enzymes is the heme centre, and is responsible for the oxidation of hydrophobic compounds to hydrophilic or more polar metabolites for subsequent excretion.

➢These are called CYP450 because the iron in reduced state can bind with high affinity to carbon monoxide and this CO-bound CYP complex shows a large absorbance at 450 nm.

➢CYPs catalyze the transfer of one atom of oxygen to a substrate producing an oxidised substrate along with a molecule of water, as shown in the equation below:



The catalytic cycle of CYP450, leading to the oxidation of substrate.



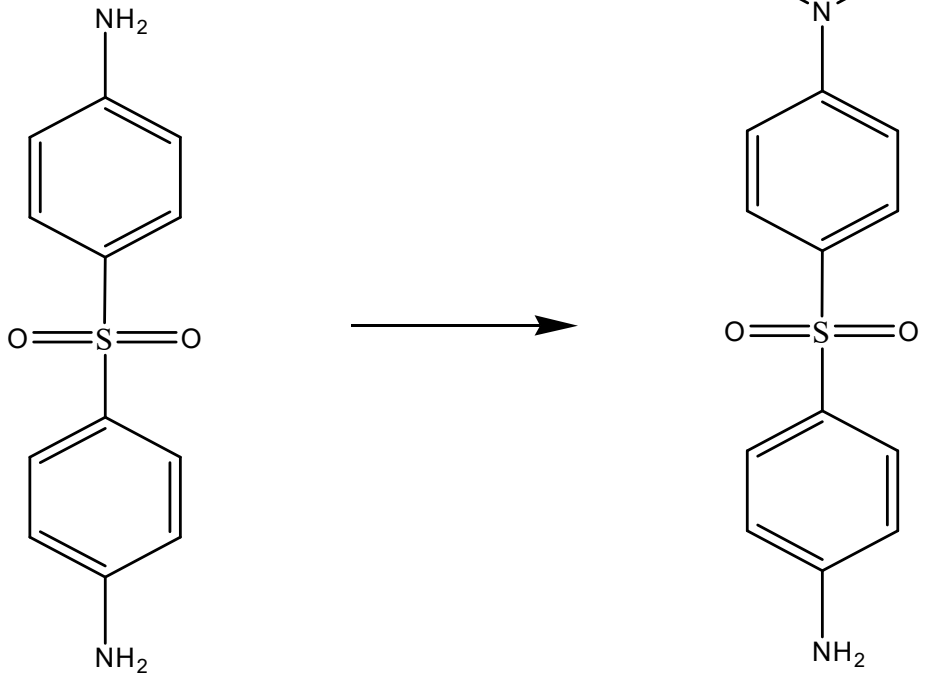
1. Oxidation Reactions– 9 types

1.OXIDATION AT NITROGEN ATOM

$$RNH_2 \xrightarrow{O} RNHOH$$

Examples:

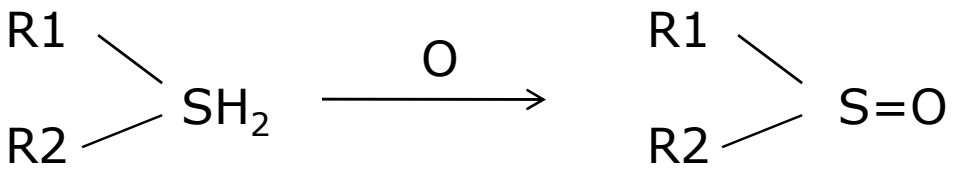
- Chlorpheniramine
- Dapsone



Dapsone

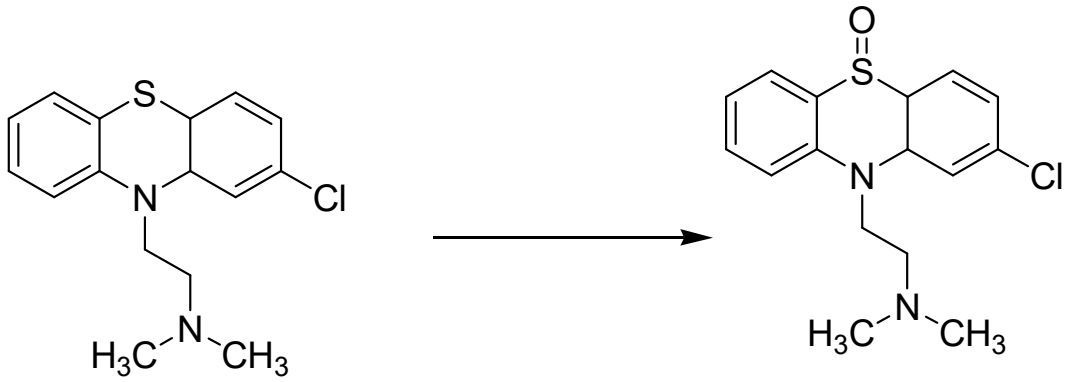
Dapsone Hydroxylamine

2.OXIDATION AT SULPHUR ATOM:



Examples:

- Chlorpromazine
- Chloramphenicol



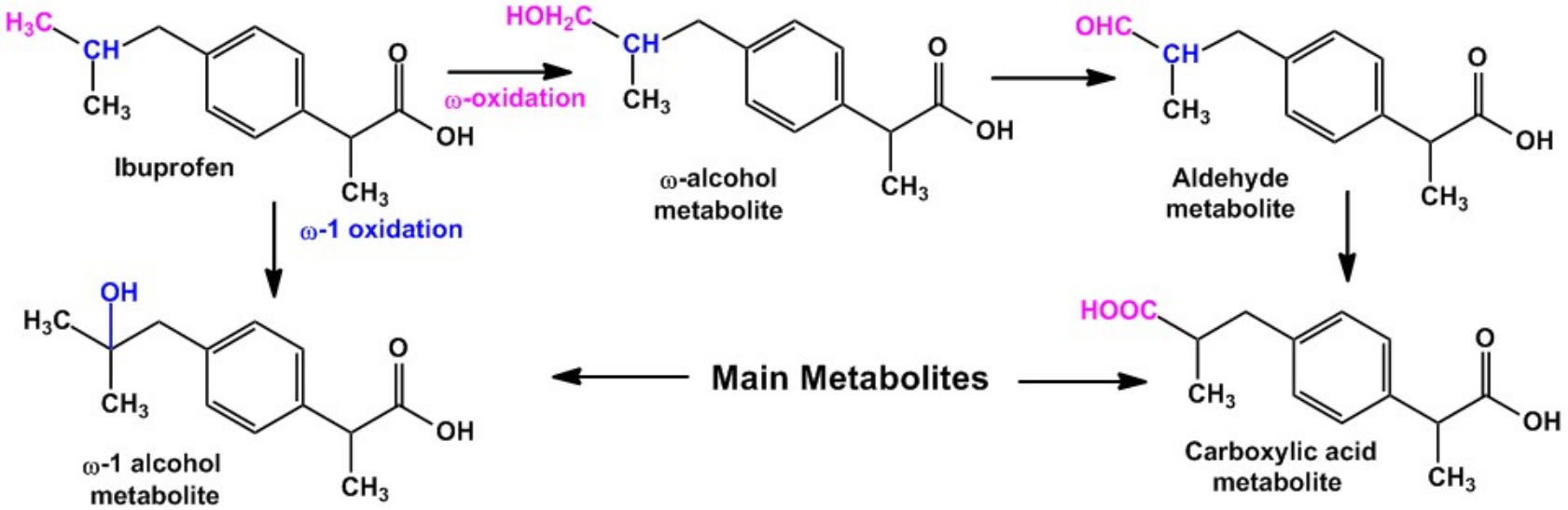
Chlorpromazine

Chlorpromazine-S-Oxide

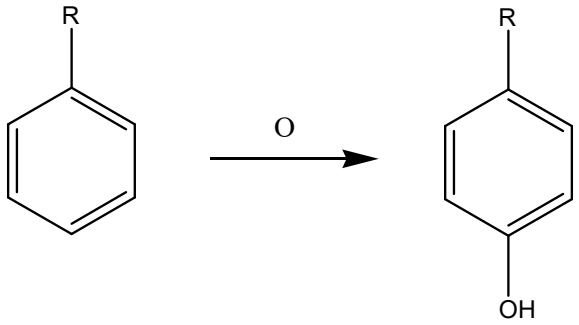
3.ALIPHATIC HYDROXYLATION: Hydroxyl group added to drug:

$$RCH_2CH_3 \xrightarrow{O} RCHOHCH_3$$

Examples:
- Salicylic acid to Gentisic acid
- Ibuprofen
- Tolbutamide, Chlorpropamide

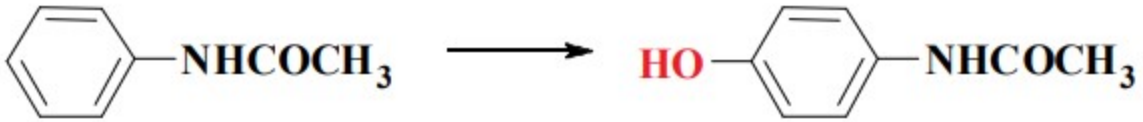


4.AROMATIC HYDROXYLATION:



Examples:

- Acetanilide
- Phenytoin
- Phenobarbitone
- Propranolol

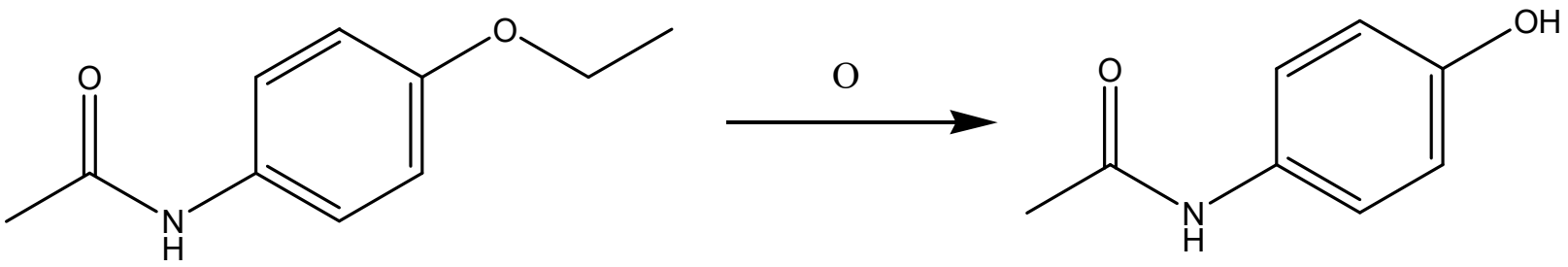


Acetanilide **Paracetamol**

5.DEALKYLATON AT OXYGEN ATOM:

$$ROCH_3 \xrightarrow{O} ROH + CH_2O$$

Examples:
Phenacetin to Paracetamol



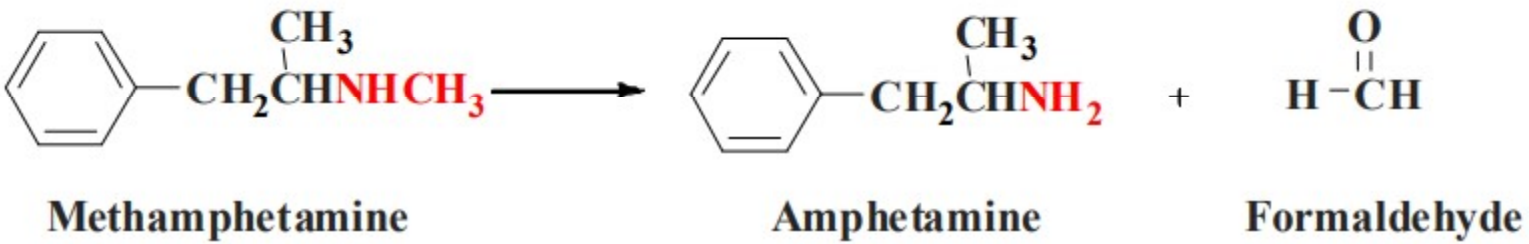
Phenacetin

Paracetamol

6.DEALKYLATON AT NITROGEN ATOM:

$$RNHCH_3 \xrightarrow{O} RNH_2 + CH_2O$$

Examples:
Methamphetamine to Amphetamine

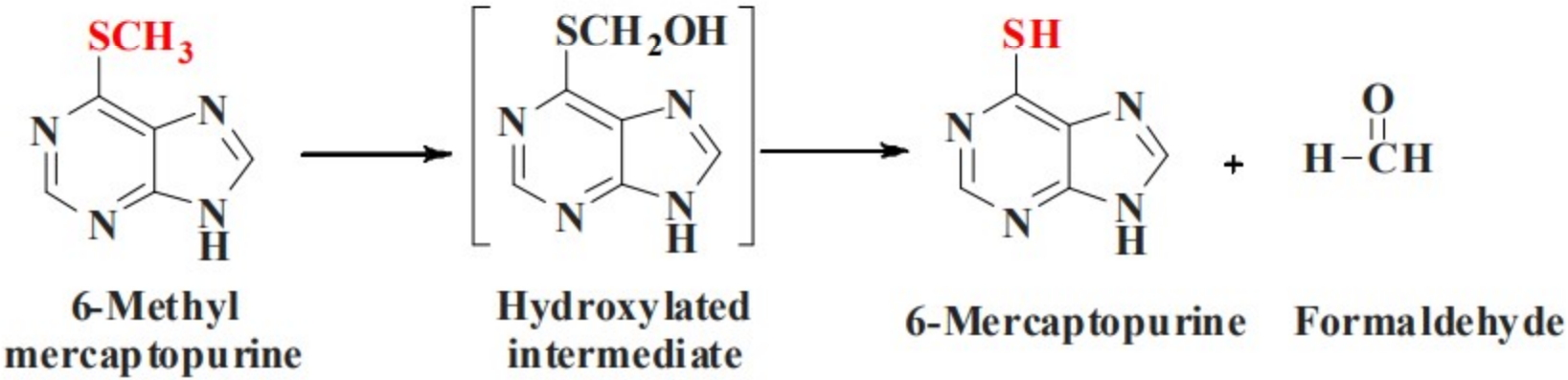


Methamphetamine Amphetamine Formaldehyde

7.DEALKYLATON AT SULPHUR ATOM:

$$RSCH_3 \xrightarrow{O} RSH + CH_2O$$

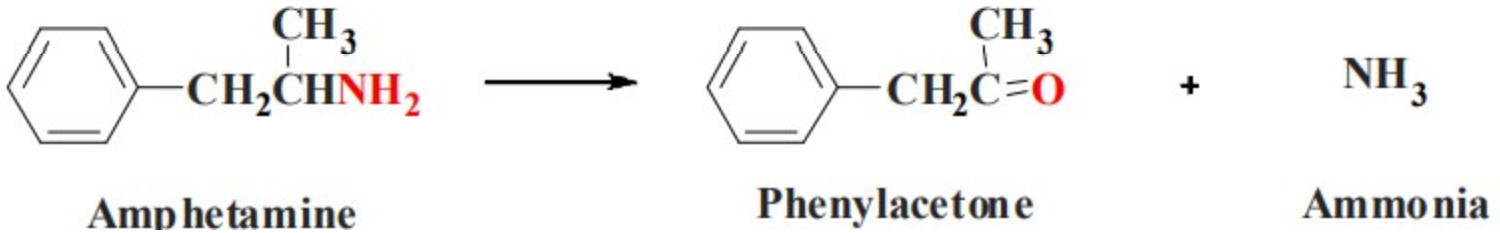
Examples:
6-Methyl mercaptopurine to Mercaptopurine



8.OXIDATIVE DEAMINATION:

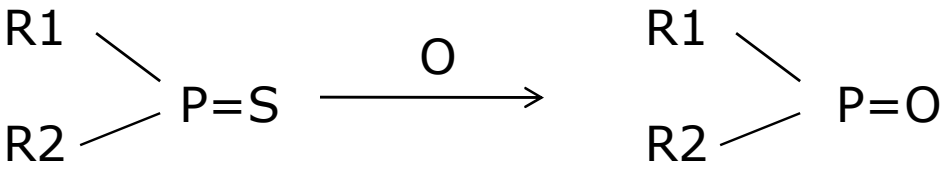
$$RCHNH_2R \xrightarrow{O} RCOR + NH_3$$

Example:
Amphetamine

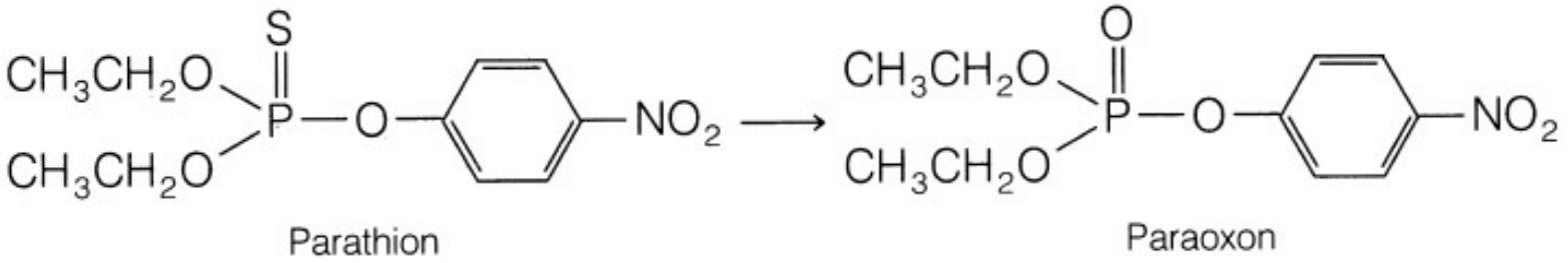


Amphetamine Phenylacetone Ammonia

9.DESULFURATION:



Examples:
Parathion to Paraoxon



Parathion Paraoxon

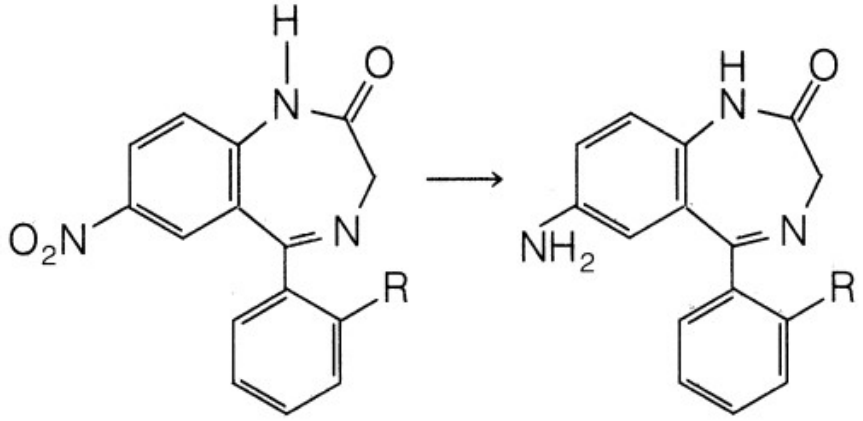
2. Reduction Reactions

This reaction is the converse of oxidation and involves cytochrome P-450 enzymes working in the opposite direction. Alcohols, aldehydes, quinones are reduced.

1. Nitro Reduction:

$$R\text{-}NO_2 \longrightarrow R\text{-}NH_2$$

Example: Clonezepam and Nitrazepam



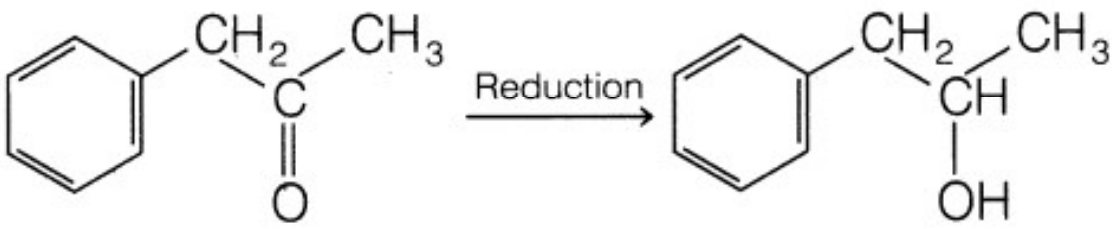
Clonazepam, R = Cl
Nitrazepam, R = H

7-Amino Metabolite

2. Keto Reduction:

R-CO-R $\longrightarrow$ R-CHOH-R

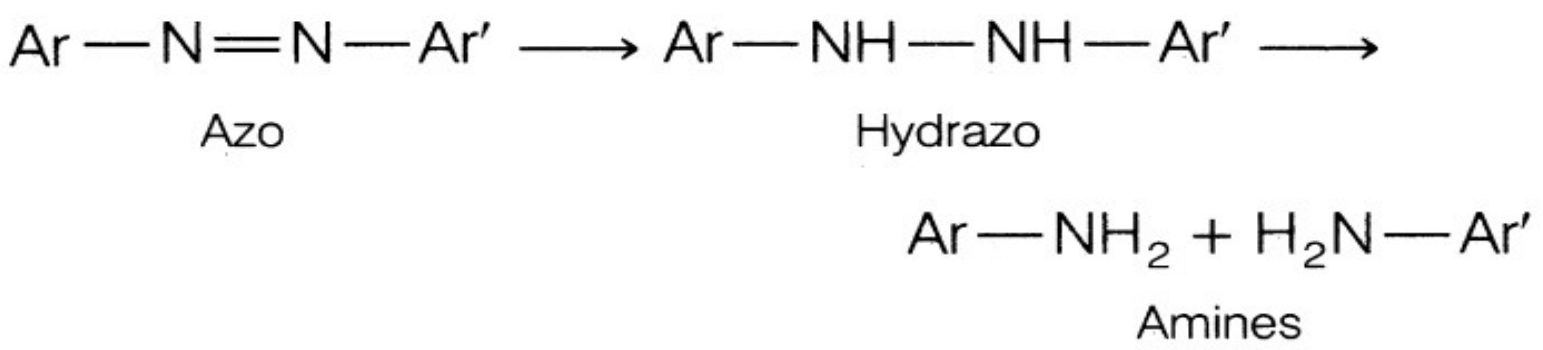
Example: Phenylacetone



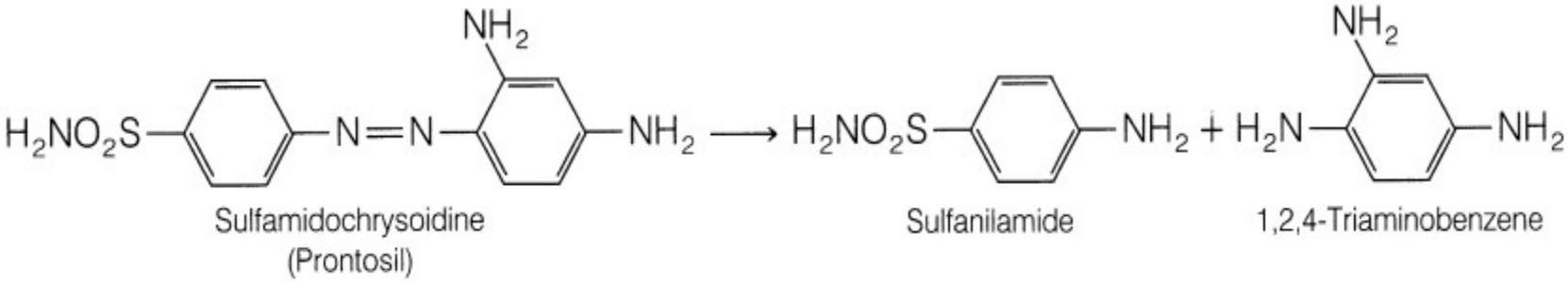
Phenylacetone

1-Phenyl-2-propanol

3. Azo Reduction:



Example: Prontosil



3. Hydrolytic Reactions

- This is cleavage of drug molecule by taking up a molecule of water.

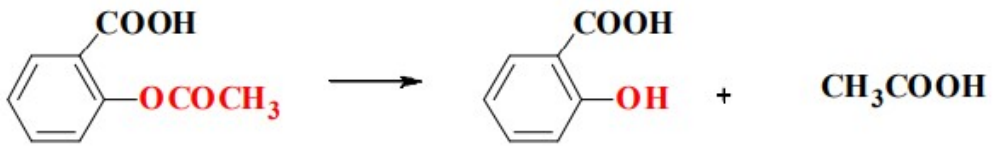
$$\text{Ester} + H_2O \xrightarrow{\textit{esterase}} \text{Acid} + \text{Alcohol}$$

- Similarly, amides and polypeptides are hydrolysed by amidases and peptidases. In addition, there are epoxide hydrolases which detoxify epoxide metabolites of some drugs generated by CYP oxygenases.

- Hydrolysis occurs in liver, intestines, plasma and other tissues.

- Examples of hydrolysed drugs are choline esters, procaine, lidocaine, procainamide, aspirin, carbamazepine-epoxide, pethidine, oxytocin.

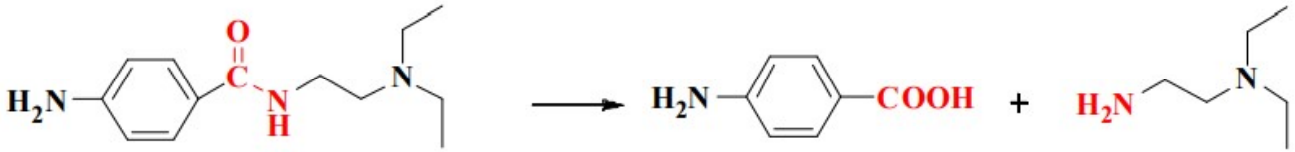
Esters and ethers



Aspirin

Salicylic acid (active)

Amides

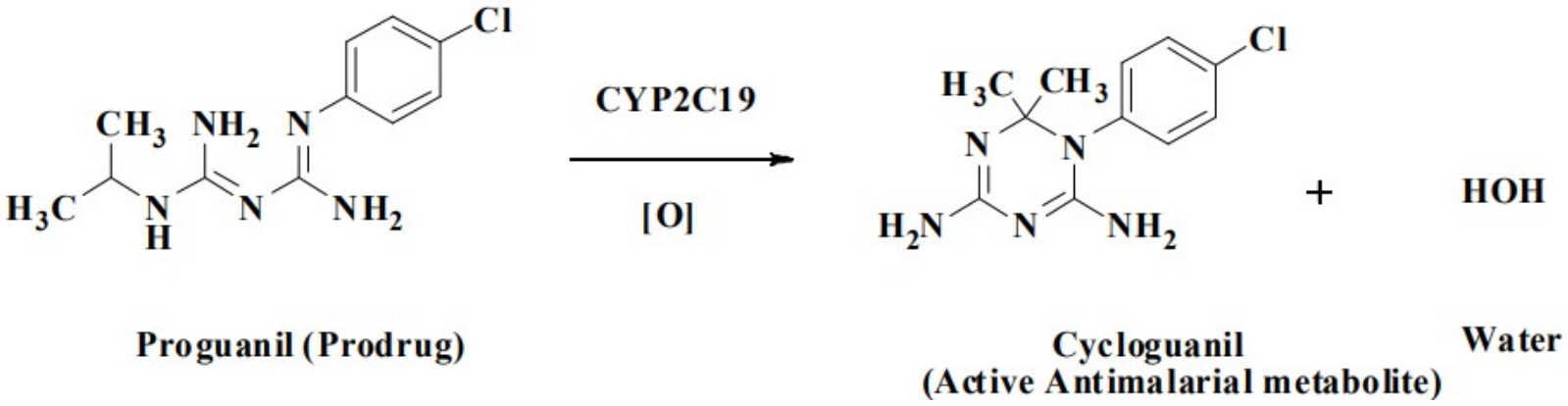


Procainamide

p-amino benzoic acid (PABA)

4. Cyclization

This is formation of ring structure from a straight chain compound, e.g. proguanil.



Proguanil (Prodrug)

Cycloguanil (Active Antimalarial metabolite)

Water

5. Decyclization

This is opening up of ring structure of the cyclic drug molecule, e.g. barbiturates, phenytoin. This is generally a minor pathway.

Synthetic/Conjugation/ Phase-II Reactions

- The Phase II reactions follow Phase I reactions, and occur mostly in the products derived from Phase I reactions.

- In these reactions, a suitable moiety such as glucuronic acid, glutathione, sulphate, glycine, etc., get conjugated to the metabolites of Phase I reaction.

- The Phase II reactions are the real drug detoxification pathways.

- These are also termed as conjugation reactions, because the metabolites are conjugated with the above-mentioned moieties which are large in size and strongly polar in nature.

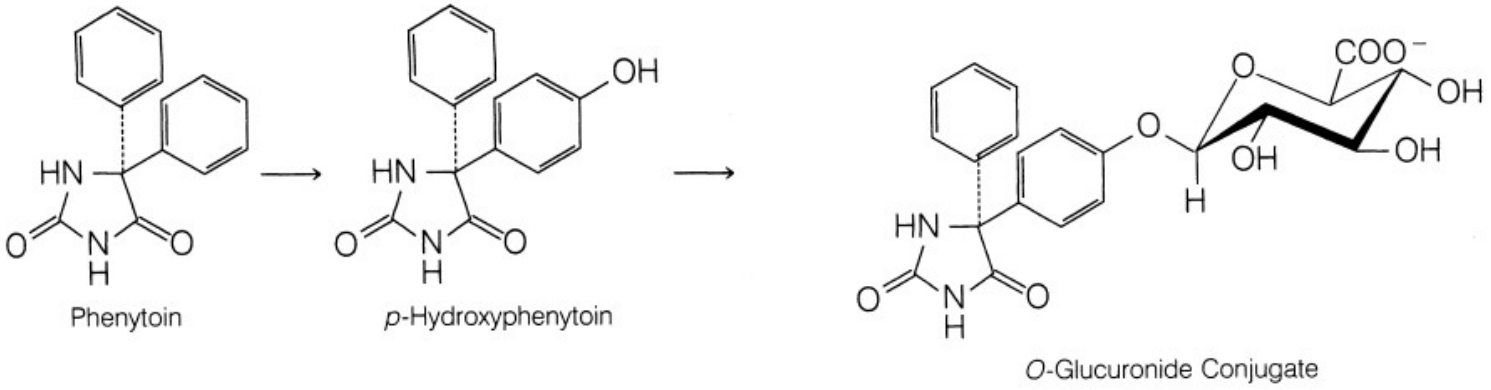
- These reactions are catalyzed by a variety of transferase enzymes, such as uridine diphosphate (UDP)-glucoronsyltransferases, sulfotransferases, glutathione transferases etc.

1. Glucuronide conjugation

➢*This is the most* important synthetic reaction carried out by a group of UDP-glucuronosyl transferases (UGTs).

➢Compounds with a **hydroxyl** or **carboxylic acid** group are easily conjugated with glucuronic acid which is derived from glucose.

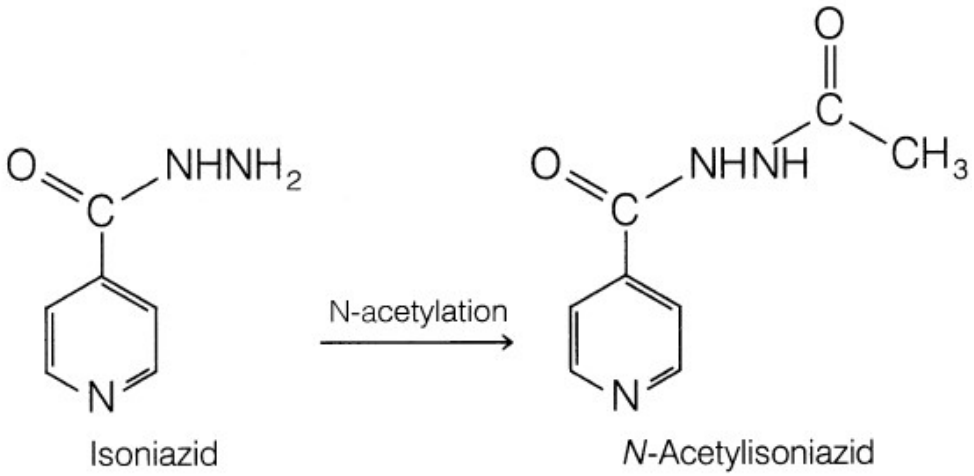
➢Glucuronidation increases the molecular weight of the drug which favours its excretion in bile.



2. Acetylation

➢*Compounds having* ***amino*** *or* **hydrazine** residues are conjugated with the help of acetyl coenzyme-A, e.g. sulfonamides, isoniazid, PAS, dapsone, hydralazine, clonazepam, procainamide.

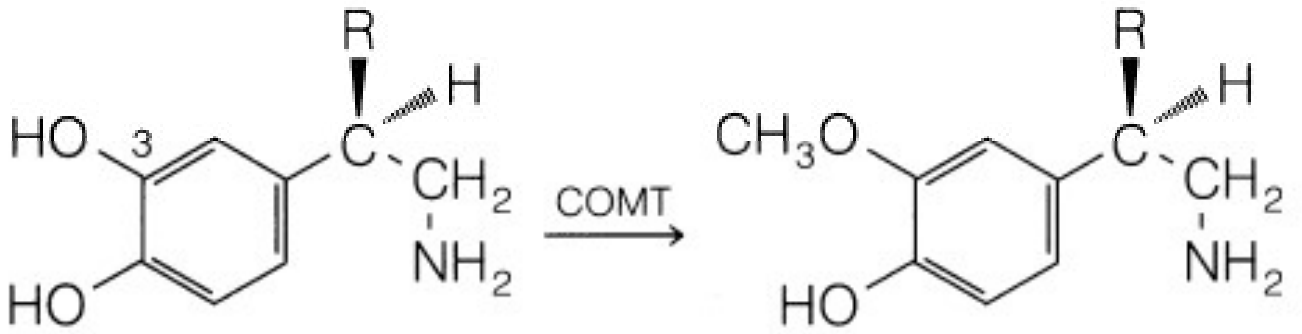
➢Multiple genes control the N-acetyl transferases (NATs), and rate of acetylation shows genetic polymorphism (slow and fast acetylators).



Isoniazid *N*-Acetylisoniazid

3.Methylation

➢The **amines** and **phenols** can be methylated by methyl transferases (MT); methionine and cysteine acting as methyl donors, e.g. adrenaline, histamine, nicotinic acid, methyldopa, captopril, mercaptopurine.



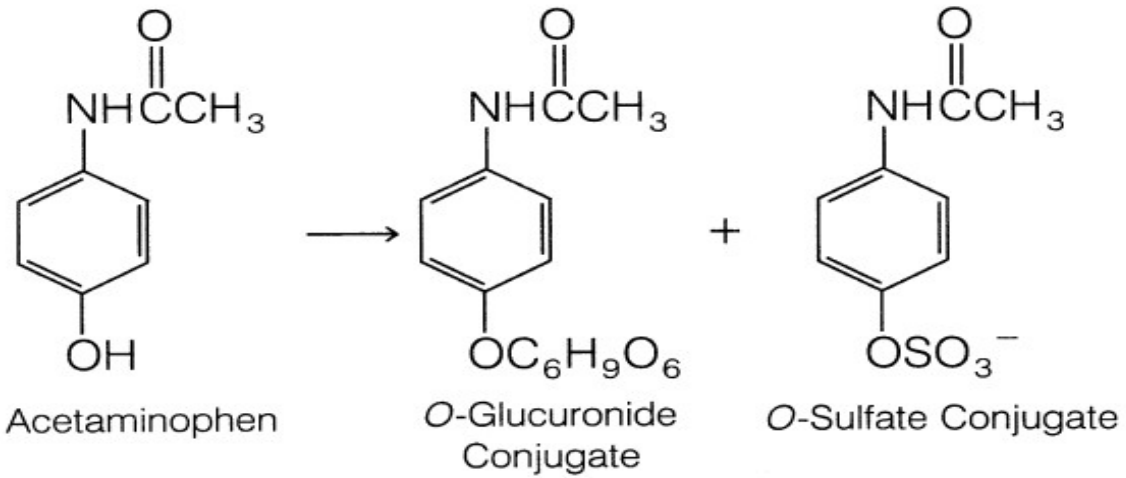
Norepinephrine, R = OH
Dopamine, R = H

Normetanephrine, R = OH
3-Methoxytyramine, R = H

COMT- Catechol-O-methyl transferase

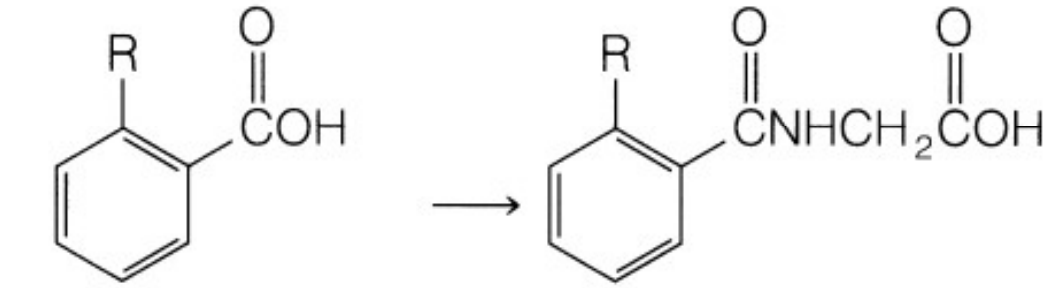
4.Sulfate conjugation

➢The **phenolic** compounds and **steroids** are sulfated by sulfotransferases (SULTs), e.g. chloramphenicol, methyldopa, adrenal and sex steroids.



5. Glycine conjugation

➢Salicylates, nicotinic acid and other drugs having **carboxylic acid group** are conjugated with glycine, but this is not a major pathway of metabolism.

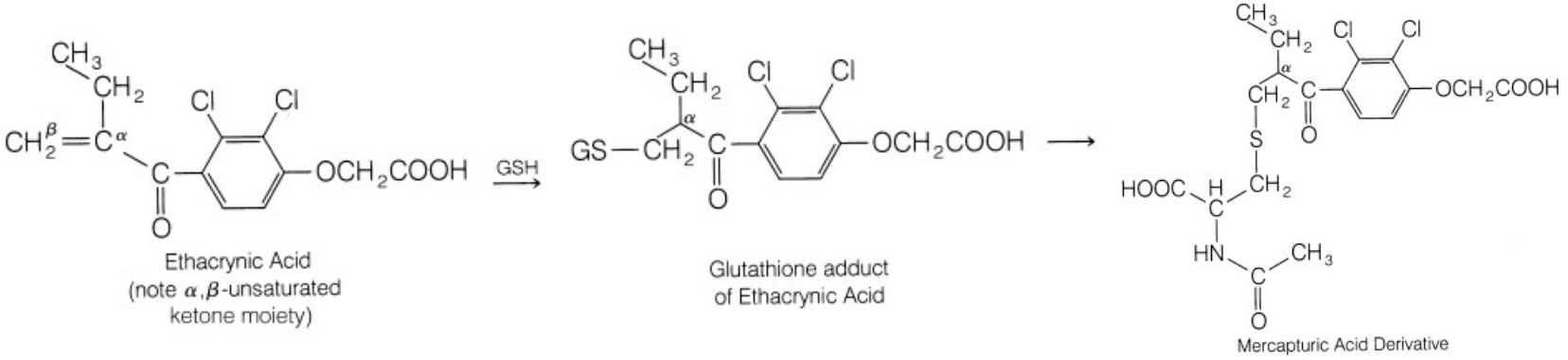


Benzoic Acid, R = H
Salicylic Acid, R = OH

Hippuric Acid, R = H
Salicyluric Acid, R = OH

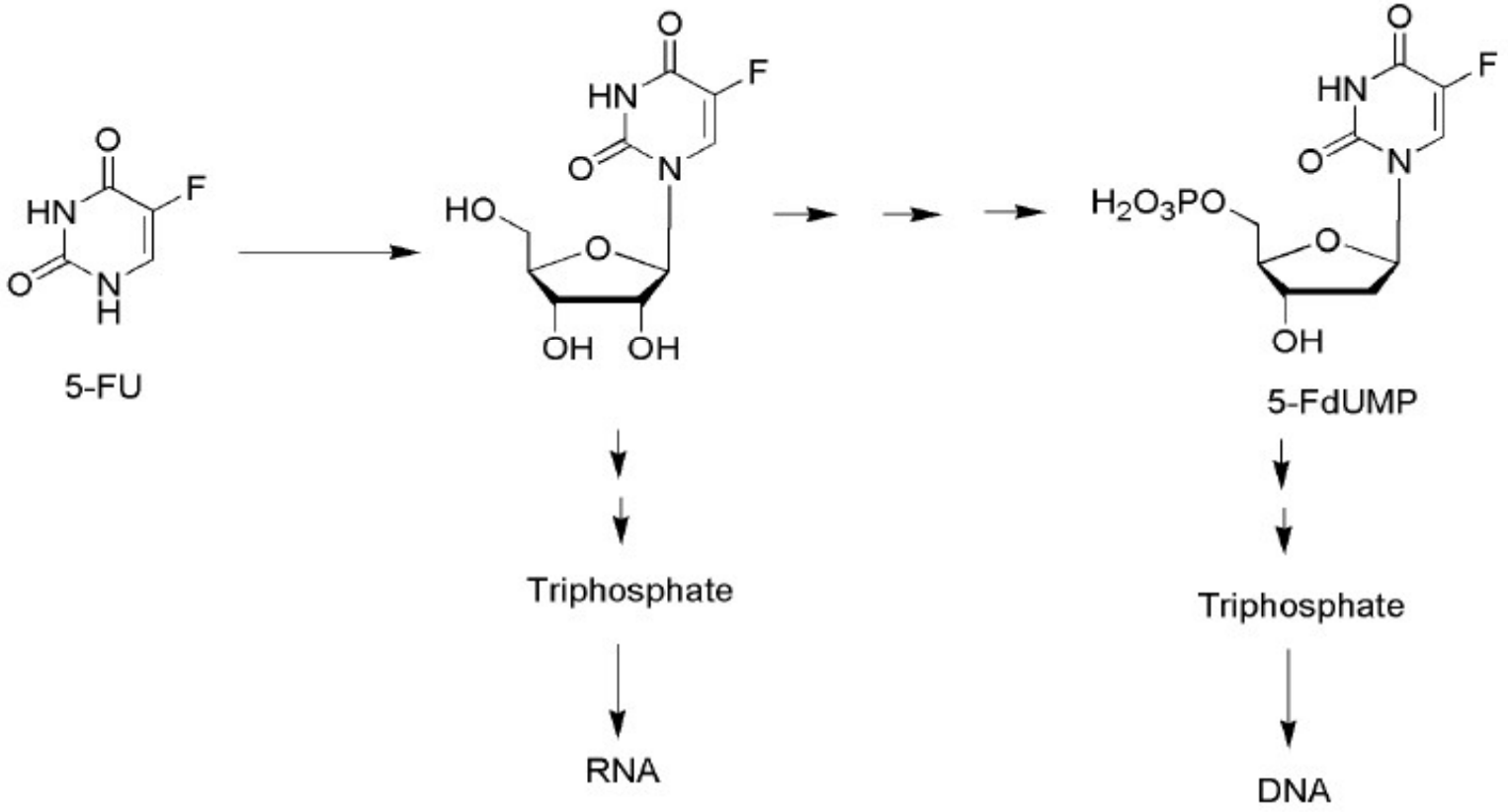
6. Glutathione conjugation

- This is carried out by glutathione-S-transferase (GST) forming a **mercapturate.**
- It is normally a minor pathway. However, it serves to inactivate highly reactive quinone or epoxide intermediates formed during metabolism of certain drugs, e.g. paracetamol.
- When large amount of such intermediates are formed (in poisoning or after enzyme induction), glutathione supply falls short—toxic adducts are formed with tissue constituents → tissue damage.



Ribonucleoside/nucleotide synthesis

➢This pathway is important for the activation of many purine and pyrimidine antimetabolites used in cancer chemotherapy.



Metabolic activation of 5-fluorouracil to 5-FdUMP (5-Fluoro deoxyuridine mono phosphate).

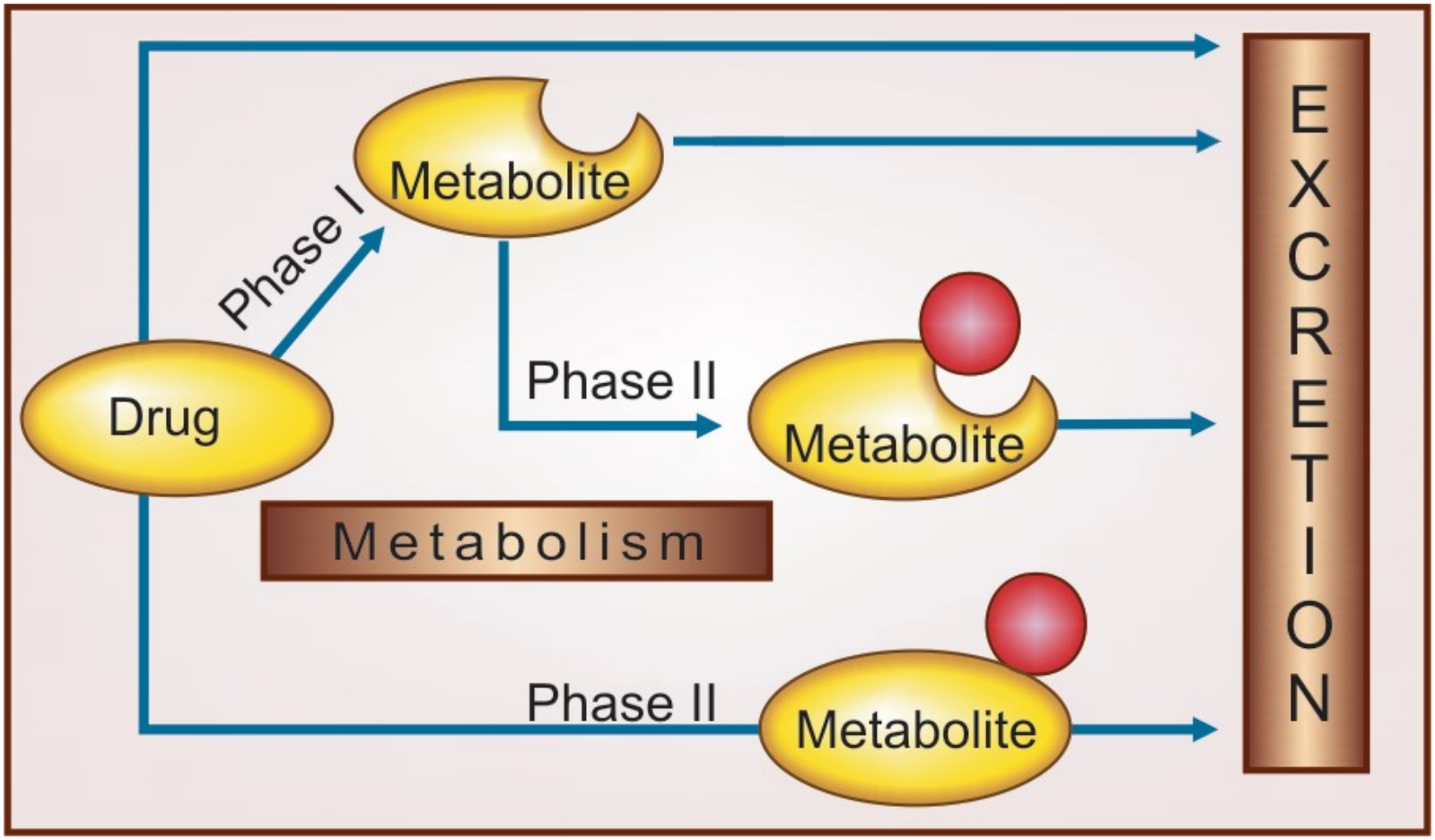


Fig. Simultaneous and/or sequential metabolism of a drug by phase I and phase II reactions

Factors affecting Drug Metabolism

Biotransformation is significantly affected by a number of factors, these include:

- Enzyme Induction
- Enzyme Inhibition
- Presystemic Metabolism/First pass effect/Route of Administration
- Genetic Variations
- Species Differences
- Exposure to Pollutants from Environment or Industry
- Age
- Sex

1.Enzyme Induction: The rate of metabolism increases as enzyme induction takes place. The drugs which bring about these changes are known as **enzyme inducers**. Some examples include anticonvulsants like phenytoin, carbomycin and chronic alcoholism. Others include various sedatives, hypnotics, tranquilizers and insecticides.

Example: Phenobarbitone is used in seizures and epilepsy. It is also an enzyme inducer. If it is administered to patients taking warfarin, therapeutic failure might occur, leading to increased bleeding tendency.

2.Enzyme Inhibition: The process in which drug metabolizing capacity of cytochrome is decreased is known as enzyme inhibition. The rate of metabolism is decreased. Drugs bringing about these changes are known as **enzyme inhibitors**. Examples include ketoconazole- antifungal drug, cimetidine and verapamil- calcium channel blocker.

Example:

•Sulfonamides decrease the metabolism of phenytoin so that its blood levels become toxic.

•Cimetidine decreases the metabolism of propanolol leading to enhanced bradycardia.

3. Presystemic Metabolism/First pass effect/Route of Administration:

•Biotransformation of drug by liver or gut enzymes before compound reaches systemic circulation

• Results in lower systemic bioavailbility of parent compound, diminished therapeutic response.

•Most of the drugs are metabolized within the liver. Changing the route of administration (IV or Sublingually.) might bypass the first pass metabolism.

•Propanolol is 80% metabolized before reaching systemic circulation.

4. Genetic Variations:

•Inter individual variations might occur; drugs behave differently in different individuals due to absent or malformed genes.

•Mostly non microsomal enzyme show genetic variations.

•Examples include succinyl choline, which is a skeletal muscle relaxant. It is metabolized by pseudocholine esterase. Some people lack this enzyme, due to which lack of metabolism of succinyl choline might occur. When administered in those individuals, prolonged apnea might result.

Different groups of populations might be classified as fast metabolizers and poor metabolizers of drugs. For certain drugs, like isoniazid, *fast acetylators* as well as *slow acetylators* are present. Fast acetylators cause rapid acetylation, while poor metabolizers metabolize less. Hepatic acetyl transferrase catalyzes acetylation. Slow acetylation might occur due to genetic malformation leading to decreased production.

5. Species Differences: Most established in animals. Some metabolize drugs rapidly. Rats and rabbits metabolize drugs more efficiently than humans. Certain species of rabbits feed on Belladonna, and have atropinase to tolerate the effects of atropine.

6. Exposure to Pollutants from Environment or Industry:

•Cigarette smokers might act as enzyme inducers.

•Chronic alcoholism might lead to enzyme induction as well. Similarly, pesticides or insecticides may act as enzyme inducers.

•In hot and humid climate biotransformation is decreased and vice versa.

•At high altitude, decreased biotransformation occurs due to decreased oxygen leading to decreased oxidation of drugs.

7. Age:

•Age plays a very important role. Extreme age groups (very young and very old) behave almost the same.

•Both microsomal and nonmicrosomal enzymes are deficient in the newborn, especially premature, making them more susceptible to many drugs, e.g. chloramphenicol, opioids.

•This deficit is made up in the first few months, more quickly in case of oxidation and other phase I reactions than in case of glucuronide and other conjugations which take 3 or more months.

8. Sex:

•Male have a higher BMR as compared to the females, thus can metabolize drugs more efficiently, e.g. salicylates (others might include ethanol, propanolol, benzodiazepines).

•Females, during pregnancy, have an increased rate of metabolism. Thus, the drug dose has to be increased. After the pregnancy is over, the dosage is decreased back to normal levels. Example includes phenytoin, whose dose has to be increased during pregnancy (specially second and third trimester).

Further Readings:

Wilson, C. O., Gisvold, O., & Doerge, R. F. (1982). Wilson and Gisvold's Textbook of organic medicinal and pharmaceutical chemistry. Philadelphia: Lippincott.

Thank You...

Keep Learning!