Biotransformation

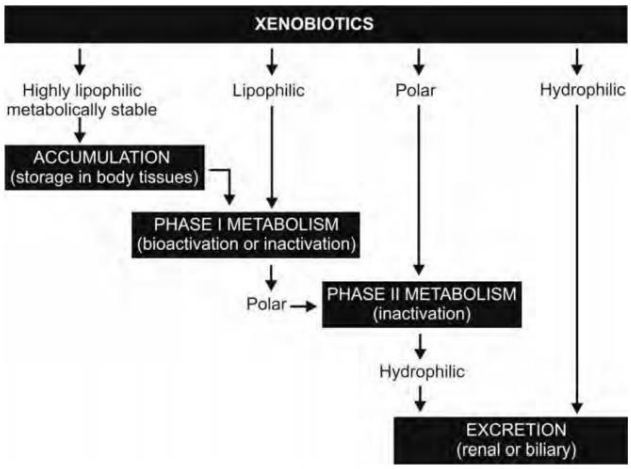
❖ **Definition**

- **Biotransformation** of drugs is defined as the chemical conversion of one form to another.
- The term is used synonymously with **metabolism**.
- The chemical changes are usually affected enzymatically in the body and thus, the definition excludes chemical instability of a drug within the body; for e.g. conversion of penicillin to penicilloic acid by the bacterial penicillinase and mammalian enzymes is metabolism but its degradation by the stomach acid to penicillenic acid is chemical instability.

❖ **Need for Drug Biotransformation**

- All chemical substances that are not nutrients for the body and enter the body through, ingestion, inhalation or absorption are called as **xenobiotics** (Greek: xenos = foreign) or **exogenous compounds**.
- Substances foreign(xenobiotics) to body include-
  i) Drugs, Processed food, Food additives, Cosmetic products, Environmental pollutants, Agrochemicals,
  ii) Phytoallexins (dietary plant toxins)

  Biotransformation needed for detoxification & protect the body from ingested toxins.
- Drugs are also xenobiotics which enter the body by virtue of their lipophilicity.
- It is interesting to note that only water-soluble agents undergo renal excretion (major route for exit of drugs from the body) whereas lipid soluble substances are passively reabsorbed from the renal tubules into the blood after glomerular filtration.
- Drugs are lipid soluble and are passively reabsorbed from the renal tubule therefore may accumulate in the body and precipitate toxic reactions.
- However, to prevent such a consequence, the body is armed with the metabolic system which transforms the water insoluble, lipophilic, nonpolar drugs into polar and water-soluble products that can be easily excreted by the kidneys and are poorly reabsorbed; for instance, hippuric acid, the metabolite of benzoic acid, is 2.5 times more water-soluble.
- Drug biotransformation is thus a **detoxification process**. However, exceptions are there when biotransformation leads to products with decreased water solubility. The N-acetyl derivatives of sulphonamides are less water-soluble than the parent drug and thus have a tendency to cause crystalluria.



❖ **Effect of Biotransformation –**

- **Normally biotransformation** results in pharmacological inactivation of drugs, i.e. it results in formation of metabolites with little or no pharmacological activity; e.g. conversion of phenytoin to p-hydroxy phenytoin.
- **Occasionally biotransformation** yields metabolites with equal activity; e.g. conversion of phenylbutazone to oxyphenbutazone.
- **Rarely biotransformation** leads to toxicological activation of drugs, i.e. it results in formation of metabolites with high tissue reactivity; e.g. conversion of paracetamol to reactive metabolites that cause hepatic necrosis.

- Inactive drugs (prodrugs) also depend upon biotransformation for activation, the process being called as **pharmacological activation**; e.g. conversion of enalapril to enalaprilat.
- In comparison with xenobiotics, the natural endogenous substances such as neurotransmitters (dopamine, GABA, epinephrine, norepinephrine, etc.), steroids (testosterone, progesterone, cortisol, etc.) and insulin which are also used as therapeutic agents, are inactivated rapidly because of the body's well developed system for metabolising such agents. These substances are therefore called as **soft drugs**. Such soft drugs do not precipitate unexpected toxicity when used in concentrations close to their normal levels.

❖ **Drug metabolising organ**

- Liver is the primary site for metabolism of almost all drugs (and other xenobiotics) because of its relative richness in possessing a large variety of enzymes in large amounts.
- Metabolism by organs other than liver (called as **extrahepatic metabolism**) is of minor importance since lower level of drug metabolising enzymes are present in such tissues. The decreasing order of drug metabolising ability of various organs is:
  Liver > Lungs > Kidneys > Intestine > Placenta > Adrenals > Skin Brain, testes, muscles, spleen, etc.

❖ **Drug Metabolising Enzymes**

- The enzymes that biotransform xenobiotics differ from those that metabolise food materials. The enzymes are broadly divided into two categories:

i) **Microsomal enzymes**
   - These are located on smooth endoplasmic reticulum, 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, certain dietary constituents, and other agencies.

ii) **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.

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

❖ **Types of biotransformation reaction**-

Biotransformation reactions can be classified into:

(a) **Nonsynthetic/Phasel/Functionalization reactions**:
   - a functional group (-OH, -COOH, -CHO, -NH_2 , -SH) is generated or exposed-
   - metabolite produce may be active or inactive.
   - Lipophilic substance become polar

(b) Synthetic/Conjugation/ Phase II reactions:
   - an endogenous radical is conjugated to the drug-
   - metabolite is mostly inactive; except few drugs, e.g. glucuronide conjugate of morphine and sulfate conjugate of minoxidil are active.
   - Polar substance become hydrophilic

1. Phase-I Reactions
   (a) Oxidation
   (b) Reduction
   (c) Hydrolysis
   (d) Cyclization
   (e) Decyclization

i) 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.
- Various oxidation reactions are:-
  - → hydroxylation;
  - → oxygenation at C, N or S atoms;
  - → N or O-dealkylation,
  - → oxidative deamination, etc

- Oxidative reactions are mostly carried out by a group of monooxygenases in the liver, which in the final step involve a cytochrome P-450 haemoprotein, NADPH, cytochrome P-450 reductase and molecular O2.
- The CYP isoenzymes important for drug metabolism in humans.
- **Cytochrome P450** (CYP) enzymes are a group of enzymes encoded by P450 genes and are expressed as membrane bound proteins mostly found in the endoplasmic reticulum of the liver.

ii) Reduction

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

iii) Hydrolysis

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

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

- Similarly, amides and polypeptides are hydrolysed by amidases and peptidases.
- Hydrolysis occurs in liver, intestines, plasma and other tissues.
- Examples of hydrolysed drugs are choline esters, procaine, lidocaine, procainamide, aspirin, indomethacin, carbamazepine-epoxide, pethidine, oxytocin

iv) Cyclization

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

v) Decyclization

This implies opening up of ring structure of the cyclic drug molecule, such as barbiturates, phenytoin. This is generally a minor pathway.

2. Phase-II reaction

- These reactions involve conjugation of the drug or its phase I metabolite with an endogenous substrate, usually derived from carbohydrate or amino acid, to form a polar highly ionized organic acid, which is easily excreted in urine or bile.
- Conjugation reactions have high energy requirement and are generally faster than phase I reactions.

***i)* Glucuronide conjugation**

- This is the most important Phase-II reaction carried out by a group of **uridine diphosphate** (UDP)-glucuronosyl transferases (UGTs).
- Compounds with a hydroxyl or carboxylic acid group are easily conjugated with glucuronic acid which is derived from glucose.
- Examples are—chloramphenicol, aspirin, paracetamol, diazepam, lorazepam, morphine, metronidazole.
- Not only drugs but endogenous substrates like bilirubin, steroidal hormones and thyroxine utilize this pathway.
- Glucuronidation increases the molecular weight of the drug which favours its excretion in bile.
- Drug glucuronides excreted in bile can be hydrolysed by bacteria in the gut—the liberated drug is reabsorbed and undergoes the same fate.
- This enterohepatic cycling of the drug prolongs its action, e.g. phenolphthalein, oral contraceptives

ii) **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.

iii) **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.

iv) **Sulfate conjugation**

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

v) **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.

vi) **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.

vii) **Ribonucleoside/nucleotide synthesis**

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

❖ **Hofmann elimination-**

- This refers to inactivation of the drug in the body fluids by spontaneous molecular rearrangement without the agency of any enzyme, e.g. atracurium.

❖ **Factors influencing drug metabolism**

- The rate of metabolism of a drug is particularly important for its pharmacological action as well as its toxicity
- For example, if the rate of metabolism of a drug is decreased, this generally increases the intensity and duration of the drug action. In addition, decreased metabolic elimination may lead to accumulation of toxic levels of the drug.
- Conversely, an increased rate of metabolism decreases the intensity and duration of action as well as the drug's efficacy.
- Many factors may affect drug metabolism like age, species and strain, genetic or hereditary factors, sex, enzyme induction, and enzyme inhibition.

1. **Age Differences**
   - Age-related differences in drug metabolism are generally quite apparent in the new born.
   - In most foetal and new born animals, undeveloped or deficient oxidative and conjugative enzymes are chiefly responsible for the reduced metabolic capability.
   - In general, the ability to carry out metabolic reactions increases rapidly after birth and approaches adult levels in about 1 to 2 months.
2. **Species and Strain Differences**
   - The metabolism of many drugs and foreign compounds is often species dependent. Different animal species may biotransform a particular xenobiotic by similar or different metabolic pathways.
   - Even within the same species, individual variations (strain differences) may result in significant differences in a specific metabolic pathway.
3. **Hereditary or Genetic Factors**
   - Individual differences in the metabolism of several drugs exist in humans.
   - Many of these genetic or hereditary factors are responsible for the large differences seen in the rate of metabolism of these drugs.

4. **Sex Differences**
    - The rate of metabolism of xenobiotics also varies according to gender in some animal species. A marked difference is observed between female and male rats.
    - Adult male rats metabolize several foreign compounds at a much faster rate than female rats (e.g., N-demethylation of aminopyrine, hexobarbital oxidation, glucuronidation of o-aminophenol).
5. **Enzyme Induction**
    - Many drugs, insecticides and carcinogens interact with DNA and increase the synthesis of microsomal enzyme protein, especially cytochrome P-450 and UGTs.
    - As a result the rate of metabolism of inducing drug itself (autoinduction) and/or some other coadministered drugs is accelerated.
6. **Enzyme Inhibition**-
    - Azole antifungal drugs, macrolide antibiotics and some other drugs bind to the heme iron in CYP450 and inhibit the metabolism of many drugs, as well as some endogenous substances like steroids, bilirubin.
    - One drug can competitively inhibit the metabolism of another if it utilizes the same enzyme or cofactors.
7. **First-pass(presystemic) metabolism**
    - It is the metabolism of a drug during its passage from the site of absorption into the systemic circulation.
    - All orally administered drugs are exposed to drug metabolizing enzymes in the intestinal wall and liver (where they first reach through the portal vein).
    - Presystemic metabolism in the gut and liver can be avoided by administering the drug through sublingual, transdermal or parenteral routes.
    - The extent of first pass metabolism differs for different drugs and is an important determinant of oral bioavailability.
    - A drug can also be excreted as such into bile. The hepatic extraction ratio (ER_{Liver}) of a drug is the fraction of the absorbed drug prevented by the liver from reaching systemic circulation. Both presystemic metabolism as well as direct excretion into bile determine ER_{Liver},

$$ER = \frac{CL_{Liver}}{Hepatic\ \ blood\ \ flow}$$

8. **Disease**
    - Many disease states alter drug metabolism especially those affecting the liver.
    - e.g amlodipine clearance decreased with hepatic disease
9. **Extrahepatic metabolism**
    - Other tissues also possess significant drug metabolising activity.
    - Extrahepatic tissues may contain different isozymes.
    - Tissues include: Lung, kidney, intestinal mucosa, skin, brain
    - Some drugs are given via different routes. i.e. skin – therefore there must be drug metabolising enzymes here.
    - Examples of these isozymes:
        i) FMO and CYP 1A1 in lung
        ii) Xanthine oxidase, CYP3A4, glucuronosyl transferase in intestine