Content

- Introduction
- History
- Phases of Metabolism
- Phase I Metabolism
- Cytochrome P family
- Phase II Metabolism
- First Pass Metabolism
- Role of Metabolism in Drug Discovery
- Factors affecting drug metabolism including stereo chemical aspects.

Introduction

- Biotransformation: Chemical alteration of the drug in body that converts nonpolar or lipid soluble compounds to polar or lipid insoluble compounds
- Consequences of biotransformation
  - Active drug → Inactive metabolite : Pentobarbitone, Morphine, Chloramphenicol
  - Active drug → Active metabolite: Phenacetin
  - Inactive drug → active metabolite: Levodopa

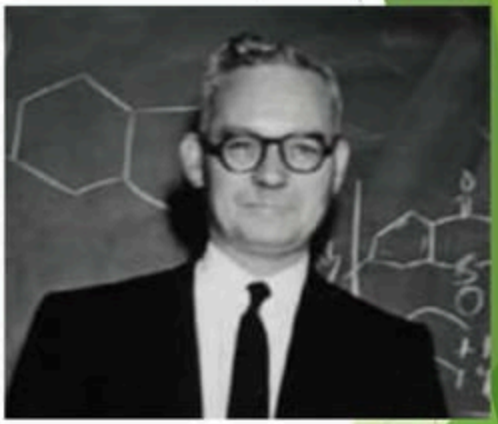
Prodrugs

- Inactive drug is converted to active metabolite
- Coined by Albert in 1958
- Advantages:
  - Increased absorption
  - Elimination of an unpleasant taste
  - Decreased toxicity
  - Decreased metabolic inactivation
  - Increased chemical stability
  - Prolonged or shortened action

History

- Welsh biochemist
- Metabolism of sulfonamides, benzene, aniline, acetanilide, phenacetin, thalidomide and stilbesterol
- Metabolism of TNT (Trinitrotoluene) with regard to toxicity in munitions (1942)

Richard Tecwyn Williams

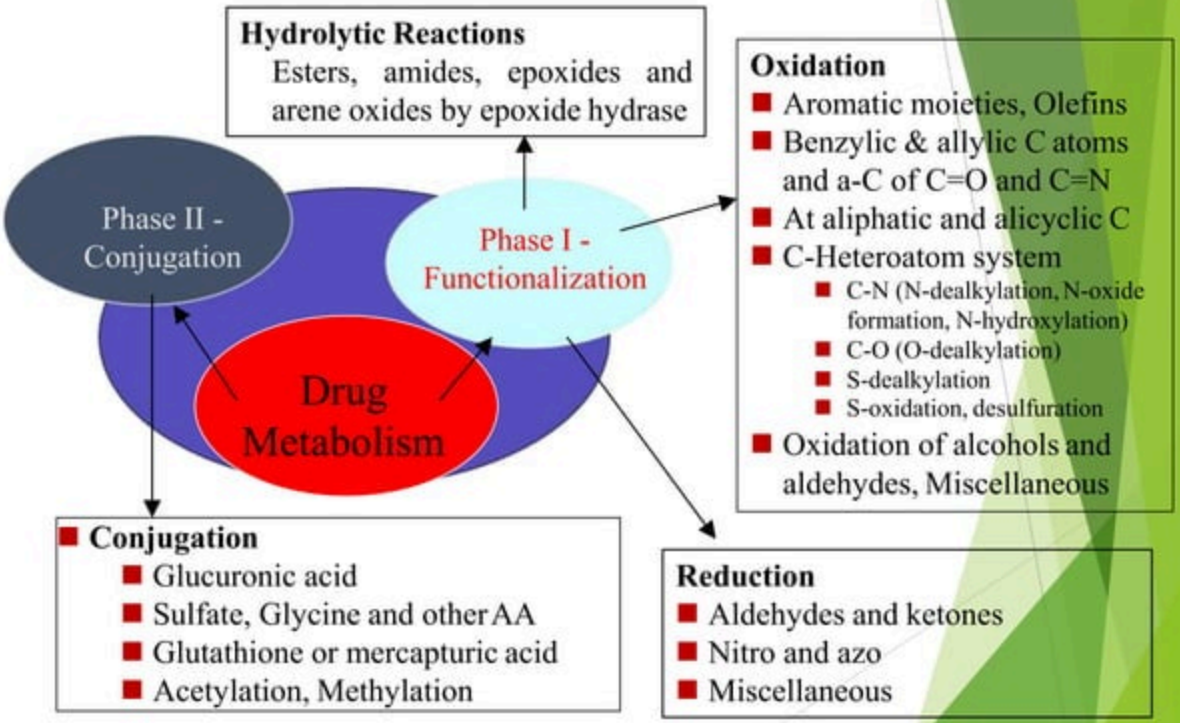


1909 - 1979

Phases of Metabolism

- **Phase I**
  - Functionalization reactions
  - Converts the parent drug to a more polar metabolite by introducing or unmasking a functional group (-OH, -NH2, -SH).
- **Phase II**
  - Conjugation reactions
  - Subsequent reaction in which a covalent linkage is formed between a functional group on the parent compound or Phase I metabolite and an endogenous substrate such as glucuronic acid, sulfate, acetate, or an amino acid

Phases of Metabolism

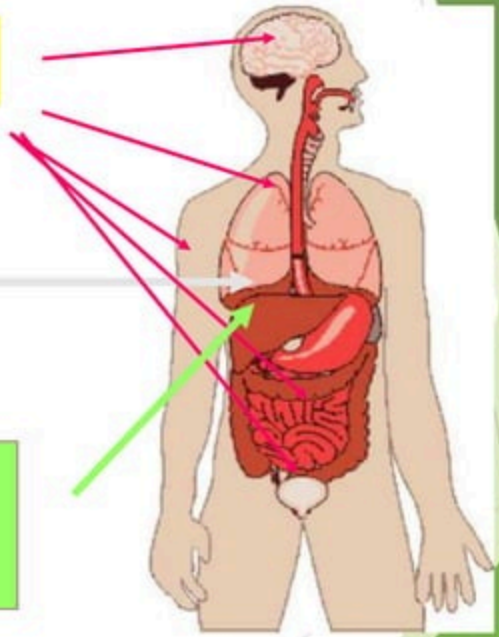


Sites of Drug Metabolism

Extrahepatic microsomal enzymes (oxidation, conjugation)

Hepatic microsomal enzymes (oxidation, conjugation)

Hepatic non-microsomal enzymes (acetylation, sulfation,GSH, alcohol/aldehyde dehydrogenase, hydrolysis, ox/red)



Phase I / Non Synthetic Reactions

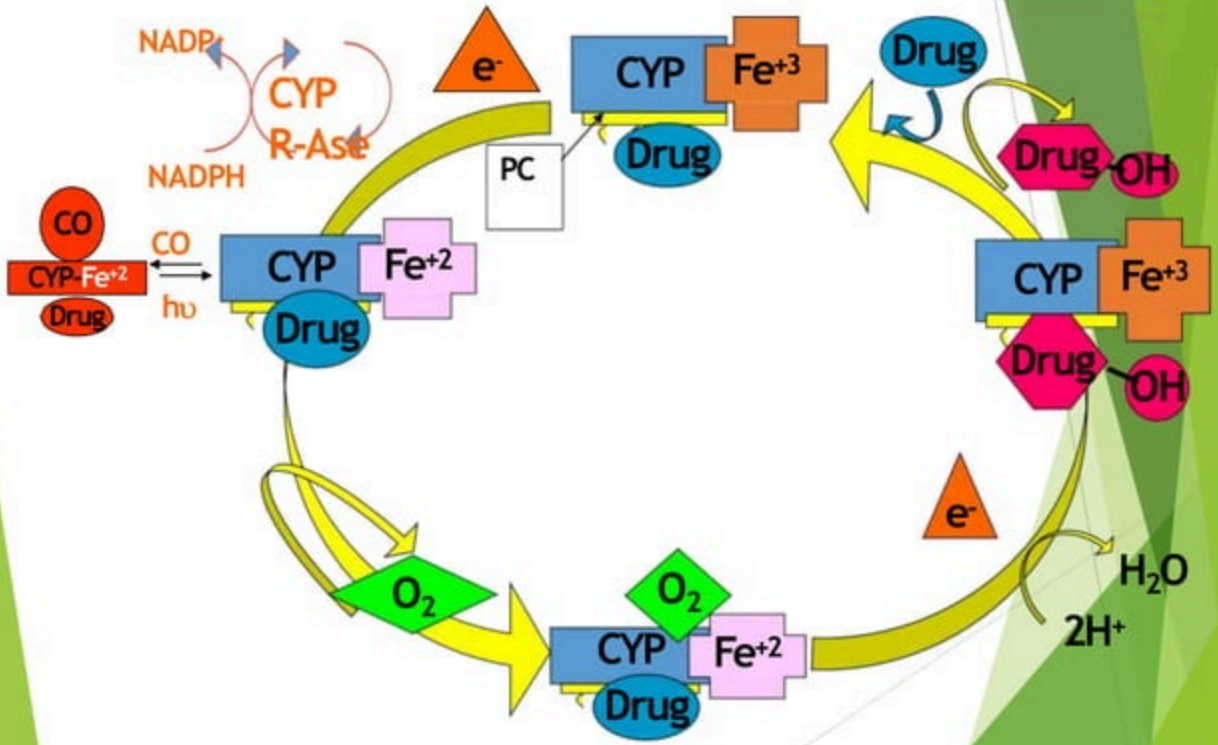
Oxidation

- Addition of oxygen/ negatively charged radical or removal of hydrogen/ positvely charged radical.
- Reactions are carried out by group of mono-oxygenases in the liver.
- Fianl step: Involves cytochrome P-450 haemoprotein, NADPH, cytochrome P-450 reductase and O2

Cytochrome P450 enzymes

- Monooxygenase enzyme family
- Major catalyst: Drug and endogenous compound oxidations in liver, kidney, G.I. tract, skin and lungs
- Oxidative reactions require: CYP heme protein, the reductase, NADPH, phosphatidylcholine and molecular oxygen
- Location: smooth endoplasmic reticulum in close association with NADPH-CYP reductase in 10/1 ratio
- The reductase serves as the electron source for the oxidative reaction cycle

Electron flow in Cytochromes



Cytochrome P family

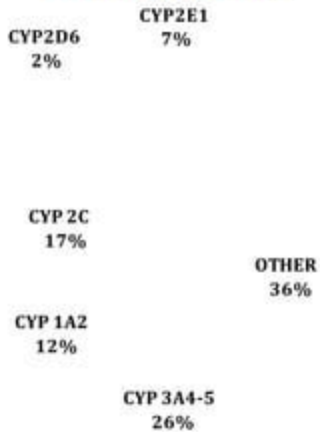
- Multiple CYP gene families have been identified in humans, and the categoriezed based on protein sequence homology
- Most of the drug metabolizing enzymes are in CYP 1, 2, & 3 families .
- Frequently, two or more enzymes can catalyze the same type of oxidation, indicating redundant and broad substrate specificity.
- CYP3A4 is very common to the metabolism of many drugs; its presence in the GI tract is responsible for poor oral availabilty of many drugs

Cytochrome families Continued….

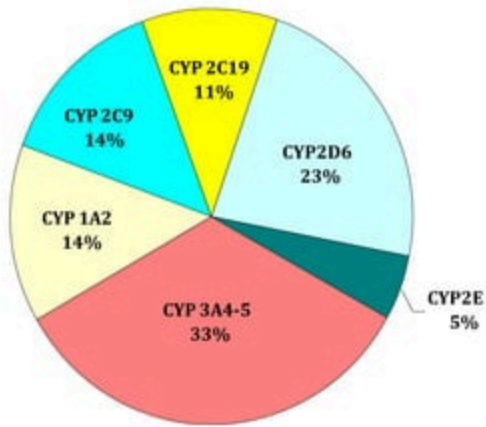
- Families: CYP plus arabic numeral (>40% homology of amino acid sequence, eg. CYP1)
- Subfamily: 40-55% homology of amino acid sequence; eg. CYP1A
- Subfamily: Additional arabic numeral when more than 1 subfamily has been identified; eg. CYP1A2
- Italics: Indicate gene (*CYP1A2*); regular font for enzyme

Role of CYP Enzymes in Hepatic Drug Metabolism

Relative Hepatic Content of CYP enzymes



Percentage of Drugs Metabolized by CYP Enzymes



Cytochromes: Metabolism of Drugs

CYP Enzyme	Examples of substrates
1A1	Caffeine, Testosterone, **R-Warfarin**
1A2	Acetaminophen, Caffeine, Phenacetin, **R-Warfarin**
2A6	17□-Estradiol, Testosterone
2B6	Cyclophosphamide, Erythromycin, Testosterone
2C-family	Acetaminophen, Tolbutamide (2C9); Hexobarbital, S- Warfarin (2C9,19); Phenytoin, Testosterone, **R- Warfarin**, Zidovudine (2C8,9,19);
2E1	Acetaminophen, Caffeine, Chlorzoxazone, Halothane
2D6	Acetaminophen, Codeine, Debrisoquine
3A4	Acetaminophen, Caffeine, Carbamazepine, Codeine, Cortisol, Erythromycin, Cyclophosphamide, S- and **R-Warfarin**, Phenytoin, Testosterone, Halothane, Zidovudin

Non-CYP Drug Oxidations

- Monoamine Oxidase (MAO), Diamine Oxidase (DAO)
  - MAO (mitochondrial) oxidatively deaminates endogenous substrates including neurotransmitters
  - Dopamine, serotonin, norepinephrine, epinephrine
- Alcohol & Aldehyde Dehydrogenase
  - Non-specific enzymes found in soluble fraction of liver
  - Ethanol metabolism
- Flavin Monooxygenases
  - Require molecular oxygen, NADPH, flavin adenosine dinucleotide (FAD)

Reduction

- Converse of oxidation
- Drugs primarily reduced are chloralhydrate, chloramphenicol, halothane.

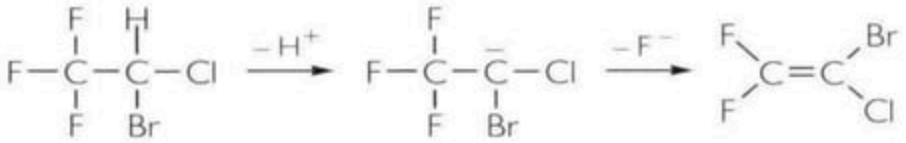


Figure 1.25 Reductive defluorination of halothane.

Hydrolysis

- Cleavage of drug molecule by taking up a molecule of water.

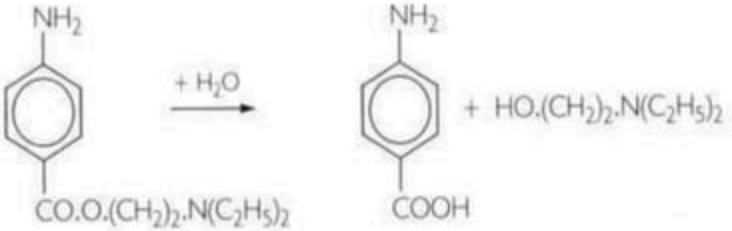


Figure 1.26 Hydrolysis of procaine.

- Sites: Liver, intestines, plasma and other tissues
- Examples: Choline esters, Procaine, Isoniazid, pethidine, oxytocin.

Cyclization and Decyclization

- **Cyclization**
  - Formation of ring structure from a straight chain compound
  - E.g. Proguanil
- **Decyclization**
  - Opening up of ring structure of the cyclic drug molecule
  - E.g. Barbiturates, Phenytoin.

Phase II/ Synthetic reactions

- Conjugation of the drug or its phase I metabolite with an endogenous substrate to form a polar highly ionized organic acid

- Types of phase II reactions
  - Glcuronide conjugation
  - Acetylation, Methylation
  - Sulfate conjugation, Glycine conjugation
  - Glutathione conjugation
  - Ribonucleoside/ nucleotide synthesis

Glucuronide Conjugation

- Conjugation to α-d-glucuronic acid
- Quantitatively the most important phase II pathway for drugs and endogenous compounds
- Products are often excreted in the bile
- Requires enzyme UDP-glucuronosyltransferase (UGT)
- Compounds with a hydroxyl or carboxylic acid group are easily conjugated with glucuronic acid which is derived from glucose

Glucuronide Conjugation Continued..

- Enterohepatic recycling may occur due to gut glucuronidases
- Drug glucuronides excreted in bile can be hydrolysed by bacteria in gut and reabsorbed and undergoes same fate.
- This recycling of the drug prolongs its action
  e.g.Phenolpthalein, Oral contraceptives
  - Examples: Chloramphenicol, aspirin, phenacetin, morphine, metronidazole

Acetylation

- Common reaction for aromatic amines and sulfonamides
- Requires co-factor acetyl-CoA
- Responsible enzyme is N-acetyltransferase
- Important in sulfonamide metabolism because acetyl-sulfonamides are less soluble than the parent compound and may cause renal toxicity due to precipitation in the kidney
- E.g. Sulfonamides, isoniazid, Hydralazine.

Sulfate Conjugation

- Major pathway for phenols but also occurs for alcohols, amines and thiols
- Sulfate conjugates can be hydrolyzed back to the parent compound by various sulfatases
- Sulfoconjugation plays an important role in the hepatotoxicity and carcinogenecity of N-hydroxyarylamides
- Infants and young children have predominating O-sulfate conjugation
- Examples include: a-methyldopa, albuterol, terbutaline, acetaminophen, phenacetin

Amino Acid Conjugation:

- ATP-dependent acid: CoA ligase forms active CoA-amino acid conjugates which then react with drugs by N-Acetylation:
  - Usual amino acids involved are:
    - Glycine. Glutamine, Ornithine, Arginine

Glutathione Conjugation:

- Glutathione is a protective factor for removal of potentially toxic compounds
- Conjugated compounds can subsequently be attacked by g-glutamyltranspeptidase and a peptidase to yield the cysteine conjugate => product can be further

First pass Metabolism

- Metabolism of a drug during its passage from the site of absorption into the systemic circulation.
- Extent of first pass metabolism differs in different drugs

Extent of first pass metabolism of important drugs

Low	Intermediate	High – not given orally	High oral dose
Phenobarbitone	Aspirin	Isoprenaline	propranolol
Phenylbutazone	Quinidine	Lignocaine	Alprenolol
Tolbutamide	Desipramine	Hydrocortisone	Verapamil
Pindolol	Nortriptyline	Testosterone	Salbutamol

Attributes of drugs with high first pass metabolism

- Oral dose is considerably higher then sublingual or parenteral dose
- Marked individual variation in the oral dose due to differences in the extent of first pass metabolism
- Oral bioavailability is apparently increased in patients with severe liver disease
- Oral bioavailability of a drug is increased if another drug competing with it.

E.G. Chloropromazine and Propranolol

Inhibition of Metabolism

- Competitively inhibit the metabolism of another drug if it utilizes the same enzyme or co factors.
- A drug may inhibit one isoenzyme while being itself a substrate of another isoenzyme

e.g. quinidine is metabolized by CYP3A4 but inhibits CYP2D6

- Inhibition of drug metabolism occurs in a dose related manner and can precipitate toxicity of the object drug.
- Blood flow limited metabolism

e.g. Propranolol reduces rate of lignocaine metabolism by decreasing hepatic blood flow.

Microsomal Enzyme Induction

- Certain drugs, insecticides and carcinogens increase the synthesis of microsomal enzyme protein.
- Different inducers are relatively selective for certain cytochrome P-450 enzyme families e.g.
  - Phenobarbitone , rifampin, glucorticoids induce CYP3A isoenzymes
  - Isoniazid and chronic alochol consumption induce CYP2E1
- Induction takes 4-14 days to reach its peak and is maintained till the inducing agent is present.

Consequences of Induction

- Decreased intensity or Increased Intensity of action of drug
- Tolerance- auto induction
- Precipitation of acute intermittent porphyria
- Interfere with adjustment of dose of another drug
- Interference with chronic toxicity

Possible Uses of Induction: Congenital non hemolytic anaemia Cushing's Syndrome

Role of Metabolism in pediatric and elderly

- New born has low g.f.r and tubular transport is immature, so the t1/2 of the drug like streptomycin and penicillin is prolonged
- Hepatic drug metabolising system is inadequate in new borns e.g. chloramphenicol can produce gray baby syndrome
- In elderly the renal function progressively declines
- Reduction of hepatic microsomal activity and liver blood flow
- Incidence of adverse drug reactions is much higher in elderly

Role of Metabolism in Drug discovery

- In drug development it is important to have an information on the enzymes responsible for the metabolism of the candidate drug
- Invitro Studies can give information about
  - Metabolite stability
  - Metabolite profile
  - Metabolite Identification
  - CYP induction/Inhibition
  - Drug/Drug interaction studies
  - CYP isoform identification