

DRUG

METABOLISM

# Outline

- Introduction
- History
- Phases of Metabolism
- Phase I Metabolism
- Cytochrome P family
- Phase II Metabolism
- First Pass Metabolism
- Ante drug
- Microsomal Enzyme Induction
- Role of Metabolism in Drug Discovery

# 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

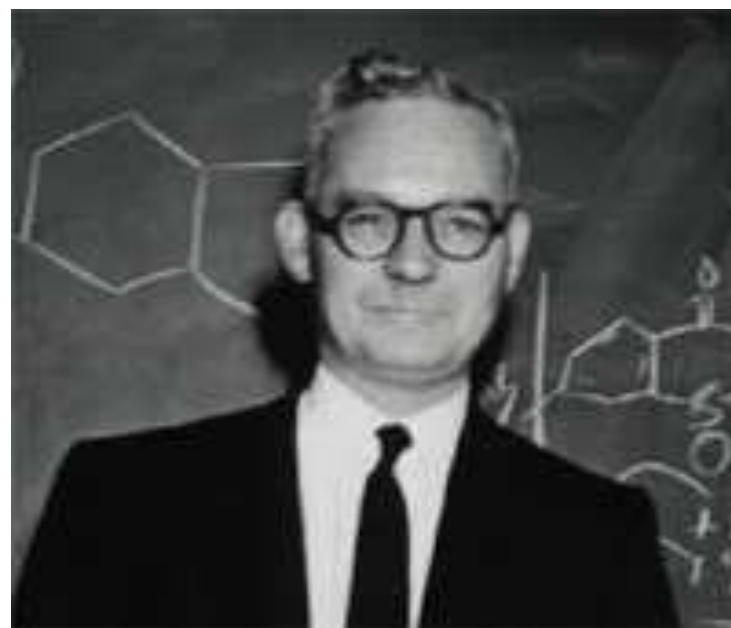
# Prodrugs

- 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

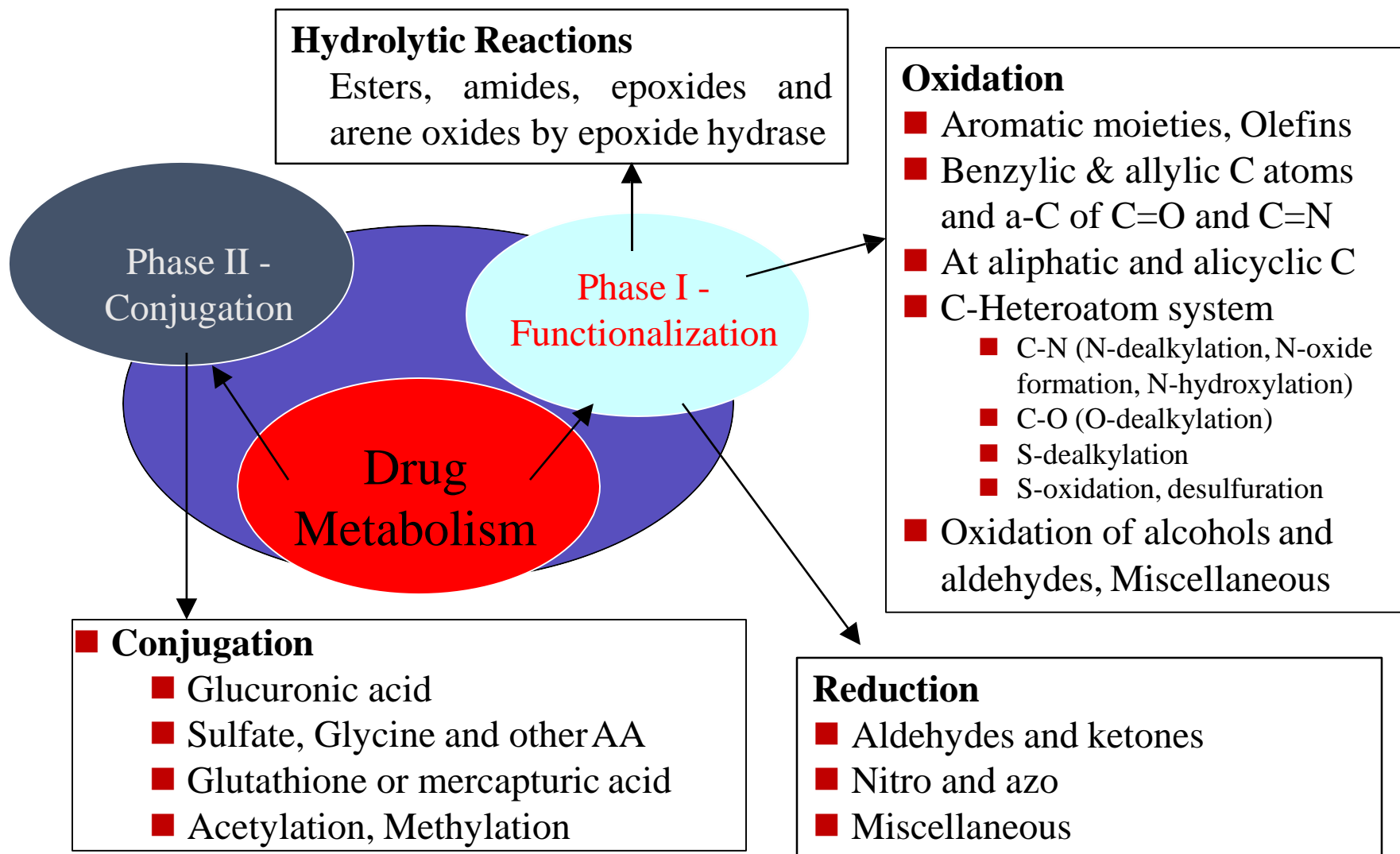
- **Phase I**

- Functionalization reactions
- Converts the parent drug to a more polar metabolite by introducing or unmasking a functional group (-OH, -NH<sub>2</sub>, -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

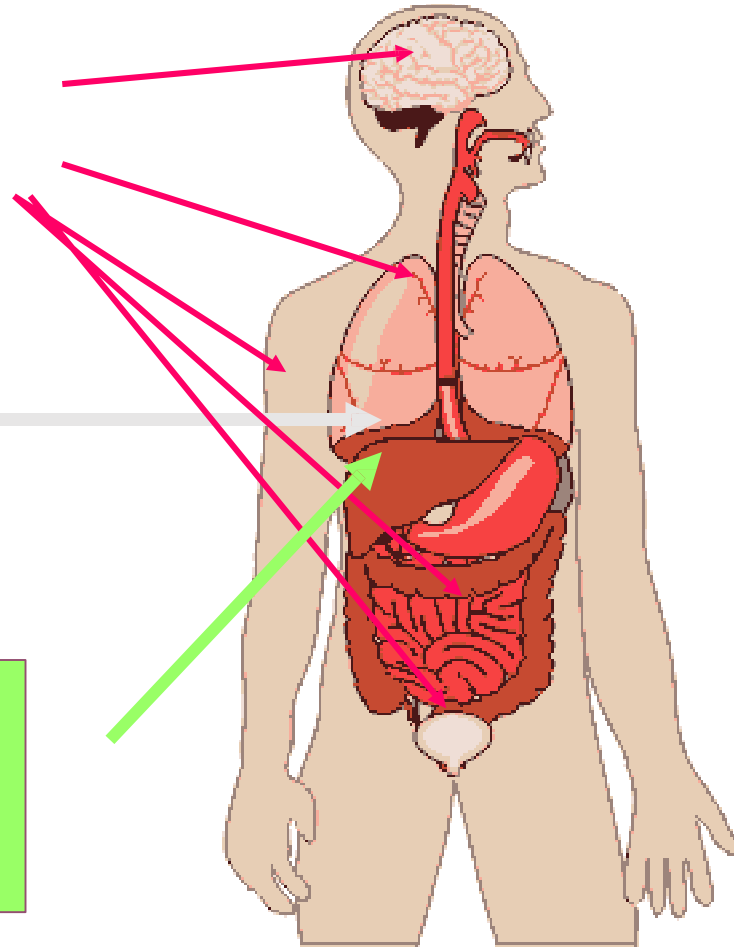


# Sites of Drug Metabolism

**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

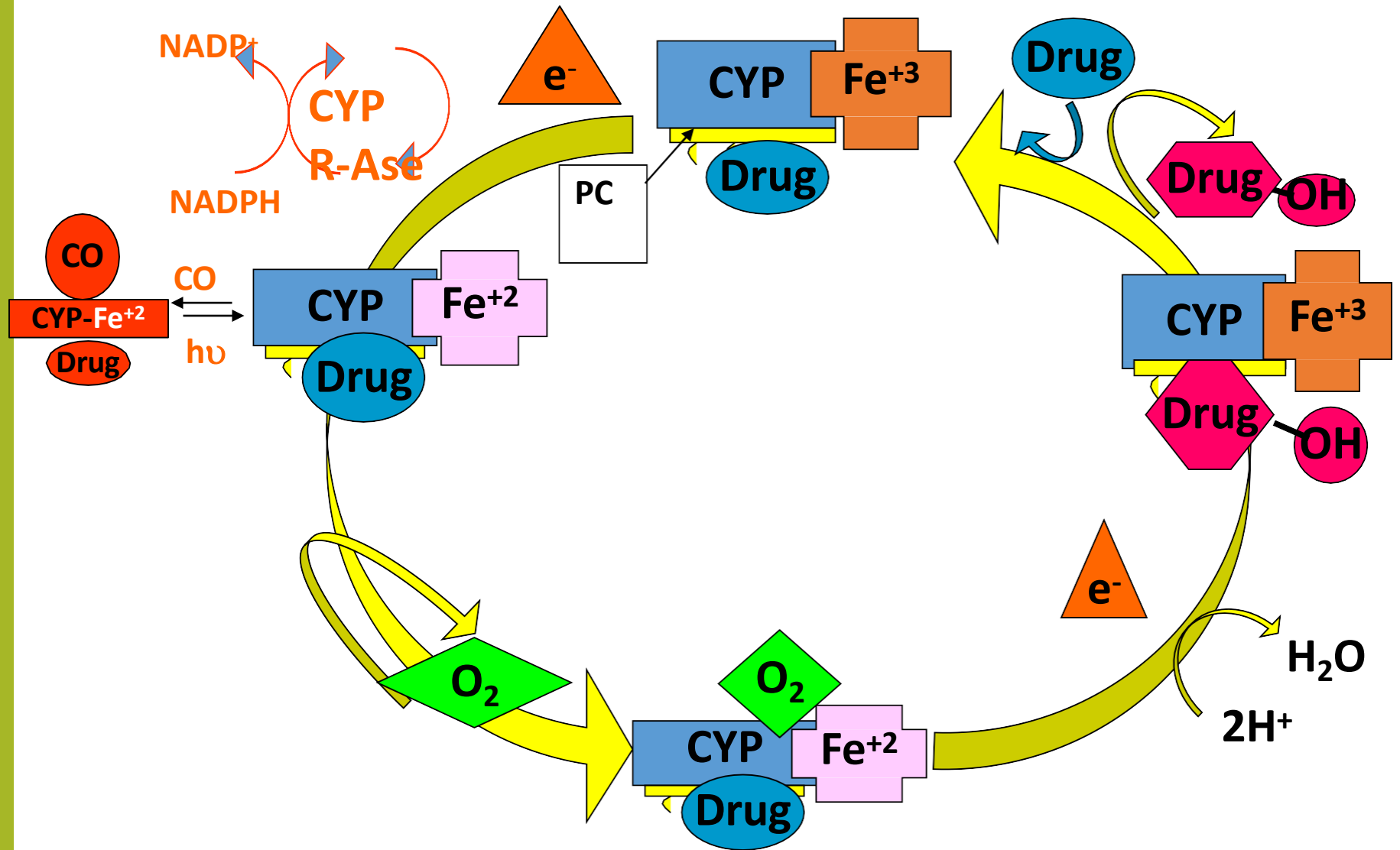
## **Oxidation**

- Addition of oxygen/ negatively charged radical or removal of hydrogen/ positively charged radical.
- Reactions are carried out by group of mono-oxygenases in the liver.
- Final step: Involves cytochrome P-450 haemoprotein, NADPH, cytochrome P-450 reductase and O<sub>2</sub>

# Cytochrome P<sub>450</sub> 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 categorized 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 availability of many drugs

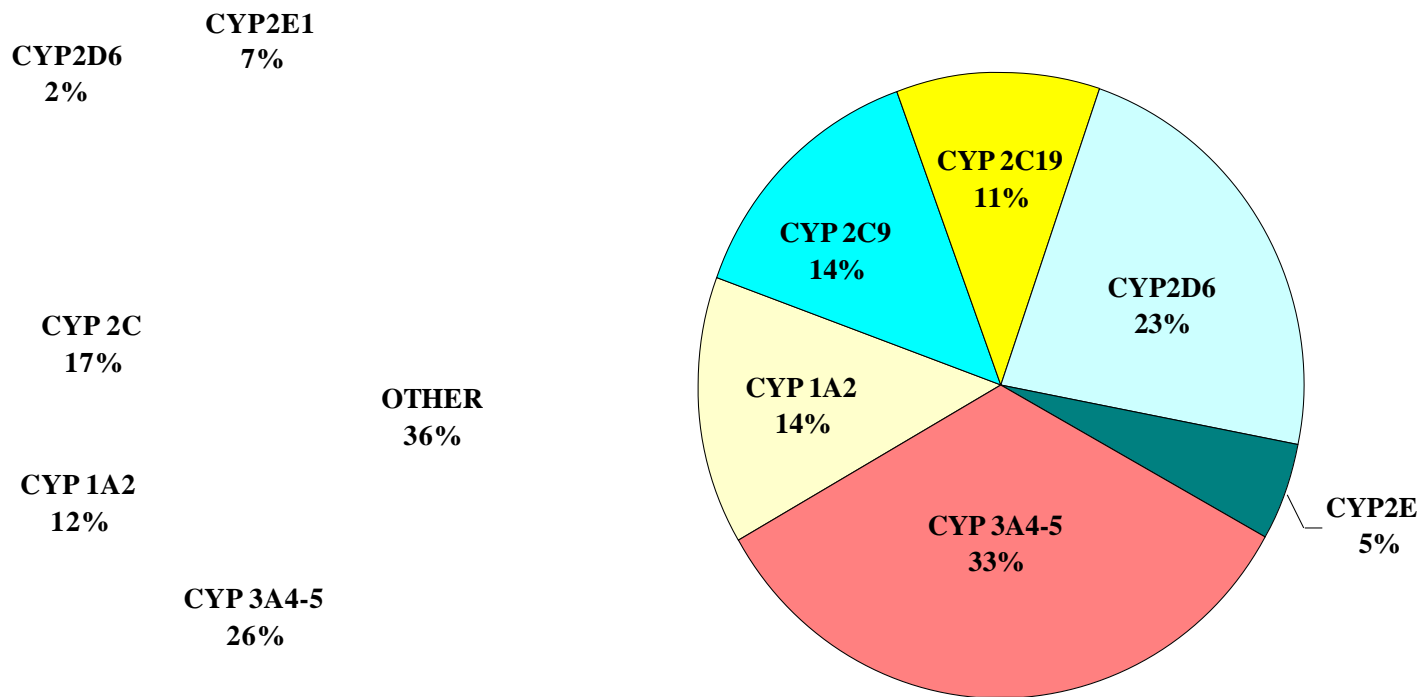
# Cytochrome families Continued....

- **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 $\beta$ -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, Zidovudine |

Adapted from S. Reuhl *Drug Metab Rev* 34: 83-448, 2002

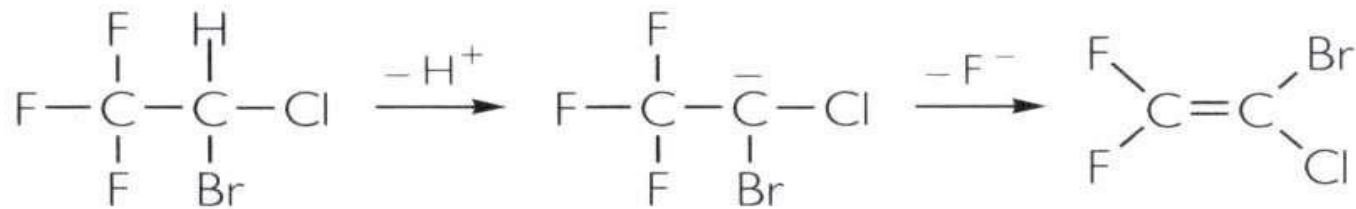
# Non-CYP Drug Oxidations

- **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

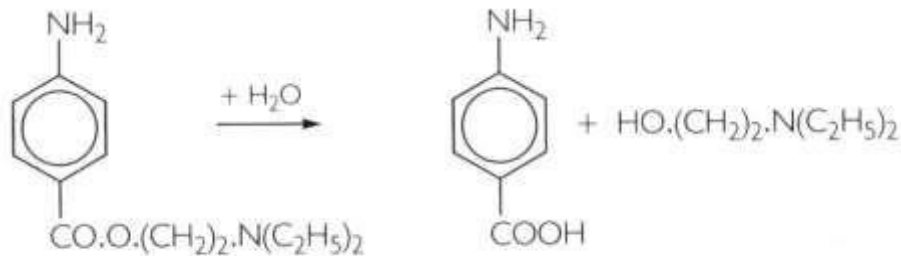
- Converse of oxidation
- Drugs primarily reduced are chloralhydrate, chloramphenicol, halothane.



**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
  - Glucuronide conjugation
  - Acetylation, Methylation
  - Sulfate conjugation, Glycine conjugation
  - Glutathione conjugation
  - Ribonucleoside/ nucleotide synthesis

# Glucuronide Conjugation

- Conjugation to  $\alpha$ -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. Phenolphthalein, 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 carcinogenicity 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  $\gamma$ -glutamyltranspeptidase and a peptidase to yield the cysteine conjugate => product can be further acetylated to N-acetylcysteine conjugate  
E.g. Paracetamol

# Hofmann elimination

Inactivation of the drug in the body fluids by spontaneous molecular re arrangement without the agency of any enzyme

e.g. Atracurium.

# 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

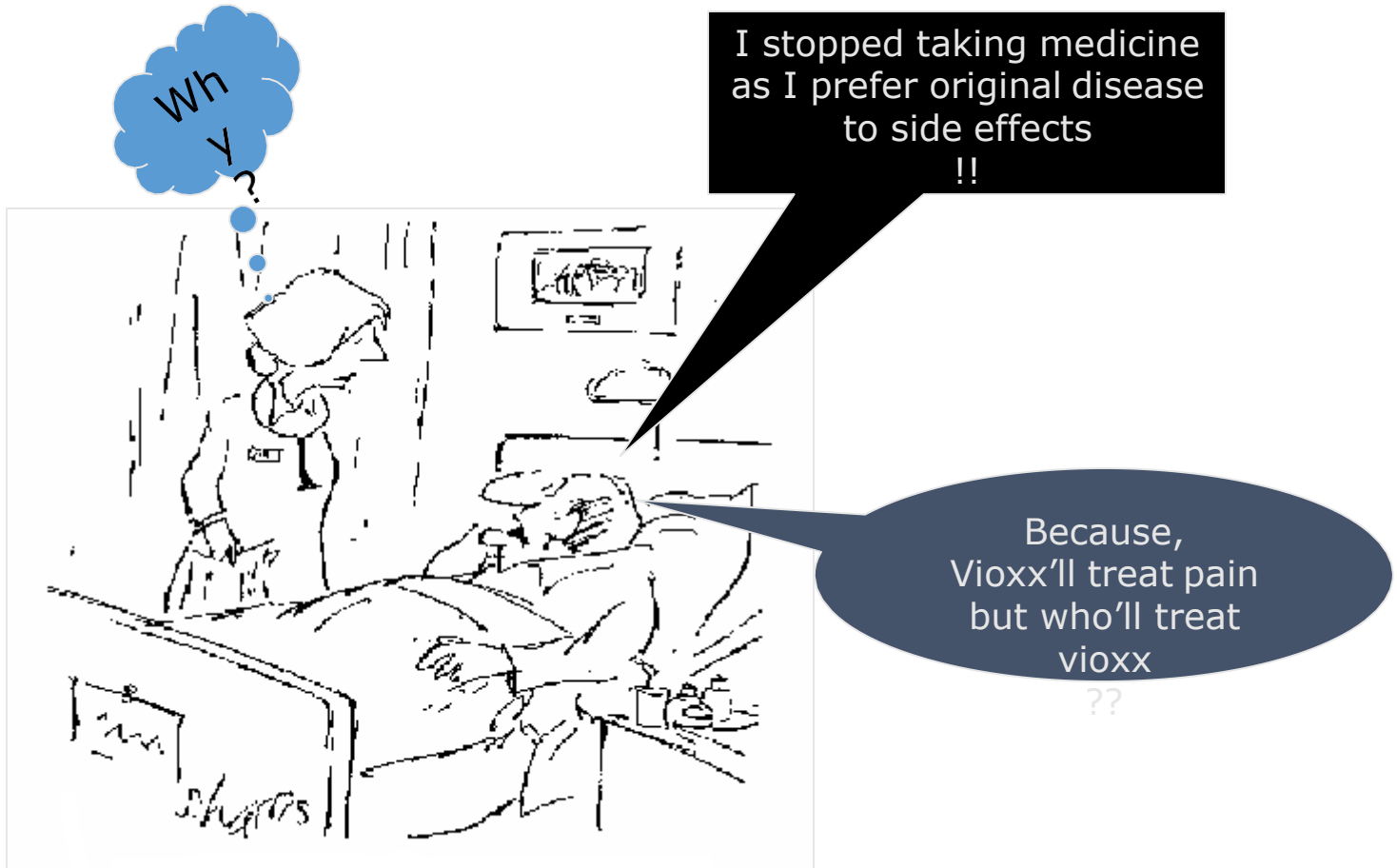
| Low            | Intermediate  | High – not given orally | High oral dose |
|----------------|---------------|-------------------------|----------------|
| Phenobarbitone | Aspirin       | Isoprenaline            | propranolol    |
| Phenylbutazone | Quinidine     | Lignocaine              | Alprenolol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

# Attributes of drugs with high first pass metabolism

- Oral dose is considerably higher than 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. Chlorpromazine and Propranolol

# Ante Drug



# What is Antedrug?

**An active synthetic drug which is inactivated by a metabolic process upon entry into the systemic circulation.**

**Therefore, a true antedrug acts only locally.**

**True Antedrug             $\longrightarrow$     Inactive Metabolite**

**Partial Antedrug        $\longrightarrow$     Less active metabolite**

**Lee HJ and Soliman MRI (1982). *Science*, 215, 989.**

# Advantages of Antedrug

- Localization of the drug effects
- Elimination of toxic metabolites, increasing the therapeutic index
- Avoidance of pharmacologically active metabolites that can lead to long-term effects
- Elimination of drug interactions resulting from metabolite inhibition of enzymes
- Simplification of PK problems caused by multiple active species

# 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, glucocorticoids induce CYP3A isoenzymes
  - Isoniazid and chronic alcohol 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- autoinduction
- 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  $t_{1/2}$  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