

DRUG METABOLISM



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INTRODUCTION

In 1949, a Welsh biochemist Richard Tecwyn Williams introduced the metabolism. He classified two categories of biotransformation, Phase I involving oxidations, reductions or hydrolysis or a combination of any of these three and Phase II consists of conjugations such as Glucuronide, Hippuric acid, and Thiocyanate formation.

The major Phase I Oxidations are cytochromes P450 reactions, peroxidase-catalyzed oxidations, alcohol dehydrogenase, etc.; Phase I Reductions are as nitro- and azo-reduction which stands alone. The resulting metabolites are often electrophilic compounds that can bind to cellular proteins or nucleic acids and alter cell functions. Phase-I metabolic reactions normally introduce or expose functional groups on the drugs, leading to increasing polarity of the compound. Phase I drug metabolism occurs in most of the tissues by microsomal enzymes located in the endoplasmic reticulum. On other hand, the primary and first pass site of metabolism occurs during hepatic circulation. Additional metabolism occurs in gastrointestinal epithelial, renal, skin, and lung tissues.

INTRODUCTION

Phase II conjugations require a nucleophilic site especially hydroxyl and amino groups in a molecule, for the reactions of electrophilic adenosine containing cofactors (Adenosine triphosphate, 3'-Phosphoadenosine-5'-phosphosulfate, Acetyl-coenzyme A, S-Adenosyl methionine etc.).

Phase II Conjugates generally required export across cell membranes and subjected to further metabolism, for example, mercapturic acid formation. Hence, use of a new term "Phase III metabolism" is popular to describe membrane transport of drug conjugates.

- **METABOLISM OR BIOTRANSFORMATION**

The conversion from one chemical form of a substance to another.

The term **Metabolism** is commonly used probably because products of drug transformation are called **metabolites**.

Metabolism is an essential **pharmacokinetic process**, which renders lipid soluble and non-polar compounds to water soluble and polar compounds so that they are excreted by various processes.

This is because only water-soluble substances undergo excretion, whereas lipid soluble substances are passively reabsorbed from renal or extra renal excretory sites into the blood by virtue of their lipophilicity.

Metabolism is a necessary biological process that limits the life of a substance in the body.

- **Biotransformation:**

- It is a specific term used for **chemical transformation of xenobiotics** (Substance which is not naturally produced) in the body/living organism.

- **Metabolism :**

- It is a general term used for chemical transformation of xenobiotics and endogenous nutrients (e.g., proteins, carbohydrates and fats) within or outside the body.

- **Xenobiotics :**

- These are all chemical substances that are not nutrient for body (foreign to body) and which enter the body through ingestion, inhalation or dermal exposure.
- They include : drugs, industrial chemicals, pesticides, pollutants, plant and animal toxins, etc.

- **Drug Metabolising Enzymes**

- These enzymes are located mainly in the liver, but may also be present in other organs like lungs, kidneys, intestine, brain, plasma, etc.
- Majority of drugs are acted upon by relatively non-specific enzymes, which are directed to types of molecules rather than to specific drugs.
- The drug metabolising enzymes can be broadly divided into two groups: **microsomal and non-microsomal enzymes**.

- **Microsomal enzymes:**

- The endoplasmic reticulum (especially smooth endoplasmic reticulum) of liver and other tissues contain a large variety of enzymes, together called microsomal enzymes
- Microsomes are minute spherical vesicles derived from endoplasmic reticulum after disruption of cells by centrifugation, enzymes present in microsomes are called microsomal enzymes).
- They catalyze glucuronide conjugation, most oxidative reactions, and some reductive and hydrolytic reactions.
- The monooxygenases, glucuronyl transferase, etc are important microsomal enzymes

- **Non-microsomal enzymes:**

- Enzymes occurring in organelles/sites other than endoplasmic reticulum (microsomes) are called non-microsomal enzymes.
- These are usually present in the cytoplasm, mitochondria, etc. and occur mainly in the liver, GI tract, plasma and other tissues.
- They are usually non-specific enzymes that catalyse few oxidative reactions, a number of reductive and hydrolytic reactions, and all conjugative reactions other than glucuronidation.
- None of the non-microsomal enzymes involved in drug biotransformation is known to be inducible.

PHASES OF METABOLISM

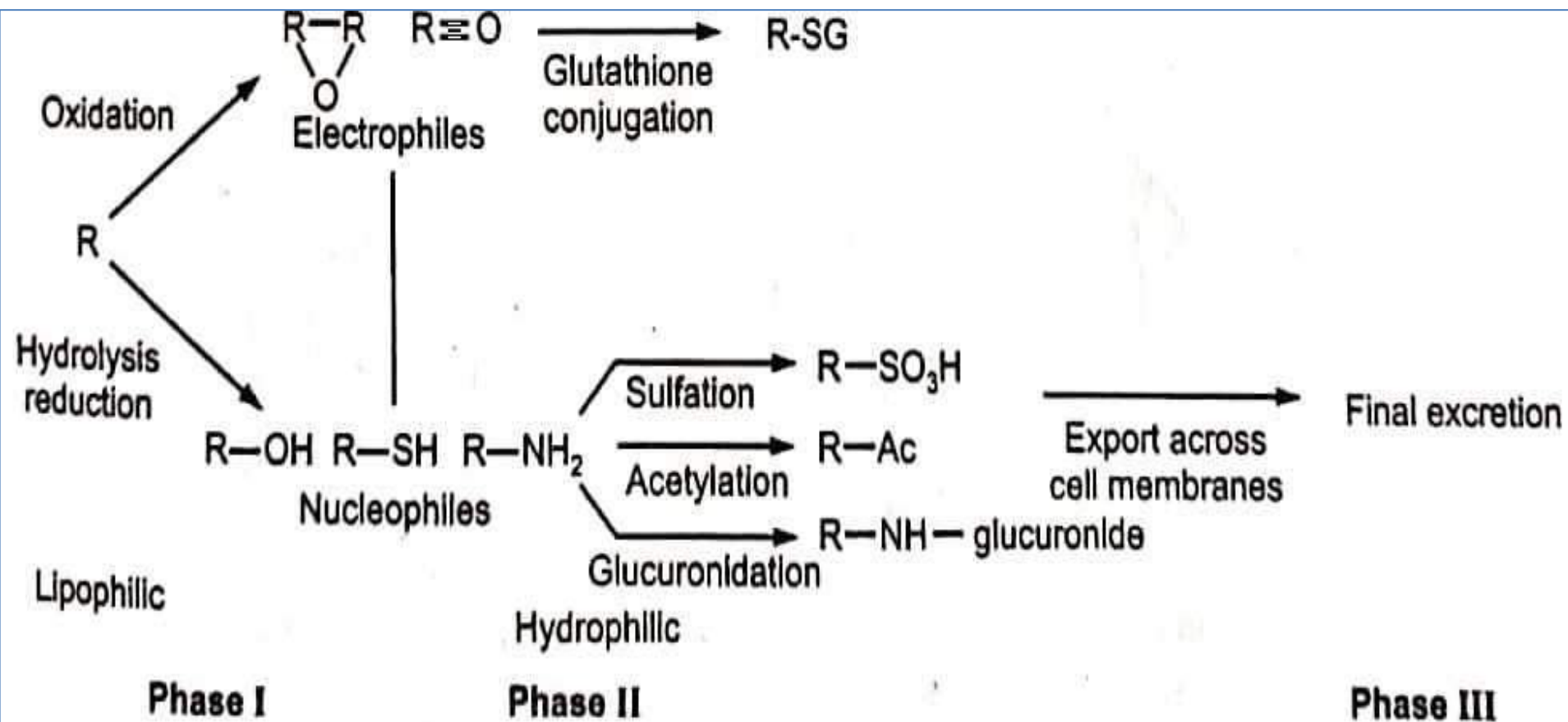
- **Phase I**

- 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 acids.

PHASE-I, II AND III DRUG METABOLISM



SITES FOR DRUG METABOLISM

Smooth endoplasmic reticulum of the liver is the major site of drug metabolism, other sites are: Intestine, Kidneys, Lungs, Adrenal Glands, Placenta, Brain and Skin.

Phases of Metabolism

Hydrolytic Reactions

Esters, amides, epoxides and arene oxides by epoxide hydrase

Phase I - Functionalization

Oxidation

- Aromatic moieties, Olefins
- Benzylic & allylic C atoms and α -C of C=O and C=N
- At aliphatic and alicyclic C
- C-Heteroatom system
 - C-N (N-dealkylation, N-oxide formation, N-hydroxylation)
 - C-O (O-dealkylation)
 - S-dealkylation
 - S-oxidation, desulfuration
- Oxidation of alcohols and aldehydes, Miscellaneous

Reduction

- Aldehydes and ketones
- Nitro and azo
- Miscellaneous

Phase II - Conjugation

Drug Metabolism

Conjugation

- Glucuronic acid
- Sulfate, Glycine and other AA
- Glutathione or mercapturic acid
- Acetylation, Methylation

- **Phase 1 reaction. (Non synthetic phase)**
 - A change in drug molecule, generally results in the introduction of a functional group into molecules or the exposure of new functional groups of molecules
 - Phase I (non-synthetic or non conjugative phase) includes reactions which catalyse oxidation, reduction and hydrolysis of drugs.
 - In phase I reactions, small polar functional groups like -OH, -NH₂, -SH, -COOH, etc. are either added or unmasked (if already present) on the lipid soluble drugs so that the resulting products may undergo phase II reactions.
 - Result in activation, change or inactivation of drug.
 - Phase I metabolism is sometimes called a “functionalization reaction,”
 - Results in the introduction of new hydrophilic functional groups to compounds.
- **Function:**
 - Introduction (or unveiling) of functional group(s) such as -OH, -NH₂, -SH, -COOH into the compounds.
- **Reaction types: oxidation, reduction, and hydrolysis**

- **Phase II reaction. (Synthetic phase)**
 - Last step in detoxification reactions and almost always results in loss of biological activity of a compound.
 - May be preceded by one or more of phase one reaction
 - Involves conjugation of functional groups of molecules with hydrophilic endogenous substrates- formation of conjugates - is formed with (an endogenous substance such as carbohydrates and amino acids.)with drug or its metabolites formed in phase 1 reaction.
 - Involve attachment of small polar endogenous molecules like glucuronic acid, sulphate, methyl, amino acids, etc., to either unchanged drugs or phase I products.
 - Products called as 'conjugates' are water-soluble metabolites, which are readily excreted from the body.
 - Phase II metabolism includes what are known as conjugation reactions.
 - Generally, the conjugation reaction with endogenous substrates occurs on the metabolite(s) of the parent compound after phase I metabolism; however, in some cases, the parent compound itself can be subject to phase II metabolism.
- **Function:**
 - conjugation (or derivatization) of functional groups of a compound or its metabolite(s) with endogenous substrates.
- **Reaction types:** glucuronidation, sulfation, glutathione-conjugation, N acetylation, methylation and conjugation with amino acids (e.g., glycine, taurine, glutamic acid).

Phase I reaction

- **Reaction types:**

- A) Oxidation
- B) Reduction
- C) Hydrolysis

Phase II reaction

- **Reaction types:**

- A) Glucuronidation
- B) Sulfation
- C) Glutathione-conjugation
- D) N –acetylation
- E) Methylation
- F) Conjugation with amino acids (e.g., glycine, taurine, glutamic acid).

• Oxidation :

- Addition of oxygen/ negatively charged radical or removal of hydrogen/ positively charged radical.
- Reactions are carried out by group of **monooxygenases** in the liver.
- Final step: Involves cytochrome P-450 haemoprotein, NADPH, cytochrome P-450 reductase and O_2
- Oxidative reactions are **most important metabolic reactions**, as energy in animals is derived by oxidative combustion of organic molecules containing carbon and hydrogen atoms.
- The oxidative reactions are **important for drugs** because they increase **hydrophilicity** of drugs by **introducing polar functional groups** such as -OH.
- **Oxidation of drugs is non-specifically catalysed** by a number of **enzymes located primarily in the microsomes**. Some of the oxidation reactions are also catalysed by **non-microsomal enzymes** (e.g., **aldehyde dehydrogenase, xanthine oxidase and monoamine oxidase**).
- The most important group of oxidative enzymes **are microsomal monooxygenases** or mixed function oxidases (MFO).
- These enzymes are located mainly in the hepatic endoplasmic reticulum and require both molecular oxygen (O_2) and reducing NADPH to effect the chemical reaction.
- Mixed function oxidase name was proposed in order to characterise the mixed function of the oxygen molecule, which is essentially required by a number of enzymes located in the microsomes.

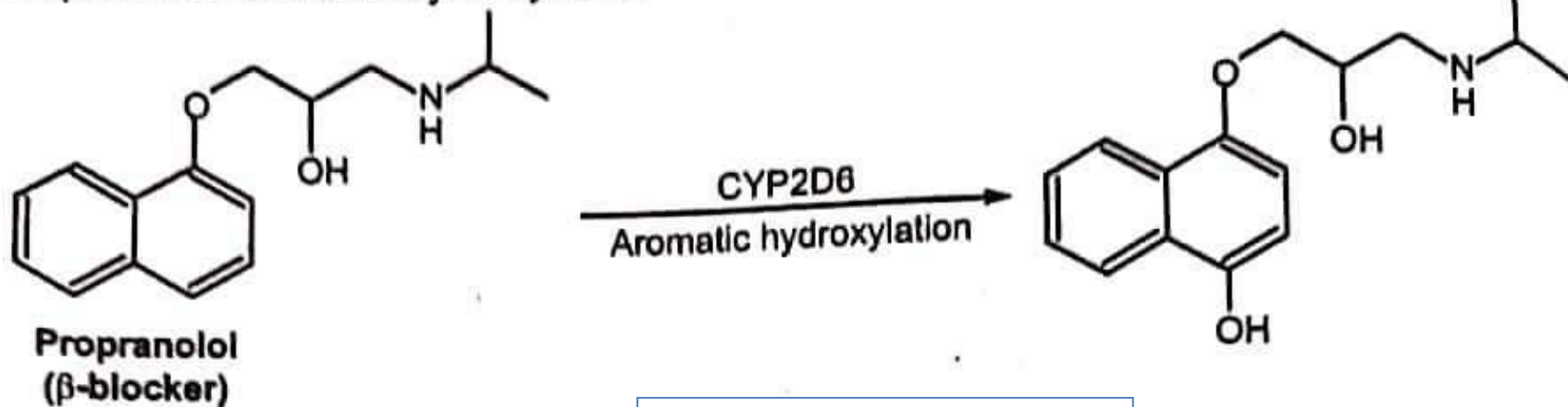
- The term monooxygenases for the enzymes was proposed as they incorporate only one atom of molecular oxygen into the organic substrate with concomitant reduction of the second oxygen atom to water.
- The overall stoichiometry of the reaction involving the substrate RH which yields the product ROH, is given by the following reaction:

MFO

- $RH + O_2 + NADPH + H^+ \longrightarrow ROH + H_2O + NADP +$
- The most important component of mixed function oxidases is the cytochrome P-450 because it binds to the substrate and activates oxygen.
- The wide distribution of cytochrome P-450 containing MFOs in varying organs makes it the most important group of enzymes involved in the biotransformation of drugs.

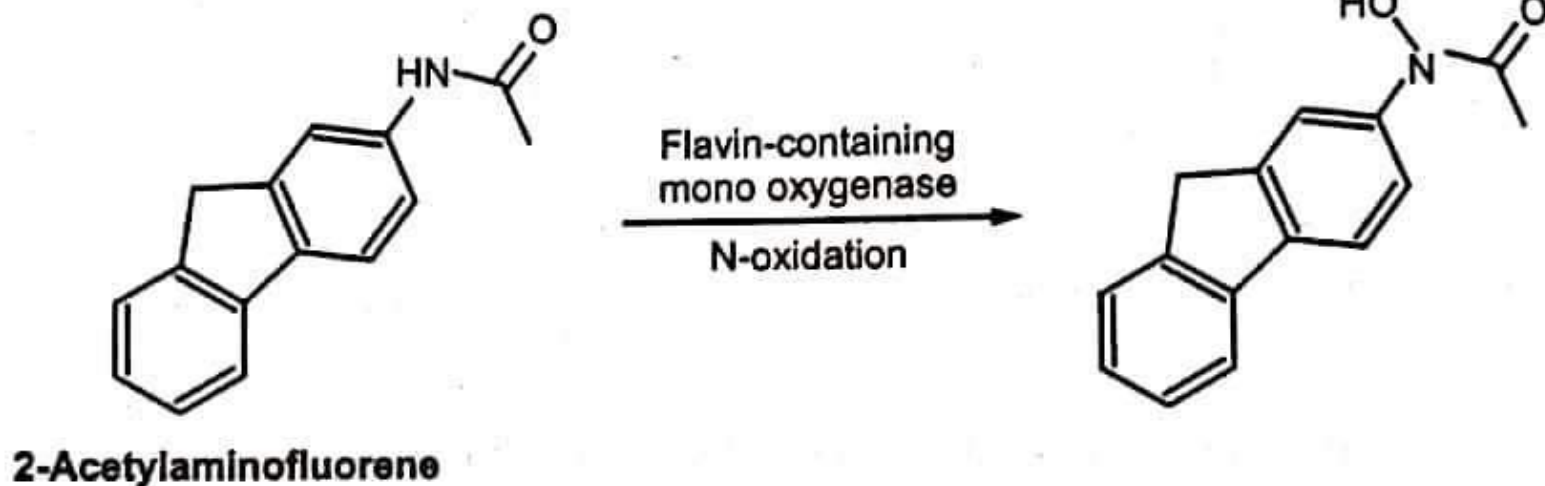
PHASE-I DRUG METABOLISM

A. Aliphatic or aromatic hydroxylation



1. OXIDATION

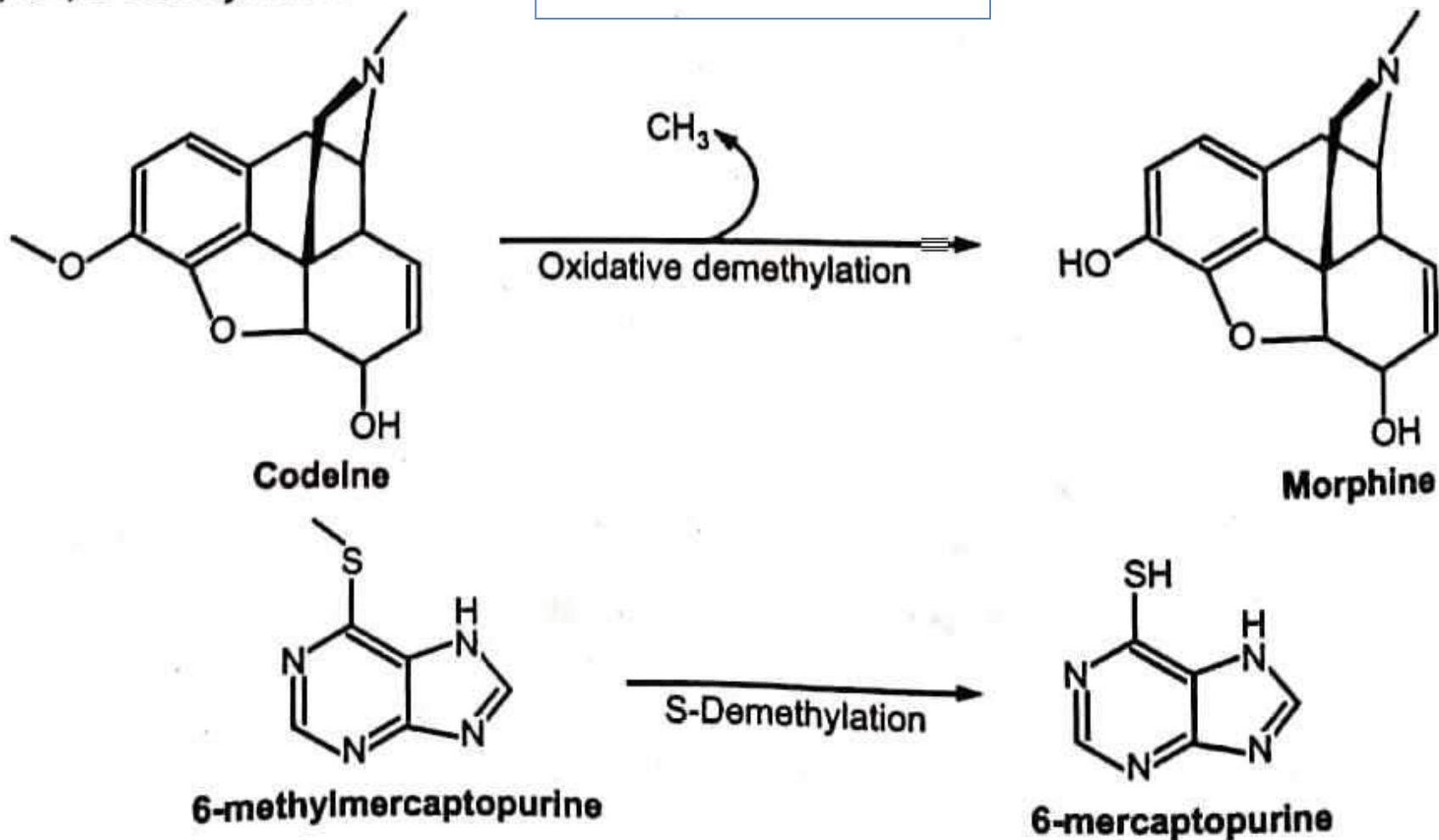
B. N-, or S-oxidation

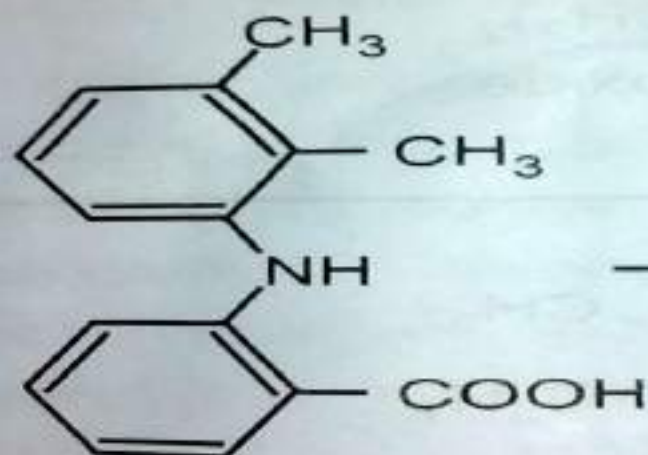


PHASE-I DRUG METABOLISM

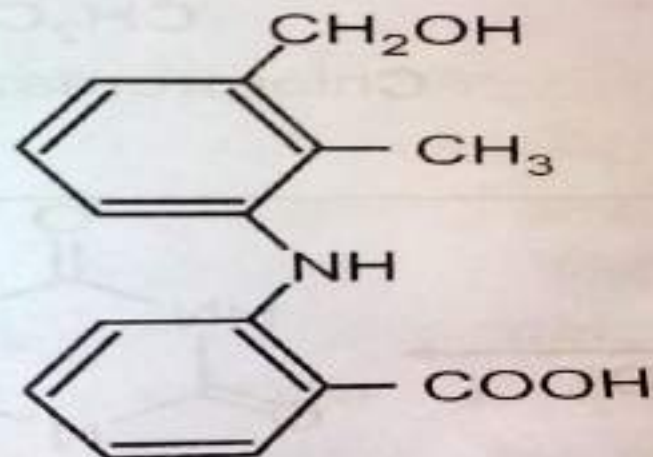
1. OXIDATION

C. N-, O-, S-dealkylation

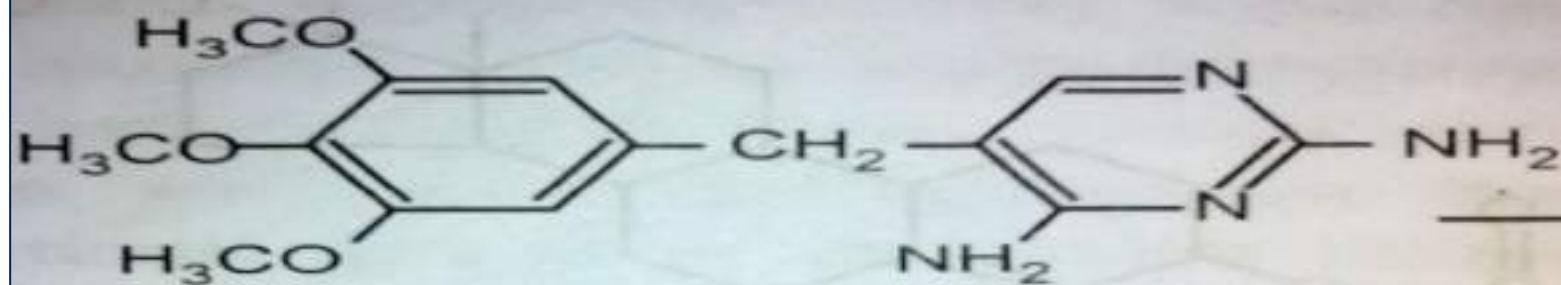




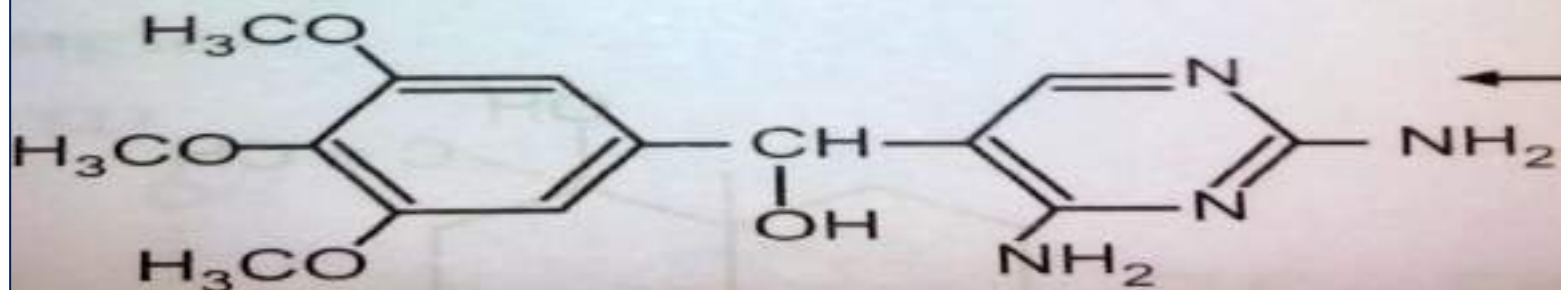
Mefenamic acid



Hydroxymethyl derivative



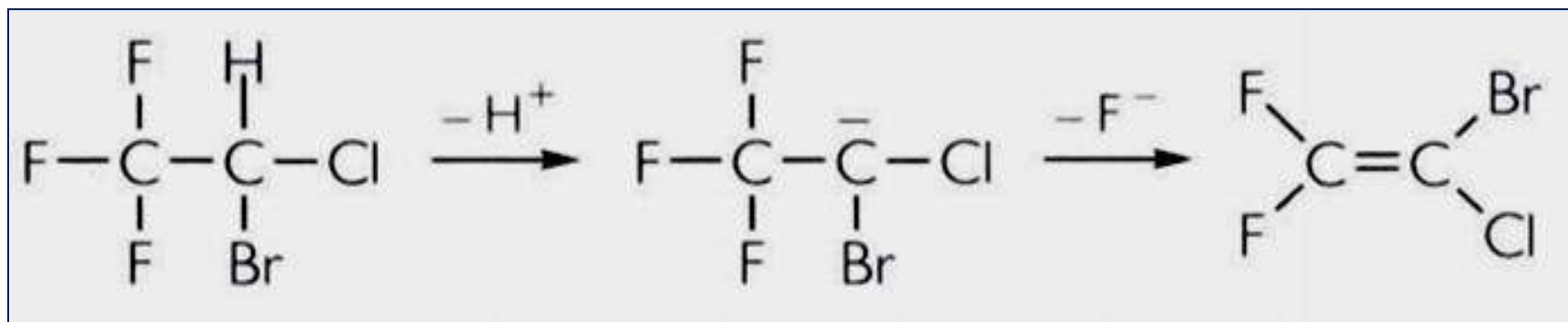
Trimethoprim



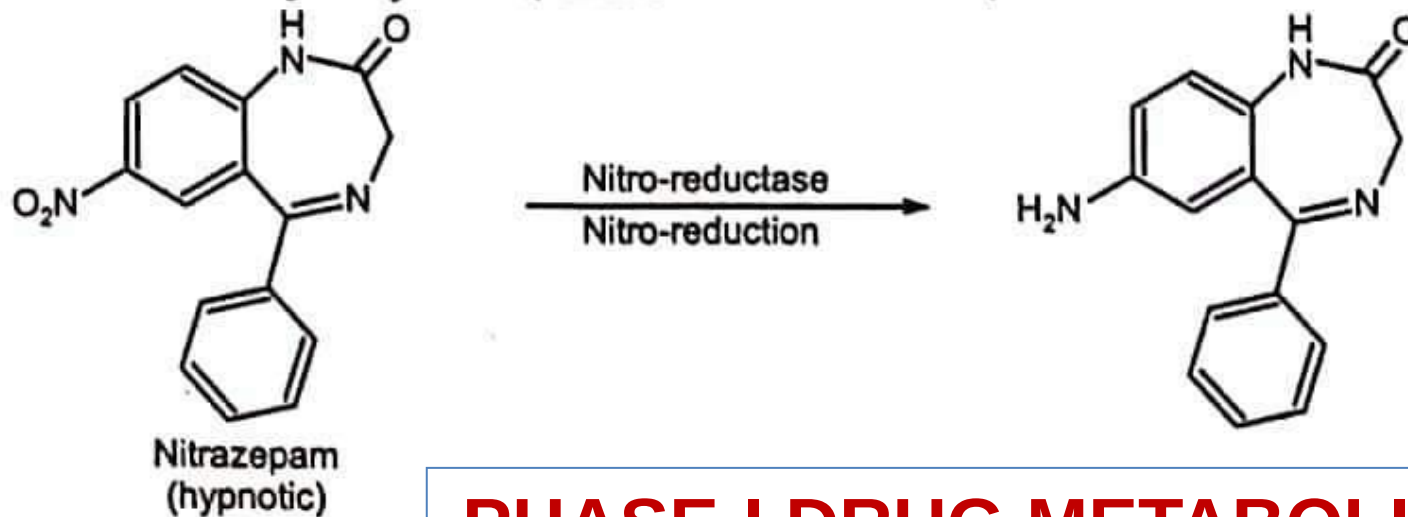
• 2. Reduction :

- Converse of oxidation
- Substrates for reductive reactions include azo- or nitro compounds, epoxides, heterocyclic compounds, and halogenated hydrocarbons:
 - (a) Azo or nitroreduction by cytochrome P450;
 - (b) Carbonyl (aldehyde or ketone) reduction by aldehyde reductase, aldose reductase, carbonyl reductase, quinone reductase
 - (c) other reductions including disulfide reduction, sulfoxide reduction, and reductive dehalogenation.
- The acceptance of one or more electron(s) or their equivalent from another substrate.
- Reductive reactions, which usually involve addition of hydrogen to the drug molecule, occur less frequently than the oxidative reactions.
- Biotransformation by reduction is also capable of generating polar functional groups such as hydroxy and amino groups, which can undergo further biotransformation.
- Many reductive reactions are exact opposite of the oxidative reactions (reversible reactions) catalysed either by the same enzyme (true reversible reaction) or by different enzymes (apparent reversible reactions).
- Such reversible reactions usually lead to conversion of inactive metabolite into active drug, thereby delaying drug removal from the body.

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- **Example:-** reductive defluorination of **halothane**



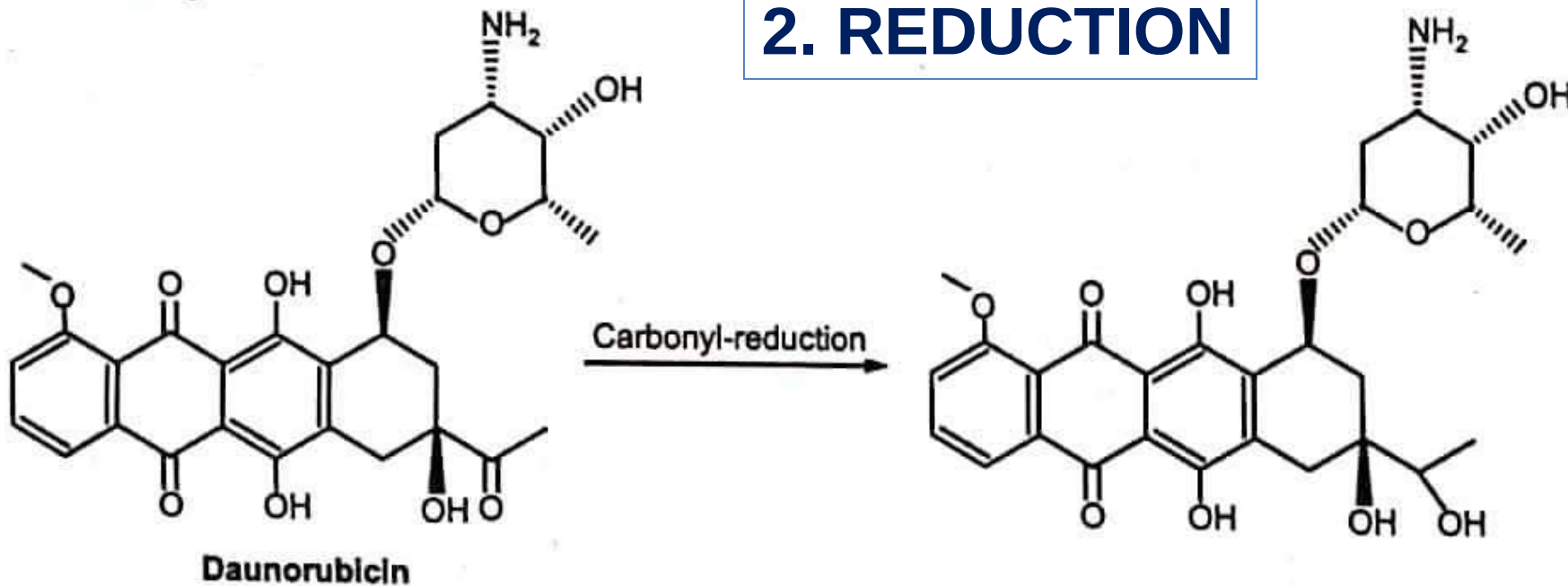
A. Nitro reduction to hydroxylamine/amine



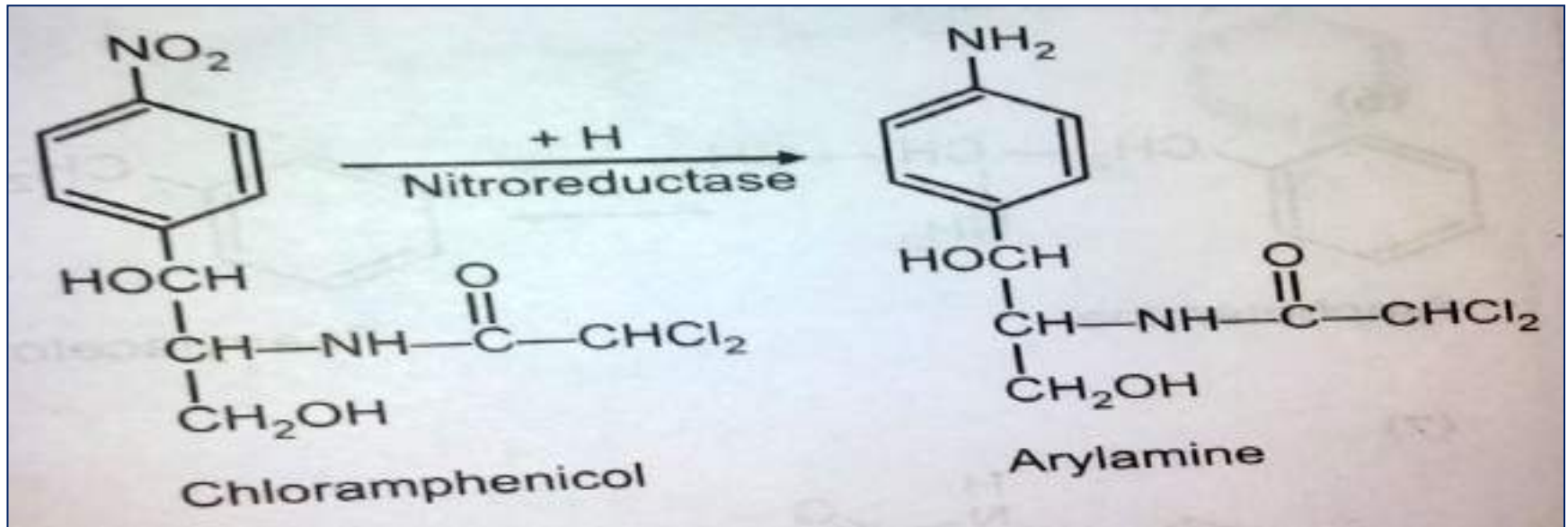
PHASE-I DRUG METABOLISM

B. Carbonyl reduction to alcohol

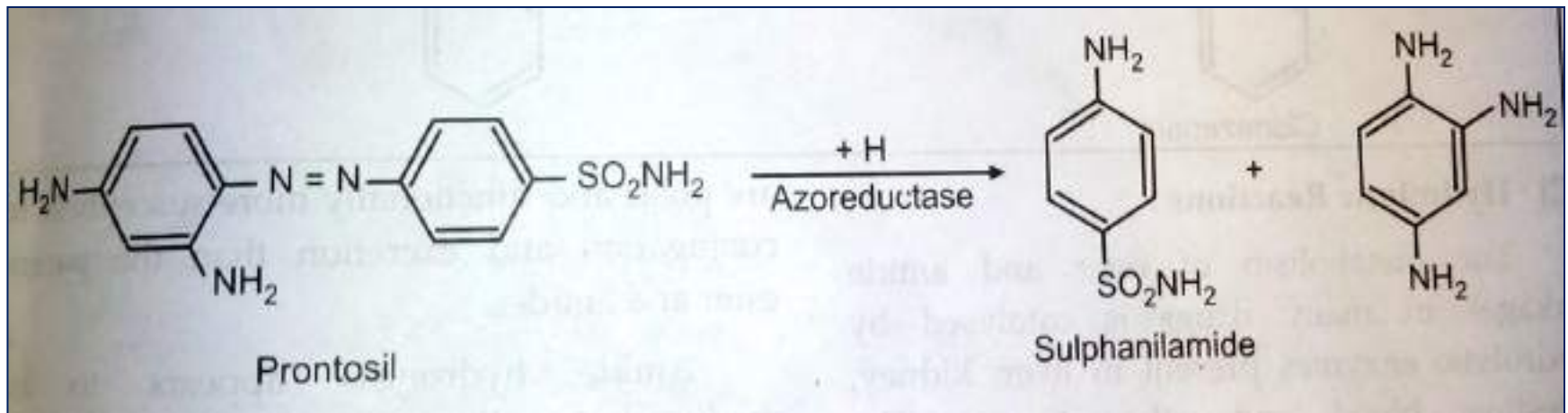
2. REDUCTION



Example. Reduction of Chloramphenicol



Example. Reduction of Prontosil



Reduction

a. Azo-reduction



M

Prontosil.

b. Nitro-reduction

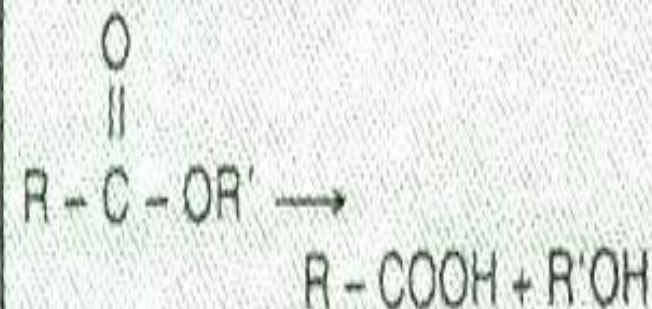


M, N

Parathion,
chloramphenicol.

Hydrolysis

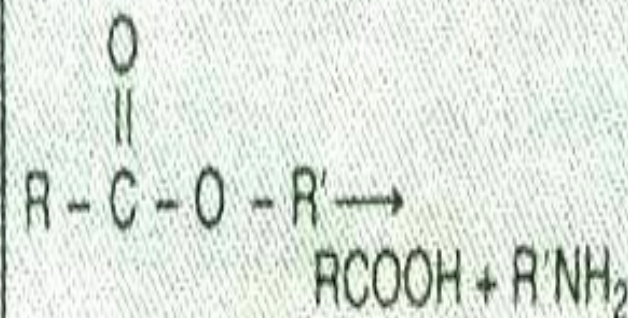
a. Hydrolysis of esters



M, N

Procaine, acetylcholine,
suxamethonium.

b. Hydrolysis of
amides



M, N

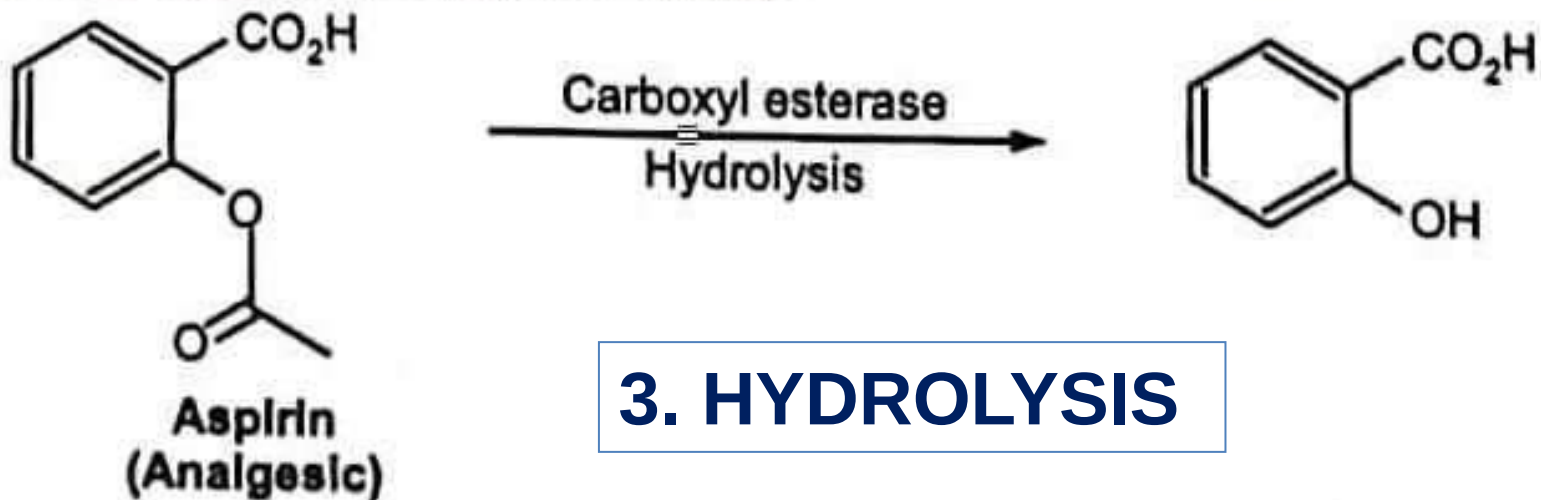
Lignocaine,
procainamide.

• 3. Hydrolysis :

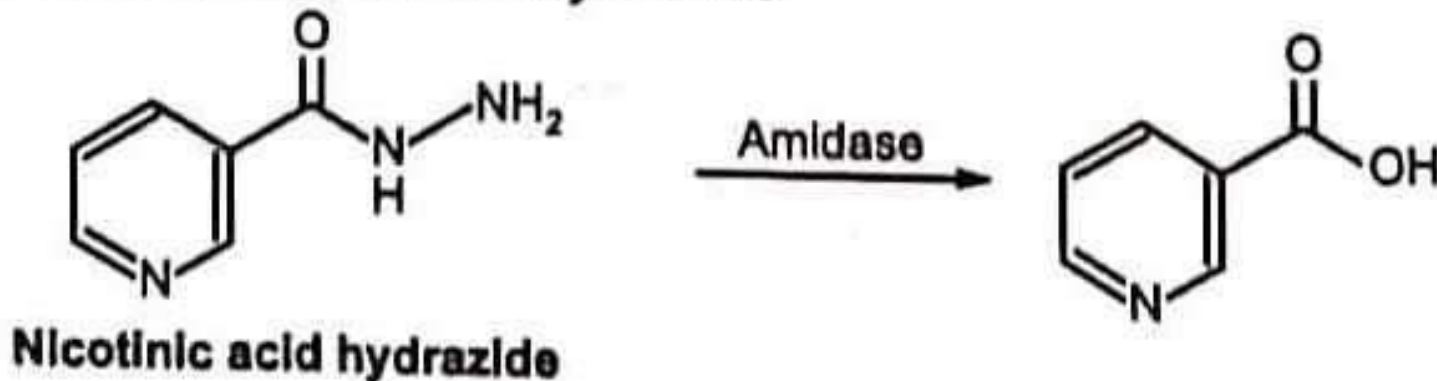
- Cleavage of drug molecule by taking up a molecule of water
- Esters, amides, hydrazides, and carbamates can be hydrolyzed by various enzymes.
- The hydrolytic reactions, contrary to oxidative or reductive reactions, do not involve change in the state of oxidation of the substrate, but involve the cleavage of drug molecule by taking up a molecule of water.
- The hydrolytic enzymes that metabolise drugs are the ones that act on endogenous substances, and their activity is not confined to liver as they are found in many other organs like kidneys, intestine, plasma, etc.
- **A number of drugs with ester, ether, amide and hydrazide linkages undergo hydrolysis. Important examples are** cholinesters, **procaine**, procainamide, and pethidine.

PHASE-I DRUG METABOLISM

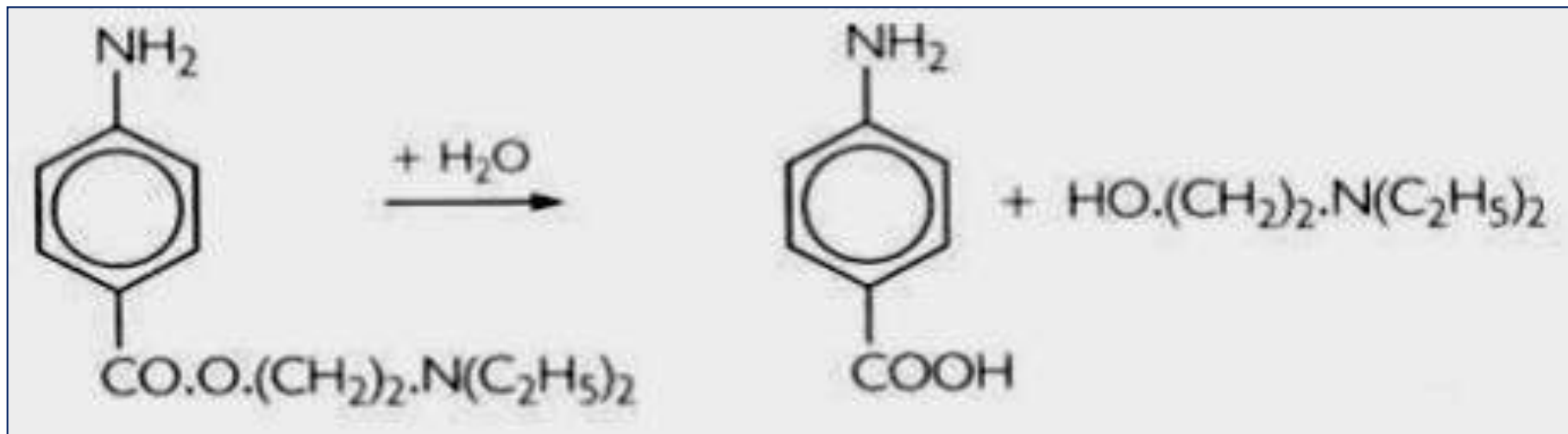
Ester or amide to acid and alcohol or amine



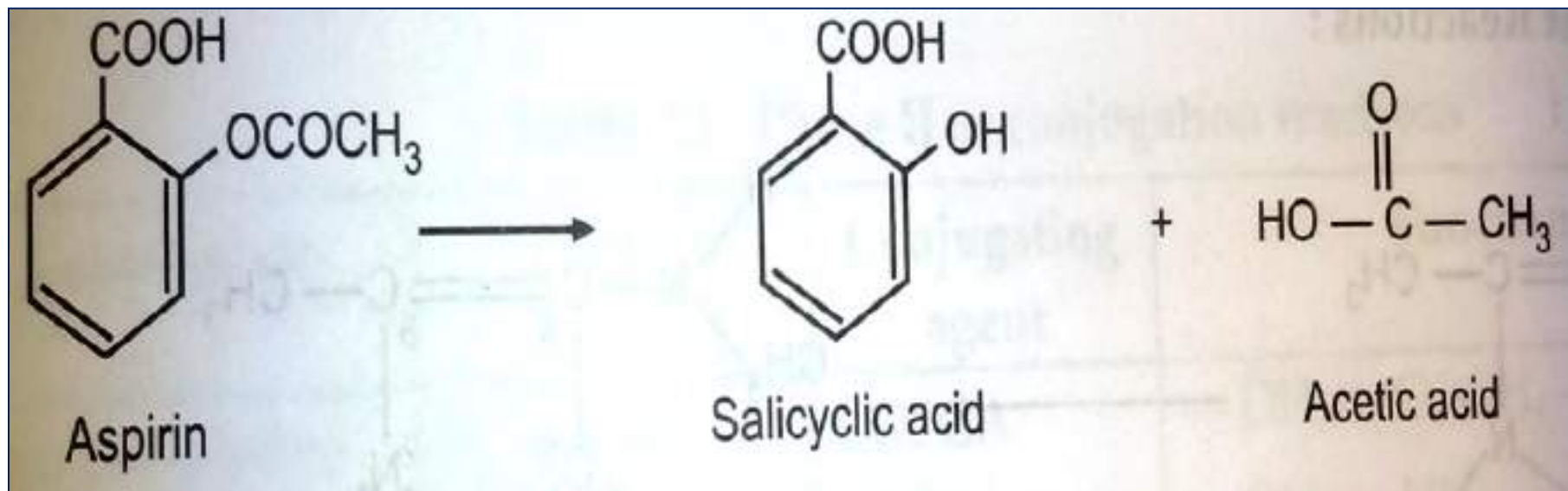
Hydrazides to acid and substituted hydrazine



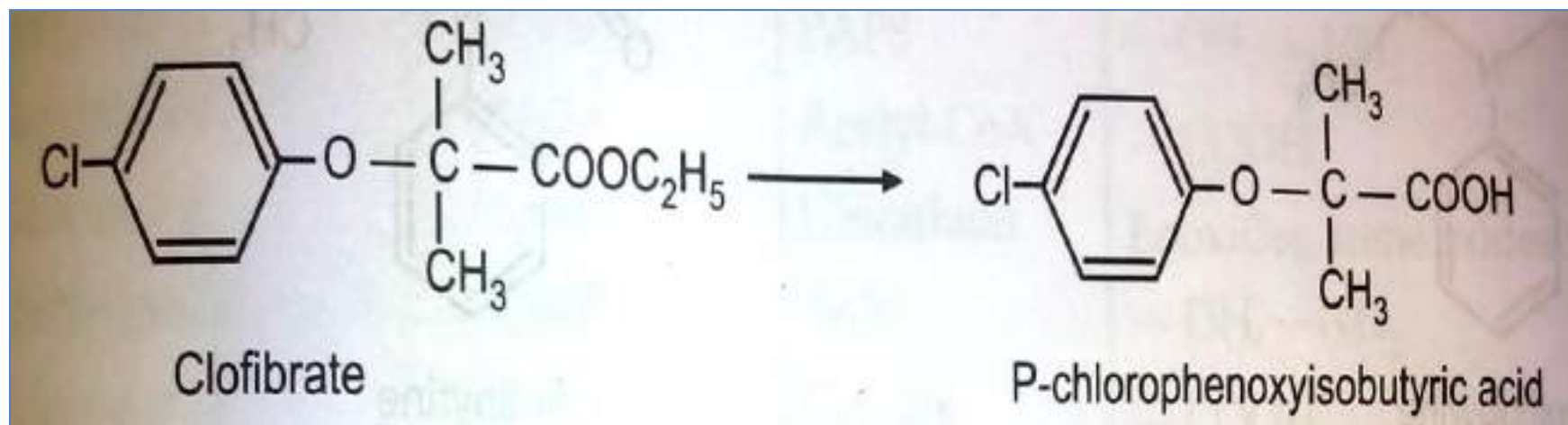
Example. Hydrolysis of Procaine



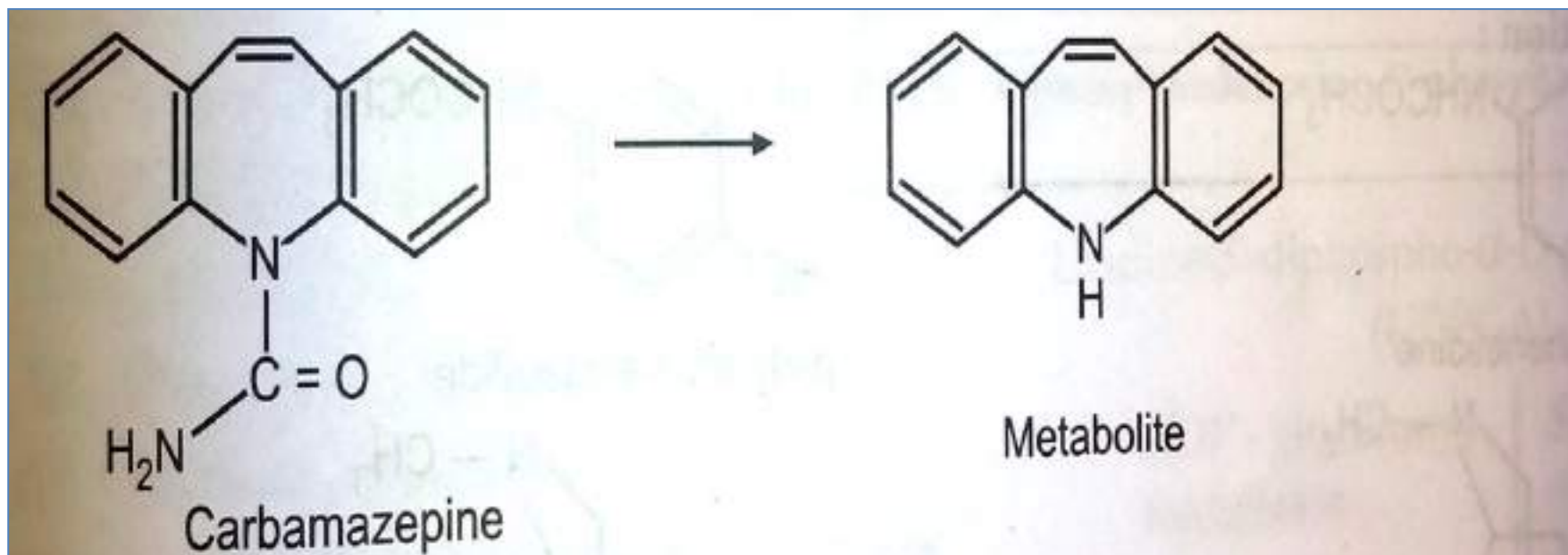
Example. Hydrolysis of Aspirin



Example. Hydrolysis of Clofibrate



Example. Hydrolysis of Carbamazepine



PHASE II REACTIONS

- Phase II or conjugation (Latin, conjugatus = yoked together) reactions involve combination of the drug or its phase I metabolite with an endogenous substance to form a highly polar product, which can be efficiently excreted from the body.
- In the biotransformation of drugs, such products or metabolites have two parts:
- Exocon, the portion derived from exogenous compound or xenobiotic,
- Endocon, the portion derived from endogenous substance.
- Conjugation reactions have high energy requirement and they often utilise suitable enzymes for the reactions.

- The **endogenous substances (endocons)** for **conjugation reactions** are **derived mainly from carbohydrates or amino acids** and usually possess large molecular size.
- **They are strongly polar or ionic in nature in order to render the substrate water-soluble. The molecular weight of the conjugate** (metabolite) is important for determining its route of excretion.
- High molecular weight conjugates **are excreted predominantly in bile (e.g., glutathione exclusively, glucuronide mainly)**, while low molecular weight conjugates are excreted mainly in the urine.
- As the availability of endogenous conjugating substance is limited, saturation of this process is possible and the unconjugated drug/metabolite may precipitate toxicity.

- **1. Conjugation with glucuronic acid /Glucuronidation**

- Conjugation with glucuronic acid (glucuronide conjugation or glucuronidation) is the most common and most important phase II reaction in vertebrates, except cats and fish.
- The biochemical donor (cofactor) of glucuronic acid is uridine diphosphate«-D-glucuronicacid (UDPGA) and the reaction is carried out by enzyme uridine diphosphate-glucuronyl transferase (UDP-glucuronyl transferase; glucuronyl transferase).
- Glucuronyl transferase is present in microsomes of most tissues but liver is the most active site of glucuronide synthesis.
- **Glucuronidation can take place in most body tissues because** the glucuronic acid donor UDPGA is present in abundant quantity in body, unlike donors involved in other phase II reactions.
- **In cats, there is reduced glucuronyl transferase activity, while in fish there is deficiency of endogenous glucuronic acid donor.**
- The limited capacity of this metabolic pathway in cats may increase the duration of action, pharmacological response and potential of toxicity of several lipid-soluble drugs (e.g., aspirin) in this species.

- A large number of drugs undergo glucuronidation including morphine, paracetamol and desipramine. Certain endogenous substances such as steroids, bilirubin, catechols and thyroxine also form glucuronides.
- Deconjugation process: Occasionally some glucuronide conjugates that are excreted in bile undergo deconjugation process in the intestine mainly mediated by β glucuronidase enzyme.
- This releases free and active drug in the intestine, which may be reabsorbed and undergo entero-hepatic cycling.
- Deconjugation is an important process because it often prolongs the pharmacological effects of drugs and/or produces toxic effects.

PHASE-II DRUG METABOLISM

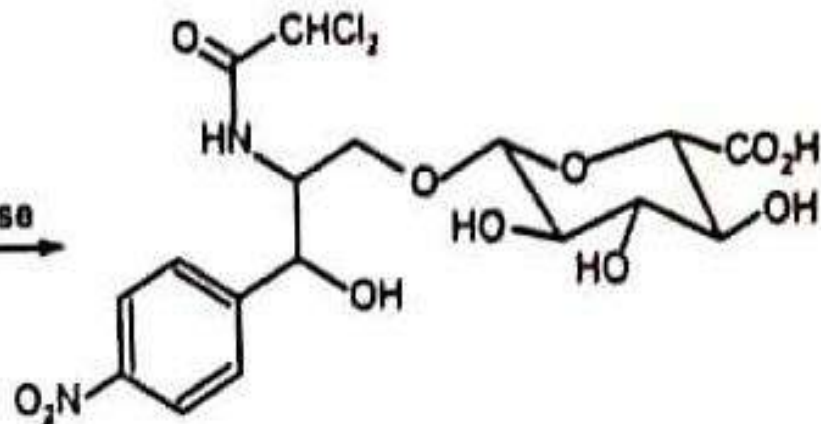
1. GLUCURONIDATION

Carboxylic acid, alcohol, phenol, amine



Chloramphenicol
(antibiotic)

Glucuronosyl transferase



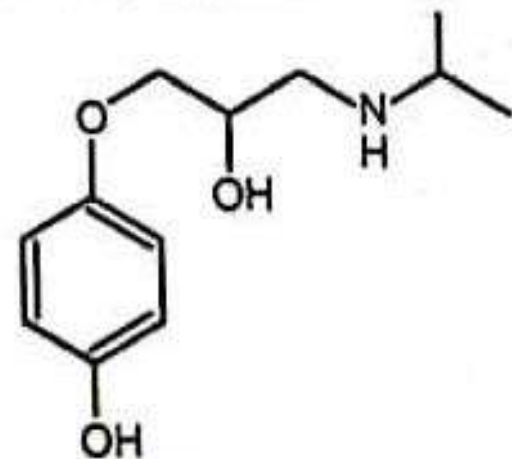
• **2. Conjugation with sulphate/ Sulphation**

- Conjugation with sulphate (sulphate conjugation, sulphoconjugation or sulphation) is similar to glucuronidation but is catalysed by non-microsomal enzymes and occurs less commonly.
- The endogenous donor of the sulphate group is 3'-phosphoadenosine-5'-phosphosulphate (PAPS), and enzyme catalysing the reaction is sulphotransferase
- The conjugates of sulphate are referred to as sulphate ester conjugates or ethereal sulphates. Unlike glucuronide conjugation, sulphoconjugation in mammals is less important because the PAPS donor that transfers sulphate to the substrate is easily depleted.
- Capacity for sulphate conjugation is limited in pigs. However in cats, where glucuronidation is deficient, sulphate conjugation is important. Functional groups capable of forming sulphate conjugates include phenols, alcohols, arylamines, N-hydroxylamines and N-hydroxyamides.
- Drugs undergoing sulphate conjugation include chloramphenicol, phenols, and adrenal and sex steroids.

PHASE-II DRUG METABOLISM

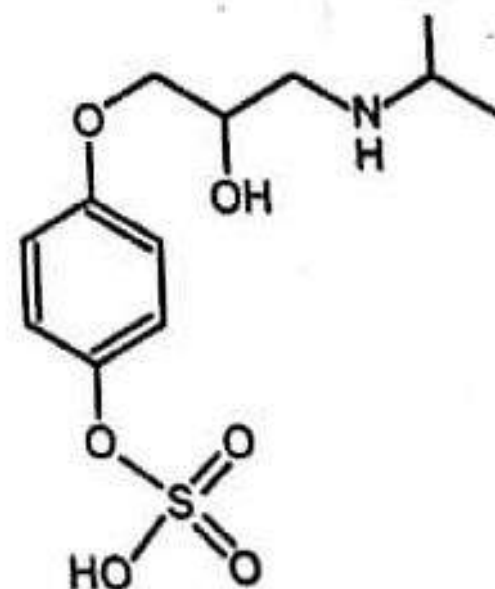
2. SULFATION

Alcohol, phenol, amine



Prenalterol
(β -blocker)

Sulfo-transferase



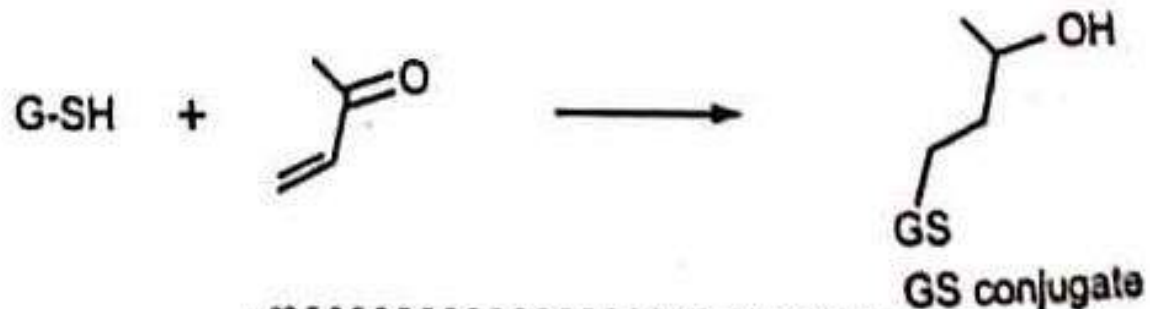
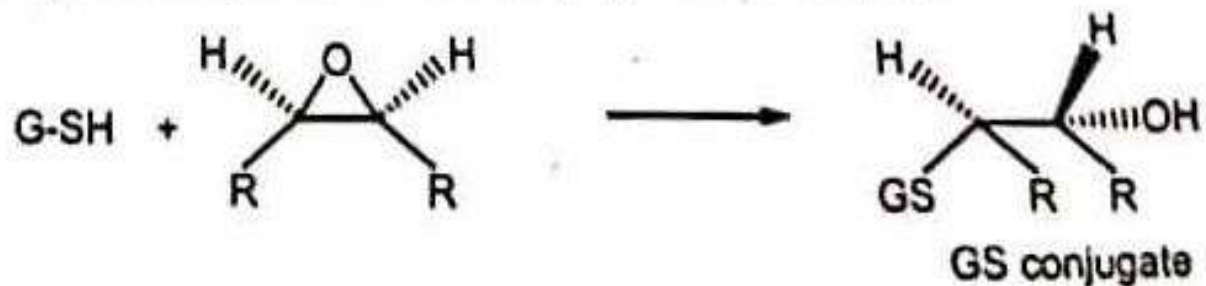
- **3. Conjugation with glutathione and mercapturic acid formation.**

- Conjugation with glutathione (glutathione conjugation) and mercapturic acid formation is a minor but important metabolic pathway in animals.
- Glutathione (GSH, G=glutathione and SH = active-SH group) is a tripeptide having glutamic acid, cysteine and glycine.
- It has a strong nucleophilic character due to the presence of a -SH (thiol) group in its structure. Thus, it conjugates with electrophilic substrates, a number of which are potentially toxic compounds, and protects the tissues from their adverse effects.
- The interaction between the substrate and the GSH is catalysed by enzyme glutathione- S-transferase, which is located in the soluble fraction of liver homogenates.
- The glutathione conjugate either due to its high molecular weight is excreted as such in the bile or is further metabolised to form mercapturic acid conjugate that is excreted in the urine.

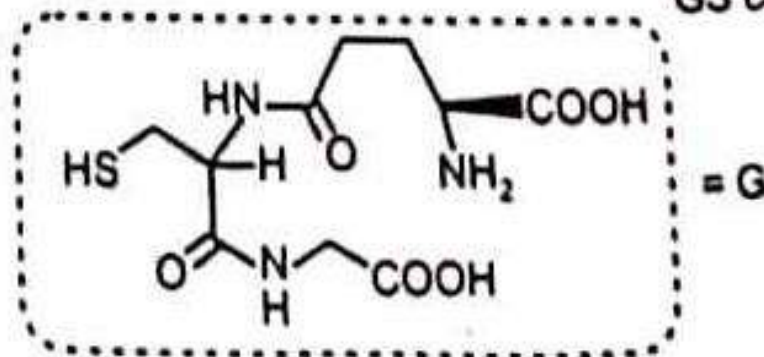
PHASE-II DRUG METABOLISM

3. GLUTATHIONE CONJUGATION- gly-cys-glu

Halo-compounds, epoxides, arene oxides, quinone-imine

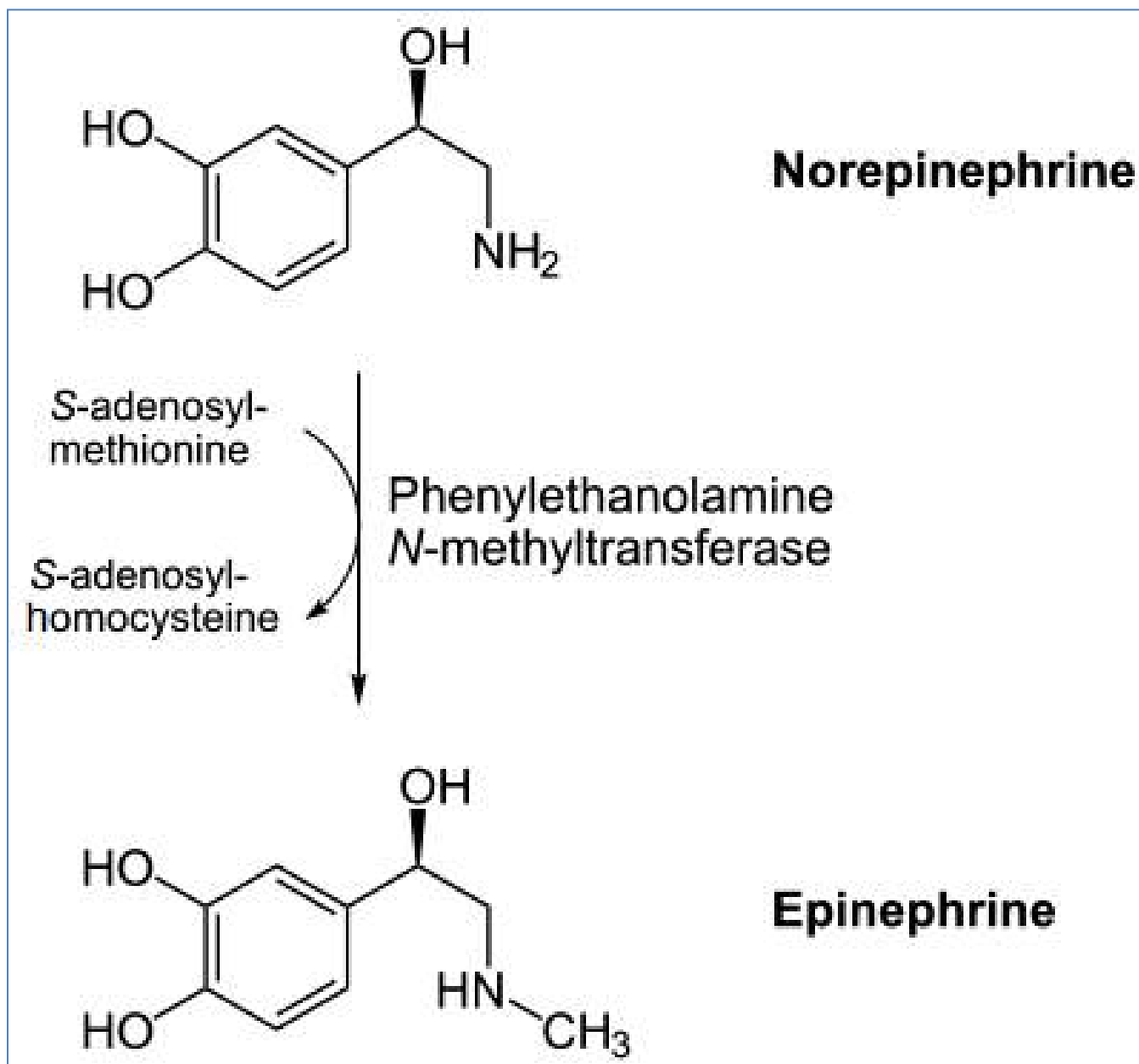


Glutathione (tripeptide-
glutamate+ cysteine+
glycine)

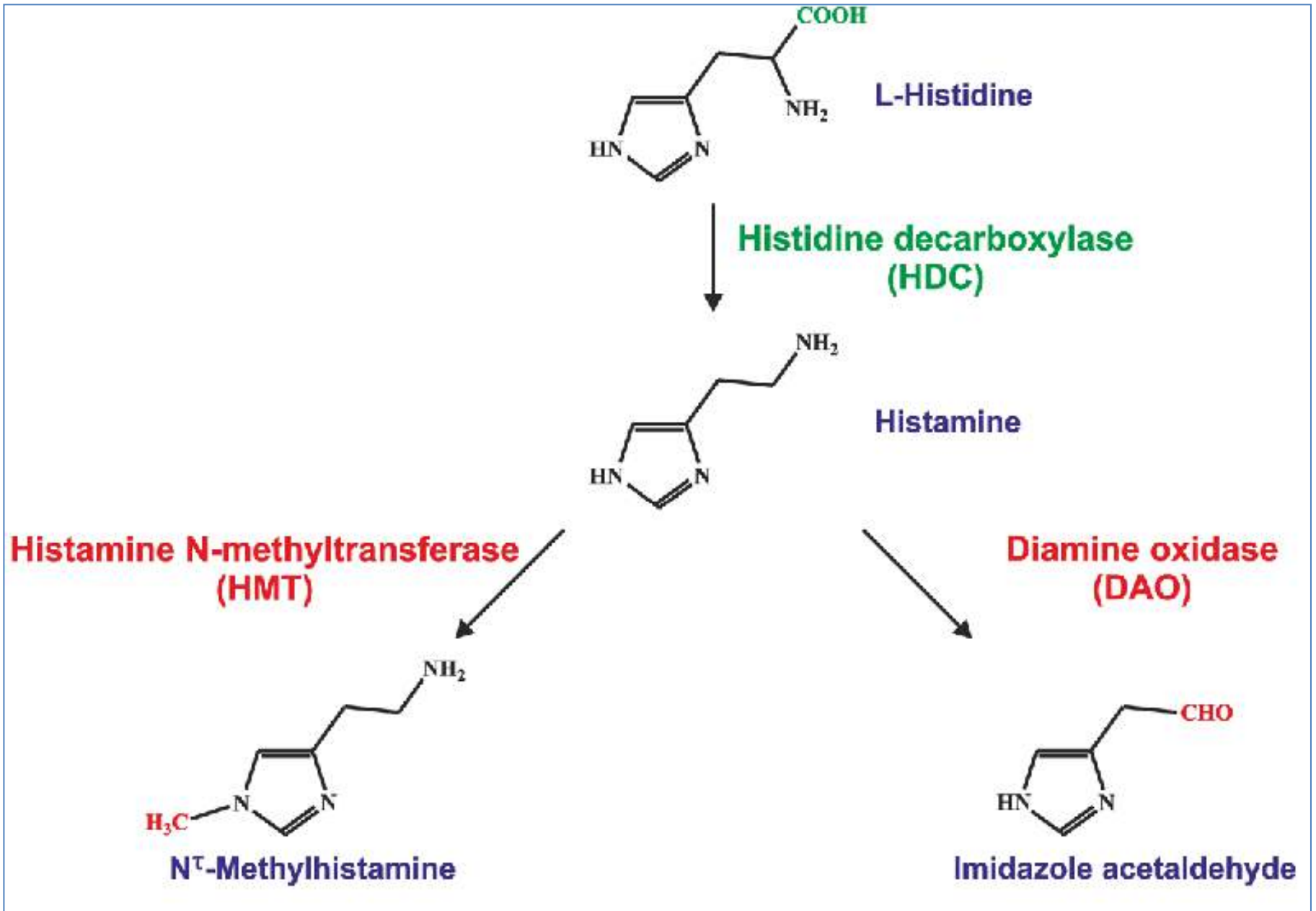


- **4. Conjugation with methyl group/ Methylation**
- Conjugation with methyl group (methyl conjugation or methylation) involves transfer of a methyl group (-CH₃) from the cofactor S-adenosyl methionine (SAM) to the acceptor substrate by various methyl transferase enzymes.
- Methylation reaction is of lesser importance for drugs, but is more important for biosynthesis (e.g., adrenaline, melatonin) and | Inactivation (e.g., histamine) of endogenous amines.
- Occasionally, the metabolites formed are not polar or water soluble and may possess equal or greater activity than the parent compound (e.g., adrenaline synthesised from noradrenaline).

Example:- Methylation of Norepinephrine



Example:- Methylation of histamine



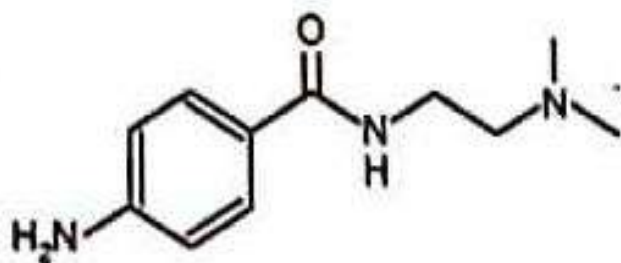
• **5. Conjugation with acetyl group/ Acetylation**

- Conjugation with acetyl group (acetylation) is an important metabolic pathway for drugs containing the amino groups.
- The cofactor for these reactions is acetyl coenzyme A and the enzymes are non-microsomal N-acetyl transferases, located in the soluble fraction of cells of various tissues.
- Acetylation is not a true detoxification process because occasionally it results in decrease in water solubility of an amine and. thus, increase in its toxicity (e.g., sulphonamides).
- Acetylation is the primary route of biotransformation of sulphonamide compounds. Dogs and foxes do not acetylate the aromatic amino groups due to deficiency of arylamine acetyltransferase enzyme.

PHASE-II DRUG METABOLISM

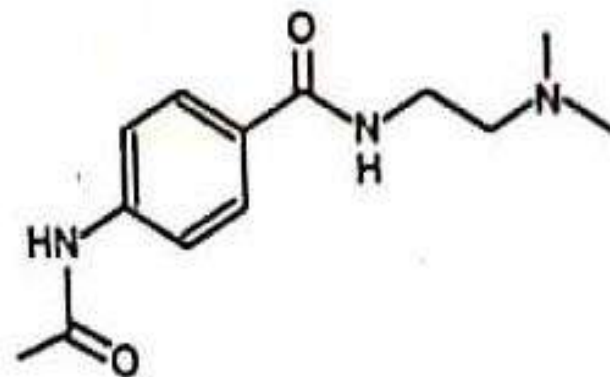
5. ACETYLATION

Amines

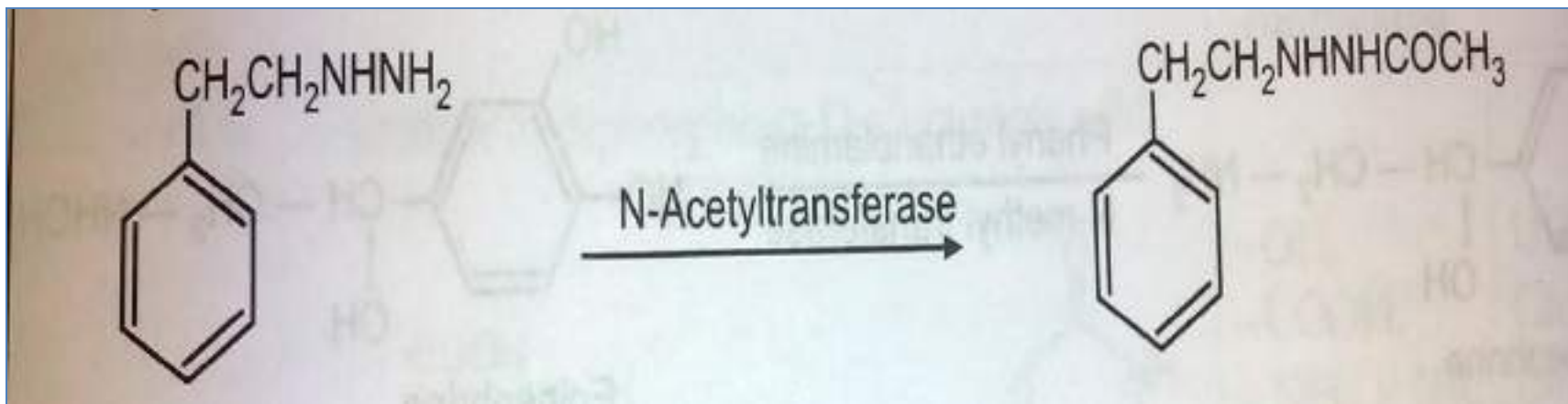


Procainamide

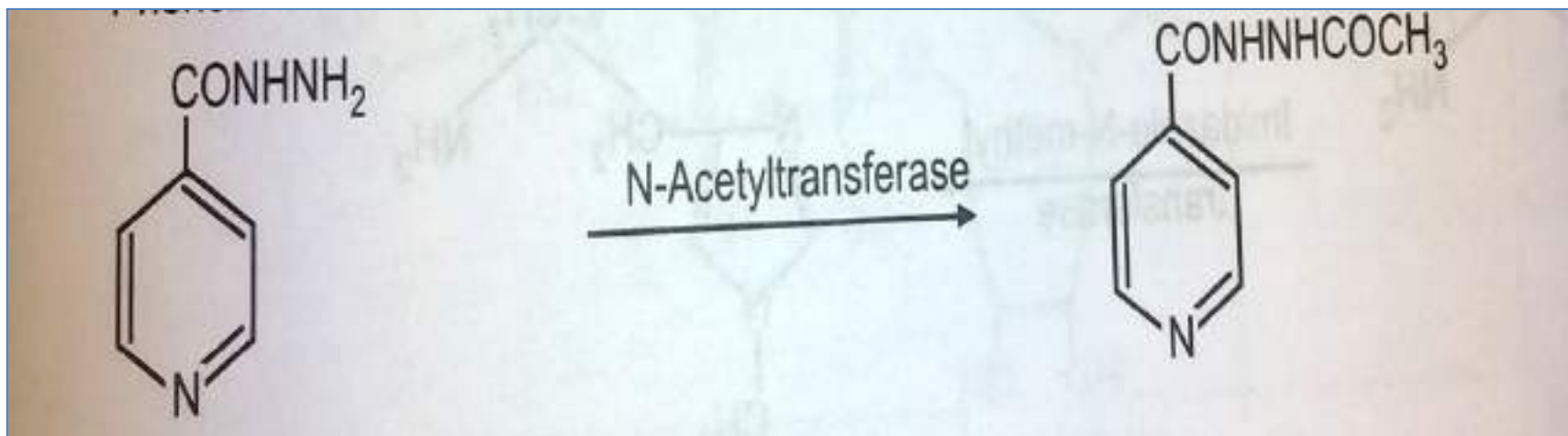
N-acetyl transferase



Example. Acetylation of phenelzine



Example. Acetylation of isoniazid



- **6. Conjugation with thiosulphate**

- **Conjugation with thiosulphate is** an important reaction in the detoxification of cyanide.
- Conjugation of cyanide ion involves transfer of sulphur atom from the thiosulphate to the cyanide ion in presence of enzyme rhodanase to form inactive thiocyanate.
- **Thiocyanate formed is much less toxic than the cyanide (true detoxification) and it is excreted in urine.**

Factors influencing metabolic pathways of the drug

- In most cases, the metabolism of a drug is a first order process which means that a constant fraction of the drug is metabolized in unit time
- Saturation of one metabolic pathway may allow for a shift in the metabolic pattern of a drug
- **Important factors that affects metabolic patterns of the drug include:**
 - 1- dose and frequency of administration of drug
 - 2- species and strain of animal used
 - 3- diet and nutritional status of animal and the weight of animal used
 - 4- route of administration
 - 5- time of administration
 - 6- interaction of other drugs and environmental contraction
 - 7- pregnancy and psychological abnormalities
 - 8- inducer of drug metabolism
 - 9- inhibitors of drug metabolism

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

Thank you..