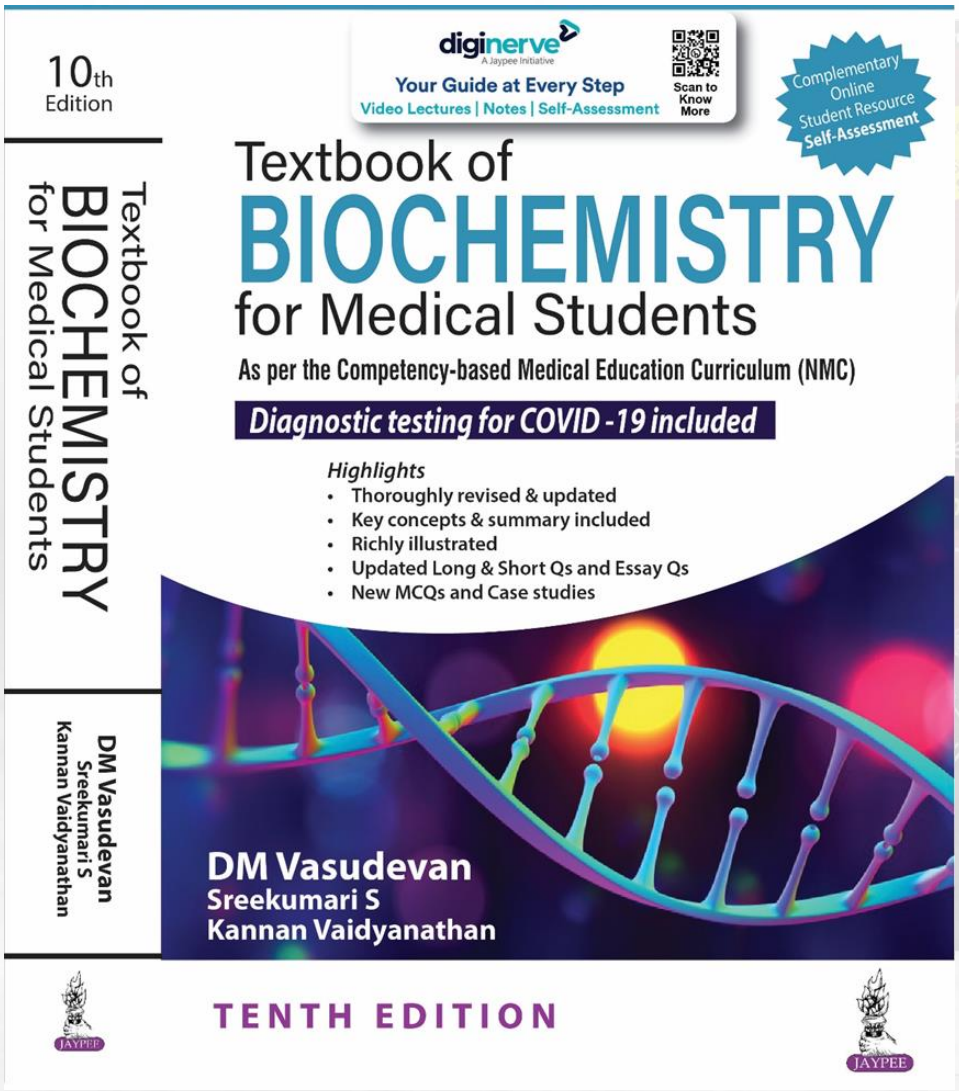
Chapter 27:

Detoxification and Biotransformation of Xenobiotics



Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

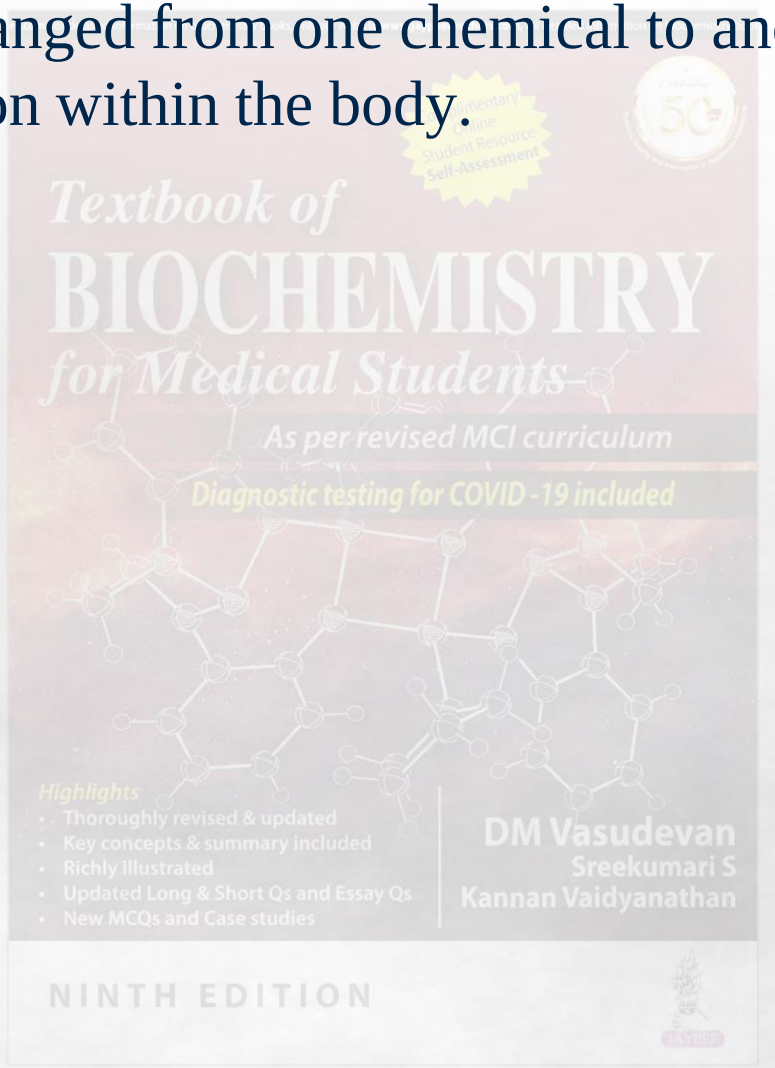
TENTH EDITION

Xenobiotics

- Compound which is foreign to the body.
- Eg:Drugs
- Chemical carcinogens
- Insecticides
- Environmental pollutants
- Compounds produced in the body by
- bacterial catabolism.
- Knowledge of metabolism of xenobiotics is basic to a rational understanding of pharmacology and therapeutics, pharmacy, toxicology and in management of cancer.

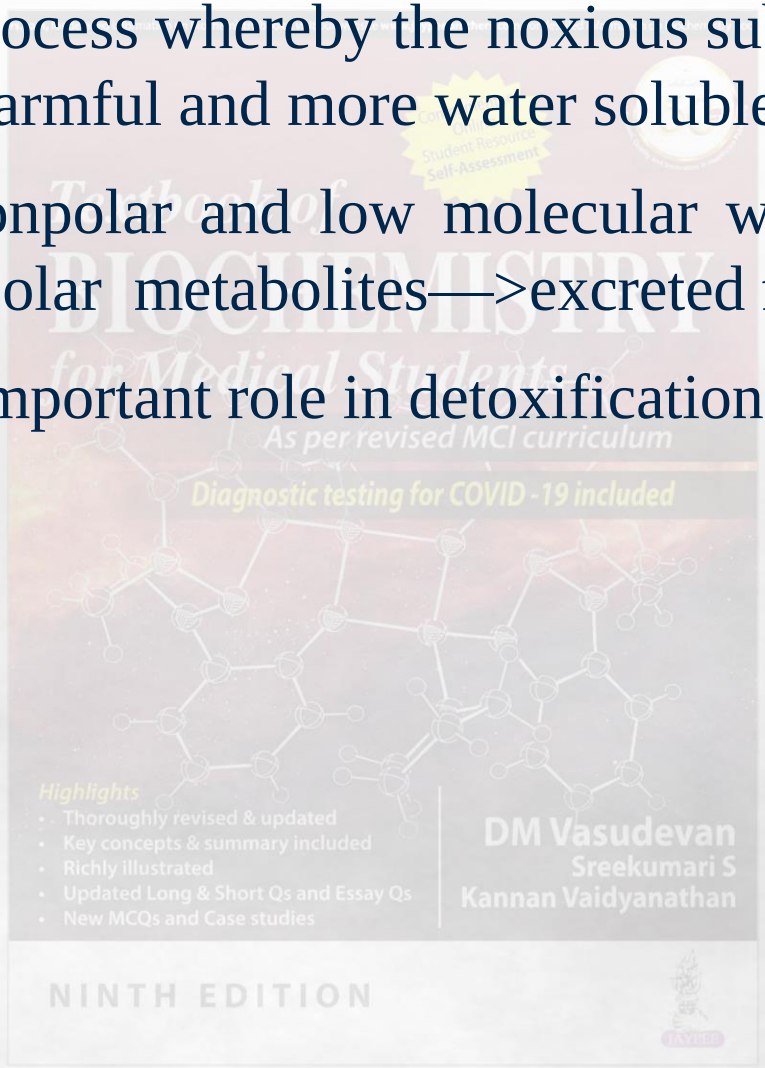
Biotransformation

- Substance is changed from one chemical to another by a chemical reaction within the body.
- Two types:
- Detoxification
- Bioactivation



Detoxification

- Biochemical process whereby the noxious substances are rendered less harmful and more water soluble.
- Lipophilic , nonpolar and low molecular weight toxicants —>hydrophilic, polar metabolites—>excreted from the body.
- LIVER plays important role in detoxification reactions.



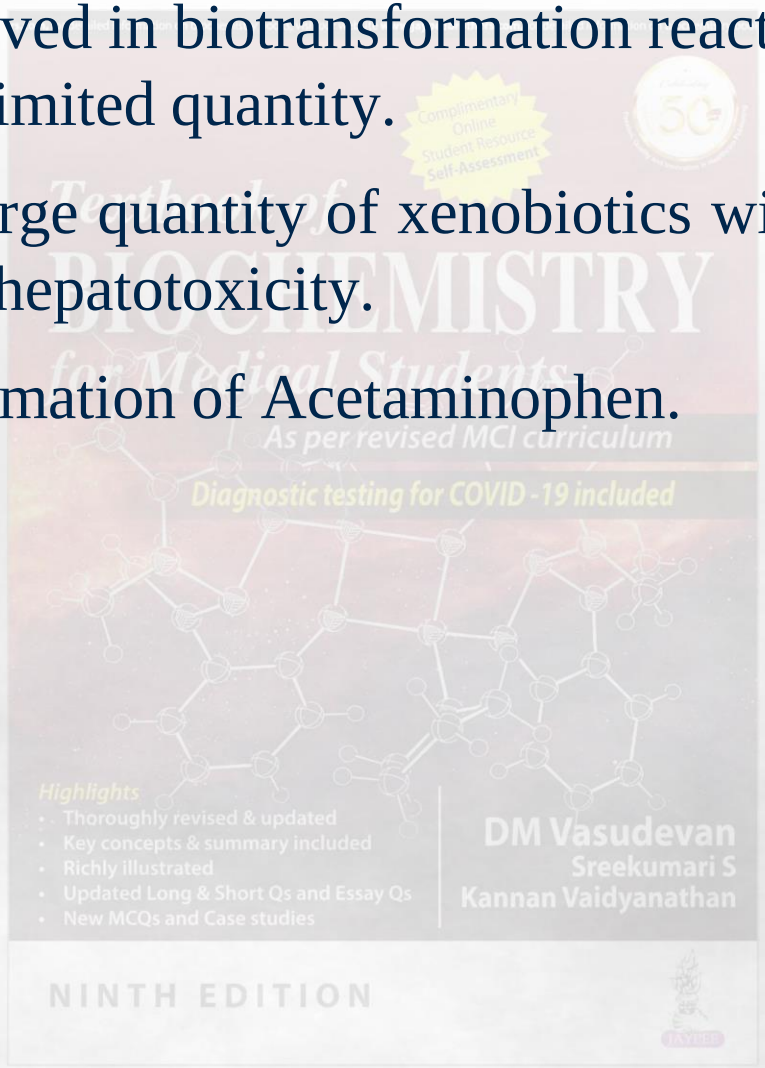
Toxication

- Bioactivation
- Xenobiotics are converted to their metabolites which are more toxic than the parent substances.
- Original compounds are called prodrugs or procarcinogens.
- Eg:Vinyl chloride.
- Compounds that are detoxified include,
  1) Compounds accidentally ingested.
  2) Drugs.
  3) Compounds produced in the body.
  4) Compounds produced by bacterial metabolism.

- Amines produced by decarboxylation of aminoacids:

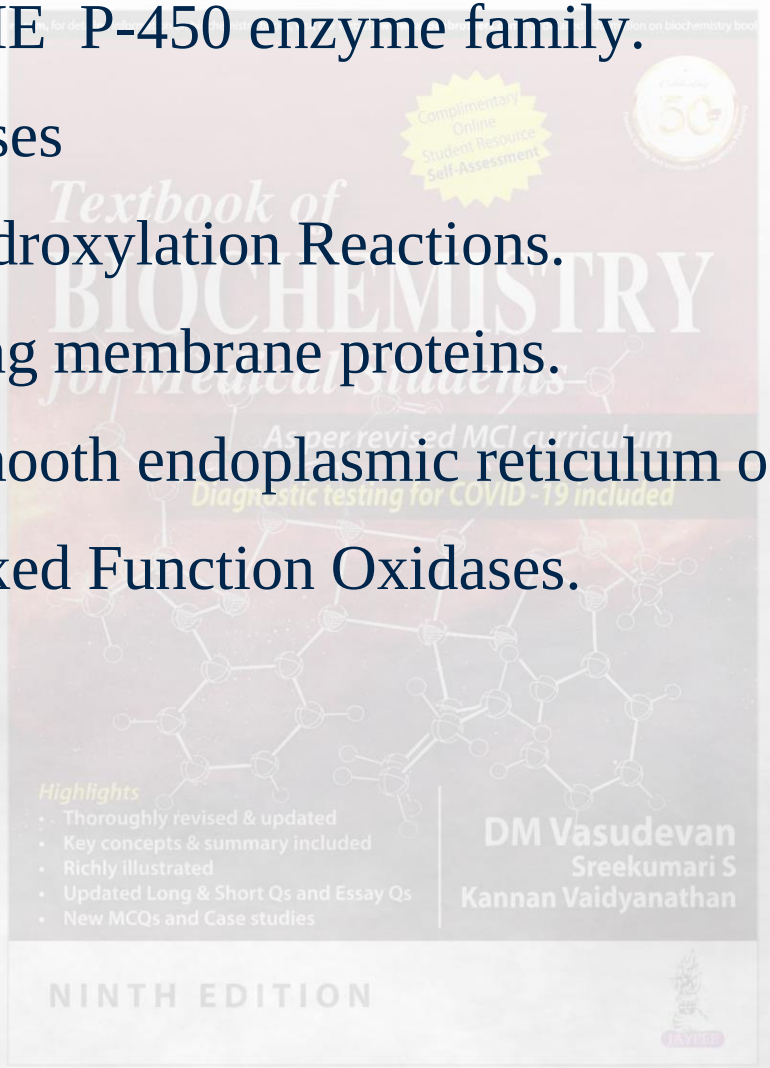
Histidine	….	Histamine
Lysine	….	Cadaverine
Ornithine	….	Putrescine
Tyrosine	….	Tyramine
Tryptophan	….	Tryptamine
Choline	….	Neurine

- Enzymes involved in biotransformation reactions are normally available in a limited quantity.
- So intake of large quantity of xenobiotics will lead to depletion of enzymes & hepatotoxicity.
- Eg:Biotransformation of Acetaminophen.



Enzyme Systems

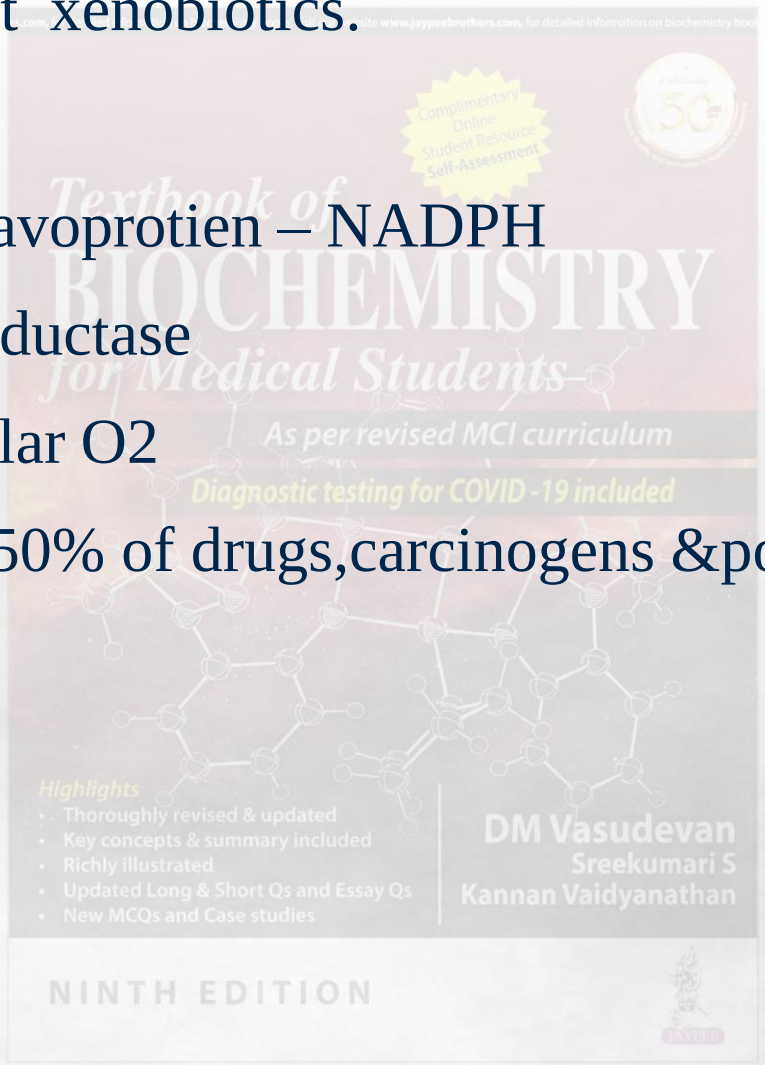
- CYTOCHROME P-450 enzyme family.
- Mono oxygenases
- Involved in Hydroxylation Reactions.
- Heme containing membrane proteins.
- Localized in smooth endoplasmic reticulum of liver.
- Also called Mixed Function Oxidases.



- $R\text{-}H + O_2 + NADPH \longrightarrow R\text{-}OH + H_2O + NADP^+$
- OR
- $RH + O_2 \longrightarrow R\text{-}OH + H_2O$

Reduced Cyt P450 → Oxidised Cyt P450

- R-H:Represent xenobiotics.
- Requires;
  - P450 flavoprotien – NADPH
  - P450 reductase
  - Molecular O2
- Metabolize ~ 50% of drugs,carcinogens &pollutants.

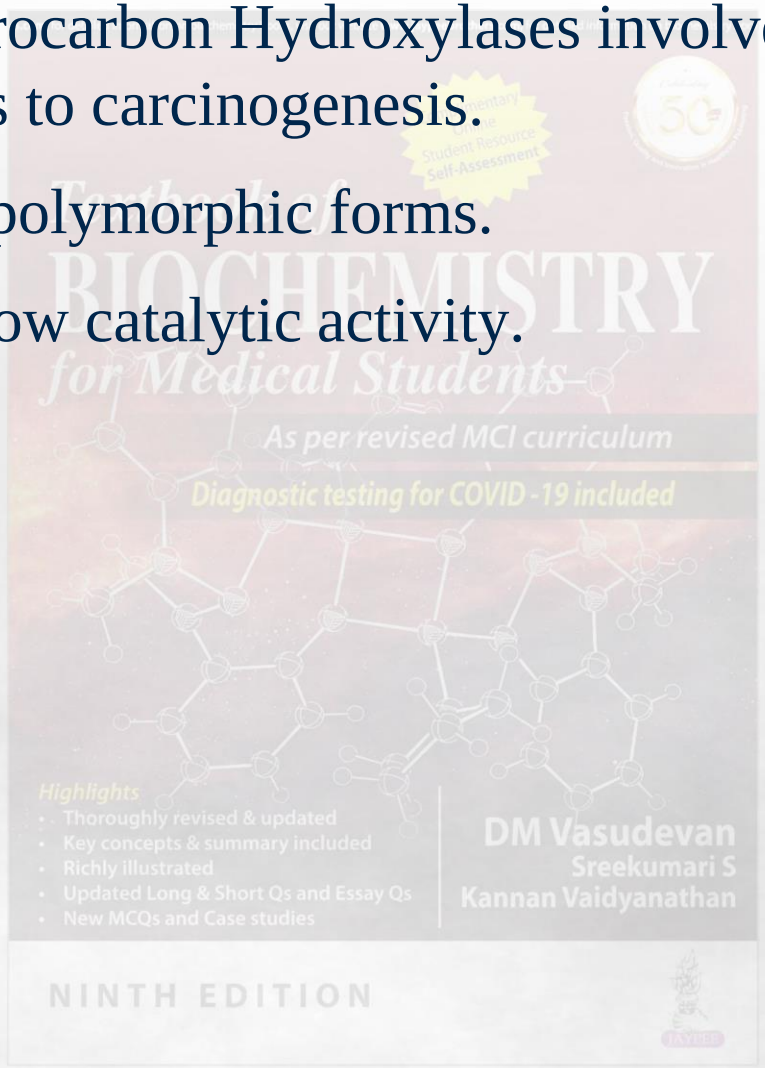


Method of Nomenclature

- Cytochrome P450 ~ CYP.
- Family-Arabic number ~ CYP1
- Subfamily-Capital letter ~ CYP1A.
- Individual P450s-Arabic numericals ~ CYP1A1.
- Gene encoding - *CYP1A1*
- Hemoproteins.
- Widely distributed across species.
- Highest amount in liver &small intestine.
- Membranes of smooth endoplasmic reticulum.
- In Adrenal gland :Smooth ER& Mitochondria

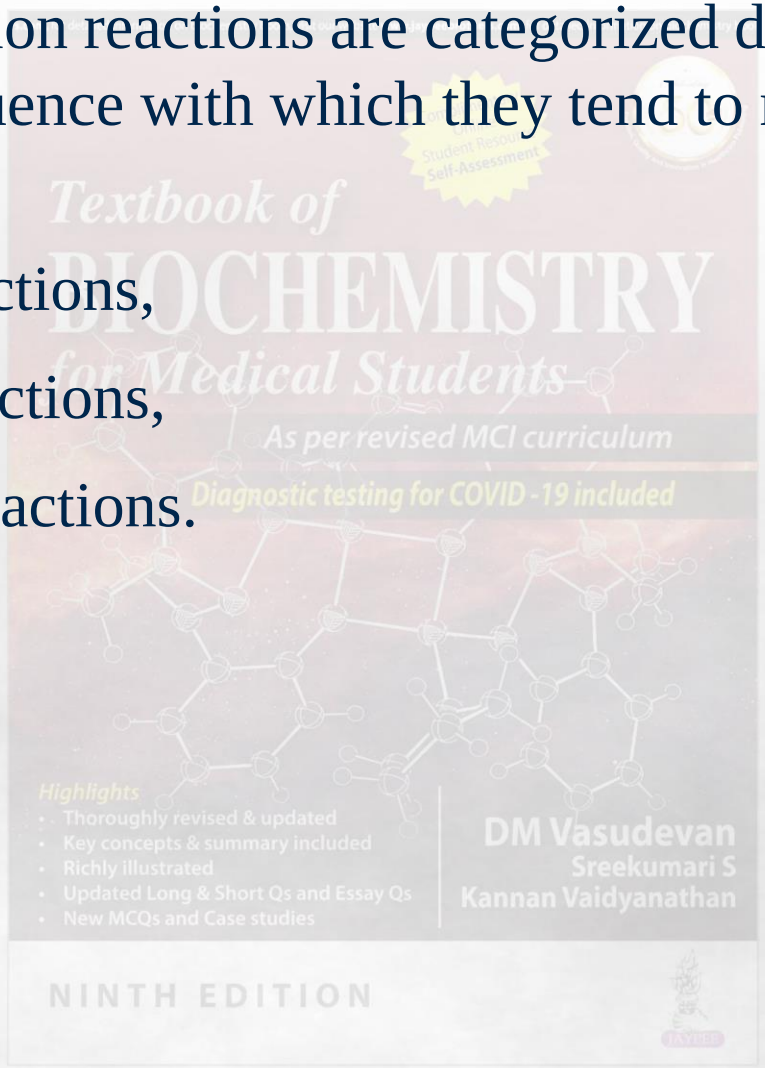
- 6 Isoforms of CYP are present.
- Show overlapping substrate specificities.
- NADPH-CYP Reductase
- Oxidised CYP ⟶ Reduced CYP
- Lipid forms component of CYP system.
- Eg:Phosphatidyl Choline.
- Most isoforms of CYP are inducible by drugs.
- Eg: Phenobarbital.
- Important clinical implications.
- CYP2E1 – Induced by Ethanol.

- Aromatic Hydrocarbon Hydroxylases involved in metabolism of PAHs.Leads to carcinogenesis.
- CYP exists in polymorphic forms.
- Some exhibit low catalytic activity.



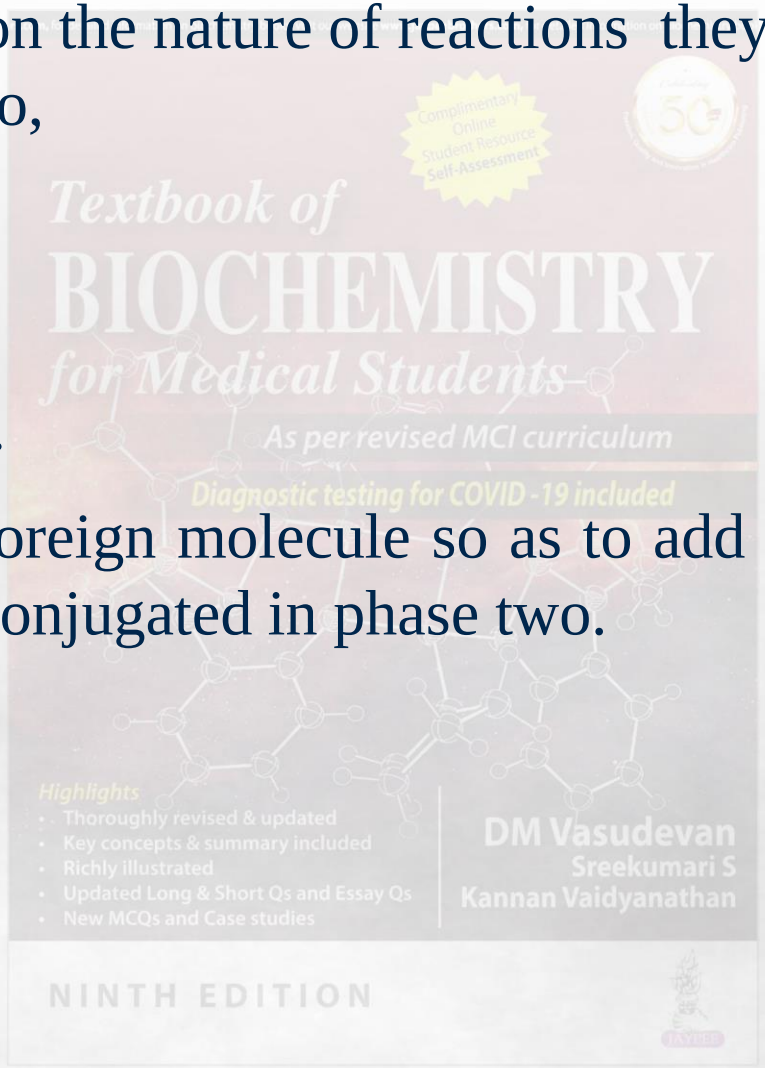
Phases of Detoxification

- Biotransformation reactions are categorized depending on the normal sequence with which they tend to react with a xenobiotic, as
- Phase One Reactions,
- Phase Two Reactions,
- Phase Three Reactions.



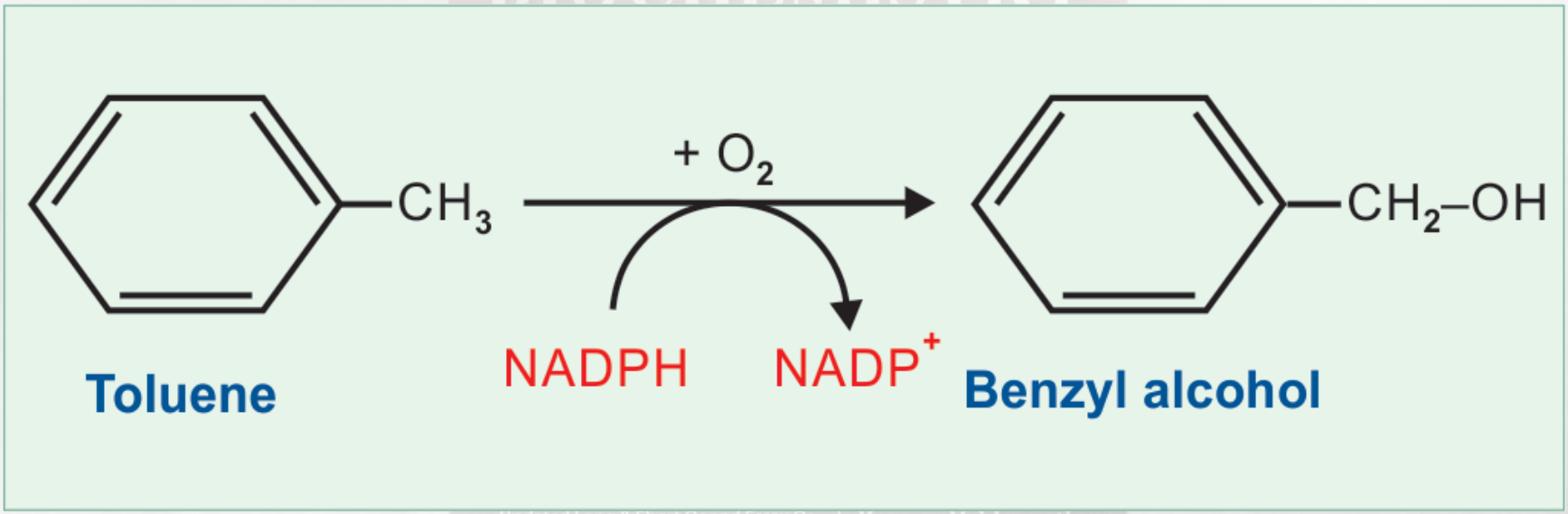
Phase One Reactions

- Depending upon the nature of reactions they are categorized into,
- Oxidation,
- Reduction,
- Hydrolysis etc.
- Alteration of foreign molecule so as to add a functional group, which can be conjugated in phase two.
- It may be
- Detoxification
- Entoxification



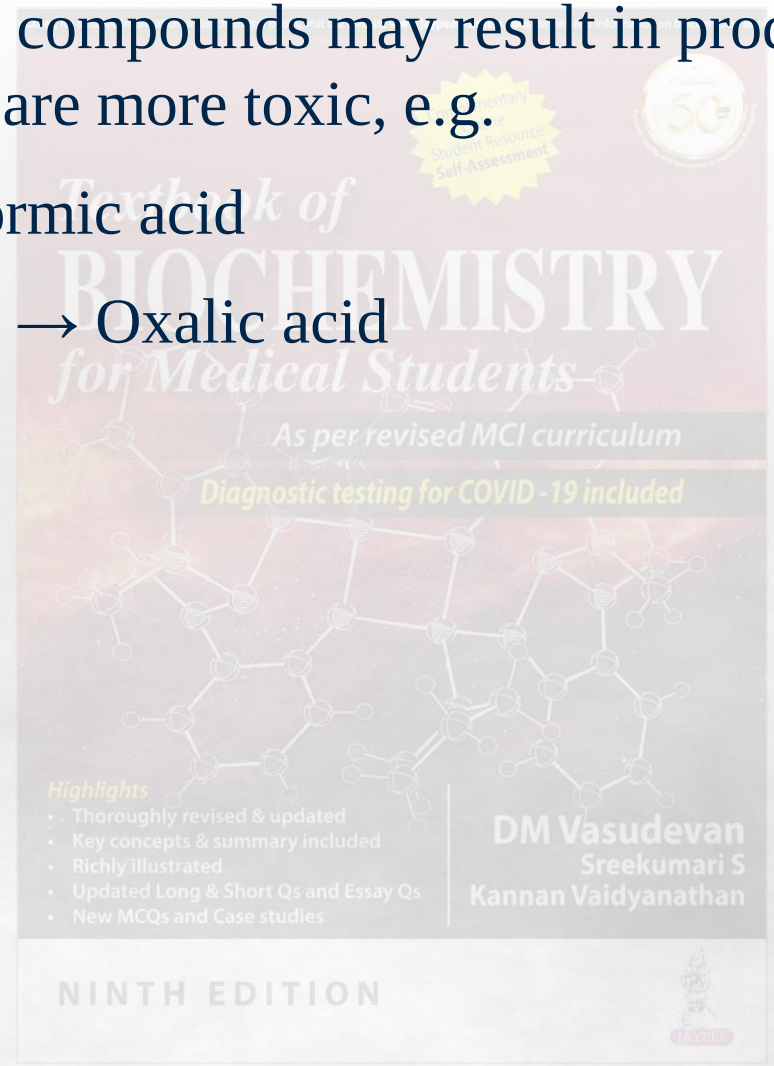
Oxidative Reactions

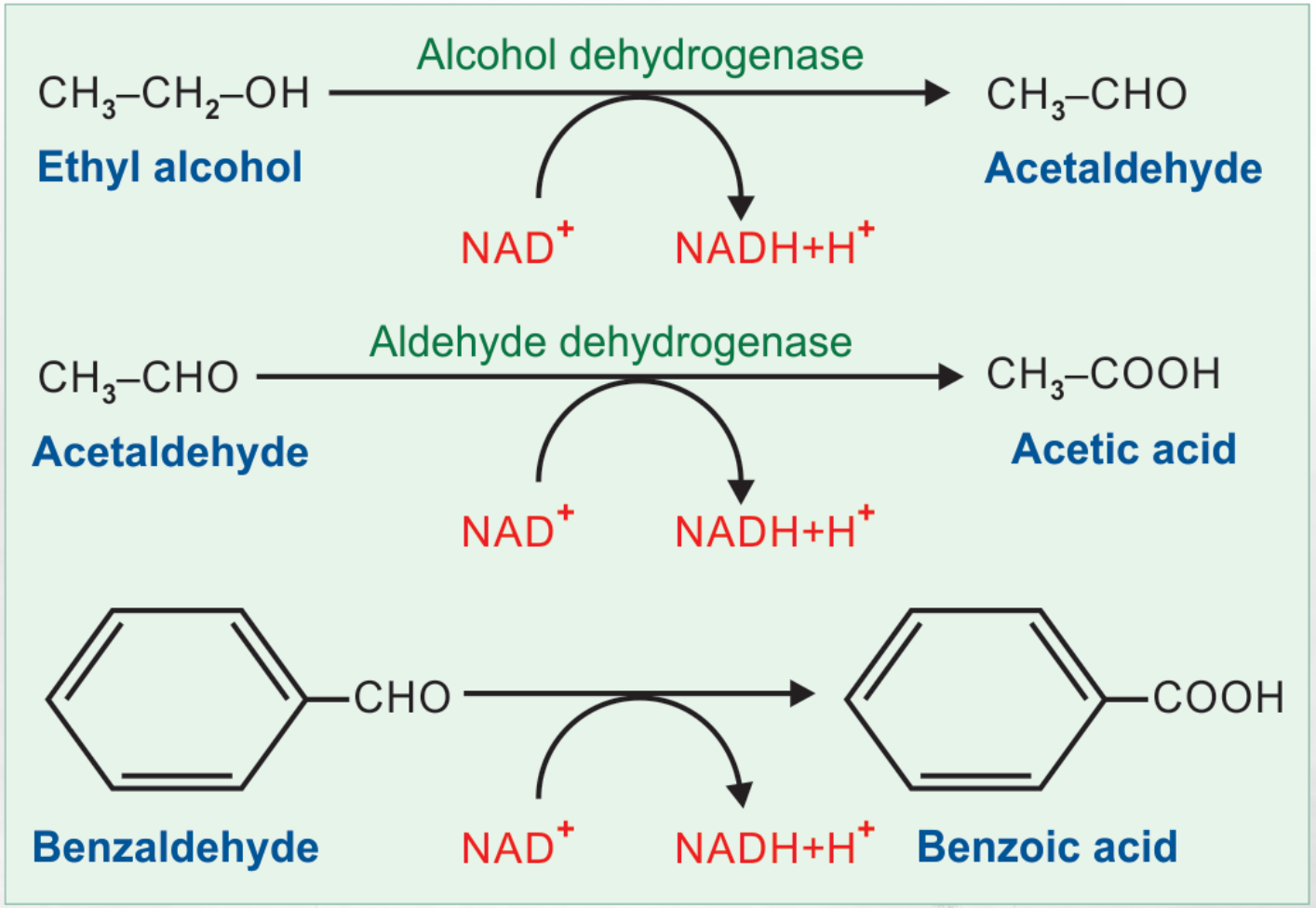
It may be either aromatic or aliphatic hydroxylation. The reactions also include sulfoxidation, N-oxidation, and epoxidation. For example, **toluene** is hydroxylated to benzyl alcohol by mixed function oxidase system



Oxidation of some compounds may result in production of substances, which are more toxic, e.g.

- Methanol → Formic acid
- Ethylene glycol → Oxalic acid



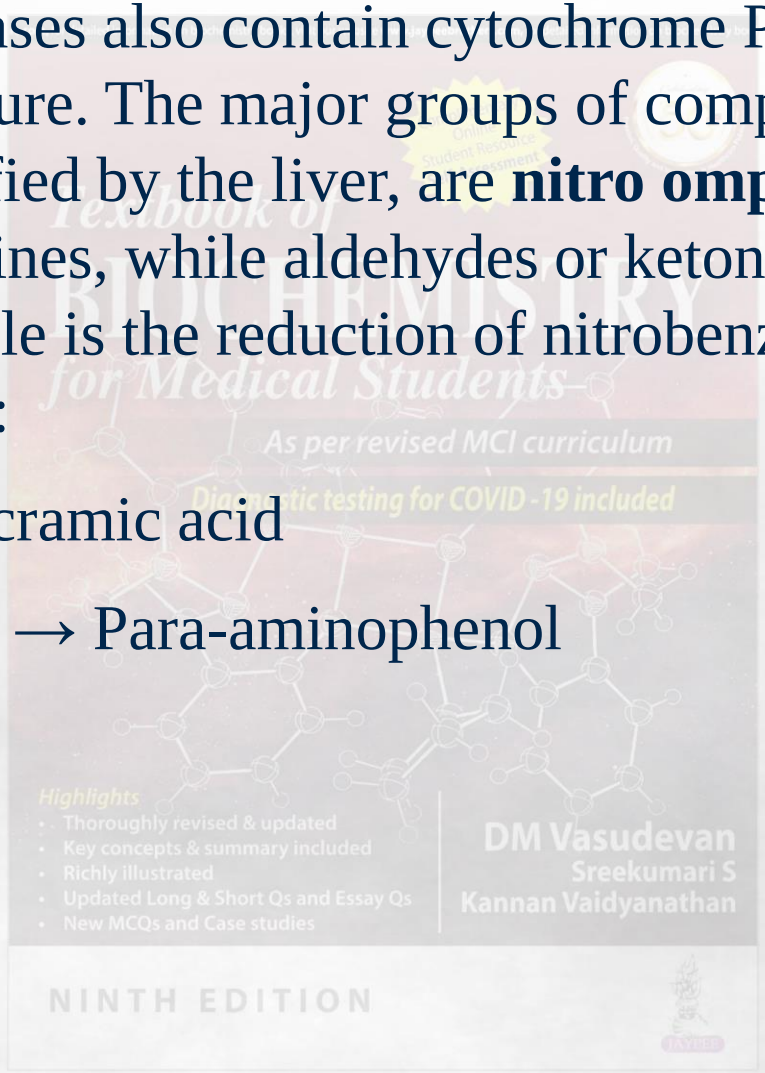


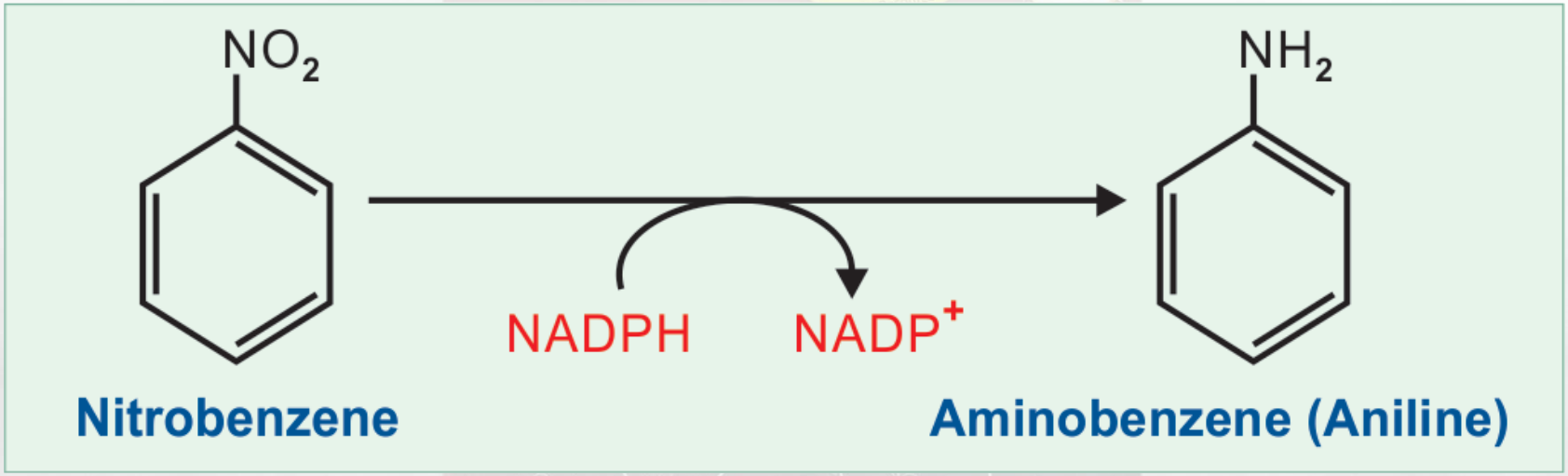
Oxidation of alcohol groups.

Reduction Reactions

Some of the reductases also contain cytochrome P450 and are flavoproteins in nature. The major groups of compounds, which are reduced and detoxified by the liver, are **nitro ompounds.** These are reduced to their amines, while aldehydes or ketones are reduced to alcohols. An example is the reduction of nitrobenzene to aniline. Other examples are:

- Picric acid → Picramic acid
- Para-nitrophenol → Para-aminophenol

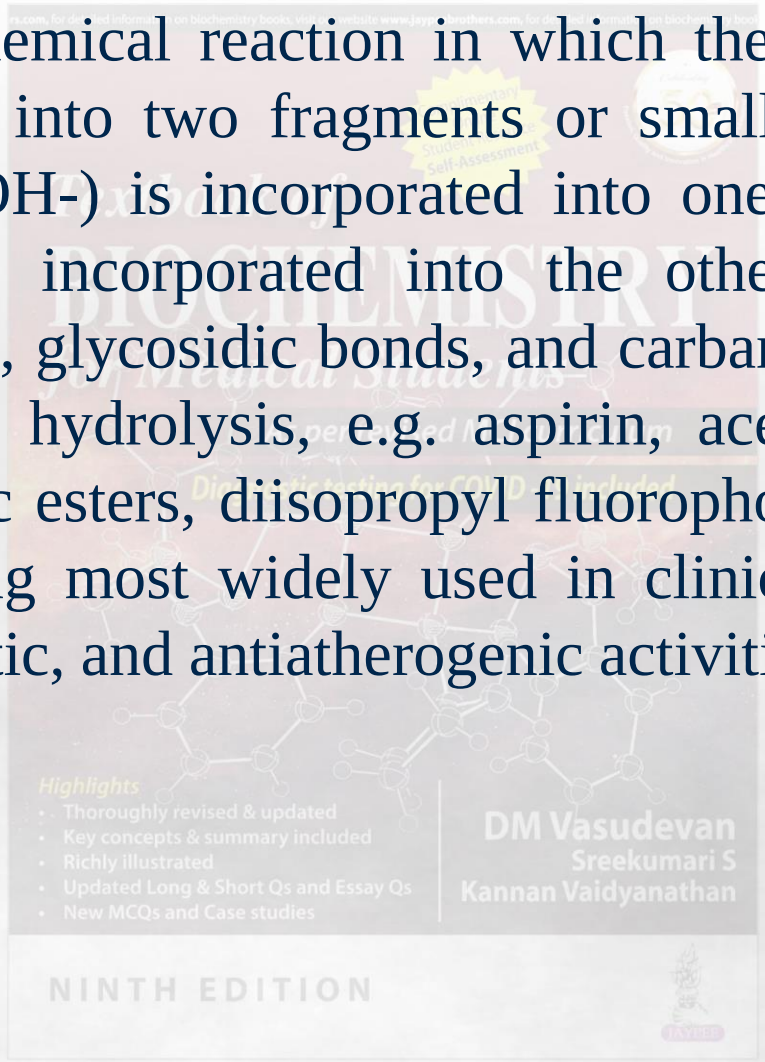


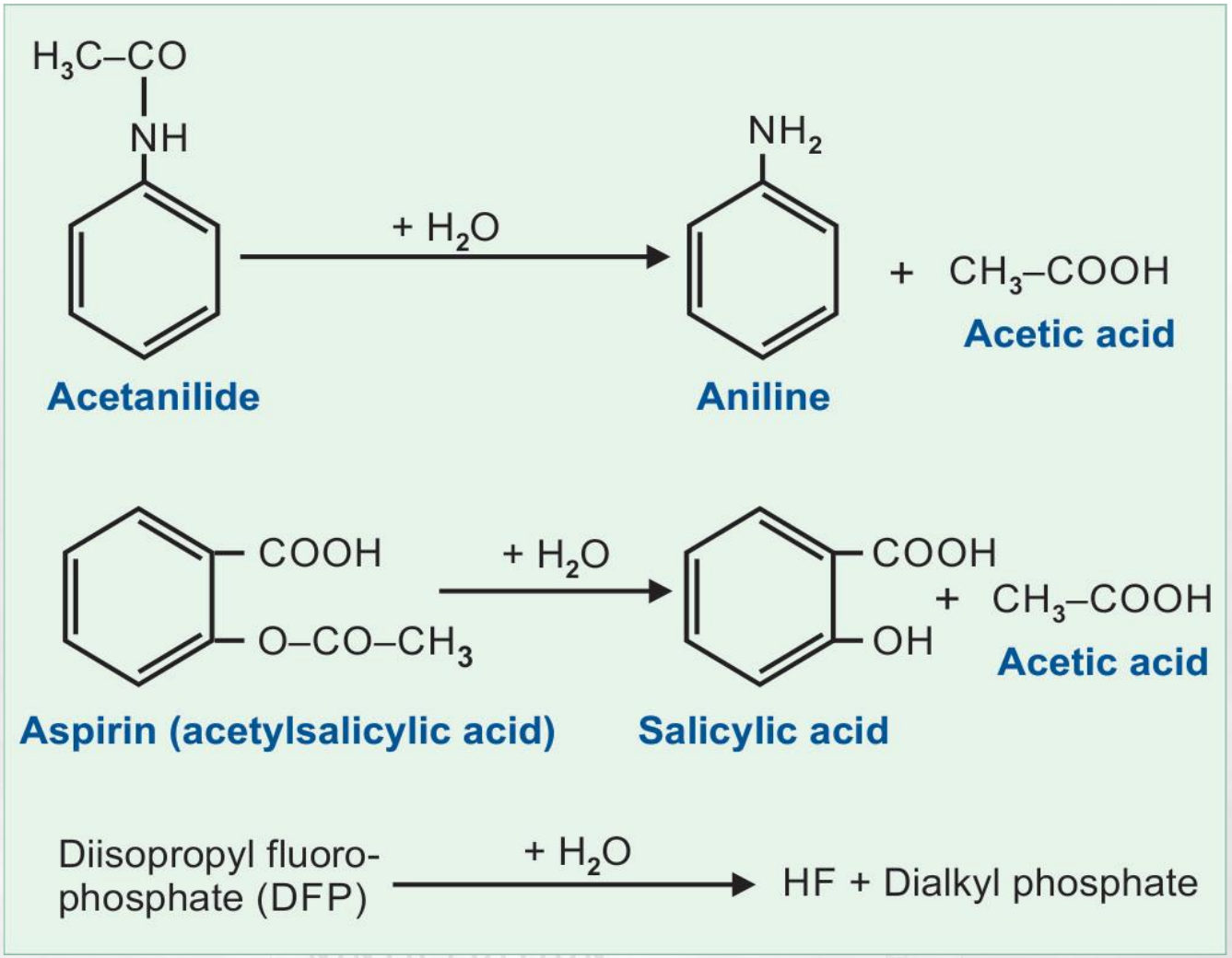


Phase one; reductive reaction.

Hydrolysis

Hydrolysis is a chemical reaction in which the addition of water splits the toxicant into two fragments or smaller molecules. The hydroxyl group (OH-) is incorporated into one fragment and the hydrogen atom is incorporated into the other. Esters, amines, hydrazines, amides, glycosidic bonds, and carbamates are generally biotransformed by hydrolysis, e.g. aspirin, acetanilide, procaine, xylocaine, aliphatic esters, diisopropyl fluorophosphate (DFP), etc. **Aspirin** is the drug most widely used in clinical practice. It has analgesic, antipyretic, and antiatherogenic activities.



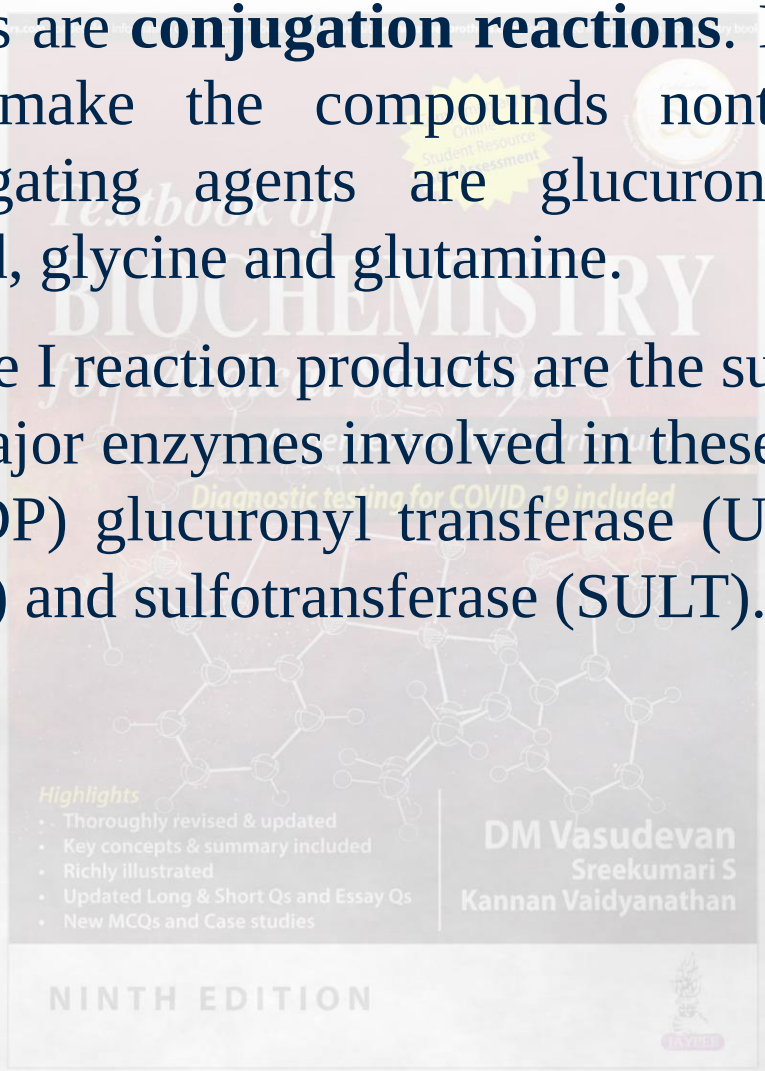


Phase one. Examples of hydrolysis.

Phase Two Reactions

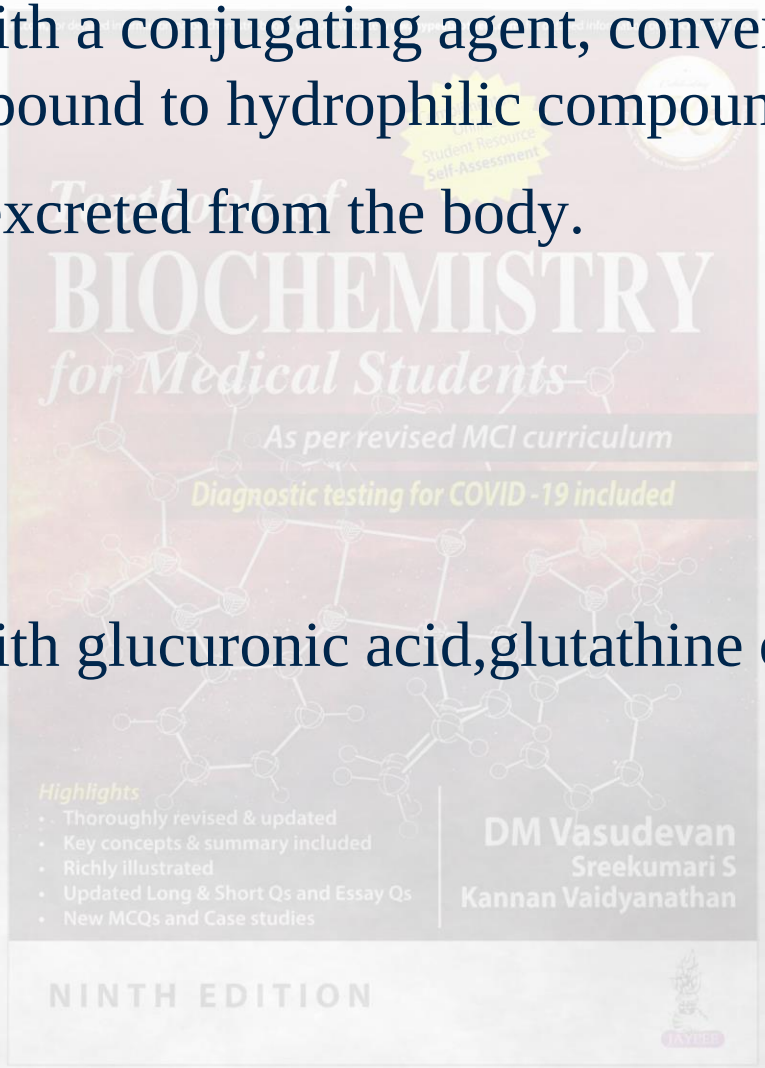
Phase two reactions are **conjugation reactions**. In most cases, the conjugation will make the compounds nontoxic and easily excretable. Conjugating agents are glucuronic acid, sulfate, cysteine, acetic acid, glycine and glutamine.

In many cases phase I reaction products are the substrates for phase II reactions. The major enzymes involved in these cases are uridine 5′-diphosphate (UDP) glucuronyl transferase (UGT), glutathione-S-transferase (GST) and sulfotransferase (SULT).



Phase Two Reactions

- Conjugation with a conjugating agent, converting lipophilic compound to hydrophilic compound.
- Can be easily excreted from the body.
- Sulfation
- Acetylation
- Methylation
- Conjugation with glucuronic acid,glutathine or glycine.



Phase Two; Usual Conjugating Agents

Conjugating agent	Active form
Glucuronic acid	UDP-glucuronic acid
Sulphate	PAPS (phospho adenosine phospho sulphate)
Cysteine	Glutathione
Acetic acid	Acetyl-CoA

Glucuronic Acid

Glucuronide conjugation is the most common Phase two reactions. **Bilirubin** is a good example for a compound conjugated and excreted as its glucuronide. Glucuronic acid can conjugate with hydroxyls (both phenolic and alcoholic), carbonyl, sulfhydryl and amino compounds. The glucuronic acid is added to xenobiotics by **UDP-glucuronyl transferases**, present in the endoplasmic reticulum.

UDP-glucuronyltransferase

UDP glucuronic acid ----------→ R-glucuronide
+ R--OH + UDP

Conjugation with Glucuronic Acid

Com-pounds	Types of bond	Products
Phenol	Glucosidic (Ether)	Phenyl glucuronide (O-glucuronide)
Benzoic acid	Ester	Glucuronic acid monobenzoate
Bilirubin	Ester with propionic acid side chain	Glucuronic acid
Steroids	Ester with OH group	Glucuronide of steroid
Amines	Amide	N-glucuronides

Sulfate Conjugation

In general, sulfation decreases the toxicity of xenobiotics. The highly polar sulfate conjugates are readily excreted through urine. Often glucuronidation or sulfation can conjugate the same xenobiotics. Phenolic and alcoholic compounds are conjugated with sulfate. The enzyme is sulfotransferase and the sulfate group is transferred from

PAPS (phosphoadenosine phosphosulfate).

$$R - OH + PAPS \rightarrow R - O - SO3 + PAP$$

Phenol + PAPS → Phenyl sulfate + PAP

Indole + PAPS → Indoxyl sulfate + PAP

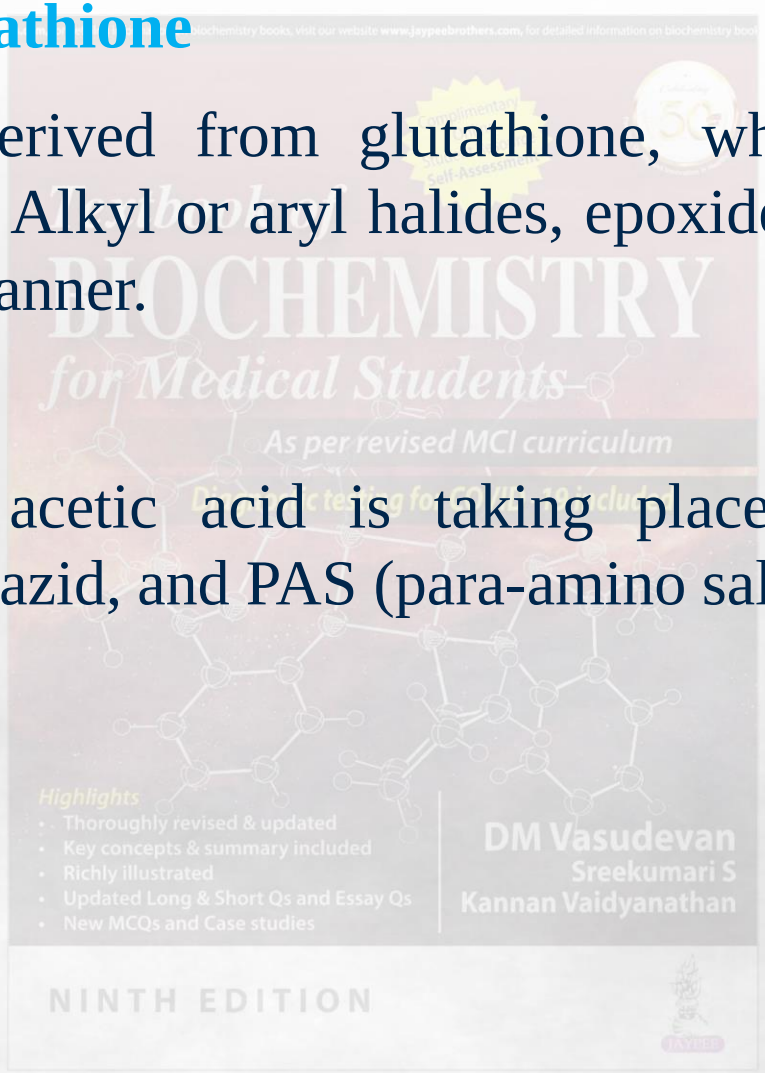
Steroids and indole compounds are excreted as their sulfates.

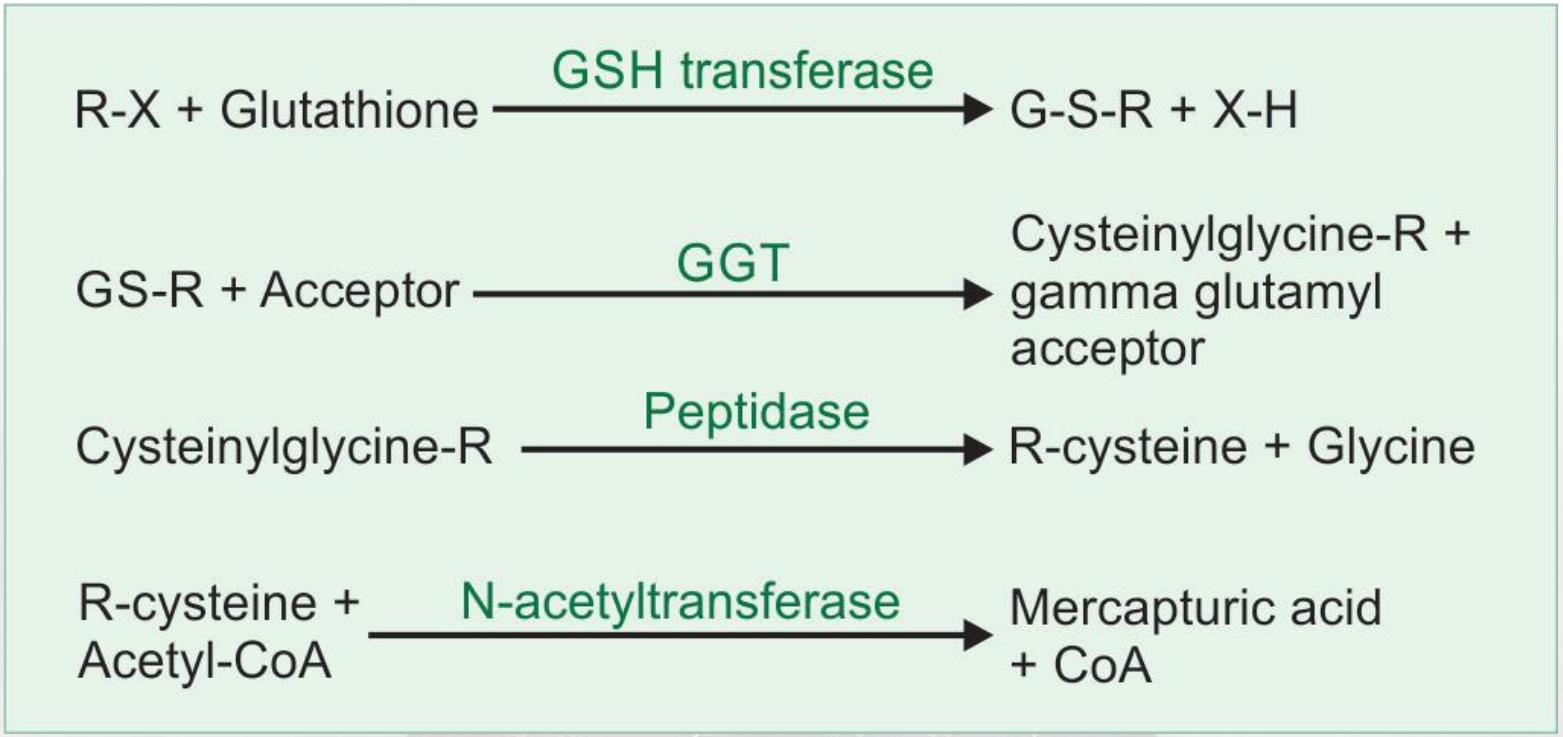
Cysteine and Glutathione

The cysteine is derived from glutathione, which is the active conjugating agent. Alkyl or aryl halides, epoxides, and alkenes are detoxified in this manner.

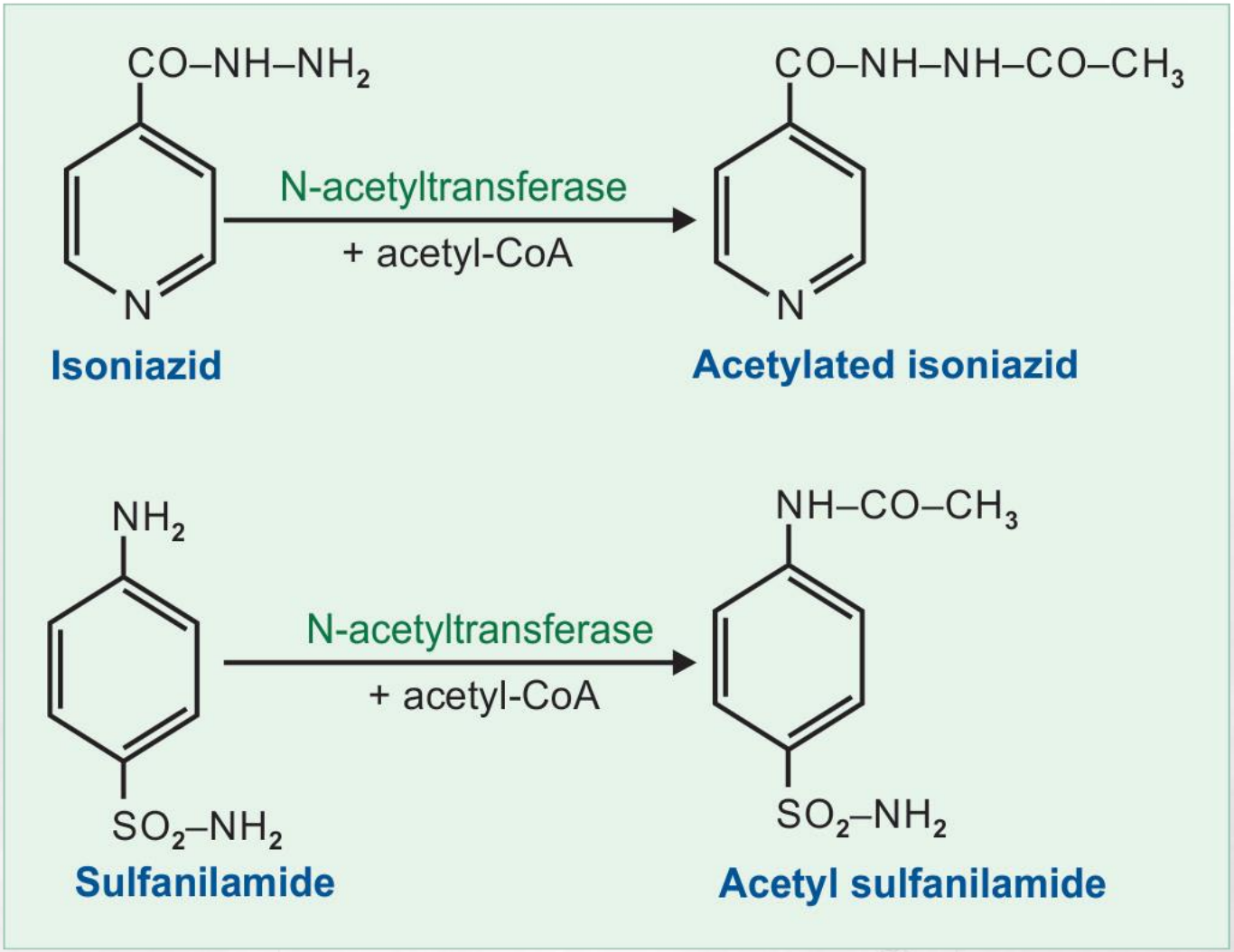
Acetylation

Conjugation with acetic acid is taking place with drugs like sulfanilamide, isoniazid, and PAS (para-amino salicylic acid).

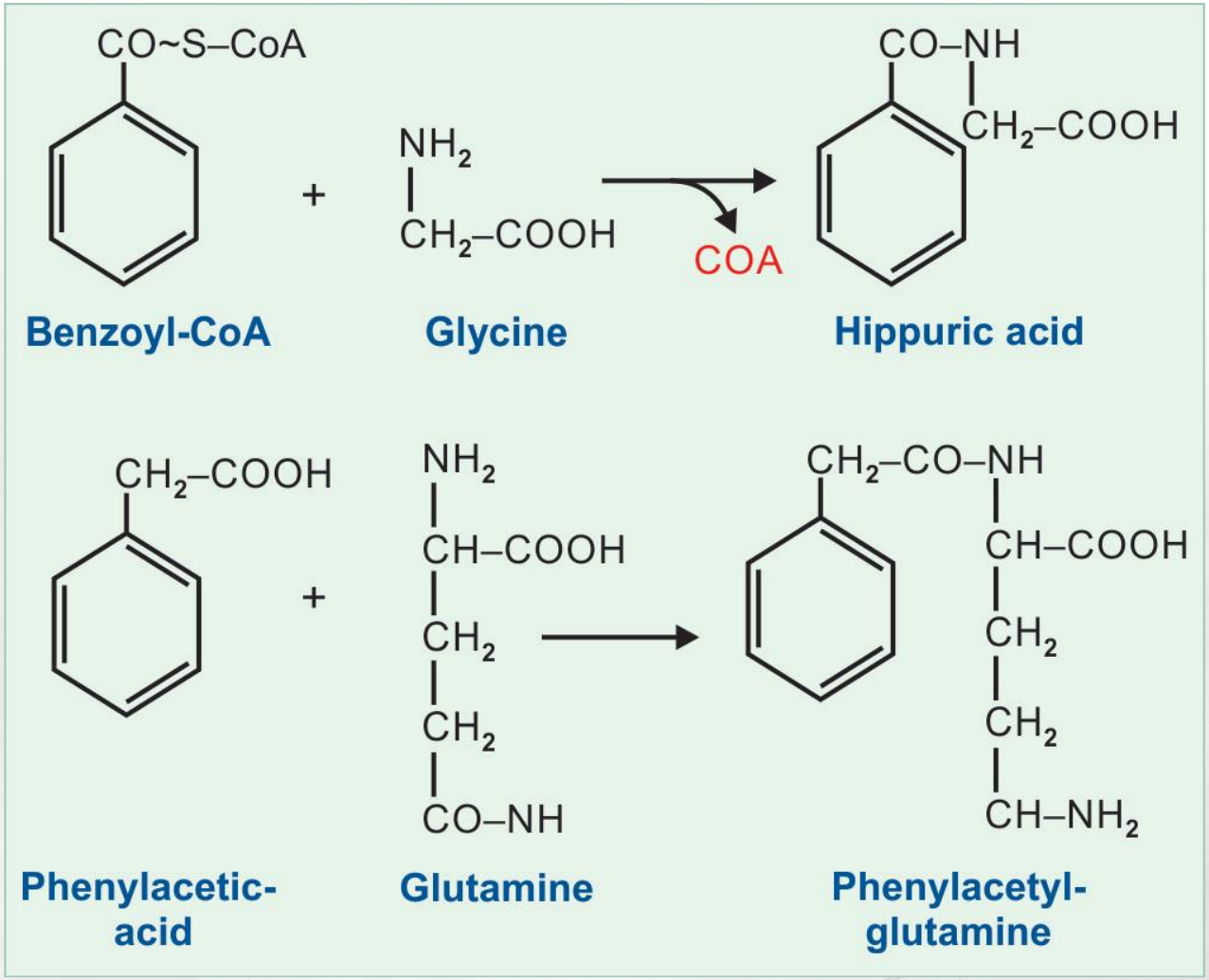




Phase two; glutathione as detoxifying agent.
(GSH: glutathione; GGT: gamma glutamyl transferase)



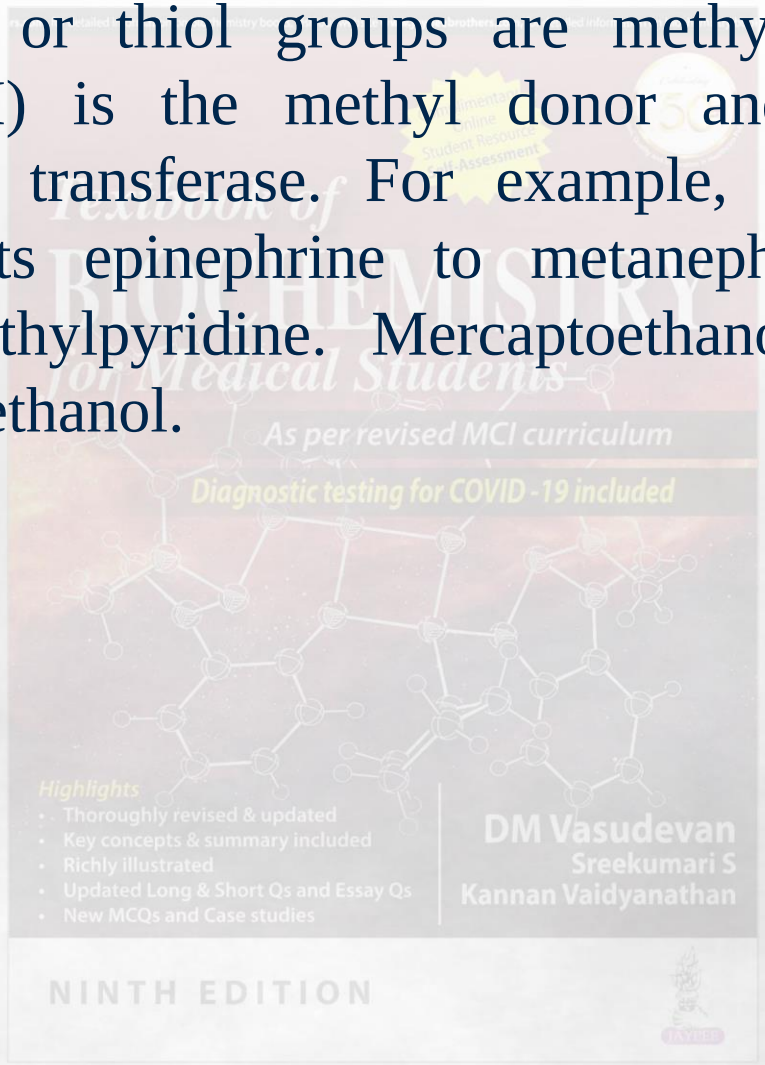
Phase two reaction; acetylation.

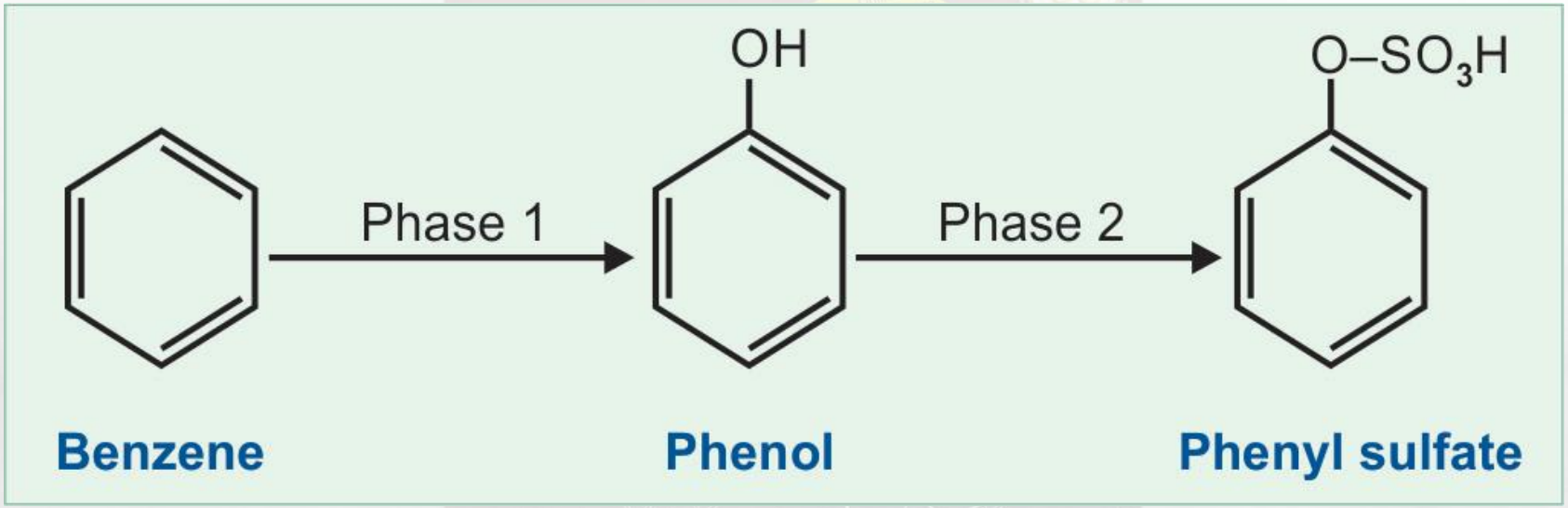


Conjugation with amino acids, Glycine and Glutamine.

Methylation Reactions

Amino, hydroxyl, or thiol groups are methylated. **S-adenosyl methionine** (SAM) is the methyl donor and the enzyme is usually O methyl transferase. For example, catechol-O-methyl transferase converts epinephrine to metanephrine. Pyridine is excreted as N-methylpyridine. Mercaptoethanol is excreted as 5-methyl mercaptoethanol.

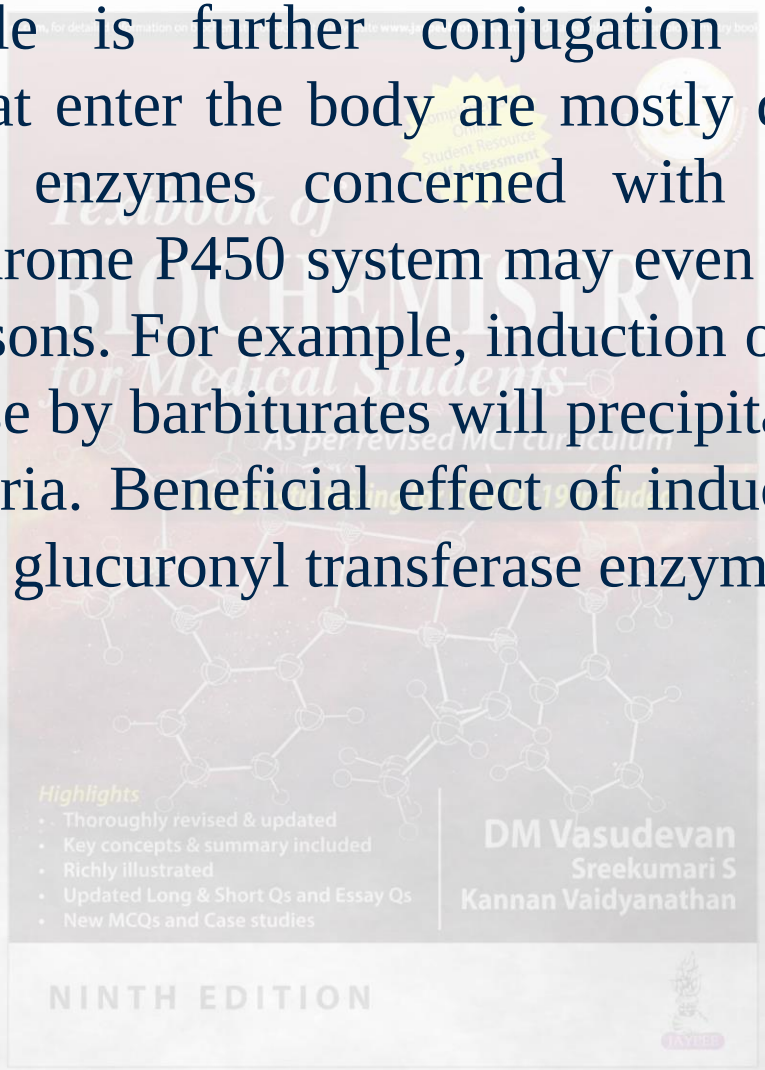




Sometimes both phase one and two reactions are needed to detoxify a compound.

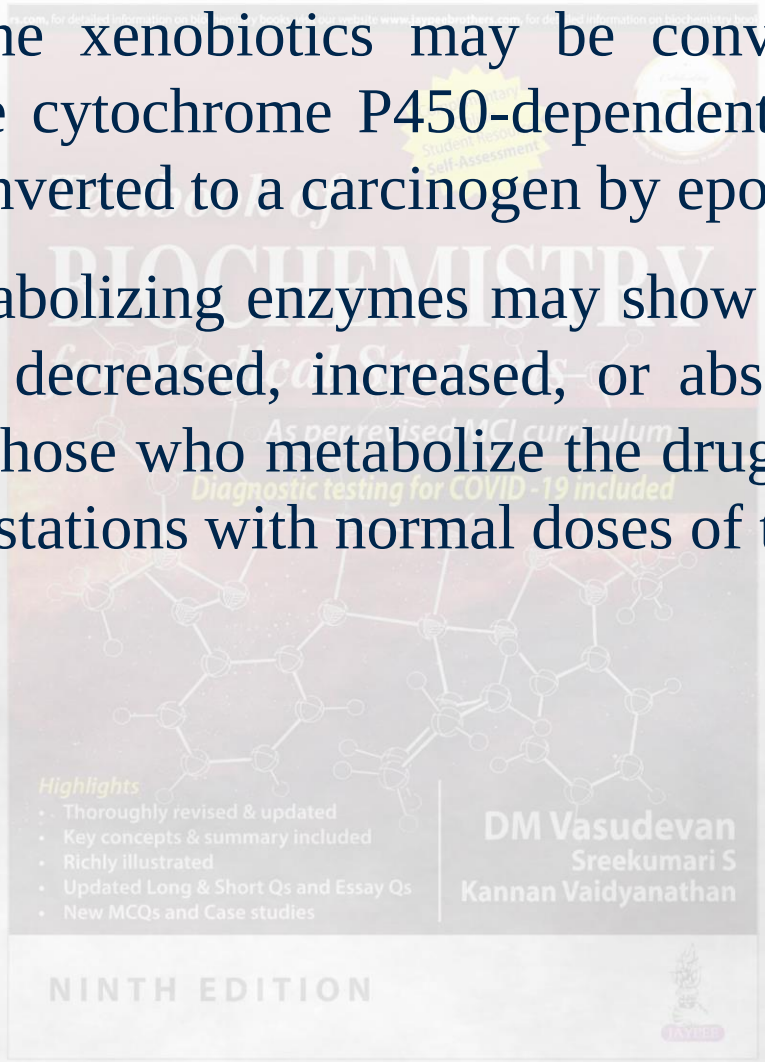
Phase Three Reactions

A typical example is further conjugation with glutathione. The xenobiotics that enter the body are mostly drugs and they are detoxified by the enzymes concerned with drug metabolism. Induction of cytochrome P450 system may even produce unwanted effects in some persons. For example, induction of δ-aminolevulinic acid (ALA) synthase by barbiturates will precipitate attacks in acute intermittent porphyria. Beneficial effect of induction is utilized in newborns to induce glucuronyl transferase enzyme by barbiturates.



In some cases, the xenobiotics may be converted to harmful compounds by the cytochrome P450-dependent oxygenases, e.g. benzopyrene is converted to a carcinogen by epoxidation.

The drug metabolizing enzymes may show genetic variation. This may lead to decreased, increased, or absent expression of enzyme activity. Those who metabolize the drugs sluggishly, may show toxic manifestations with normal doses of the drug.



Paraoxonase