What it is….?

- Xenobiotic (Gk xenos "stranger") are chemical substances foreign to the body excluding antigens.
- This includes food additives, pesticides, cosmetics, environmental pollutants and most important drugs.
- Xenobiotics can produce a variety of biological effects including
  - Pharmacological responses
  - Toxicity
  - Immunological responses
  - Cancers

SOURCES	EXAMPLES
Industrial chemicals	Solvents (benzene, carbon tetra chloride) detergents, bleaching agents
Air Pollutants	Tobacco Smoke, Automobile exhaust
Food Additives and Contaminants	Colors (butter yellow, azo dyes) sweeteners (saccharin), insecticides
Bacterial metabolites	Bacterial toxins
Cosmetics	Hair dyes, body spray, lipstick
Drugs	Aspirin, tranquilizers, OCPs etc.

Biotransformation and detoxification

- All the biochemical reactions involved in the conversion of foreign, toxic and water insoluble molecules to non toxic, water soluble and excretable forms are called Detoxification / Biotransformation reactions
- In most cases, biotransformation lessens the toxicity of xenobiotics
- The term "detoxification" is sometimes used for many of the reactions involved in the metabolism of xenobiotics

- Biotransformation is not exactly synonymous with detoxification, since in many cases, the metabolites are more toxic than the parent substance. This is known as BIOACTIVATION OR TOXICATION.

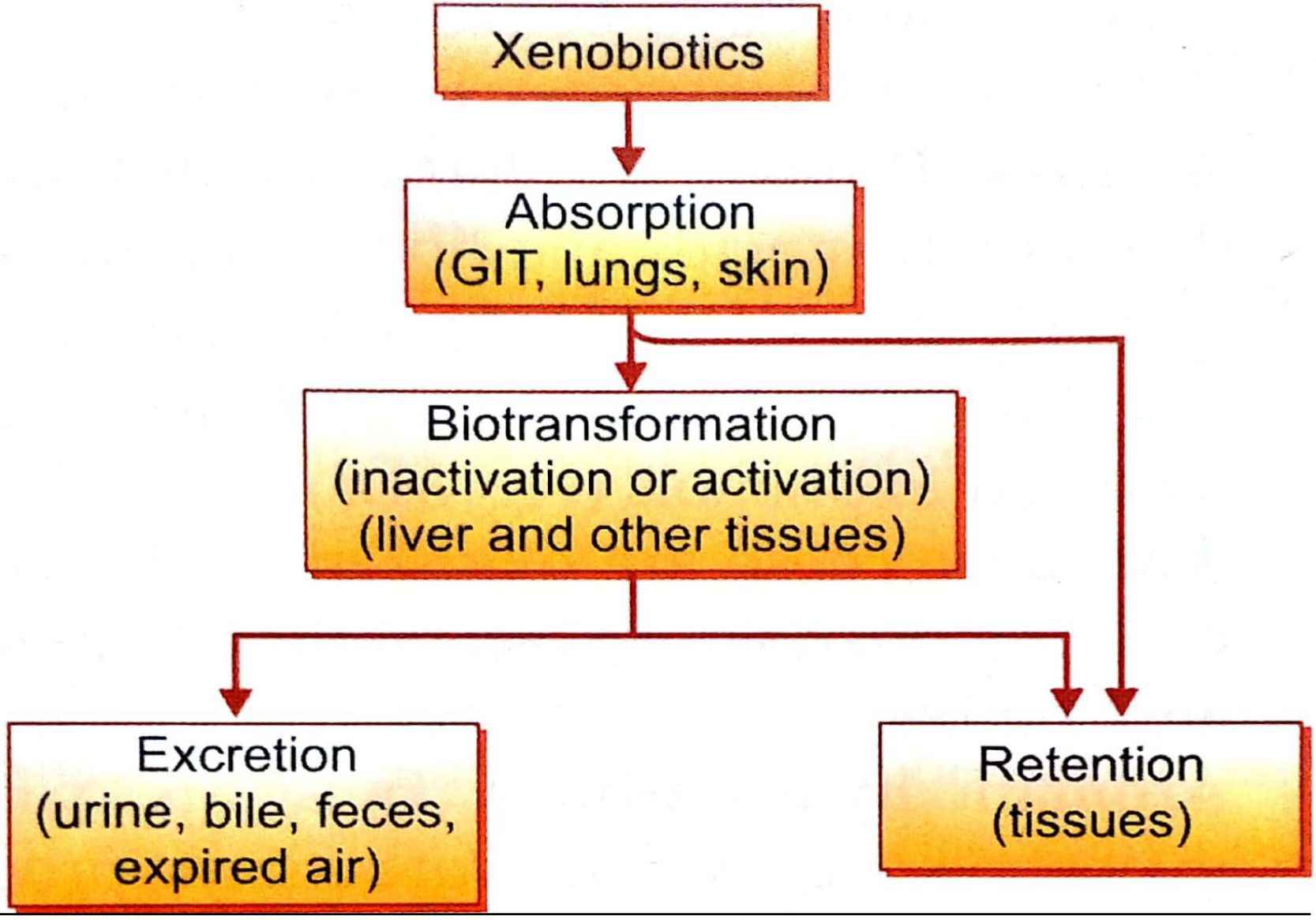
Example: biotransformation of vinyl chloride to vinyl chloride epoxide, which covalently binds to DNA and RNA, a step leading to cancer of the liver.

- Certain xenobiotics e.g. some drugs, are administered as a precursor (prodrug) which is **activated** in the body to active drug. This is an example of biotransformation but not detoxification.

Biotransformation reactions

- Purpose
  - Converts lipophilic to hydrophilic compounds
  - Facilitates excretion
- Consequences
  - Changes in solubility characteristics
  - Detoxification
  - Metabolic activation

SITES OF METABOLISM OF XENOBIOTICS



MECHANISM OF METABOLISM OF XENOBIOTICS

- Several biochemical transformation are used by the liver for the detoxification of xenobiotics and are classified into two groups

PHASE I REACTION

- The major reaction involved is Oxidation or hydroxylation and are catalyzed by Cytochrome P450 enzymes also called mono-oxygenases.
- In addition to hydroxylation, a wide range of reactions also take place including

Hydrolysis, Reduction
Dehalogenation, Desulfuration,
Deamination, Epoxidation, Peroxygenation

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- PHASE II REACTION

Reactions mainly involve further modification and conjugation to make the phase I species more water soluble. It involves

-conjugation with

a. Glucuronic acid

b. Sulfate

c. Acetate

d. glutathione

e. methyl

g. certain amino acids

XENOBIOTICS METABOLISM

PHASE I

- Oxidation
- Hydrolysis
- Reduction
- Dehalogenation,
- Desulfuration,
- Deamination
- Epoxidation,
- Peroxygenation

PHASE II

Conjugation with

a. Glucuronic acid
b. Sulfate
c. Acetate
d. glutathione
e. methyl
g. certain amino acids

Xenobiotic-Metabolizing Enzymes (XME)

Phase I

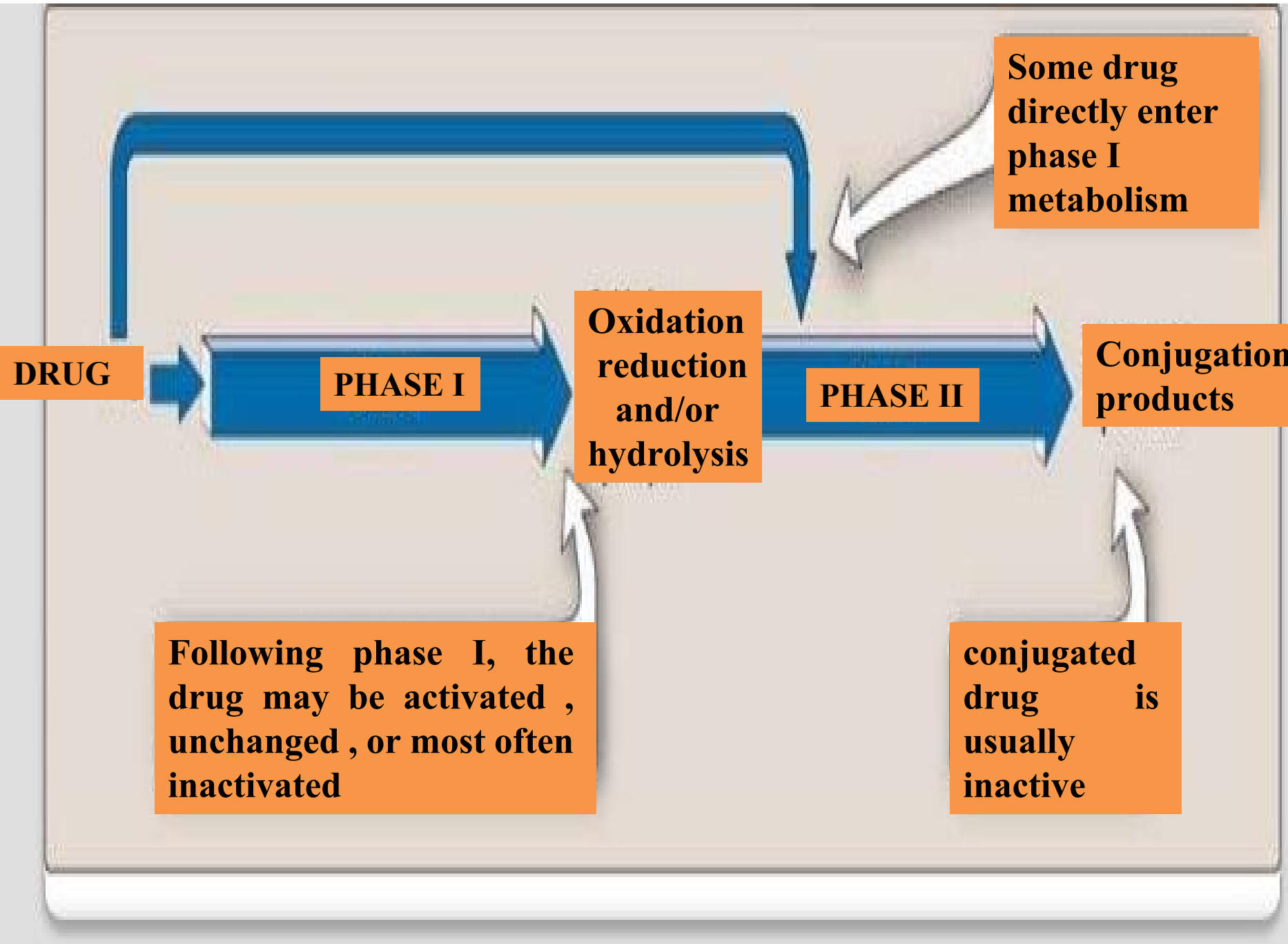
- Cytochromes P450
- Flavin Containing Monooxygenase
- Epoxide Hydrolase
- Alcohol /Aldehyde Dehydrogenases
- Monoamine Oxidases
- Xanthine oxidase

Phase II (Transferases")

-Sulfotransferases (ST)

- UDP-glucuronosyl-transferases (UGT)

- Glutathione S-transferases (GST)



Role of Liver

- Main organ involved
- Hepatocytes contain wide variety of enzymes to process xenobiotics
- Enzymes are present in cytosol, endoplasmic reticulum and to lesser extent in other organelles
- Each enzyme represents a large family of gene product
- Each gene product may be induced by different xenobiotics

Cytochrome P450

1. Superfamily of heme enzymes (many isoforms) can catalyze different reaction types, mainly hydroxylation
2. They are so named, because they absorb light at wave length of 450 nm, when exposed to carbon monoxide
3. Occur in most tissues (except of muscles and erythrocytes)
4. the highest amount in the liver (ER) and enterocytes.
5. In the liver , present in membrane of SER, which constitute microsomal fraction.
6. In hepatic microsomes, Cyt P450 can compromise as much as 20% of the total protein.
7. exhibit genetic polymorphism (atypical biotransformations)

Cytochrome P450

8. They are mono-oxygenases: R-H + O2 + NADPH + H+ → R-OH + H2O + NADP+
9. NADPH (and not NADH) is the co-enzyme for all the P450 enzymes.
10. Electrons are transferred from NADPH to cytochrome P450. This leads to the reductive activation of molecular oxygen. One atom of oxygen is inserted into the substrate.
11. can be induced and inhibited
12. At least half of the common drugs we ingest are metabolized by isoforms of cytochrome P450

- Warfarin, **a** drug to prevent blood clotting. is metabolized by CYP2C9 which is induced by phenobarbital
- Grapefruit contains a variety of furanocoumarins, which inhibit cytochrome P450
- Ethanol induces CYP2E1, which metabolises many carcinogens. Thus, the risk of carcinogenicity is increased after the use of ethanol.

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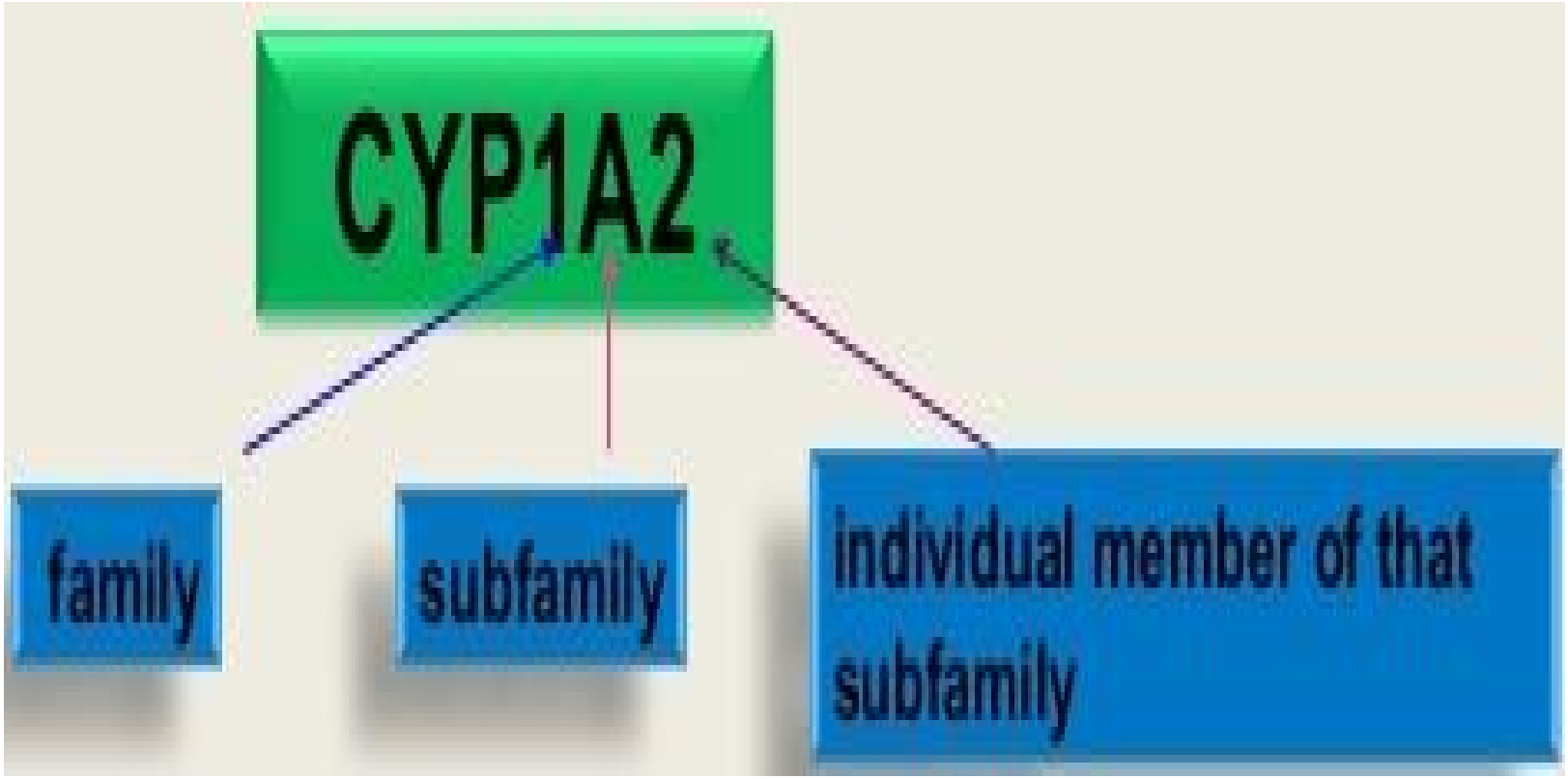
- 

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PHASE I REACTIONS

Phase I reactions include:

A. Oxidation

B. Reduction

C. Hydrolysis reactions

A. Oxidation

- A large number of foreign substances are destroyed by oxidation in the body.

  Examples

  - Oxidation of methyl group containing compounds Methyl group- is oxidized to acid through formation of alcohol and aldehyde

- $CH_3 \longrightarrow CH_2OH \longrightarrow CHO \longrightarrow COOH$

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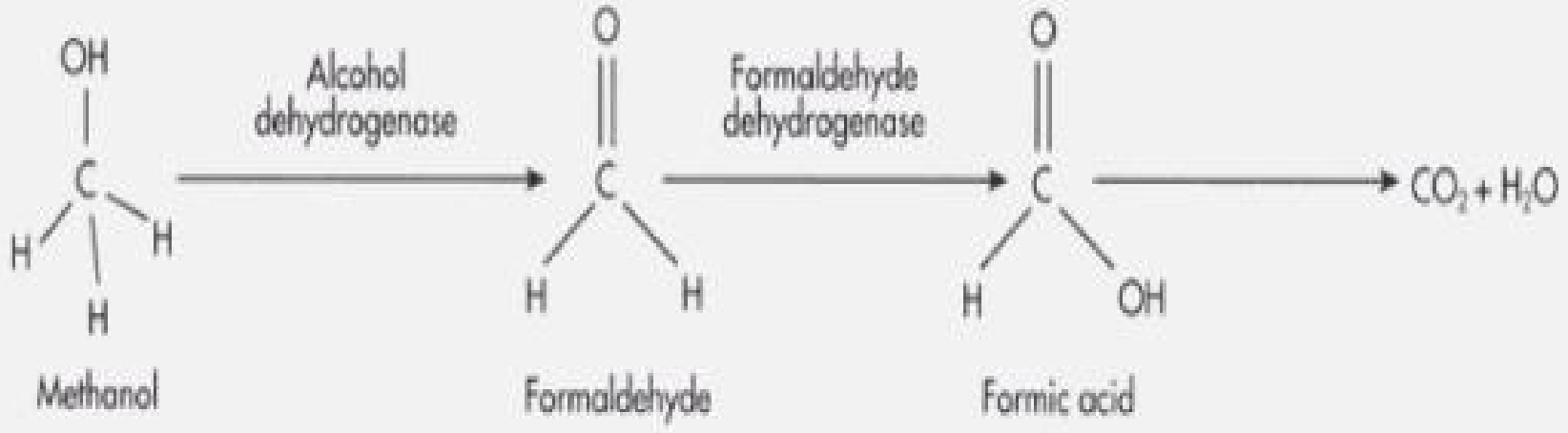
Oxidation of Alcohols-

Primary aliphatic and aromatic alcohols are oxidized to corresponding acids

- Methanol ---→Formaldehyde---→ Formic acid
- Ethanol ---→Acetaldehyde---→ Acetic acid
- BenzoyalAlcohol--→ Benzaldehyde --→Benzoic acid

METHANOL TOXICITY



It is an example of Entoxification

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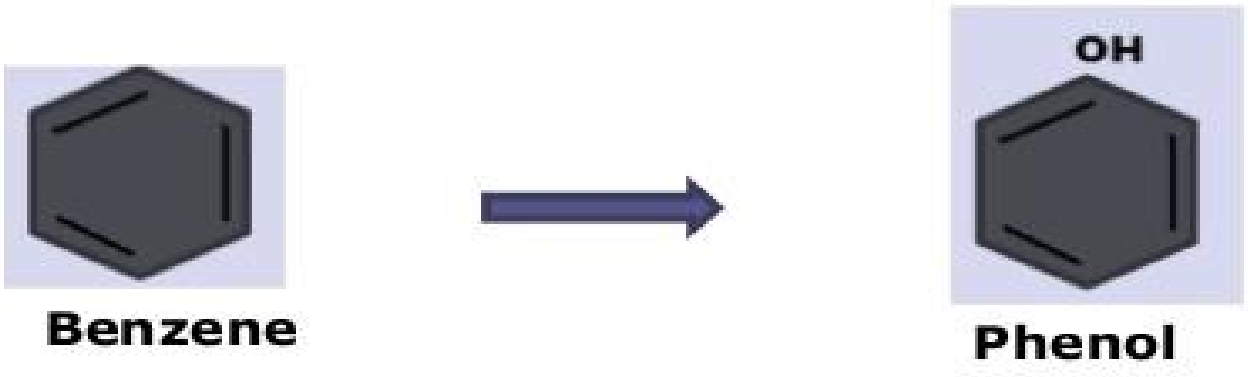
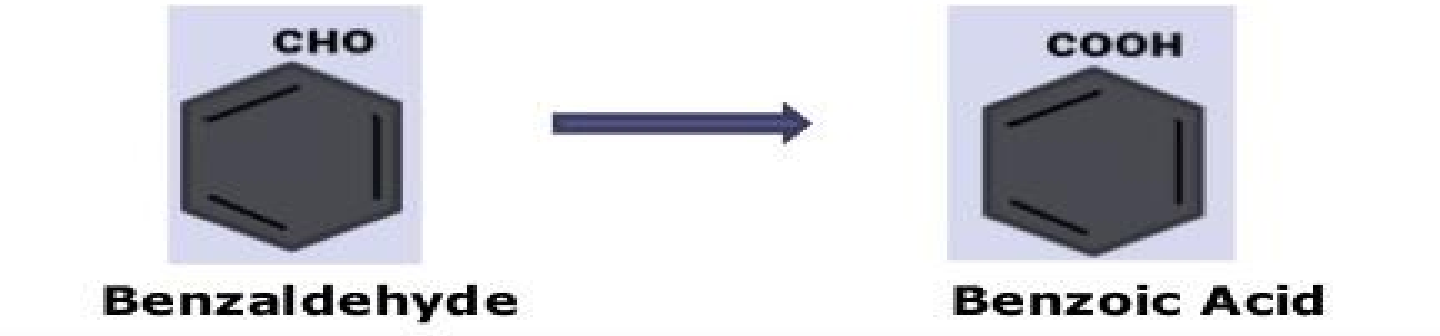
Oxidation of Aromatic Hydrocarbons

Aromatic hydrocarbons are oxidized to phenolic compounds, which can further be conjugated with Glucuronic acid or Sulfuric acid in phase 2 reactions so as to be excreted through urine.



Oxidation of Aldehydes

- Aldehydes are oxidized to corresponding acid. Acid thus formed is further conjugated in phase 2; e.g.
- Benzoic acid is conjugated with Glycine to form Hippuric acid.
- This reaction exclusively takes place in liver.
- Hippuric acid excretion test is undertaken to determine the detoxification functions of liver.



Oxidation of Anilides

Anilides are oxidized to corresponding phenols e.g.- Acetanilide is a constituent of analgesic drug. It is oxidized in the body to form p-Acetyl amino phenol.

Acetanilide ⟶ p-Acetyl -Amino phenol

Oxidation of Amines

- Many primary aliphatic amines undergo oxidation to form the corresponding acids and nitrogen is converted to urea.
- Benzyl amine  Benzoic acid + Urea
- Aromatic amines like Aniline is oxidized to corresponding phenol.

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- Oxidation of certain compounds may result in the production of more toxic compounds (Entoxification). Therefore their formation is prevented.

For example

- Methanol → Formic acid
- Halogenated Alcohol → Halogenated Acid
- Ethylene Glycol → Oxalic Acid

B) Reduction

- Some of the reductases also contain cytochrome P-450 and are flavoproteins in nature.
- The major group of compounds which are reduced and detoxified by the liver are nitro compounds.
- These are reduced to their amines, while aldehydes or ketones are reduced to alcohols

E.g.

- p- nitrobenzene → p- Amino benzene
- p- nitro phenol → p-Aminophenol
- Picric Acid → Picramic Acid

C)Hydrolysis

- Certain therapeutic compounds undergo hydrolysis,

Examples

Acetyl Salicylic acid ⟶ Acetic acid + Salicylic acid
(Aspirin)

Atropine ⟶ Tropic acid + Tropine

Procaine ⟶ p- Amino Benzoic acid + Diethyl amino ethanol

Phase II - Conjugation

- Conjugation is a process by which the foreign molecules and their metabolites are coupled with a conjugating agent and are converted to soluble, non toxic derivatives which are easily excreted in urine
- Conjugation reactions can occur independently or can follow phase 1(hydroxylation) reactions
- Conjugation takes place primarily in liver but can occur in kidney also
- After conjugation the products are generally rendered non toxic but in certain conditions they are left unchanged or become more toxic.

Types of Phase 2 Reactions

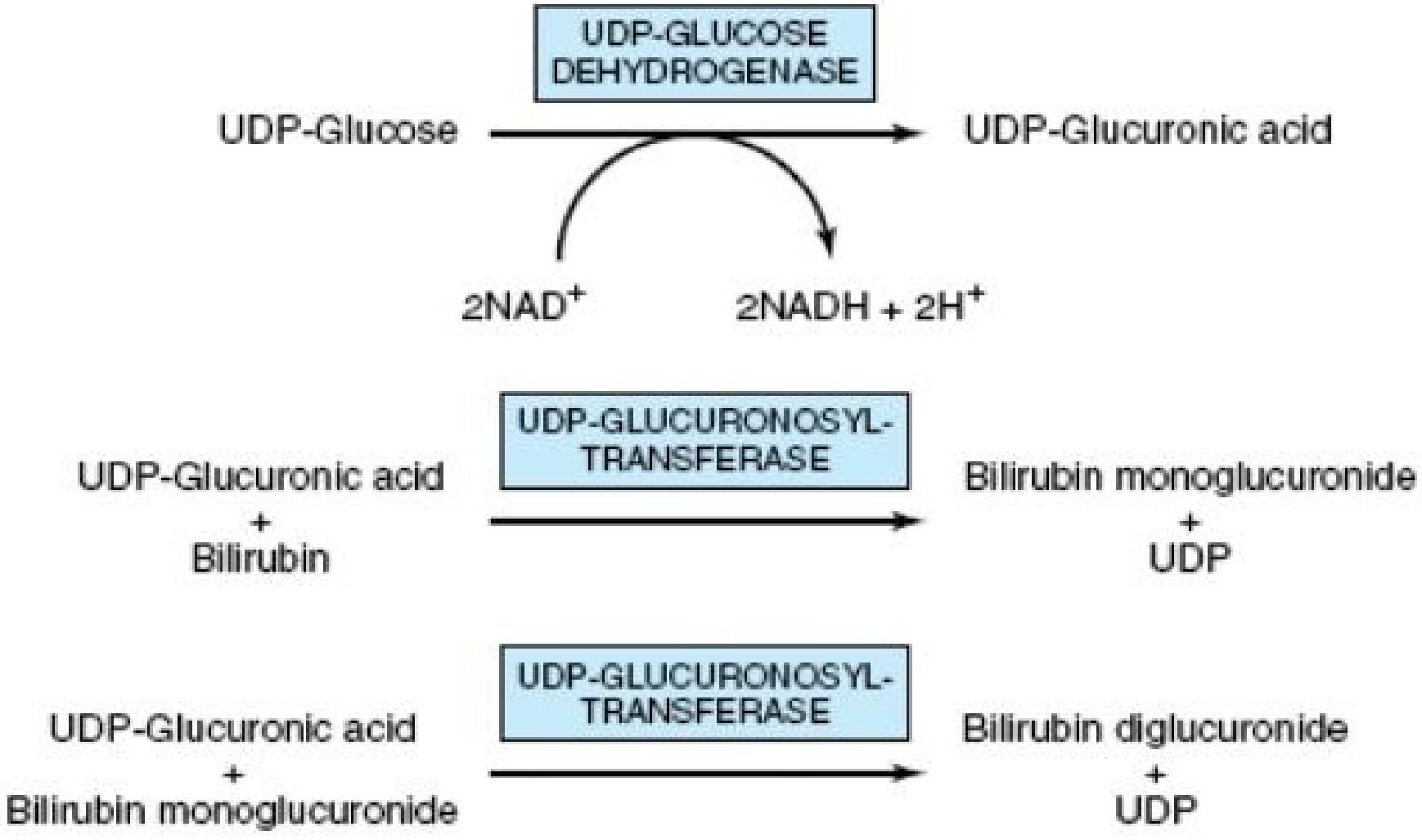
1. Glucuronidation
2. Sulfation
3. Acetylation
4. Methylation
5. Conjugation with Amino acids
6. Conjugation with G-SH (Glutathione)

1) Glucuronidation

- Glucuronidation is the most frequent conjugation reaction.
- UDP-glucuronic acid , is the Glucuronyl donor, which is formed in the uronic acid pathway of Glucose metabolism
- The glucuronic acid is added to xenobiotics by UDPglucuronyl-transferases, present in the endo plasmic reticulum. .
- Glucuronic acid can conjugate with hydroxyls (both phenolic and alcoholic), carbonyl, sulfhydryl and amino compounds.

Glucuronidation

Glucuronidation of Bilirubin



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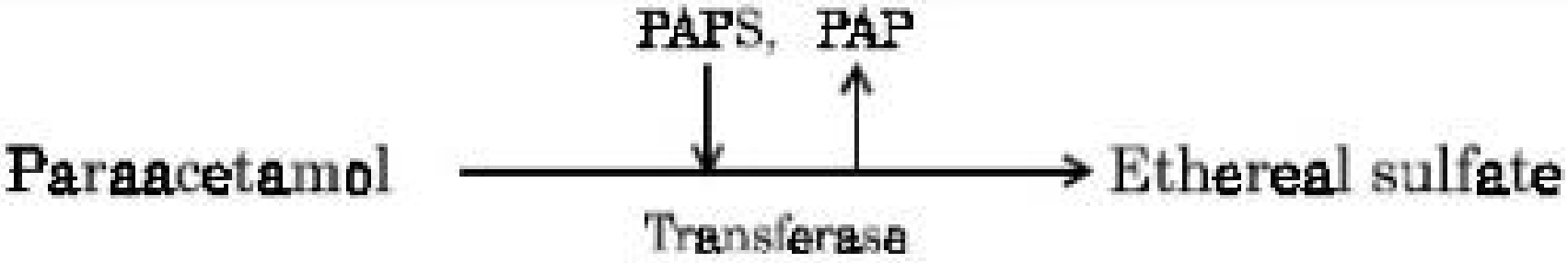
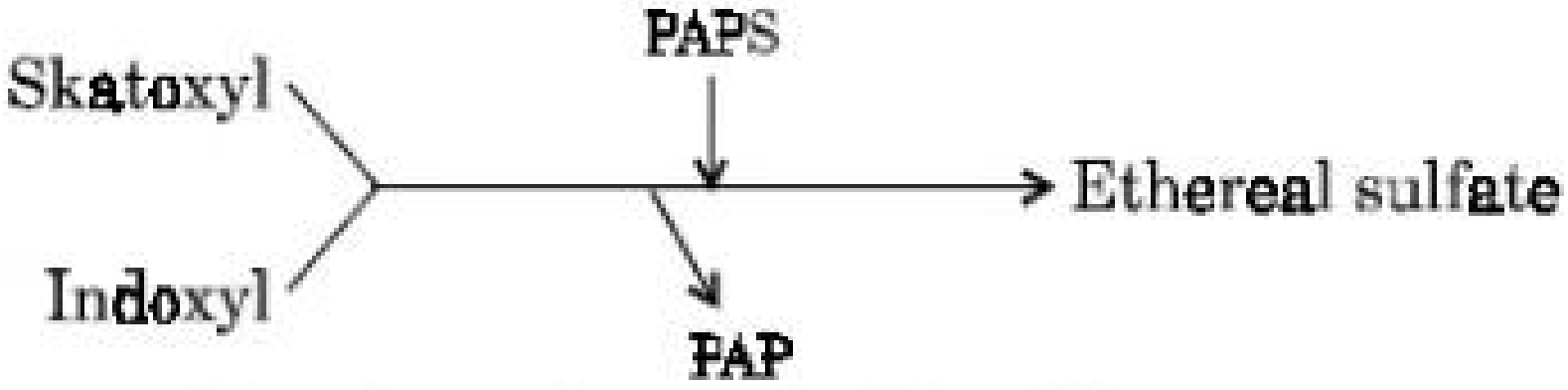
- Most of the bilirubin excreted in the bile of mammals is in the form of bilirubin diglucuronide.
- Bilirubin-UGT activity can be induced by a number of clinically useful drugs, including Phenobarbital

2) Sulfation

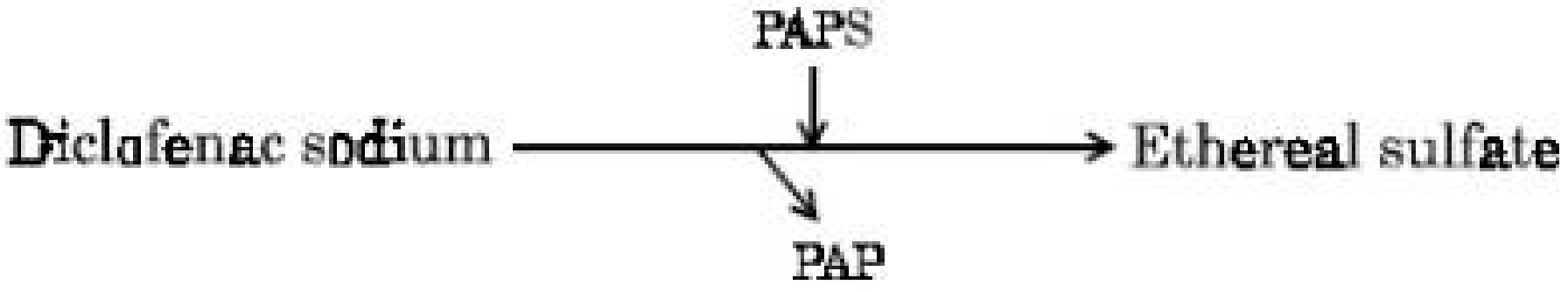
- The highly polar sulfate conjugates are readily excreted through urine.
- The sulfate donor is adenosine 3'-phosphate-5'phosphosulfate (PAPS) this compound is called "active sulfate—
- The enzyme is sulfo transferase
- Compounds which are conjugated with sulphate are 1. Phenols 2. Cresols 3. Indole

  4. Steroids 5. Oestrogen and Androgens



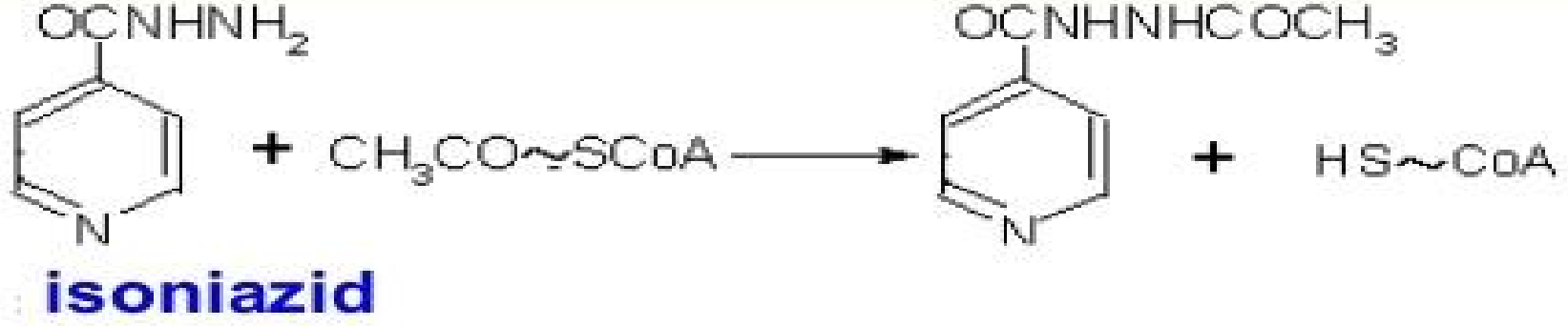


diclofenac sodium is conjugated with sulfate.



3) Acetylation

- Conjugation with acetic acid is taking place with drugs like sulfanilamide, isoniazid and PAS (para amino salicylic acid)
- Acetyl-CoA (active acetate) is the acetyl donor.
- These reactions are catalyzed by acetyltransferases present in the cytosol of various tissues, particularly liver



isoniazid

$$X + \text{Acetyl-CoA} \rightarrow \text{Acetyl-}X + \text{CoA}$$

- Polymorphic types of acetyltransferases exist, resulting in individuals who are classified as slow or fast acetylators, and influence the rate of clearance of drugs from blood.
- Slow acetylators are more subject to certain toxic effects of drug because the drug persists longer in these individuals.

Compounds conjugated by Acetylation

- Sulphanilamide
- PABA (Para Amino Benzoic Acid)
- Isoniazid

PABA Acetylated PABA

4) Methylation

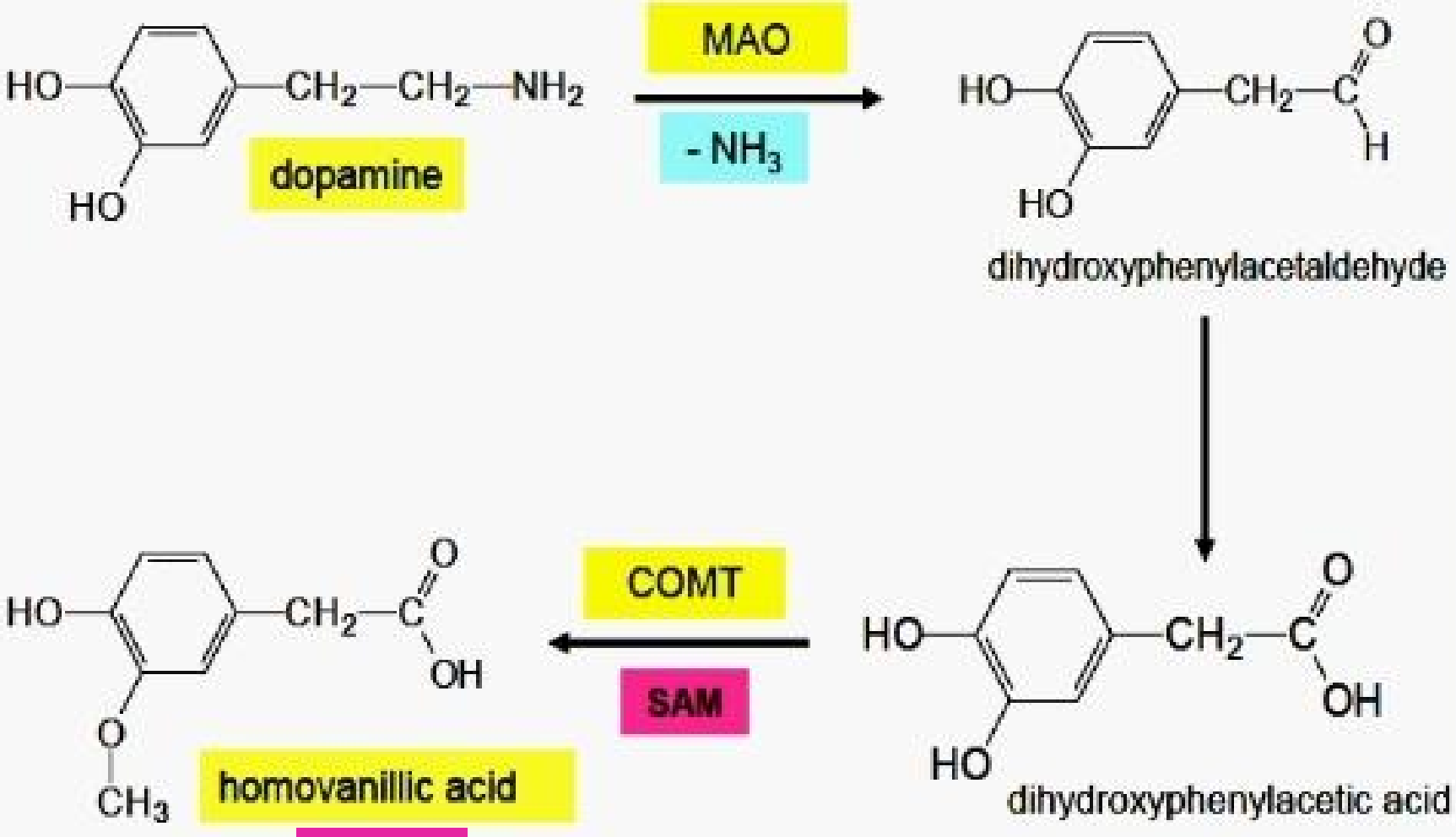
- Amino, hydroxy or thiol groups are methylated.
- S- Adenosyl Methionine-SAM (Active Methionine) acts as a Methyl group donor
- Reactions are called Transmethylation reactions
- Enzymes catalyzing the reactions are Methyl transferases

Catechol-O-Methyl Transferase

Epinephrine+ S-Adenosyl Methionine → S-Adenosyl Homocysteine + Metanephrine

Methylations are involved in the inactivation of catecholamines

MAO monoamine oxidase, COMT catechol-*O*-methyltransferase



Inactivation can proceed in the reverse order: first COMT, then MAO, product is the same.

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- Methylation decreases the water solubility rather than increasing it. Metals like mercury may be methylated, making them more lipophilic, increasing permeability and causing neurotoxicity

5) Conjugation with Amino acids

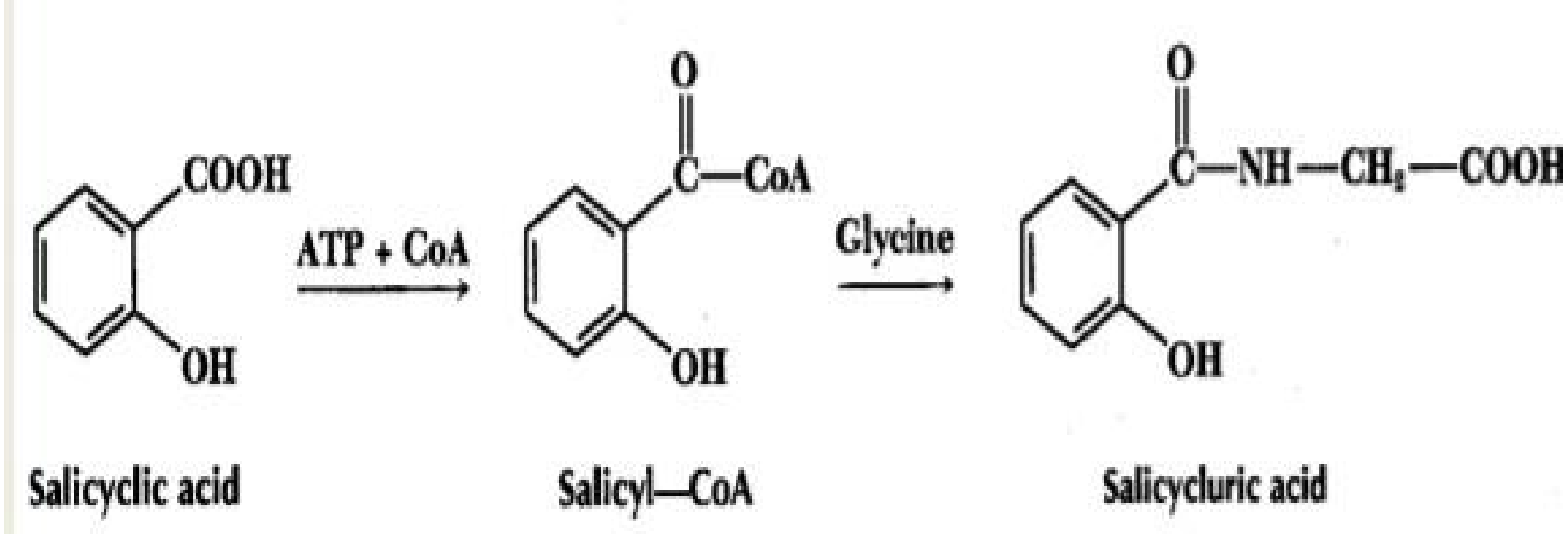
A) Conjugation with Glycine

Benzoic acid + Glyine ⟶ Hippuric acid
(Excreted in urine)

Nicotinamide + Glycine ⟶ Nicotinuric Acid

- Cholic and deoxy Cholic acid are conjugated to form Glyco cholic acid and Glycodeoxy cholic acid

. Approximately 76% of aspirin is metabolized through amino acid conjugation Salicyluric acid, the glycine conjugate of salicyclic acid, is the main metabolite of aspirin



ASPIRIN

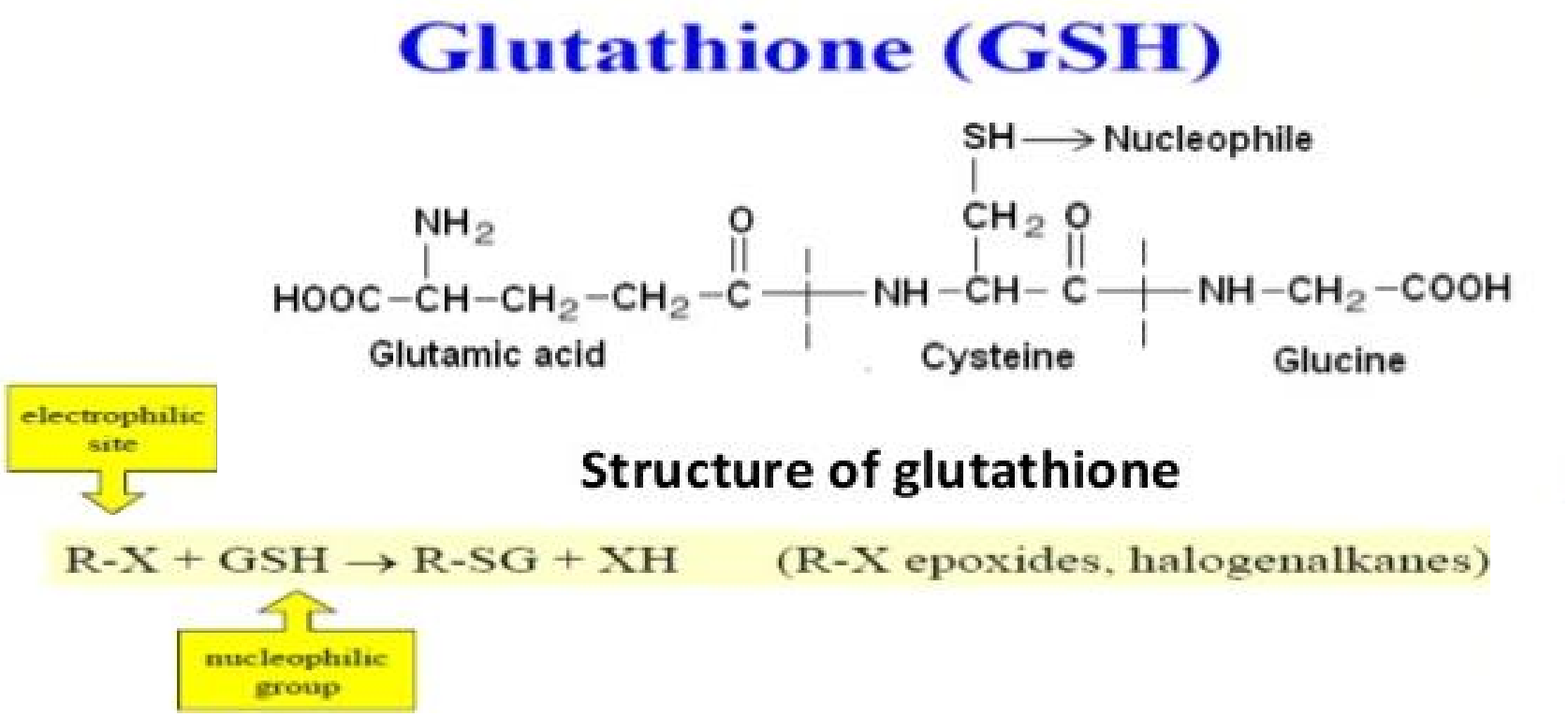
B) Conjugation with Glutamine

Phenyl Acetic acid + Glutamine ⟶ Phenyl Acetyl Glutamine

This reaction is important in patients of Phenyl ketonuria, since excess of Phenyl acetic acid leads to formation of excess of Phenyl acetyl glutamine, which is excreted in urine, that imparts a mousy odor to the urine

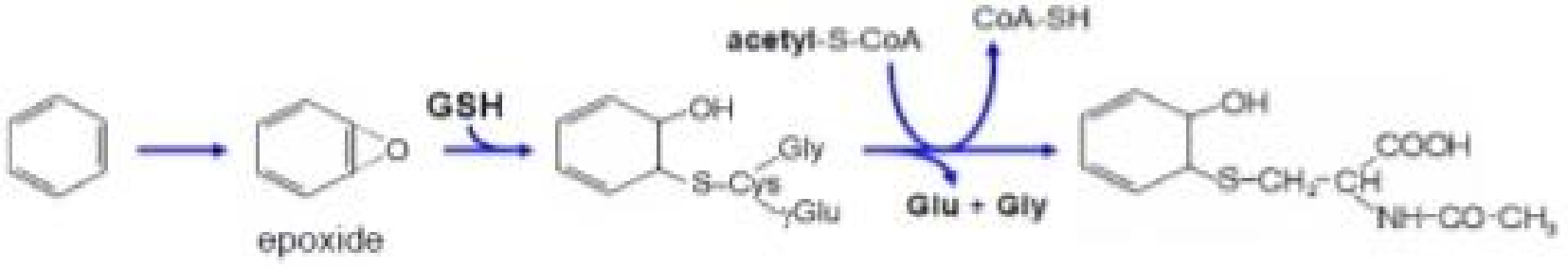
6. Conjugation with Glutathione

- Glutathione (γ-glutamyl-cysteinylglycine) is a tripeptide consisting of glutamic acid, cysteine, and glycine It detoxify electrophilic chemicals

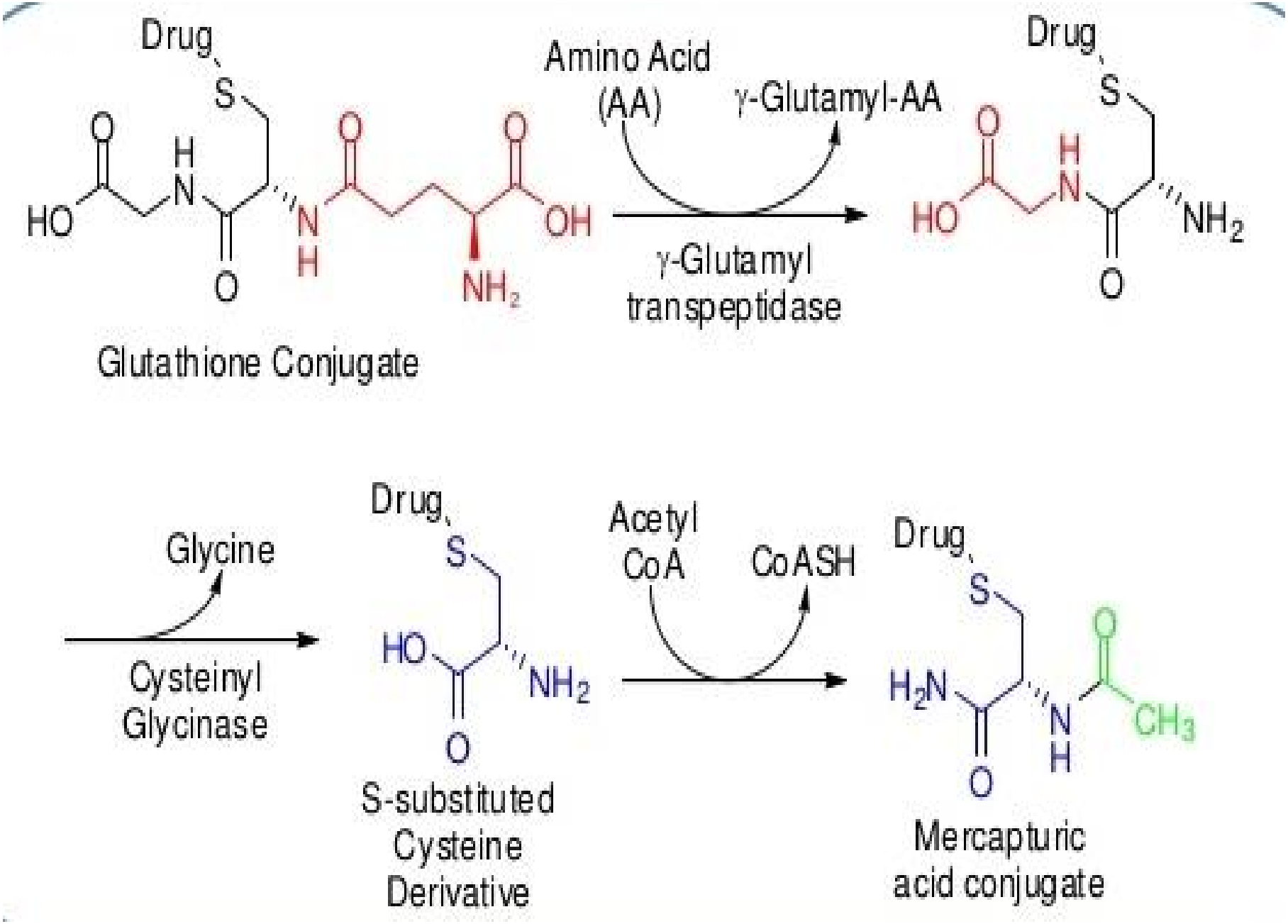


- The glutamyl and glycinyl groups belonging to glutathione are removed by specific Enzymes
- acetyl group (donated by acetyl- CoA) is added to the amino group of the remaining cysteinyl moiety
- The resulting compound is a mercapturic acid, a conjugate of L acetylcysteine, which is then excreted in the urine

R-SG sulfide is converted to mercapturic acids and excreted



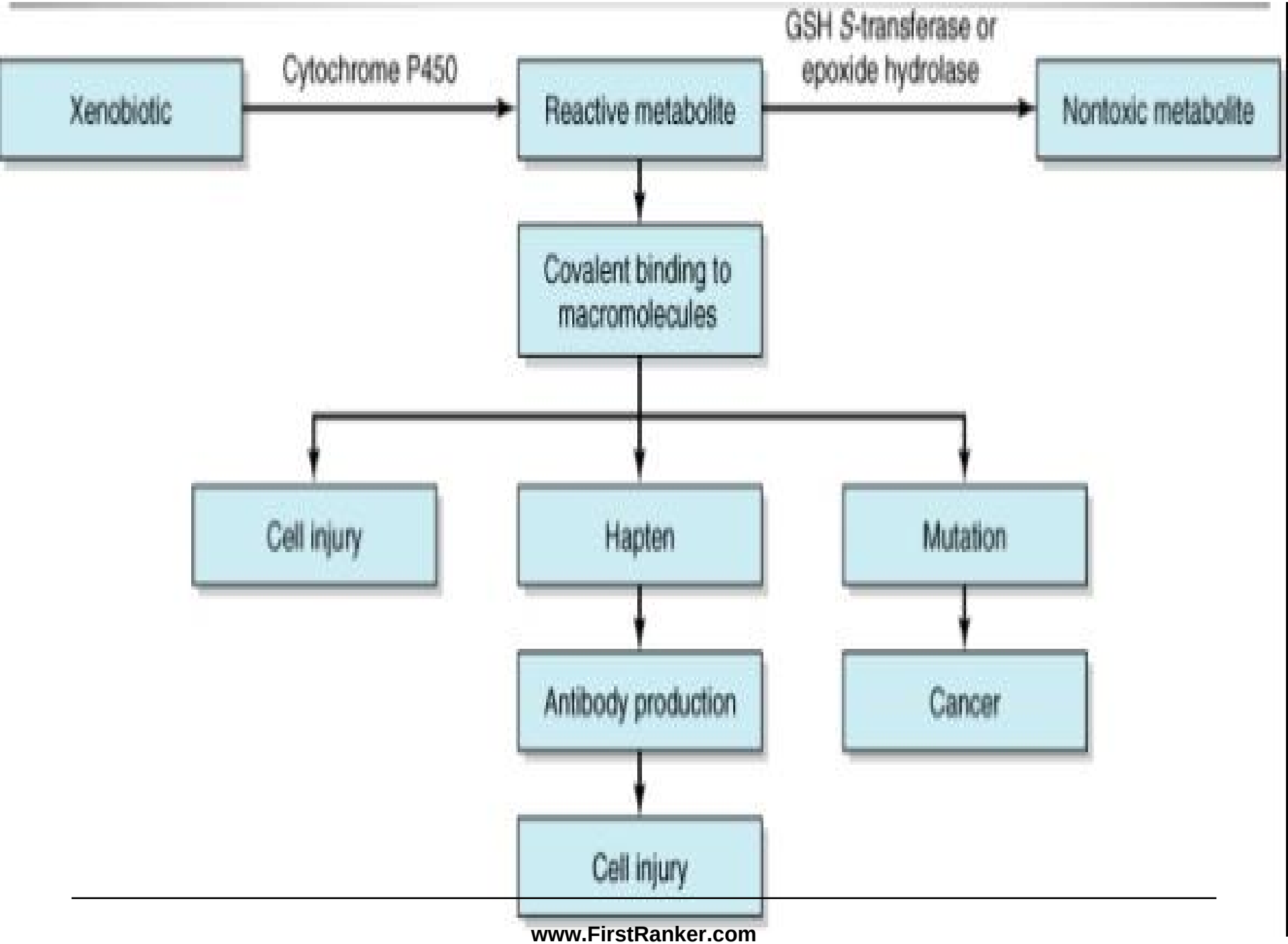
N-acetyl-*S*-substituted cysteine
(mercapturic acid)



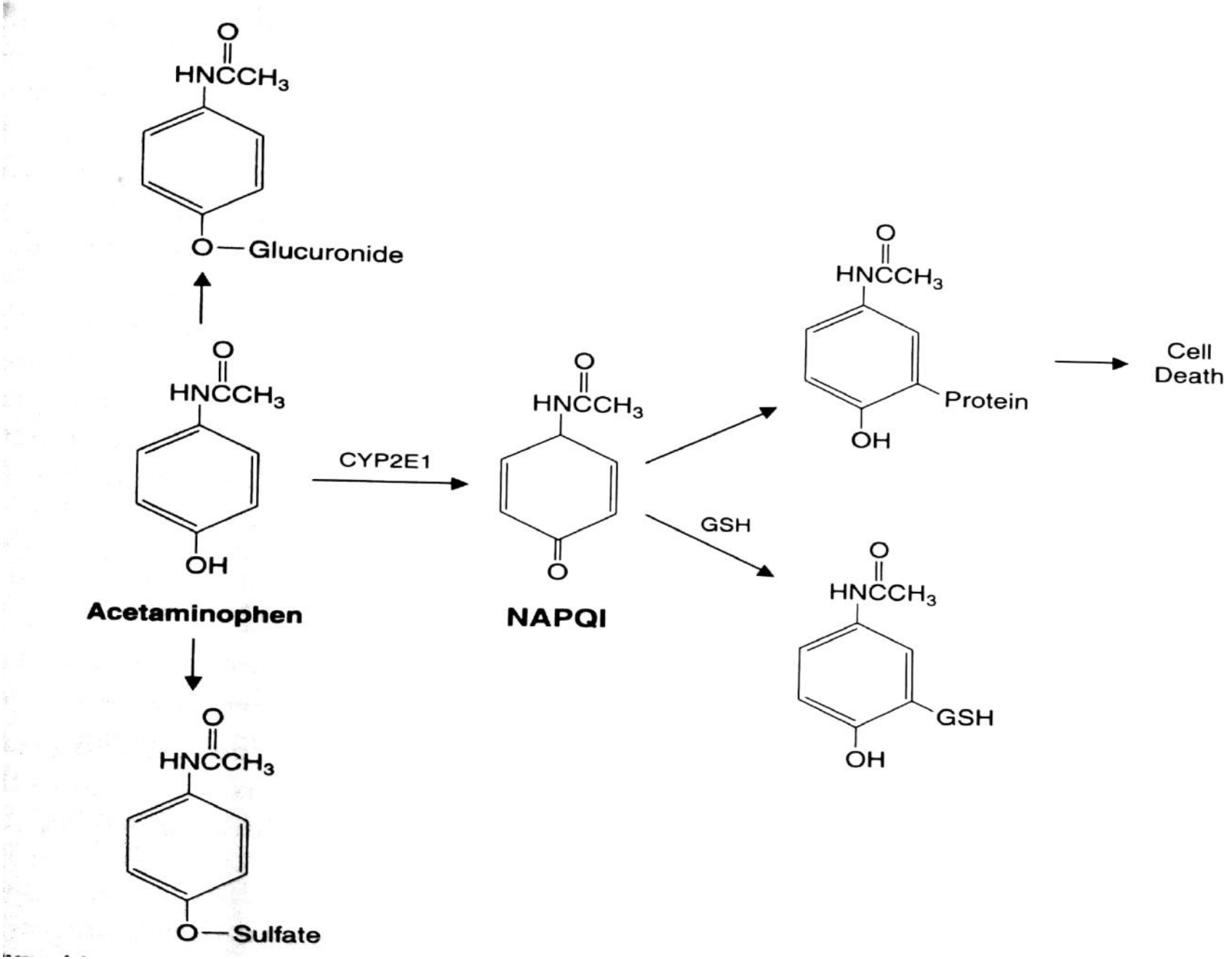
- If the potentially toxic xenobiotics were not conjugated to GSH, they would be free to combine covalently with DNA, RNA, or cell protein and could thus lead to serious cell damage.
- GSH is therefore an important defense mechanism against certain toxic compounds, such as some drugs and carcinogens.

Effects of Xenobiotics

- Metabolism of a xenobiotic can result in cell injury, immunologic damage, or cancer.
- Cell injury (cytotoxicity), can be severe enough to result in cell death.
- These macromolecular targets include DNA, RNA, and protein.
- The reactive species of a xenobiotic may bind to a protein, altering its antigenicity.
- The resulting antibodies can then damage the cell by several immunologic mechanisms that grossly perturb normal cellular biochemical processes.



- Reactions of activated species of chemical carcinogens with DNA are of great importance in chemical carcinogenesis
- Some chemicals (eg, benzo[α]pyrene) require activation by monooxygenases in the endoplasmic reticulum to become carcinogenic (they are thus called indirect carcinogens).
- The products of the action of certain monooxygenases on some procarcinogen substrates are epoxides.
- Epoxides are highly reactive and mutagenic or carcinogenic or both.
- Epoxide hydrolase—like cytochrome P450acts on these compounds, converting them into much less reactive dihydrodiols.



Summary

- Xenobiotics are chemical compounds foreign to the body, such as drugs, food additives, and environmental pollutants
- Xenobiotics are metabolized in two phases. The major reaction of phase 1 is hydroxylation catalyzed by a variety of monooxygenases, also known as the cytochrome P450s.
- In phase 2, the hydroxylated species are conjugated with a variety of hydrophilic compounds such as glucuronic acid, sulfate, or glutathione.
- The combined operation of these two phases renders lipophilic compounds into water-soluble compounds that can be eliminated from the body.
- Xenobiotics can produce a variety of biologic effects, including pharmacologic responses, toxicity, immunologic reactions, and cancer