BIOTRANSFORMATION/ DETOXIFICATION REACTIONS

METABOLISM OF XENOBIOTICS

Biochemistry for Medics

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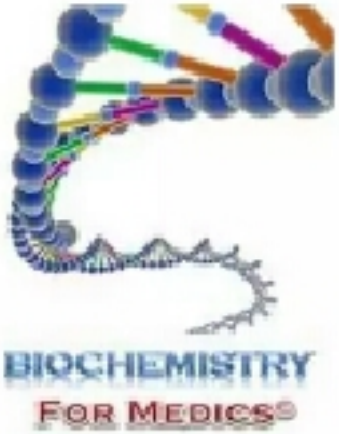


Xenobiotics

A **xenobiotic** (Gk *xenos* "stranger") is a compound that is foreign to the body.

Xenobiotics can produce a variety of biological effects including-

- Pharmacological responses
- Toxicity
- Immunological responses
- Cancers

Xenobiotics

Xenobiotics can be-

a) **Exogenous-** The foreign molecules which are not normally ingested or utilized by the organism but they gain entry through dietary food stuffs, or in the form of certain medicines/ drugs used for a therapeutic cause or are inhaled through environment .

Examples- Drugs, food additives, pollutants, insecticides, chemical carcinogens etc.

Xenobiotics

Xenobiotics can be-

b) **Endogenous** – Though they are not foreign substances but have effects similar to exogenous xenobiotics. These are synthesized in the body or are produced as metabolites of various processes in the body.

Examples-Bilirubin, Bile acids, Steroids, Eicosanoids and certain fatty acids.

Metabolism of Xenobiotics

Metabolism of xenobiotics occurs in two phases-
Phase 1, the major reaction involved is **hydroxylation.** In addition to hydroxylation, a wide range of reactions also take place including-

- Deamination,
- Dehalogenation,
- Desulfuration,
- Epoxidation,
- Peroxygenation, and
- Reduction

Metabolism of Xenobiotics

Phase 2, the hydroxylated or other compounds produced in phase 1 are converted by specific enzymes to various polar metabolites by **conjugation** with-

- Glucuronic acid,
- Sulfate, acetate,
- Glutathione, or
- Certain amino acids, or
- By **methylation.**

Biotransformation/ Detoxification Reactions

- 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**
- The overall purpose of the two phases of metabolism of xenobiotics is to increase their **water solubility (polarity)** and thus **excretion** from the body.
- In certain situations these reactions may instead increase the toxicity of a foreign compound, then these are called, **Entoxification reactions**

Biotransformation reactions

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

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

Overview of biotransformation reactions

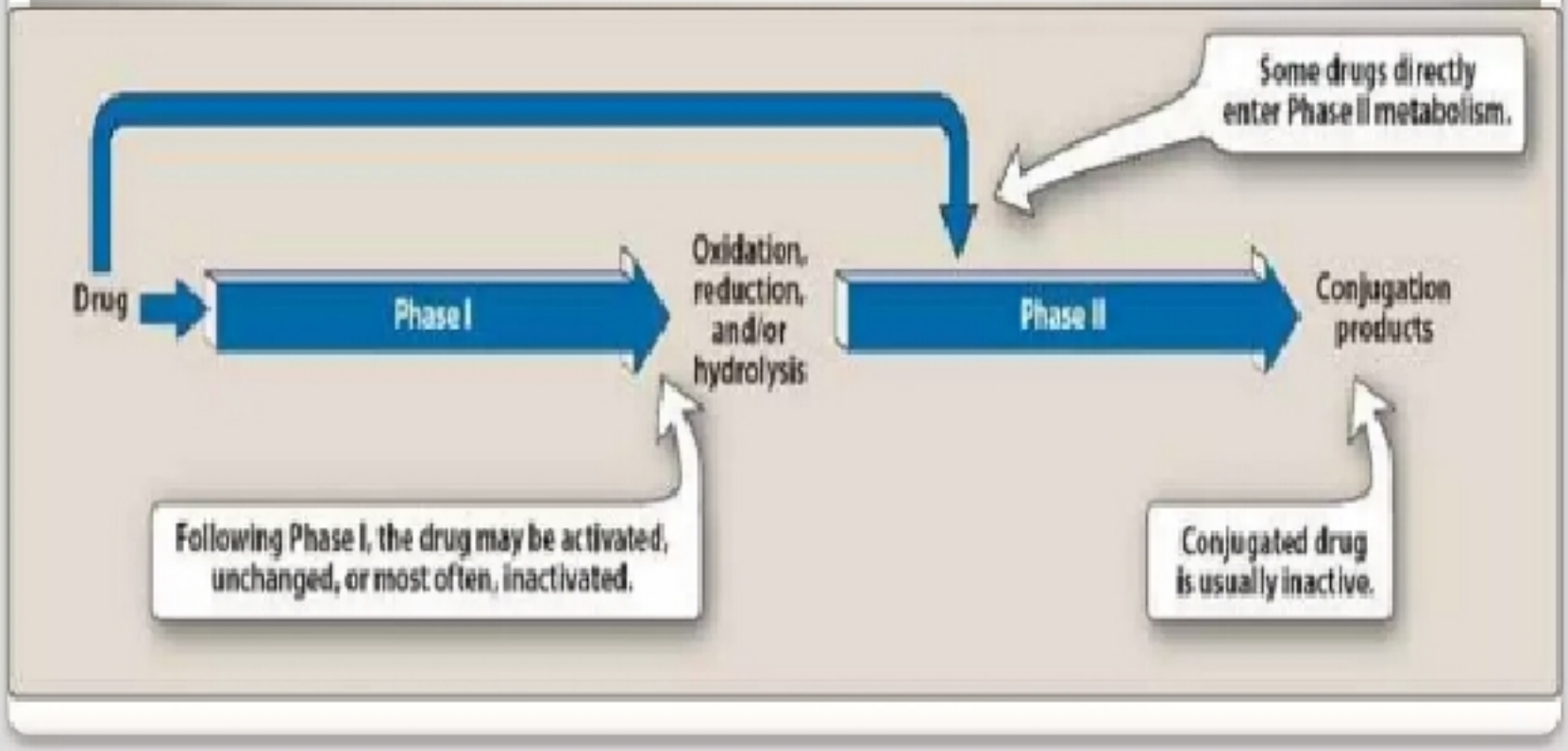
- Phase 1 reactions can limit the toxicity of a drug.
- Phase 1 reactions can also convert xenobiotics from **inactive** to **biologically active** compounds (**Metabolic activation**). In these instances, the original xenobiotics are referred to as **"prodrugs"** or **"procarcinogens."**
- Phase 2/conjugation reactions can convert the active products of phase 1 reactions to less active or inactive species, which are subsequently excreted in the urine or bile.
- In a very few cases, conjugation may actually increase the biologic activity of a xenobiotic **(Metabolic activation).**

Biotransformation

Potentially toxic xenobiotic → **Detoxification** → **Inactive metabolite**

Relatively harmless → **Metabolic activation** → **Reactive intermediate**

Overview of detoxification reactions



Comparing Phase I & Phase II

Enzyme	Phase I	Phase II
Types of reactions	Hydrolysis Oxidation Reduction	Conjugations
Increase in hydrophilicity	Small	Large
General mechanism	Exposes functional group	Polar compound added to functional group
Consquences	May result in metabolic activation	Facilitates excretion

Factors affecting Biotransformation of drugs

- **Prior administration** of the drug or Co administration of other drugs
- **Diet**
- **Hormonal status**
- **Genetics**
- **Disease** (e.g., decreased in cardiac and pulmonary disease)
- **Age** and **developmental status**
- **Functional status** of Liver and Kidney

Phase 1 reactions - Overview

- **Phase I reactions include:**
  - **Oxidation**
  - **Reduction**
  - **Hydrolysis reactions**

- They are also called **Hydroxylation reactions** since they introduce or expose a functional group (e.g., -OH) that serves as the active center for sequential conjugation in a phase II reaction.

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

A) Oxidation

- **Oxidation of Alcohols-** Primary aliphatic and aromatic alcohols are oxidized to corresponding acids

Methanol ⟶ Formaldehyde ⟶ Formic acid

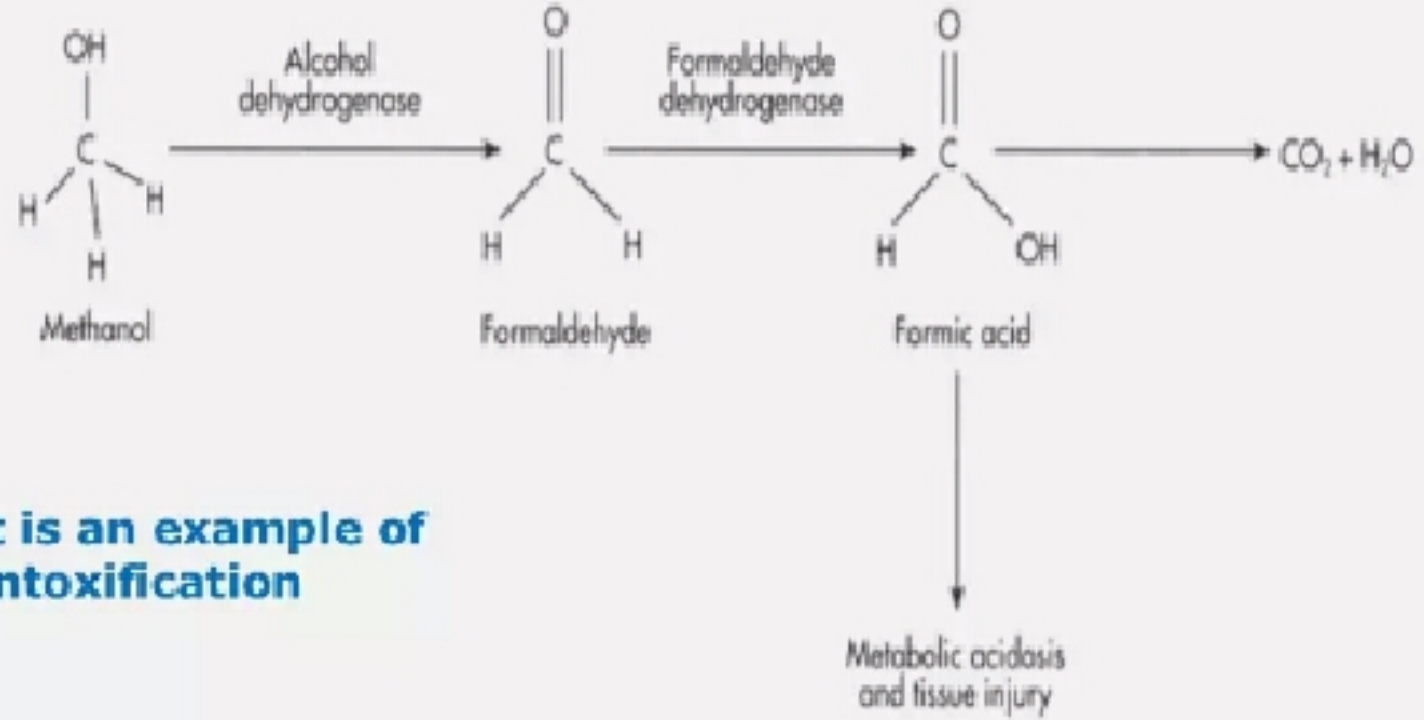
Ethanol ⟶ Acetaldehyde ⟶ Acetic acid

Benzoyal Alcohol ⟶ Benzaldehyde ⟶ Benzoic acid

Methanol toxicity

- Methanol has a relatively low toxicity.
- Methanol is metabolized in the liver.
- In the first step of degradation, methanol is transformed **to formaldehyde** via the enzyme **alcohol dehydrogenase (ADH).**
- Transformation of formaldehyde to **formic acid** via the enzyme **aldehyde dehydrogenase** is faster
- The metabolism of formic acid is very slow; thus, it often accumulates in the body, which results in **metabolic acidosis.**
- The major damage occurs to the **optic nerve.**
- **Ethanol is given as an antidote**, since it is the true substrate of Alcohol dehydrogenase, methanol is spared .

Methanol toxicity



It is an example of Entoxification

A) Oxidation

- **Oxidation of Aromatic Hydrocarbons**

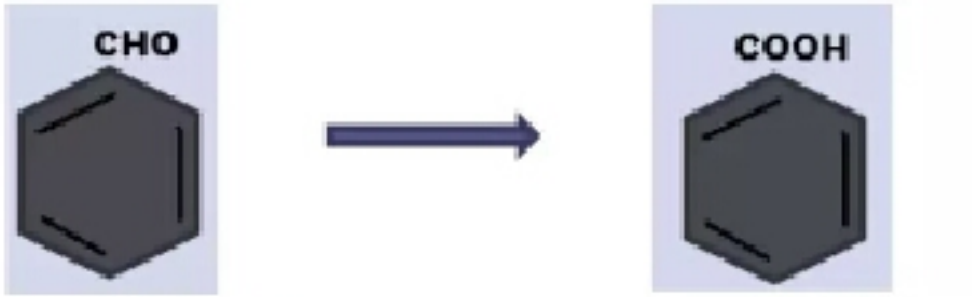
Aromatic hydrocarbons are oxidized to phenolic compounds, which can further be conjugated with Glucuronic acid or Sulfuric acid in phase 2 reactions so as to be excreted through urine.

A) Oxidation

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.



Benzaldehyde **Benzoic Acid**

A) Oxidation

- **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

A) Oxidation

- **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.

A) Oxidation

- **Oxidation of Sulphur containing compounds**

The sulphur present in organic compounds is oxidized to Sulphate (SO_4^{-2})

- **Oxidation of Drugs**

Meprobamate ⟶ **OH- Meprobamate**

Chloral ⟶ **Trichloracetic acid**

A) Oxidation

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

Reduction does not occur extensively in human beings

Examples-

- **Reduction of Aldehydes**

Chloral ⟶ **Trichloroethanol**

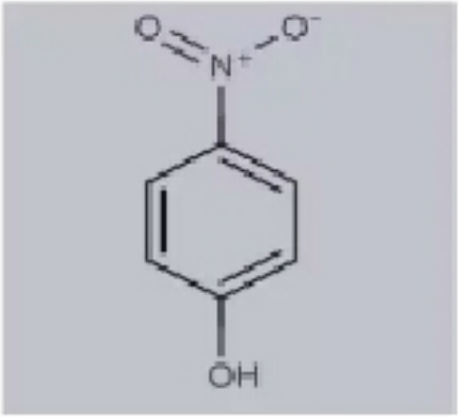
Trichloroethanol is excreted after conjugation with D-Glucuronic acid as corresponding glucuronide.

B) Reduction

- Reduction of Nitro compounds

p- nitrobenzene ⟶ p- Amino benzene

p- nitro phenol ⟶ p-Aminophenol



p- nitro phenol

C)Hydrolysis

Certain therapeutic compounds undergo hydrolysis,

Examples-

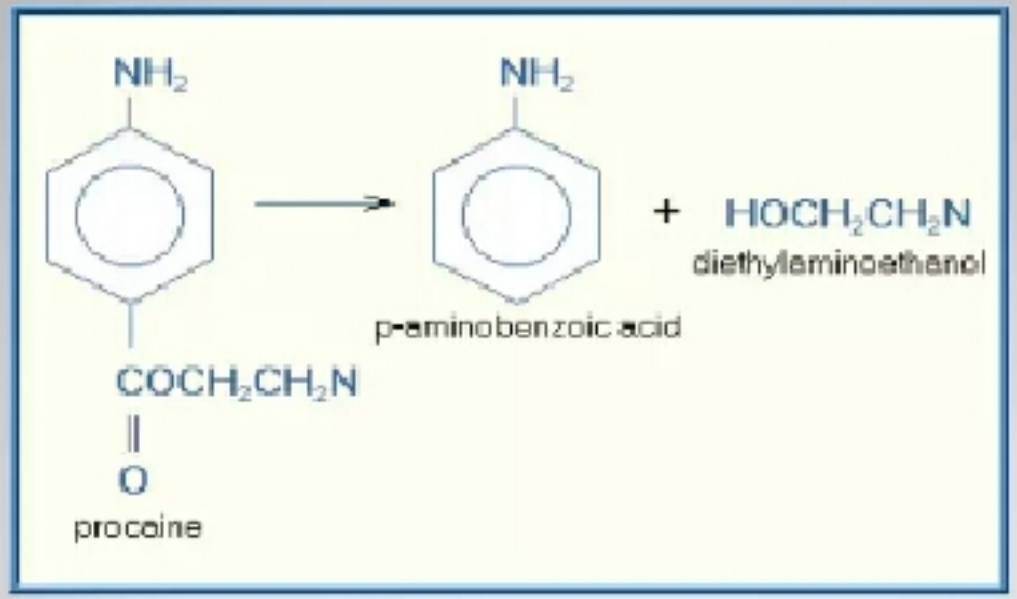
Acetyl Salicylic acid → **Acetic acid + Salicylic acid**

Atropine → **Tropic acid + Tropine**

Digitalis → **Sugar + Aglycone**

Procaine → **p- Amino Benzoic acid + Diethyl amino ethanol**

C)Hydrolysis



Phase 1 reactions- Enzymes

- Mainly Catalyzed by members of a class of enzymes referred to as **Monooxygenases, Mixed Function oxidases** or **Cytochrome P450s.**
- **Other enzymes of significance are-**
  - Aldehyde and alcohol dehydrogenase
  - Deaminases
  - Esterases
  - Amidases
  - Epoxide hydrolases

Cytochrome P450 Enzyme system

- The reaction catalyzed by a monooxygenase (cytochrome P450) is as follows:

$$RH + O_2 + NADPH + H^+ \rightarrow R-OH + H_2O + NADP$$

- RH above can represent a very wide variety of xenobiotics, including drugs, carcinogens, pesticides, petroleum products, and pollutants (such as a mixture of PCBs).
- In addition, **endogenous compounds,** such as certain steroids, Eicosanoids, fatty acids, and retinoids, are also substrates.
- The substrates are generally **lipophilic** and are rendered more **hydrophilic** by hydroxylation.

Properties of Human Cytochrome P450s

- Involved in phase I of the metabolism of innumerable xenobiotics
- Involved in the metabolism of many **endogenous compounds**
- All are **haemoproteins**
- Exhibit **broad substrate specificity**, thus act on many compounds
- Extremely **versatile catalysts**, perhaps catalyze about 60 types of reactions
- Basically they catalyze reactions involving **introduction of one atom of oxygen into the substrate and one into water**

Properties of Human Cytochrome P450s(Contd.)

- Liver contains highest amounts, but found in most if not all tissues, including small intestine, brain, and lung
- Located in the **smooth endoplasmic reticulum or in mitochondria (steroidogenic hormones)**
- In some cases, their products **are mutagenic** or carcinogenic
- Many have a molecular mass of about 55 kDa
- Many **are inducible**, resulting in one cause of drug interactions
- Many are inhibited by various drugs or their metabolic products, providing another cause of drug interactions
- Some exhibit **genetic polymorphisms**, which can result in atypical drug metabolism

Phase 2 - 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

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

- **Glucuronosyl transferases,** present in both the endoplasmic reticulum and cytosol, are the catalysts.

- The glucuronide may be attached to **oxygen, nitrogen, or sulfur** groups of the substrates.

1) Glucuronidation

Compounds conjugated with Glucuronic acid are-

1) Bilirubin
2) Aromatic acids- Benzoic acid
3) Phenols, Secondary and Tertiary aliphatic alcohols
4) Antibiotics like Chloramphenicol
5) Hormones- Thyroid hormone, derivatives of corticosteroids and sex hormone metabolites
6) 2-Acetylaminofluorene (a carcinogen)
7) Aniline
8) Meprobamate (a tranquilizer)

Glucuronidation

Glucuronidation of Bilirubin

1) UDP Glucose —(UDP- G dehydrogenase; 2 NAD⁺ → 2 NADH +2H⁺)→ UDP Glucuronic acid

2) UDP Glucuronic acid + Bilirubin

↓ UDP- Glucuronyl Transferase

Bilirubin Monoglucuronide +UDP

Glucuronidation

Glucuronidation of Bilirubin

Bilirubin Monoglucuronide+ UDP Glucuronic acid

UDP- Glucuronyl Transferase

↓

Bilirubin Diglucuronide + UDP

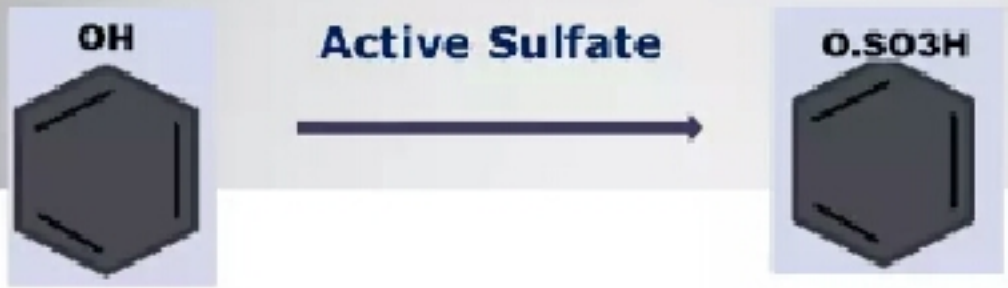
Most of the bilirubin excreted in the bile of mammals is in the form of bilirubin diglucuronide.

Bilirubin-UGT activity can be **induced** by a number of clinically useful drugs, including Phenobarbital.

2) Sulfation

- The **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 as follows-
  - Phenols
  - Cresols
  - Indole
  - Steroids
  - Oestrogen and Androgens
  - Tyrosine to form Tyrosine-O- Sulphate, which is required for the formation of Fibrinogen
  - Glycosaminoglycans, glycolipids, and glycoproteins

2) Sulfation



Phenol **Phenyl Sulfuric Acid**

Sulfotransferases are localized in the cytosol and transfer sulphate moiety mainly to OH group. The donor of sulphate is PAPS(Active sulphate) which is synthesized from 2 mol of ATP and one mol of sulphate.

3) Acetylation

- Acetylation is represented by

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

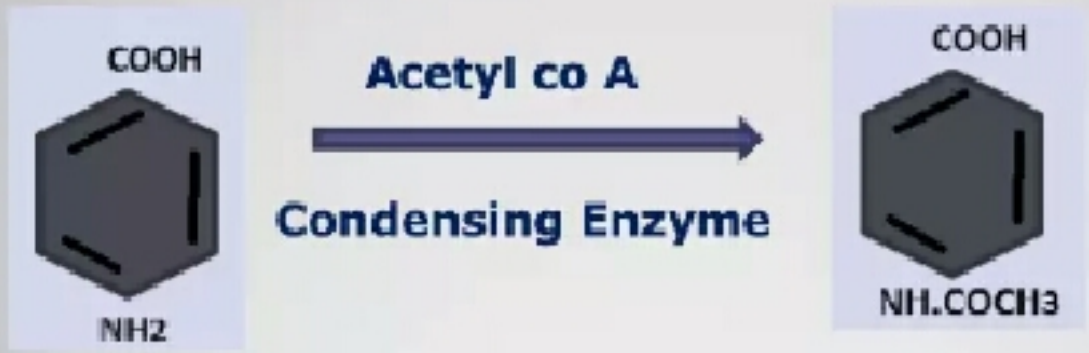
where X represents a xenobiotic.

- **Acetyl-CoA** (active acetate) is the acetyl donor.
- These reactions are catalyzed by **acetyltransferases** present in the cytosol of various tissues, particularly liver
- **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.

3) Acetylation

Compounds conjugated by Acetylation-

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



PABA

Acetylated PABA

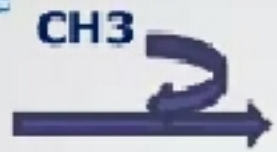
4) Methylation

- Methylation is limited in the body
- S- Adenosyl Methionine (Active Methionine)acts as a Methyl group donor
- Reactions are called Transmethylation reactions
- Enzymes catalyzing the reactions are Methyl transferases

4) Methylation

Compounds conjugated by Methylation are-

- Nicotinamide



Nicotinamide → N- Methyl Nicotinamide

- p- Methyl Amino Azo benzene

p- Dimethyl Amino Azo Benzene (Hepatic Carcinogen)

- O- Methylation of

estrogen, norepinephrine, epinephrine and their metabolites.

5) Conjugation with Amino acids

1) Conjugation with Glycine

- Benzoic acid + Glycine ⟶ Hippuric acid
- Nicotinamide + Glycine ⟶ Nicotinuric Acid
- Cholic and deoxy Cholic acid are conjugated to form Glyco cholic acid and Glycodeoxy cholic acid

5) Conjugation with Amino acids

2) Conjugation with Cysteine

A few aromatic compounds are conjugated with Cysteine in the presence of Acetic acid to form Mercapturic acid

- **Bromo Benzene + Cysteine + Acetic acid**

↓

Bromo phenyl Mercapturic acid

- **Naphthalene + Cysteine + Acetic Acid**

↓

Naphthyl Mercapturic acid

5) Conjugation with Amino acids

3) Conjugation with Glutamine

Phenyl Acetic acid + Glutamine

Phenyl Acetyl Glutamine

This reaction is important in patients of Phenyl ketonuria, since excess of Phenyl acetyl glutamine is excreted in urine, that imparts a mousy odor to the urine.

6)Conjugation with Glutathione

- Glutathione (Y-glutamyl-cysteinylglycine) is a **tripeptide** consisting of glutamic acid, cysteine, and glycine
- Glutathione is commonly **abbreviated GSH** (because of the sulfhydryl group of its cysteine, which is the business part of the molecule).
- A number of potentially toxic electrophilic xenobiotics (such as certain carcinogens) are conjugated to the nucleophilic GSH in reactions that can be represented as follows:

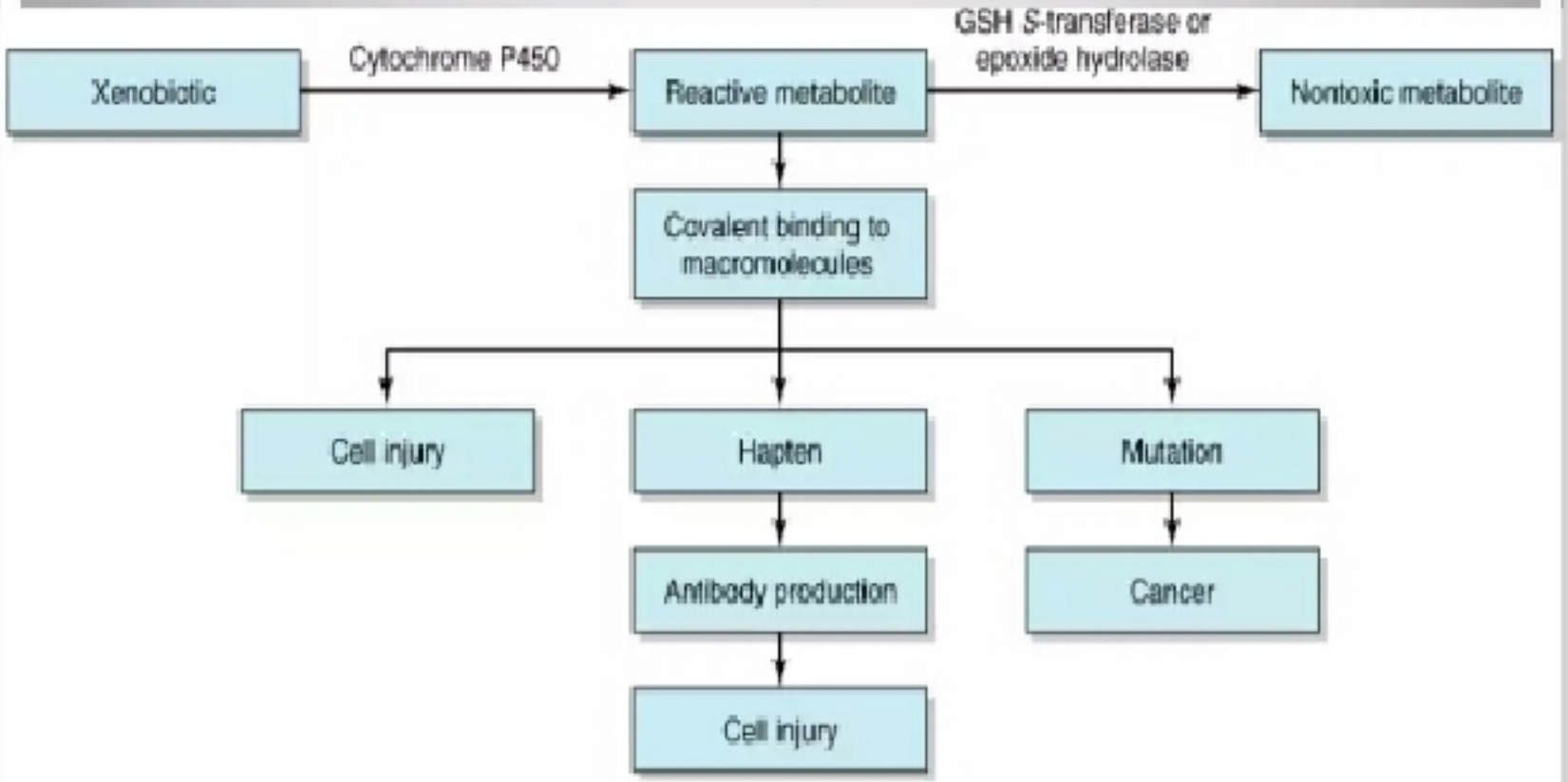
$$R + GSH \rightarrow R - S - G$$

where R = an electrophilic xenobiotic.

6)Conjugation with Glutathione

- The enzymes catalyzing these reactions are called **glutathione S-transferases**
- A variety of glutathione S-transferases are present in human tissue. They exhibit different substrate specificities and can be separated by electrophoretic and other techniques.
- 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



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.

Effects of Xenobiotics

- Reactions of activated species of chemical carcinogens with **DNA** are of great importance in **chemical carcinogenesis**
- Some chemicals (eg, benzo[a]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.

You're welcome!