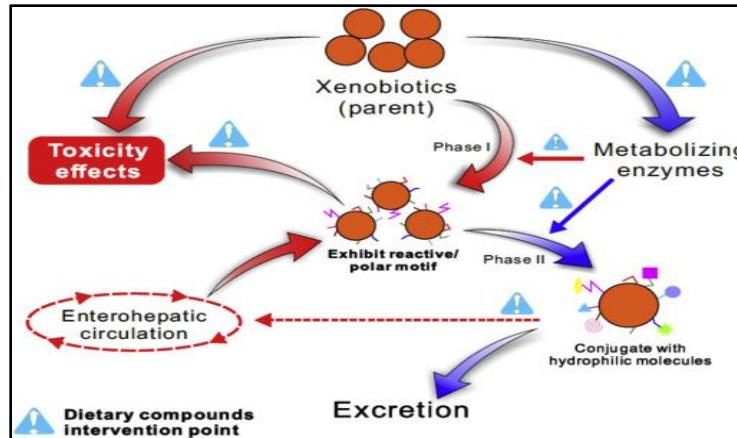


Metabolism of Xenobiotics/ Biotransformation/ Detoxification



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Xenobiotics

- A xenobiotic (Greek *xenos* “stranger”) is a compound that is foreign to the body. or
- A xenobiotic is a foreign chemical substance found within an organism that is **not normally naturally** produced by or expected to be present within.
- **Metabolism of Xenobiotics is Metabolism of Foreign compounds.**
- Increasingly, humans are subjected to exposure to various foreign chemicals (Xenobiotics)—drugs, food additives, pollutants, etc.

Detoxification/ Detoxication

- The series of biochemical reactions occurring in body to convert foreign (**often toxic**) compounds to **non-toxic or less toxic more easily excretable forms** (which are mainly eliminated through urine & bile).
- Word DETOXIFICATION IS MISLEADING
- Detoxified products may be more toxic than original form but more water soluble (polar) and easily excretable compounds.

Eg: Methanol → Formaldehyde

Procarcinogens → Carcinogens

Site of Detoxification

- ❑ Takes place **Mainly** in the “Liver”. Hepatocytes contain wide variety of enzymes (are present in cytosol & endoplasmic reticulum) to process xenobiotics.
- ❑ **Extra-hepatic** metabolism sites :
 - Intestinal wall : Sulfate conjugation and Esterase and lipases - important in prodrug metabolism
 - Lungs, kidney, placenta, brain, skin, adrenal glands
- ✓ Kidney and Intestines are involved to a lesser extent (via urine & feces).
- ✓ Lungs & Skin (via expired air & sweat)

Biotransformation

- Biotransformation is the process whereby a substance is changed from one chemical to another by a chemical reaction in body.
Or Biotransformation is Conversion of **lipophilic to water soluble chemicals catalyzed by enzymes in the liver and other tissues .**
- In most cases, biotransformation **lessens the toxicity** of Xenobiotics.
- But In certain situations these reactions may instead increase the toxicity of a foreign compound, then these are called, **Entoxification reactions.**

Biotransformation reactions

Significance:

- **Facilitates excretion:** Converts lipophilic to hydrophilic compounds
- **Detoxification/inactivation:** converts chemicals to less toxic forms
- **Metabolic activation:** converts chemicals to more toxic active forms or converts inactive drug to its active form

Consequences:

- Changes in solubility characteristics
- Detoxification
- Metabolic activation

The overall **purpose** of the metabolism of xenobiotics is to increase their water solubility (polarity) and thus excretion from the body.

Biotransformation

Potentially toxic xenobiotic

Relatively harmless

Detoxification

Metabolic activation

Inactive metabolite

Reactive intermediate

Biomedical Importance

Knowledge of the metabolism of xenobiotics is basic to a rational understanding of:

- Pharmacology and Toxicology
- Pharmacy and Therapeutics
- Management of Cancer
- Drug addiction

All these areas involve administration of, or exposure to, xenobiotics

Compounds that are detoxified include-

- **Foreign chemicals (xenobiotics)** – drugs (antibiotics, cardiac drugs, steroids), food additives, insecticides, pollutants, etc.
- Compounds produced in the **body** which are to be eliminated – bilirubin, steroids, NH₃, etc.
- Compounds produced in the **intestine** by bacterial putrefaction and fermentation – indole and skatole (from tryptophan), histamine (from histidine), tyramine (from tyrosine), etc.

Carcinogens – food dyes, preservatives, artificial sweeteners, alcohols, chemicals & cosmetics etc.

The Process of Detoxification and Elimination

Toxins Enter Through:



Fat-Soluble Toxins
(e.g. pesticides, hormones, heavy metals)

Have Affinity For:

Fat Cells

Bone Marrow

Liver

Central Nervous System/Brain

Liver
(Primary Detoxifying Organ)

Excess Toxins Get Stored In:

Toxins Exit Through:



Water-Soluble Toxins
(e.g. nicotine, vapors, other chemicals)

Have Affinity For:

Joints

Blood

Tissues

Muscles

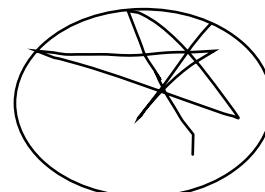
Side Effects & Signs of Toxicity

acne/skin rashes - allergies - arthritis/joint pain - autoimmune disorders - cardiovascular disease - chronic fatigue - constipation - diabetes - diarrhea - fibromyalgia - headaches - hormone imbalance - inflammatory disorders - IBS - neurologic disorders - obesity/overweight

TYPES OF XENOBIOTICS

Exogenous:

- Drugs
- Food additives
- Pollutants
- Insecticides
- Carcinogens



Endogenous:

- Bilirubin,
- Bile acids
- Steroids



- As part of normal metabolism, body produces toxins which have to be eliminated.
- Humans are constantly exposed to exogenous & endogenous toxins.

Exogenous Xenobiotics

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.

Endogenous Xenobiotics

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.

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

Biotransformation Reactions

Metabolism of foreign compounds occurs as a results phase-I & phase-II reactions.

- **Phase-I reactions are**
 - Oxidation
 - Reduction
 - Hydrolysis
 - They are also called **Hydroxylation reactions** since they introduce or expose a functional group (e.g., -OH i.e. increase a molecule's polarity) that serves as the active center for sequential conjugation in a phase II reaction.
- **Phase II is conjugation reactions of phase I compounds.**
 - Direct conjugation can also occur.

Oxidation followed by Conjugation is most common / frequent process in the metabolism of xenobiotics.

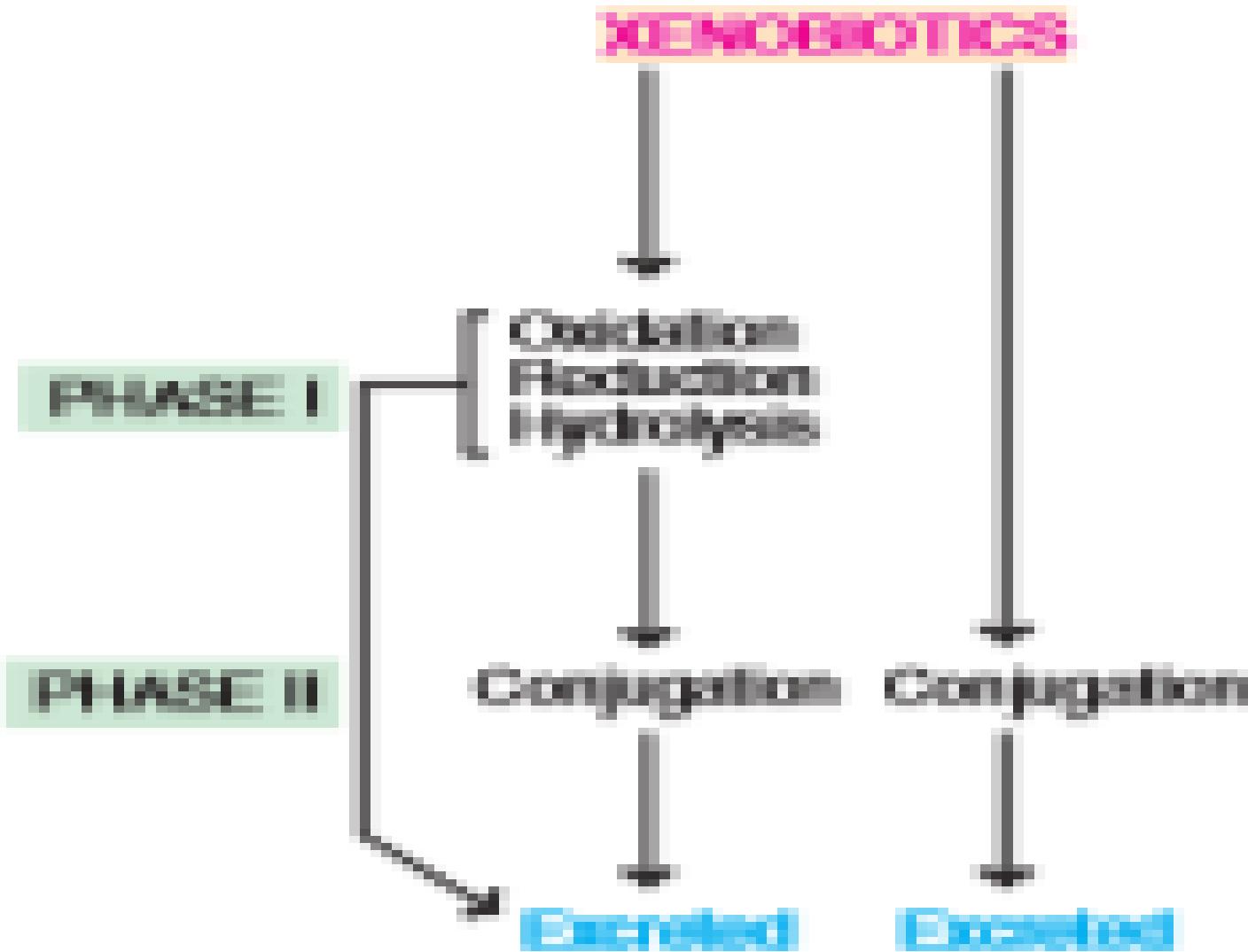


Fig. 30.7 • Phase I and phase II metabolisms in the overall form of metabolism.

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 biological activity of a xenobiotic (**Metabolic activation**).



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
Consequences	May result in metabolic activation	Facilitates excretion

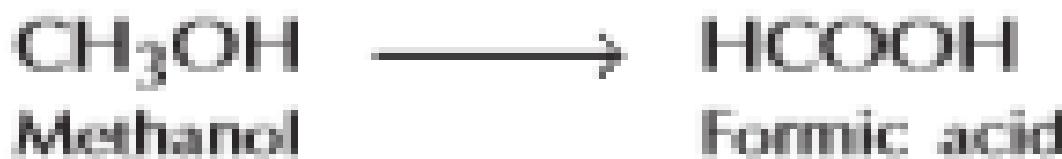
Phase-I

A) Oxidation

- A large number of foreign substances are destroyed by oxidation in the body.
- These include alcohols, aldehydes, amines, aromatic hydrocarbons and sulfur compounds. In general, aliphatic compounds are more easily oxidized than aromatic ones.
- Examples: Oxidation of methyl group containing compounds. Methyl group- is oxidized to acid through formation of alcohol and aldehyde.

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





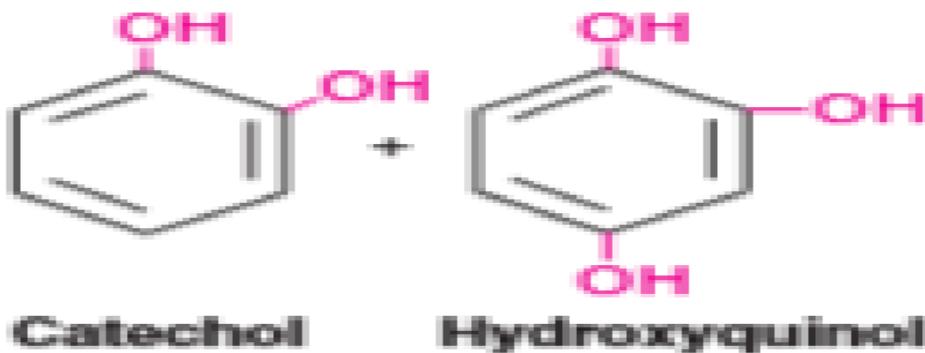
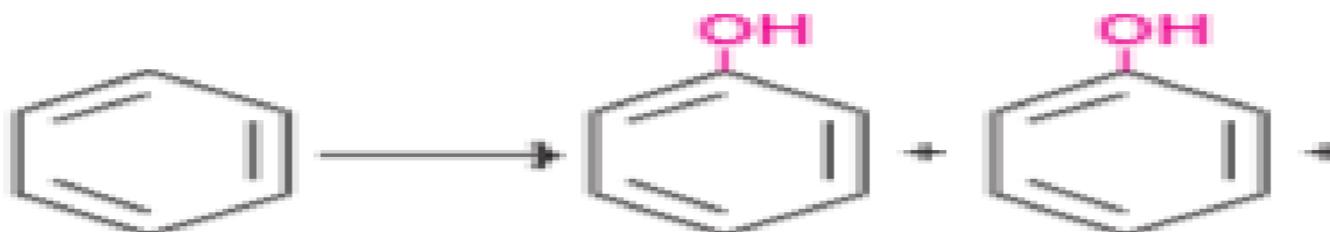
Aldehydes : Aldehydes are oxidized to produce the corresponding acids.



Amines and their derivatives : Aliphatic
amines are converted to the corresponding acids,
liberating urea while aromatic amino acids are
oxidized to phenols.



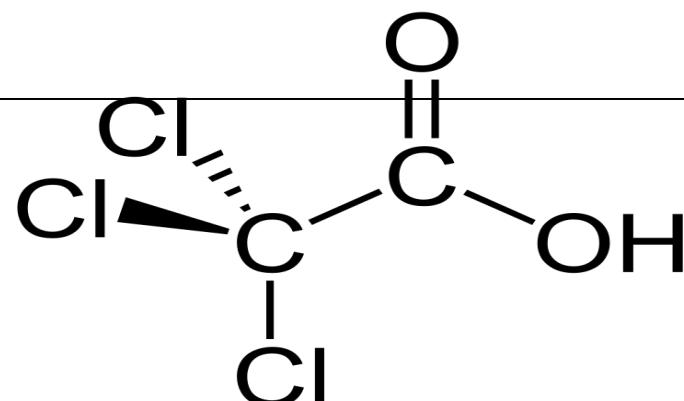
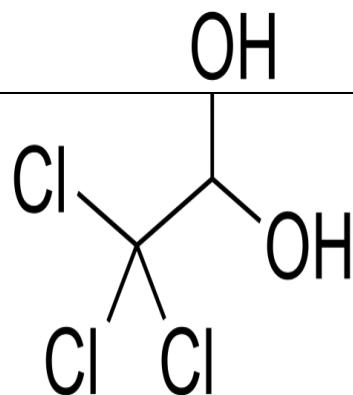
Aromatic hydrocarbons : Benzene may be oxidized to mono, di- and trihydroxy phenols as shown below



Sulfur compounds : Organic sulfur is oxidized to sulfuric acid.

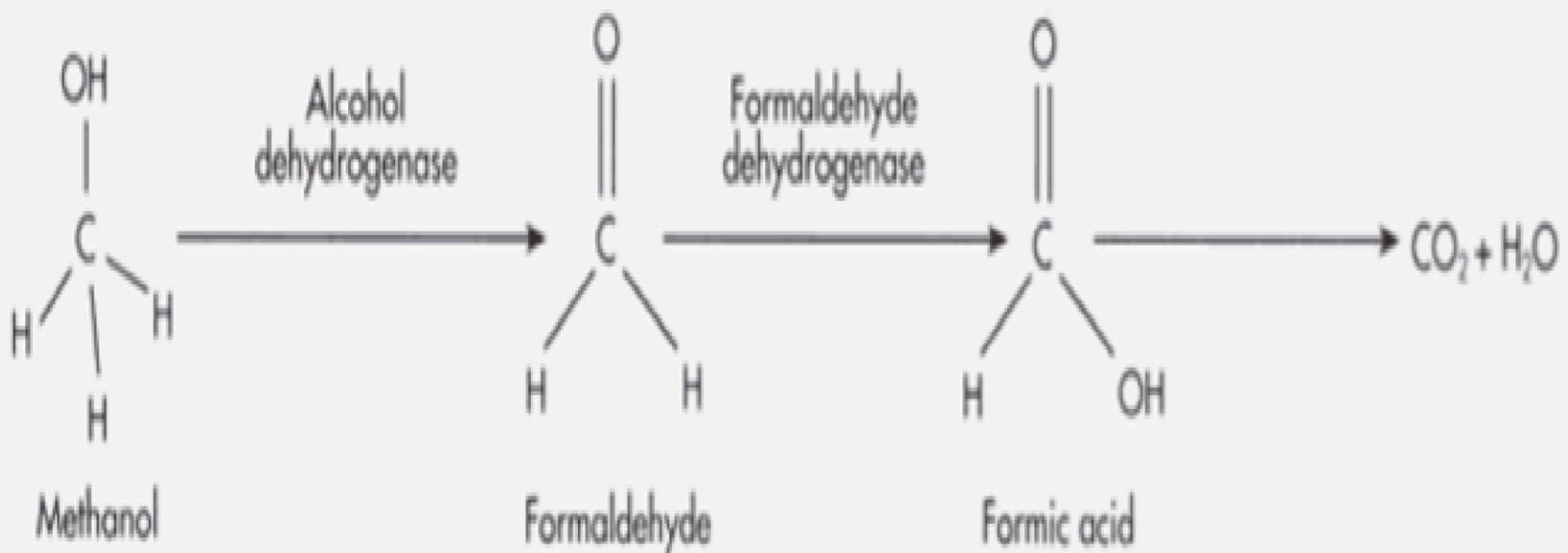
Drugs : Meprobamate is a tranquilizer. It is oxidized to hydroxymeprobamate and excreted in urine.

❖ **Oxidation of Drugs**



Methanol toxicity (Entoxification)

- 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 .



**It is an example of
Entoxification**

↓
**Metabolic acidosis
and tissue injury**

Reduction

A few examples of detoxification by reduction are given.



Picric acid

Picramic acid



Chloral

Trichloroethanol



Nitrobenzene

Hydrolysis

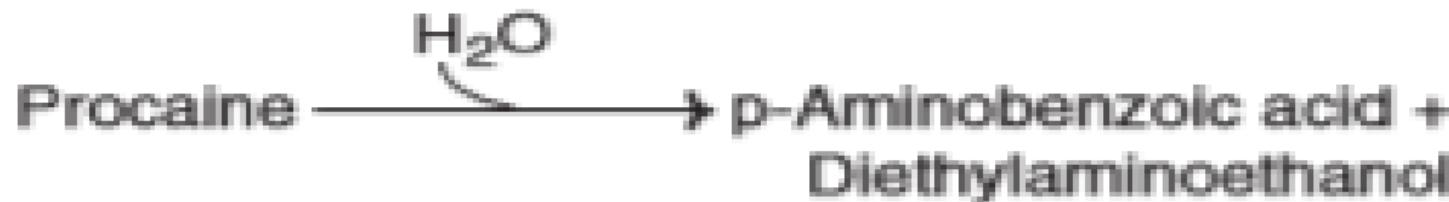
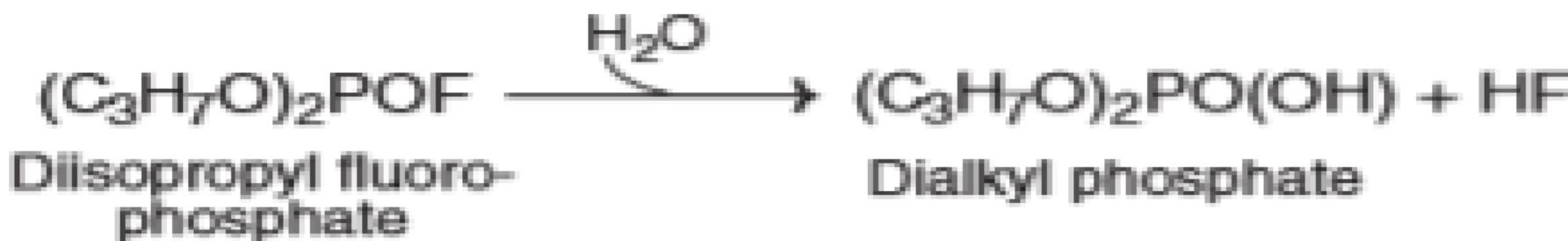
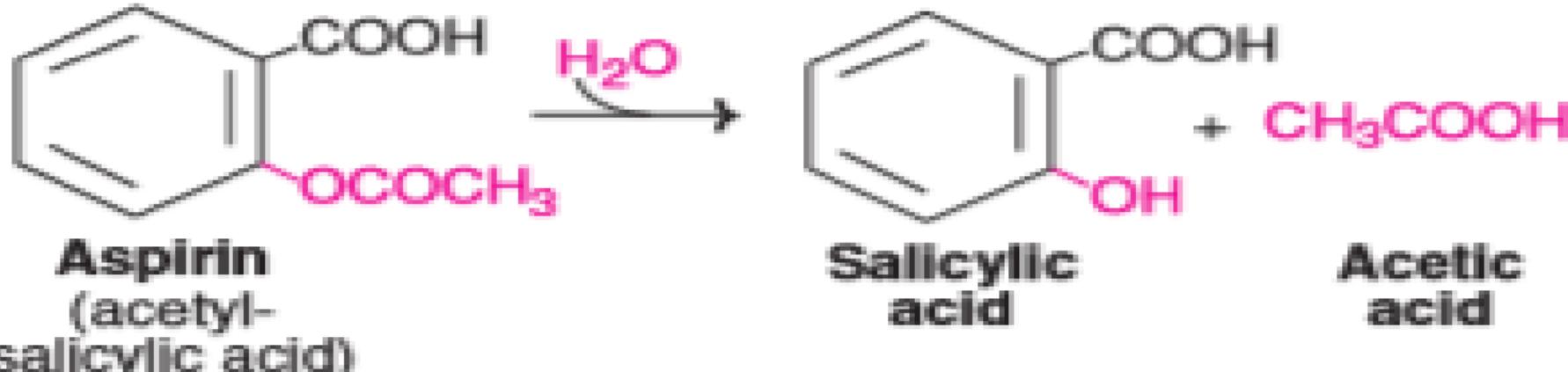
- The hydrolysis of the bonds such as ester, glycoside and amide is important in the metabolism of xenobiotics.
- Several compounds undergo hydrolysis during the course of their detoxification.
- These include aspirin, acetanilide, diisopropylfluorophosphate, atropine and procaine.

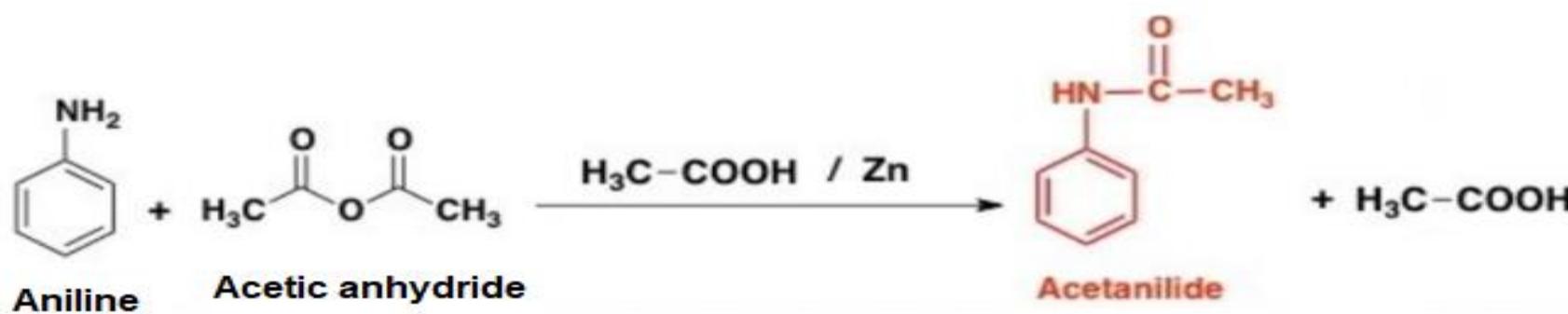
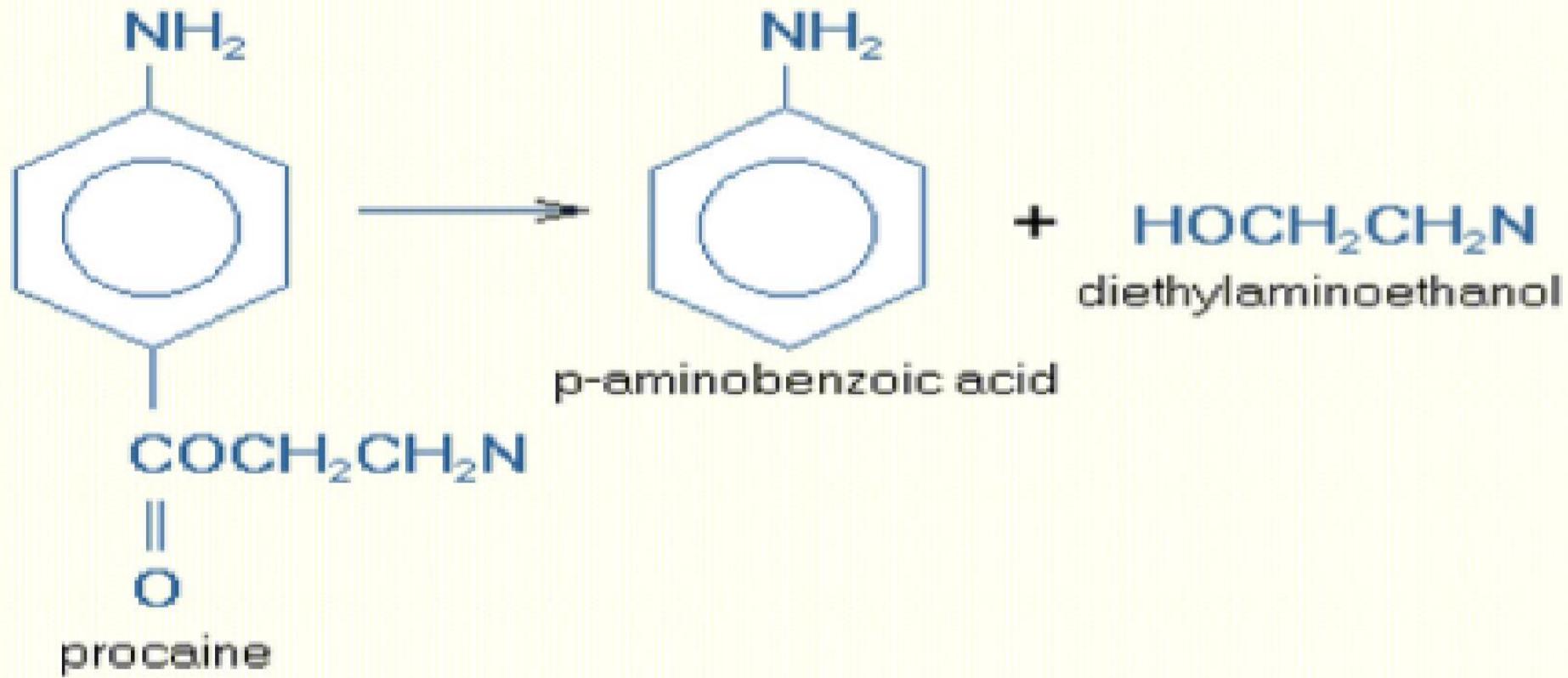
DETOXIFICATION BY HYDROLYSIS

- (1) Aspirin (Acetyl Salicylic acids) → Salicylic acid + Acetic acid
- (2) Acetanilide → Aniline + Acetic Acid
- (3) Di isopropyl Fluorophosphate (DFP) → HF + Di -Alkyl phosphate
(Nerve gas → Serine Protease → Acetyl Choline Esterase inhibitor)
- (4) Atropine → Tropic acid + Tropine
- (5) Procaine (anesthetia) → P- Amino Benzoic acid + Diethylamino Ethanol
- (6) Digitalis → Sugar + (Aglycan)
- (7) Detoxification of Arsenic ,Mercury, Cadmium (Xeno biotics)
↓
BAL (BRITISH ANTI LEWISITE) IN WORLD WAR

BAL+ METAL EXCRETED IN URINE

Active enzyme with Cystein at active site + Heavy Metal → Inactive Enzyme + BAL (ANTIDOTE)





REACTION	ENZYME	LOCALIZATION
<i>Phase I</i>		
<i>Hydrolysis</i>	Esterase	Microsomes, cytosol, lysosomes, blood
	Peptidase	Blood, lysosomes
	Epoxide hydrolase	Microsomes, cytosol
<i>Reduction</i>	Azo- and nitro-reduction	Microflora, microsomes, cytosol
	Carbonyl reduction	Cytosol, blood, microsomes
	Disulfide reduction	Cytosol
	Sulfoxide reduction	Cytosol
	Quinone reduction	Cytosol, microsomes
	Reductive dehalogenation	Microsomes
<i>Oxidation</i>	Alcohol dehydrogenase	Cytosol
	Aldehyde dehydrogenase	Mitochondria, cytosol
	Aldehyde oxidase	Cytosol
	Xanthine oxidase	Cytosol
	Monoamine oxidase	Mitochondria
	Diamine oxidase	Cytosol
	Prostaglandin H synthase	Microsomes
	Flavin-monooxygenases	Microsomes
	Cytochrome P450	Microsomes

Phase 1 reactions- Enzymes

- Mainly Catalyzed by members of a class of enzymes referred to as **Monoxygenases, 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 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.
-
- Most of the reactions of cytochrome P450 involve the addition of a hydroxyl group to aliphatic or aromatic compounds which may be represented as above reaction.
 - Polychlorinated biphenyls (PCBs)

Role of Cytochrome P450

- The usage P450 refers to the absorption peak (at 450 nm), exhibited by the enzyme when exposed to carbon monoxide.
- They are present in highest amount in liver and small intestine but are probably present in all tissues.
- In liver and most other tissues, they are present mainly in the membranes of the smooth endoplasmic reticulum (**Microsomes**).
- In the adrenal, they are found in mitochondria as well as in the endoplasmic reticulum.
- Approximately 50% of the drugs are metabolized by isoforms of cytochrome P 450.

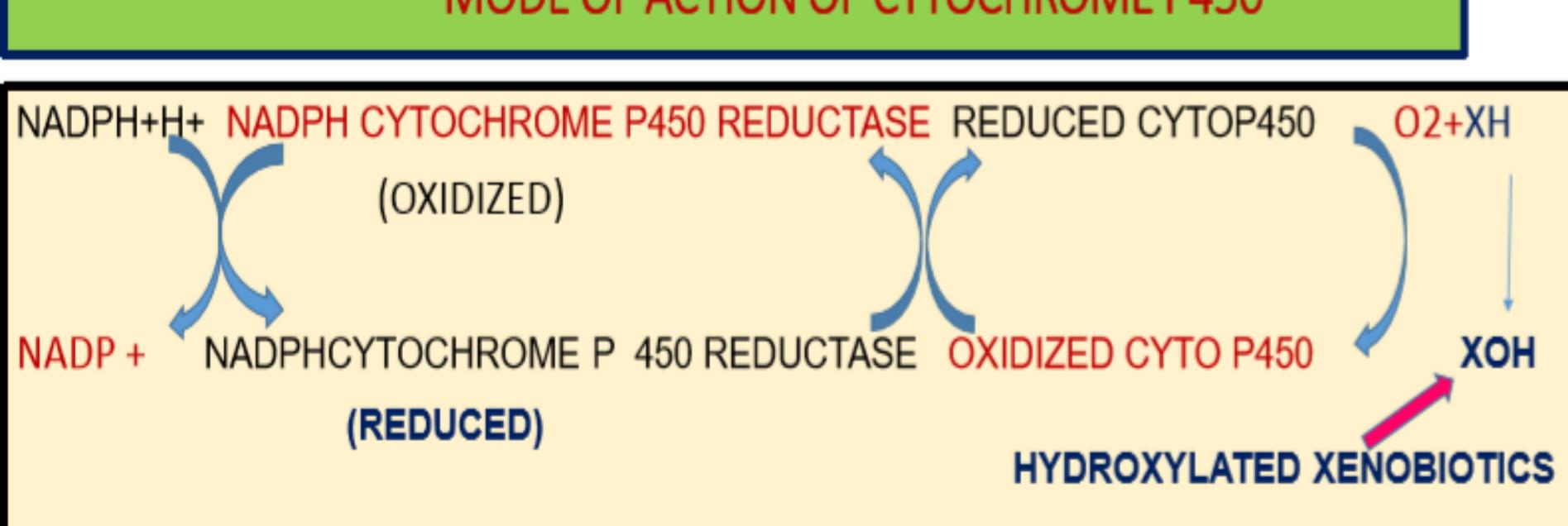
Salient features of Cytochrome P450

1. Multiple forms of cytochrome P450 are believed to exist, ranging from 20 to 200. At least six isoforms of cytochrome P450 have been isolated.
2. They are all hemoproteins , containing heme as the prosthetic group.
3. Cytochrome P450 species are found in the highest concentration in the microsomes of liver. In the adrenal gland, they occur in mitochondria.
4. The mechanism of action of cytochrome P450 is complex and is dependent on NADPH.
5. The phospholipid-phosphatidylcholine is a constituent of cytochrome P450 system which is necessary for the action of this enzyme.
6. Cytochrome P450 is an **inducible enzyme**. Its synthesis is increased by the administration of drugs such as phenobarbitol.

Cytochrome P450

- A distinct species namely cytochrome P448 (with absorption peak at 448 nm) is specific for the metabolism of polycyclic aromatic hydrocarbons, hence it is also known as **aromatic hydrocarbon hydroxylase**.
- Some exhibit genetic polymorphisms, which can result in atypical drug metabolism

MODE OF ACTION OF CYTOCHROME P450



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.
- At least 8 different conjugating agents have been identified in the body. These are glucuronic acid, glycine, cysteine (of glutathione), glutamine, methyl group, sulfate, acetic acid and thiosulfate.

Types of Phase 2 Reactions

1. Glucuronidation
2. Sulfation
3. Acetylation
4. Methylation
5. Conjugation with Amino acids
6. Conjugation with Glutathione (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.
- Microsomal enzyme **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.

Compounds conjugated with Glucuronic acid are:

- 1) Bilirubin
 - 2) Aromatic acids- Benzoic acid
 - 3) Phenols, Secondary and Tertiary aliphatic alcohols (phenyl glucuronide forms)
 - 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)
- Certain drugs (e.g. barbiturates) when administered induce glucuronyltransferase and this increases the glucuronide formation.
 - Glucuronic acid conjugation may occur with compounds containing hydroxyl, carbonyl, sulphydryl or amino groups.
 - In general, conjugation with glucuronic acid produces strongly acidic compounds which are more water soluble at physiological pH, and therefore, more easily excreted.

Glucuronidation

Glucuronidation of Bilirubin

UDP- G dehydrogenase



2) UDP Glucuronic acid + Bilirubin

UDP- Glucuronyl Transferase



Bilirubin Monoglucuronide + UDP

Glucuronidation

Glucuronidation of Bilirubin

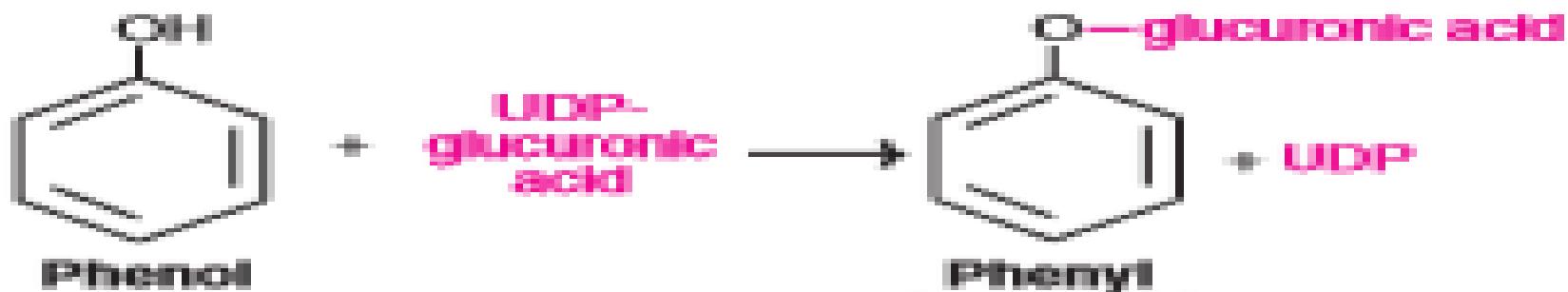
Bilirubin Monoglucuronide + UDP Glucuronic acid



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.

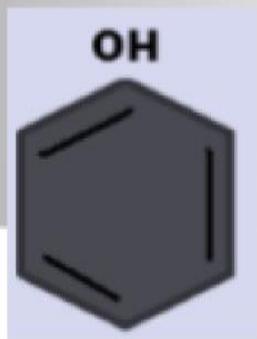


2) Sulfation

- 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

PAPS: 3' –phosphoadenosine 5'-phosphosulfate

2) Sulfation



Active Sulfate



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



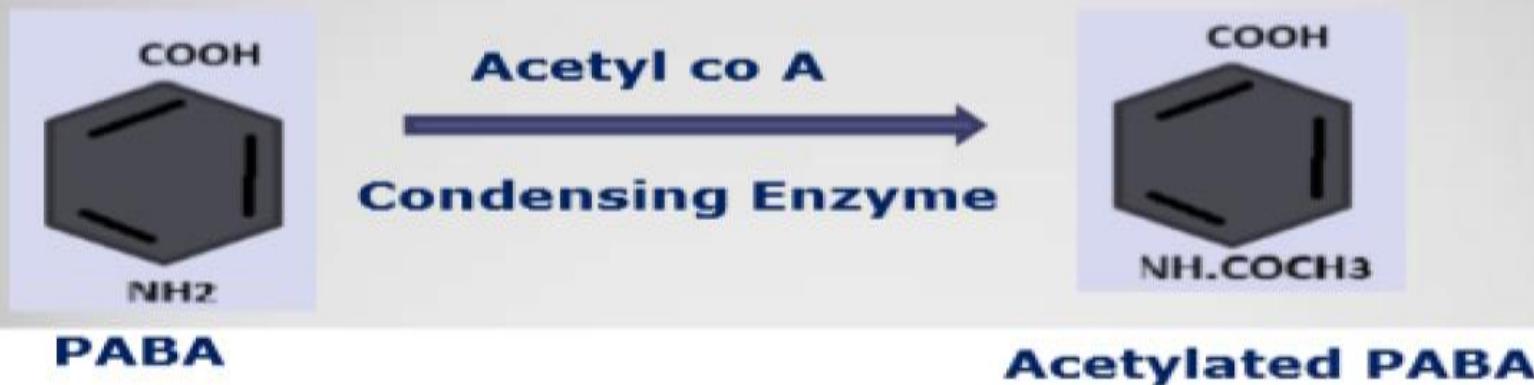
where X represents a xenobiotic.

- **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.

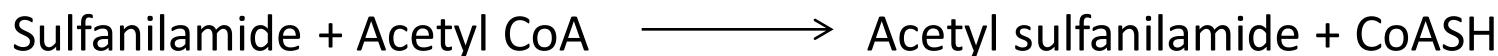
3) Acetylation

Compounds conjugated by Acetylation-

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



- Acetyl CoA is the active form of acetic acid that takes part in conjugating reactions.
- Drugs such as sulfanilamide are converted to acetyl derivatives.



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



- p- Methyl Amino Azo benzene

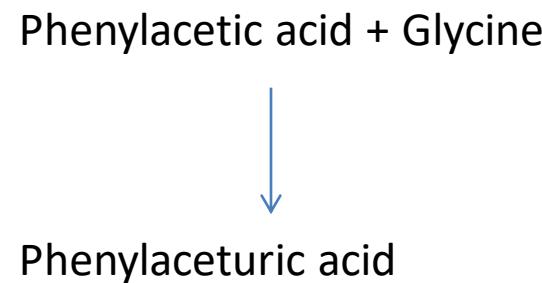
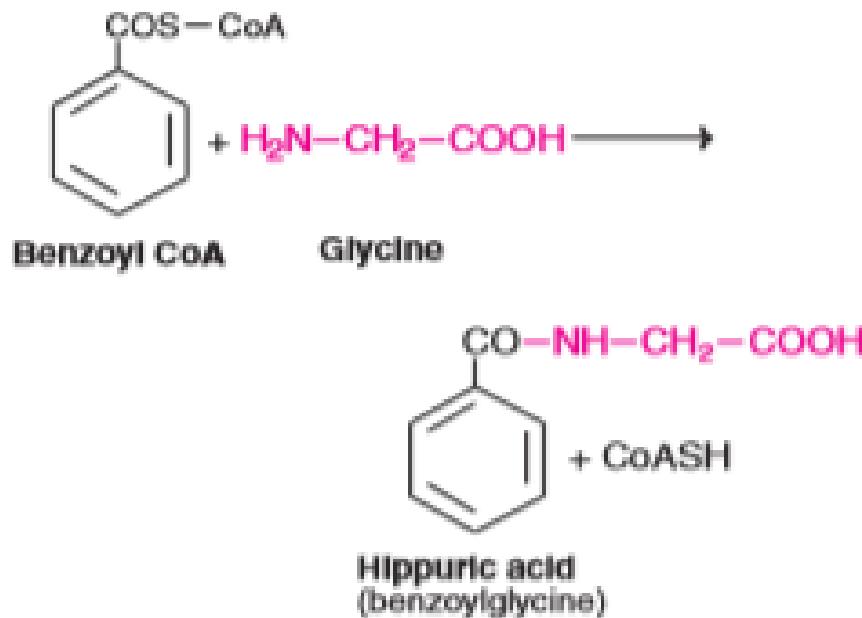


- O- Methylation of estrogen, norepinephrine, epinephrine and their metabolites.

5) Conjugation with Amino acids

1) Conjugation with Glycine

- Benzoic acid + Glycine \longrightarrow Hippuric acid
- Nicotinamide + Glycine \longrightarrow 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 (γ -glutamyl-cysteinylglycine) is a **tripeptide** consisting of glutamic acid, cysteine, and glycine
- Glutathione is commonly **abbreviated GSH** (because of the sulphydryl 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:



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.

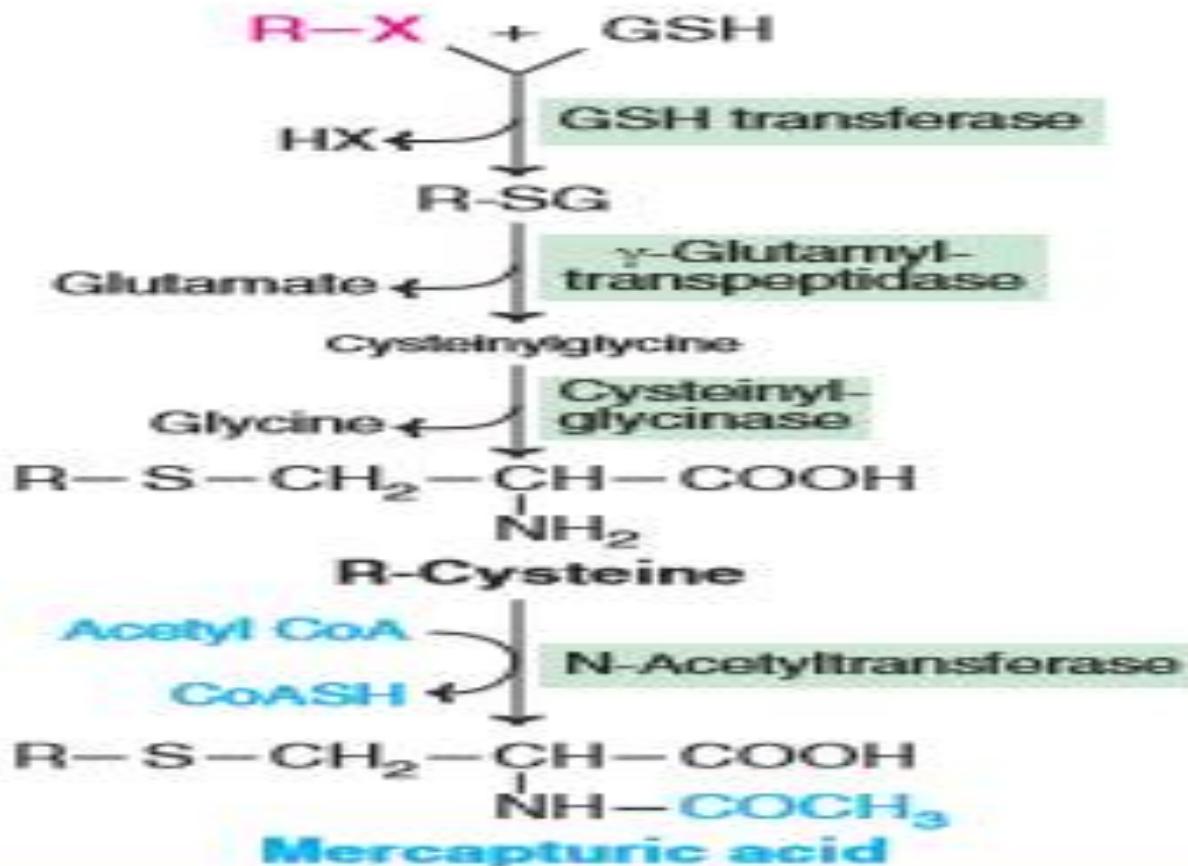


Fig. 31.2 : Role of glutathione in conjugation to form mercapturic acid (R-X—A xenobiotic; GSH—Glutathione).

- A wide range of organic compounds such as alkyl or aryl halides, alkenes, nitro compounds and epoxides get conjugated with cysteine of glutathione .
- The formation of mercapturic acid is depicted in figure .
- The glutamate and glycine of glutathione are removed and an acetyl group is added to the cysteine residue.

7) Conjugated with Thiosulfate

The highly toxic cyanides are conjugated with thiosulfate to form less toxic thiocyanate.



Conjugated Reactions and Reagents

Reaction	Reagent	Group in substrate
Glucuronidation	UDP-glucuronate	-OH, -COOH, -NH ₂
Sulfation	PAPS	-OH, -NH ₂ , -SH
Methylation	SAM	-OH, -NH ₂
Acetylation	acetyl-CoA	-OH, -NH ₂
Sulfide formation	glutathione	Ar-halogen, Ar-epoxide
Amide formation	glycine, taurine	-COOH

Figure 1 - Detoxification (Biotransformation) Pathways

Toxins

(fat-soluble)

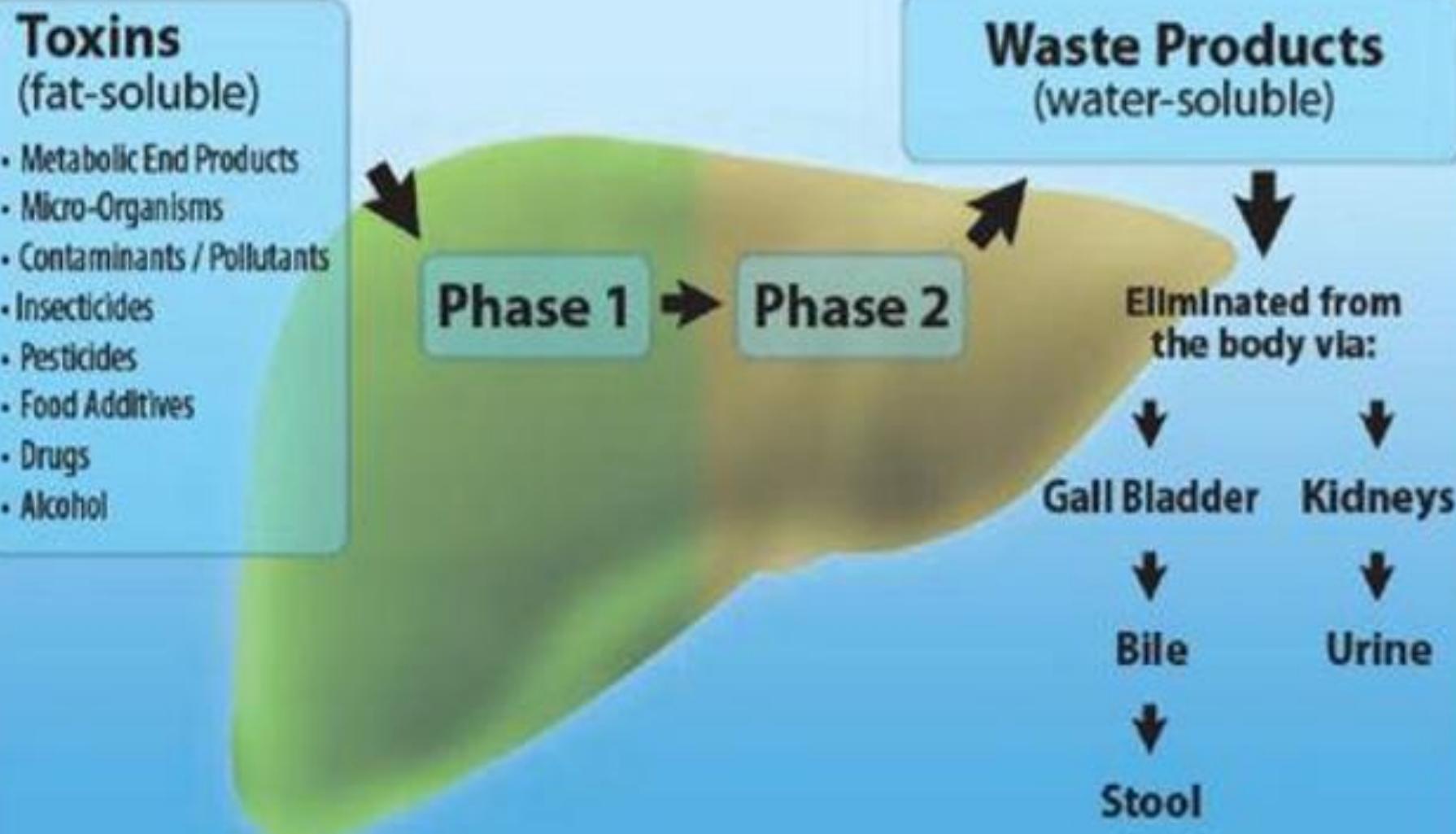
- Metabolic End Products
- Micro-Organisms
- Contaminants / Pollutants
- Insecticides
- Pesticides
- Food Additives
- Drugs
- Alcohol

Waste Products

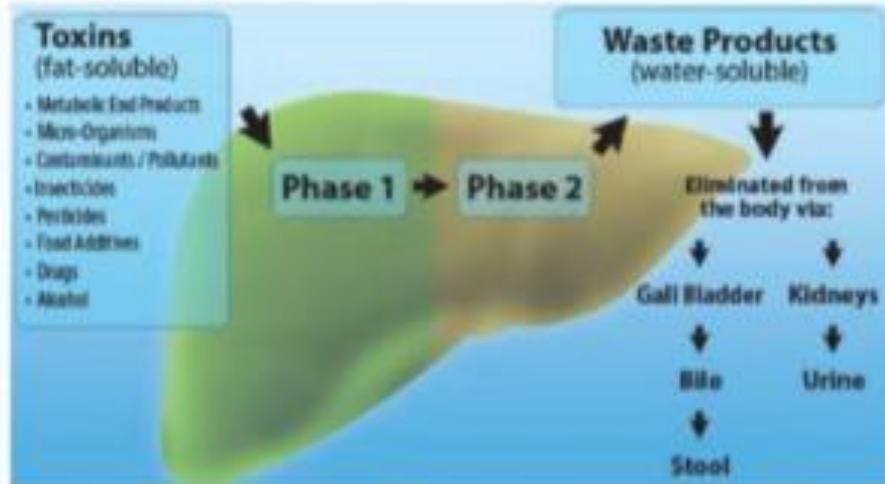
(water-soluble)

Phase 1

Phase 2



Role of liver in detoxification



- liver detoxifies harmful substances such as: alcohol, drugs & toxins that have entered the blood stream by breaking them into harmless forms for excretion
- drugs such as the antibiotics penicillin & erythromycin and sulphonamides are also broken down into harmless wastes which are then excreted
- hormones such as thyroid hormone, and steroid hormones such as oestrogen, testosterone, & aldosterone are similarly inactivated, ready for removal from the blood through the kidney

Figure 3. Phase I and II Liver Detoxification

FAT-SOLUBLE **TOXINS**

Phase 1 →

(Cytochrome P450 Enzymes)

- Oxidation
- Reduction
- Hydrolysis
- Hydration
- Dehalogenation

Nutrients Needed

- Vitamins B2, B3, B6, B12
- Folic Acid
- Glutathione
- Flavonoids

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WATER-SOLUBLE **WASTE**

Phase 2 →

(Conjugation Pathways)

- Sulfation
- Glucoronidation
- Glutathione Conjugation
- Acetylation
- Amino Acid Conjugation
- Methylation

Eliminated via:

- Urine
- Bile
- Stool

Nutrients Needed

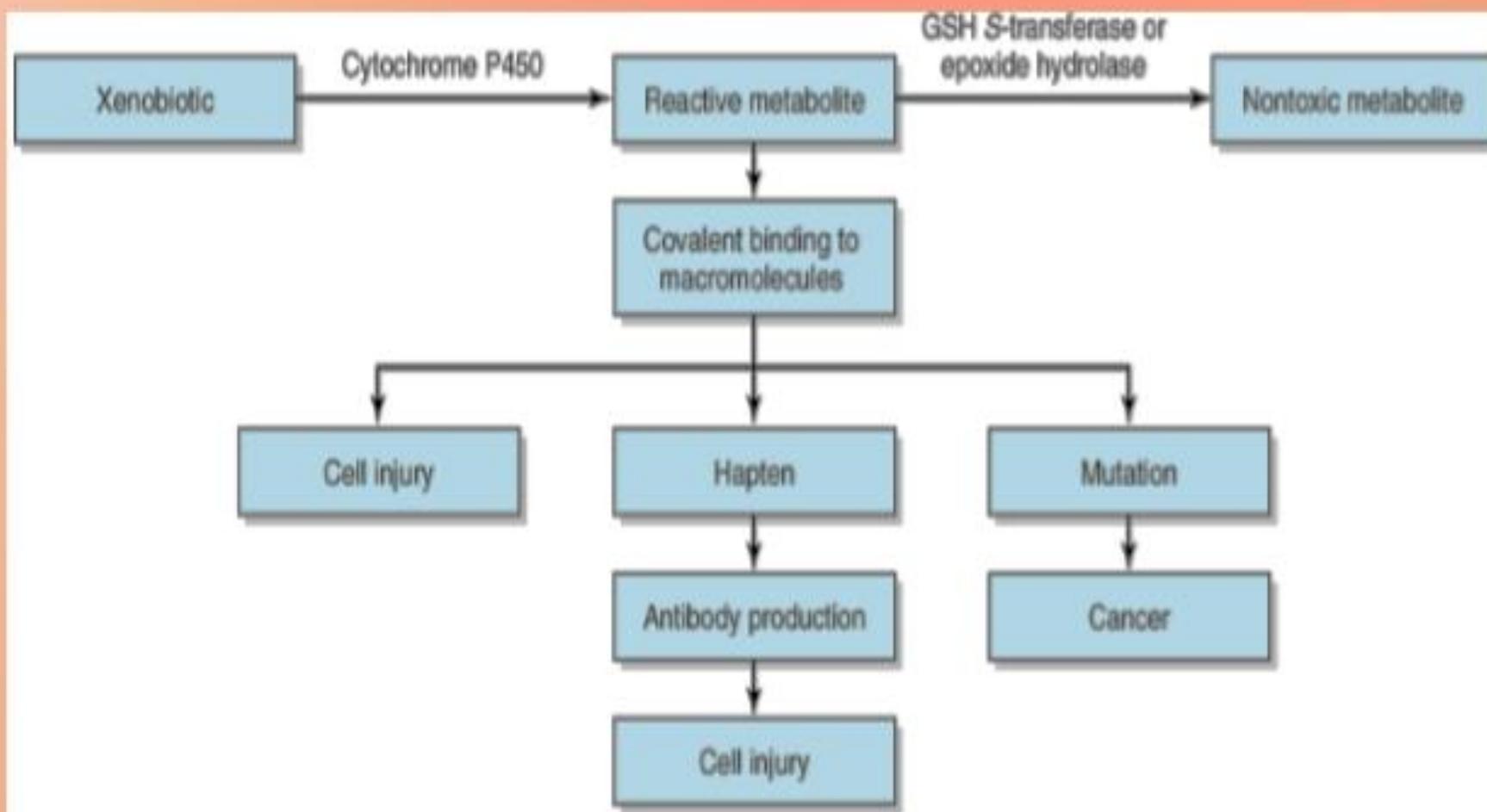
- Methionine
- Cysteine
- Magnesium
- Glutathione
- Vitamin B5, B12
- Vitamin C
- Glycine
- Taurine
- Glutamine
- Folic Acid
- Choline

Effects of Xenobiotics

Xenobiotics can produce a variety of biological effects including:

- Pharmacological responses
- Toxicity (cell injury)
- Immunological responses (antibodies)
- Cancers (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 P450 acts on these compounds, converting them into much less reactive dihydrodiols.

Effects of Xenobiotics



THANKS