BIOKIMIA
BIOTRANSFORMATION/ DETOXIFICATION REACTIONS

METABOLISM OF XENOBIOTICS

Xenobiotics

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

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 fattymetabolites acids.

Xenobiotics

Xenobiotics can produce a variety of **biological effects** including:

- **Pharmacological responses**
  - **Activation of Pro-drug**
  - **Termination of drug action**
- **Immunological responses**
- **Toxicity**
- **Cancers**
- **Teratogenic**

Activation of Pro-drug

L-dopa ⟶ Dopamine

Termination of Drug Action

atropine ⟶ tropic acid and tropine

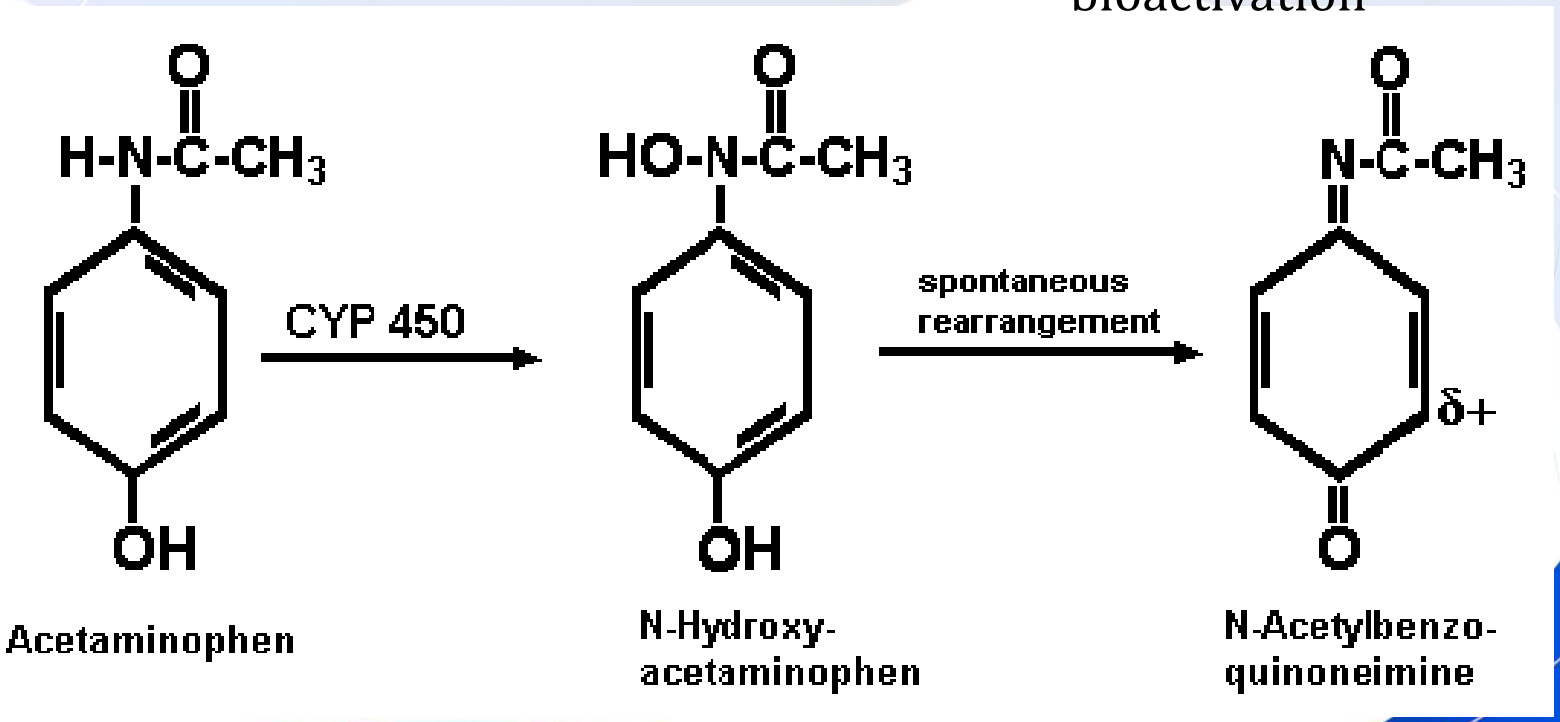
Some Xenobiotics Are Metabolized to Carcinogenic Agents

- 3,4-Benzopyrene
- Aflatoxin
- N-Acetylaminofluorene

Metabolites of these agents interact with DNA

Small Amounts of **Acetaminophen** is Converted to the Reactive Metabolite **N-Acetylbenzoquinoneimine**

bioactivation



Bioactivation of acetaminophen; under certain conditions, the electrophile N-acetylbenzoquinoneimine reacts with tissue macromolecules, causing **liver necrosis.**

Thalidomide is a Teratogen

- THALIDOMIDE: Fetal malformations in humans, monkeys, and rats occur due to metabolism of the parent compound to a teratogen. This occurs very early in gestation.

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**

Role of Liver

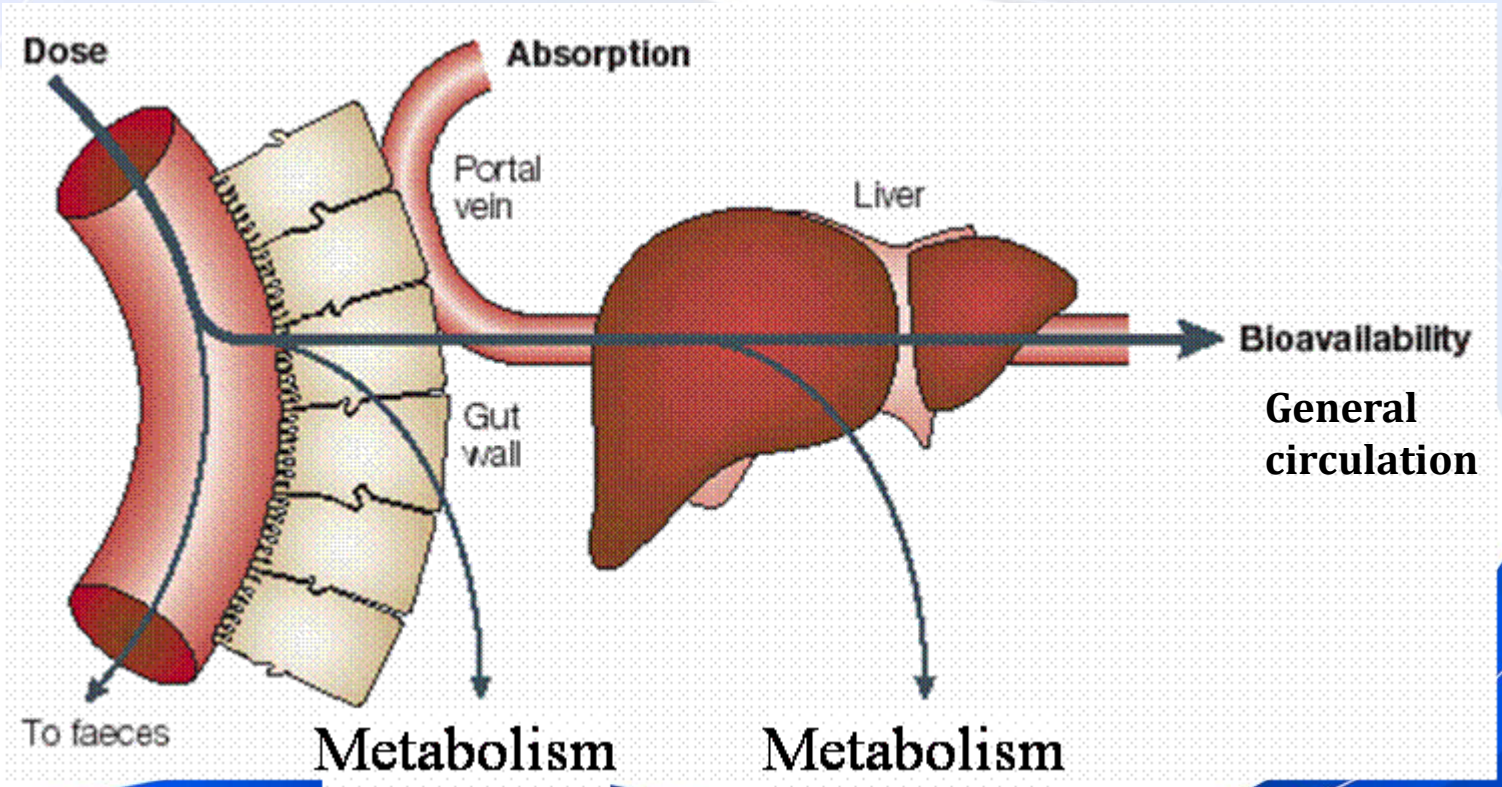
- **Main organ** involved
- Hepatocytes contain **wide variety of enzymes** to process xenobiotics
- Enzymes are present in **cytosol**, **endoplasmic reticulum** and to lesser extent in other **organelles**
- Each enzyme represents a large family of gene product
- Each gene product may be induced by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

First-pass Effect

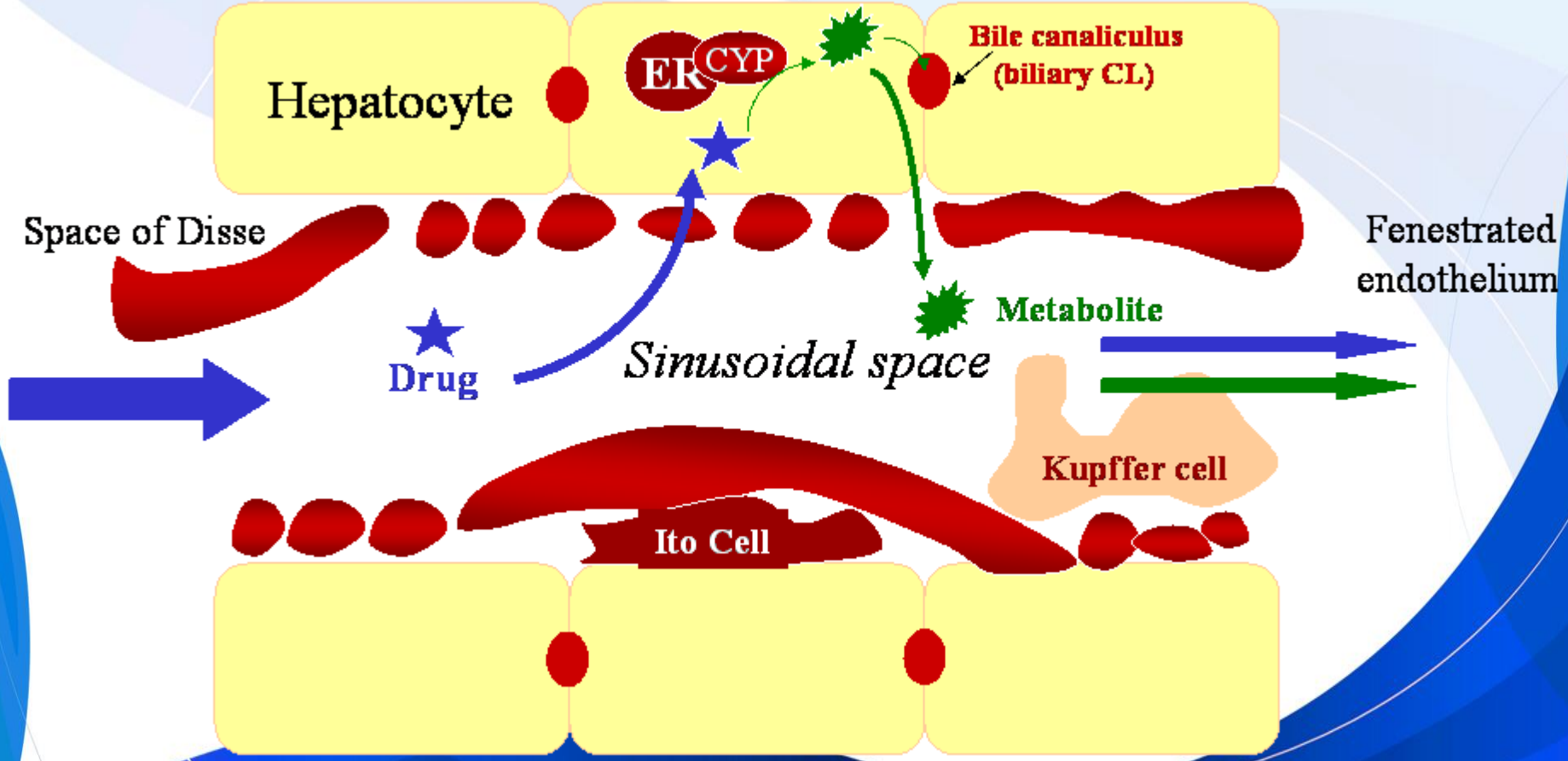
- The first-pass effect is the term used for the **hepatic metabolism** of a **pharmacological agent** when it is absorbed **from the gut** and delivered **to the liver** via the **portal circulation**.

- The greater the first-pass effect, the less the agent will reach the systemic circulation when the agent is administered orally

Drug Metabolism



Hepatic Metabolism



CYPs are found in the smooth endoplasmic reticulum (ER). Hepatocytes contain the full complement of the major DMEs including cytosolic (e.g. Sulfotransferases, Aldehyde Dehydrogenase, Xanthine Oxidase), membrane-bound (CYPs, UGTs, FMOs) and mitochondrial (e.g. MAOs)

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**

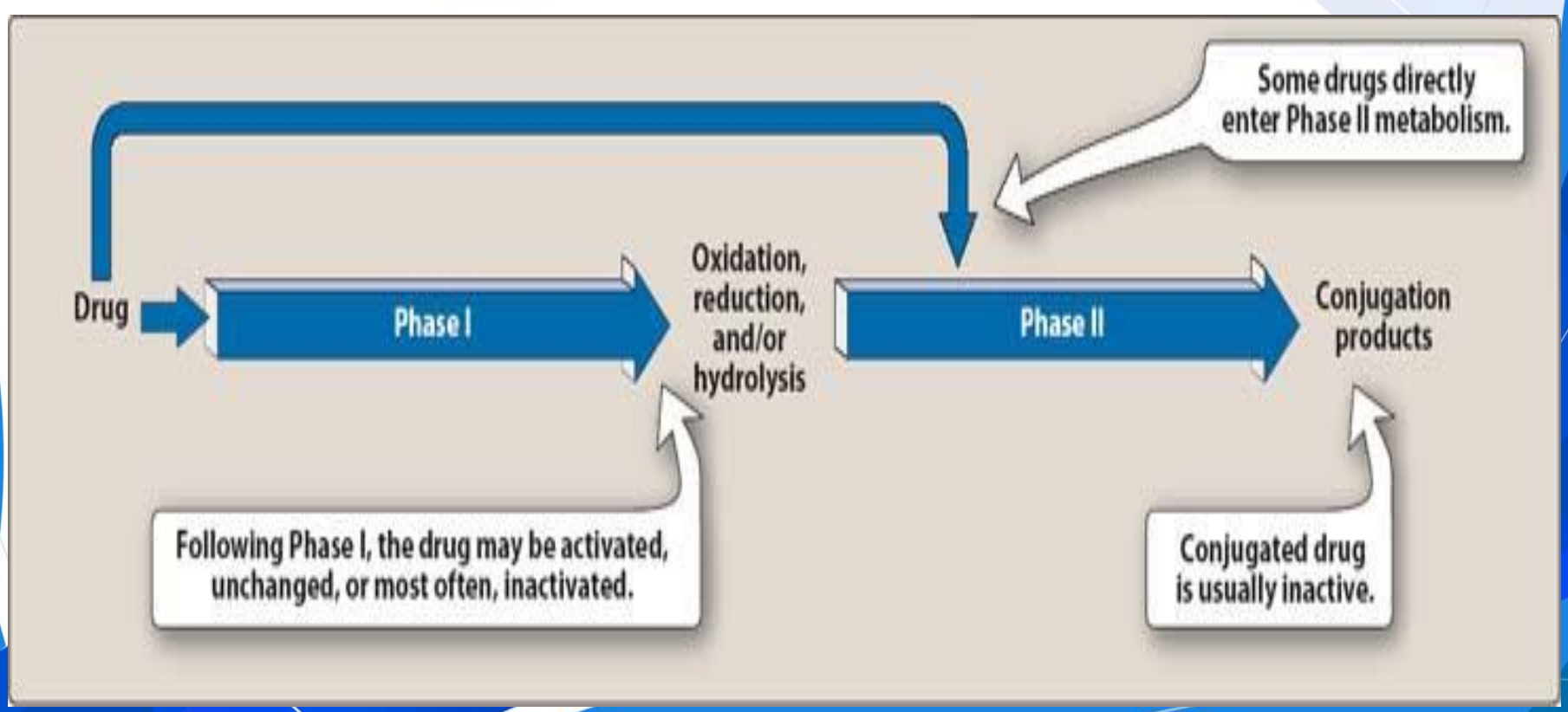
Inactive metabolite

Relatively harmless

↓ **Metabolic activation**

Reactive intermediate

Overview of detoxification reactions



Comparing Phase I & Phase II

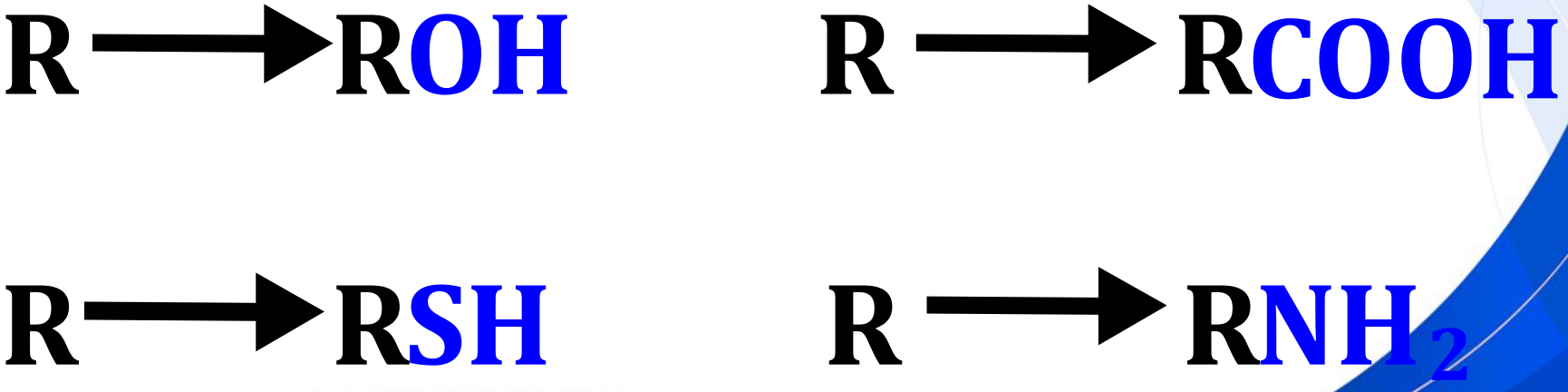
Enzyme	Phase I	Phase II
Types of reactions	Hydrolysis Oxidation Reduction	Conjugations
Increase in hydrophilicity	Small	Large
General mechanism	Exposes functional group	Polar compound added to functional group
Consquences	May result in metabolic activation	Facilitates excretion

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 I Metabolism

Polar groups are exposed on or introduced to a molecule



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.

Phase 1 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 → CH_2OH → CHO → COOH

Phase 1 reactions

A) Oxidation

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

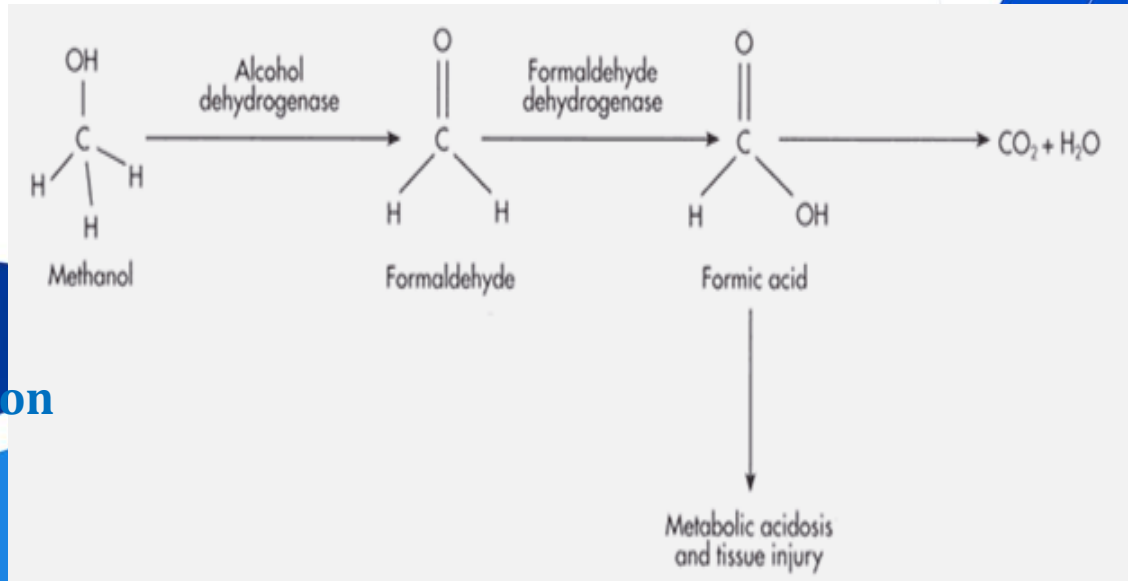
Methanol → Formaldehyde → Formic acid

Ethanol → Acetaldehyde → Acetic acid

Benzoyl Alcohol → Benzaldehyde → Benzoic acid

Methanol toxicity

- Methanol has a relatively low toxicity; 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.**



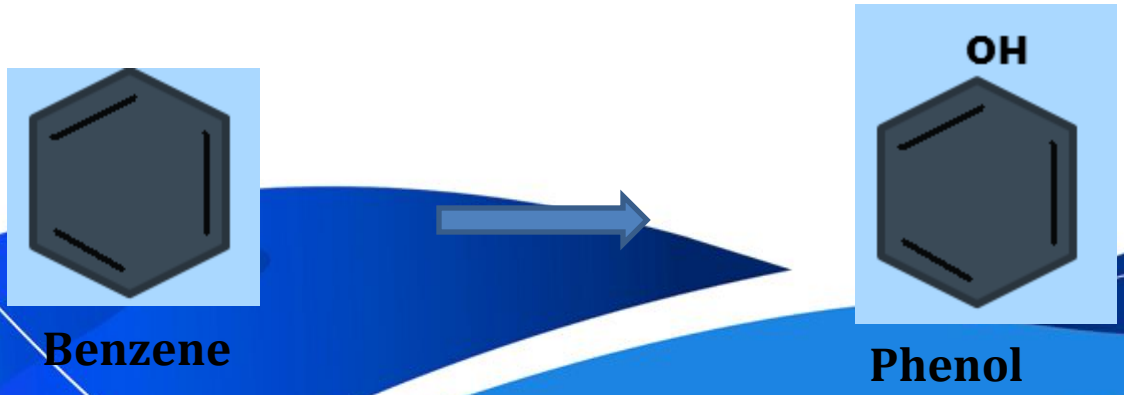
It is an example of Entoxification

Phase 1 reactions

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.



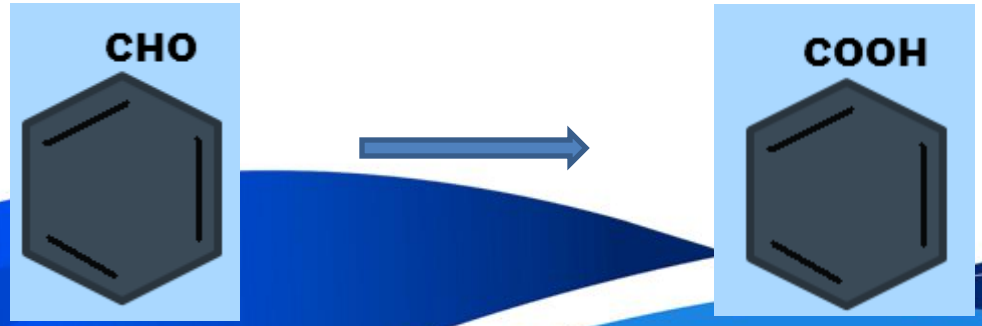
Phase 1 reactions

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**

Phase 1 reactions

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**

Phase 1 reactions

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.

Phase 1 reactions

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

Phase 1 reactions

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

Phase 1 reactions

B) Reduction

Reduction does not occur extensively in human beings

Examples-

- **Reduction of Aldehydes**

Chloral → Trichloroethanol

Trichloroethanol is excreted after conjugation with D-

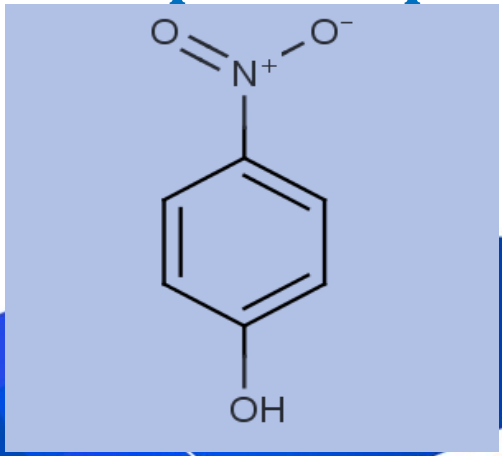
Phase 1 reactions

B) Reduction

- ❖ **Reduction of Nitro compounds**

- **p- nitrobenzene → p-Amino benzene**

- **p- nitro phenol → p-Aminophenol**



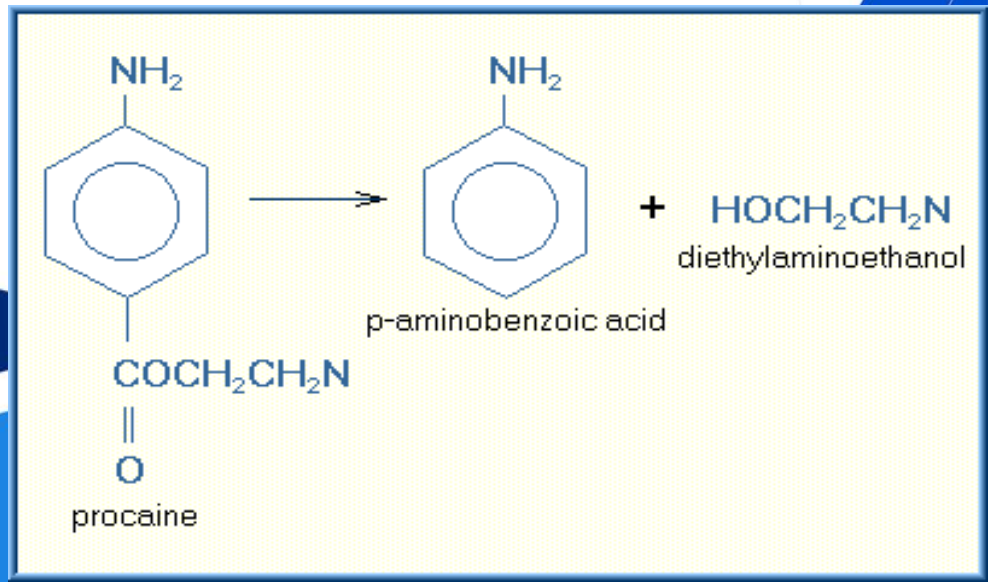
p-nitrophenol

Phase 1 reactions

C) Hydrolysis

Certain therapeutic compounds undergo hydrolysis,

- **Examples-**
- Acetyl Salicylic acid → Acetic acid + Salicylic acid
- Atropine → Tropic acid + Tropine
- Digitalis → Sugar + Aglycone



Phase 1 reactions- Enzymes

- Mainly Catalyzed by members of a class of enzymes referred to as **Monooxygenases, Mixed Function oxidases** or **Cytochrome P450s.**

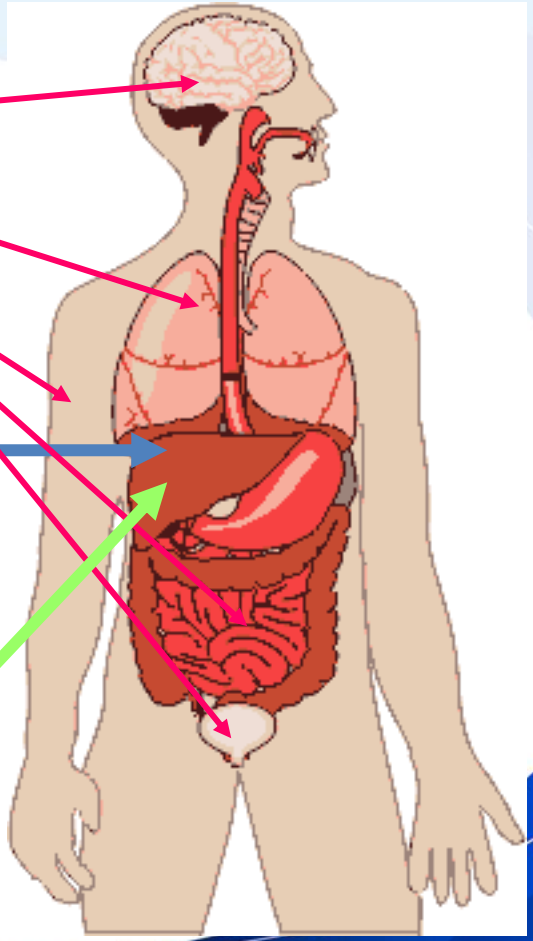
- **Other enzymes of significance are-**
  - Aldehyde and alcohol dehydrogenase
  - Deaminases
  - Esterases
  - Amidases
  - Epoxide hydrolases

Enzim yang berperan pada fase 1

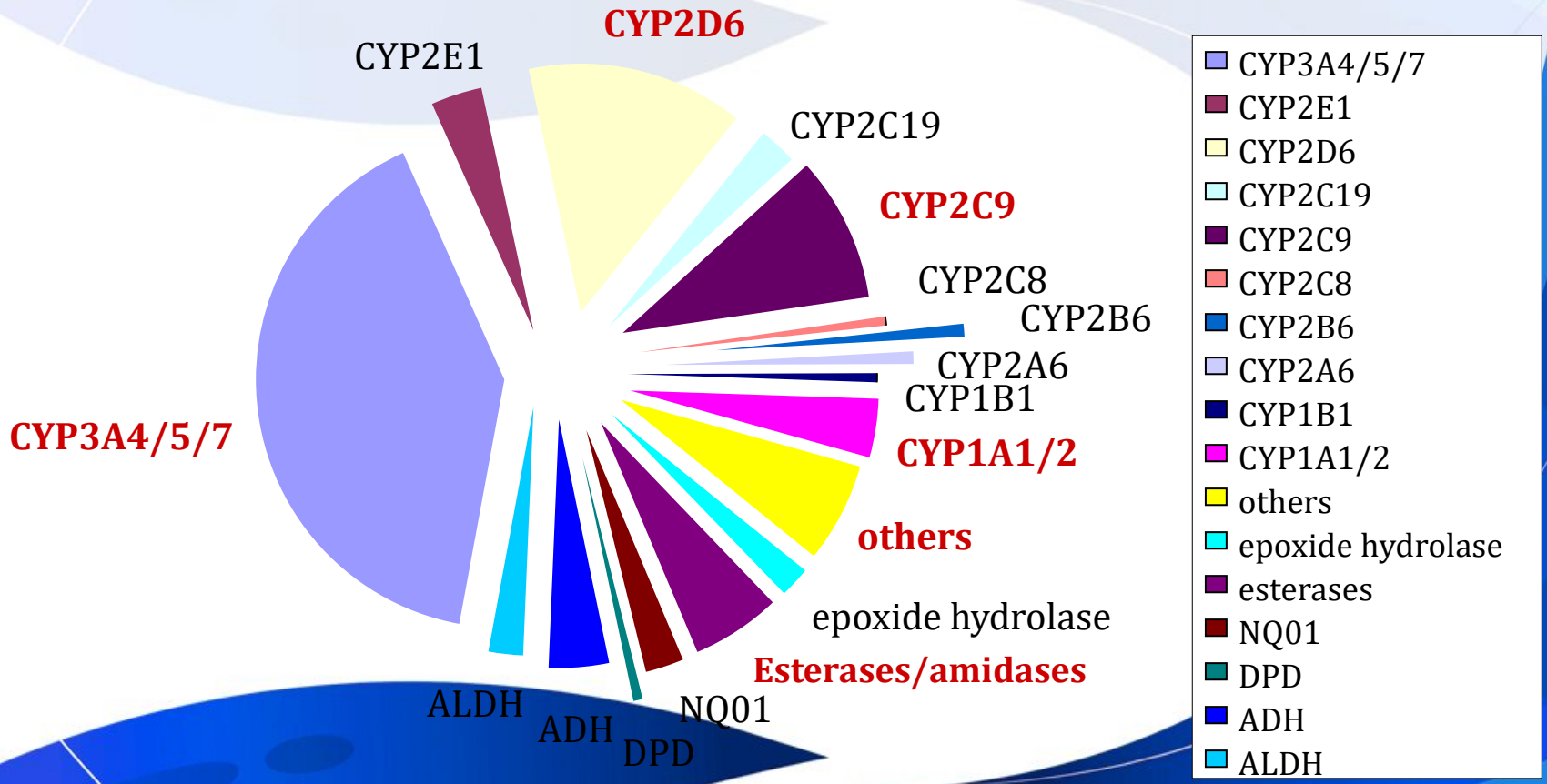
Extrahepatic microsomal enzymes
(oxidation, conjugation)

Hepatic microsomal enzymes
(oxidation, conjugation)

Hepatic non-microsomal enzymes
(acetylation, sulfation, GSH, alcohol/aldehyde dehydrogenase, hydrolysis, ox/red)



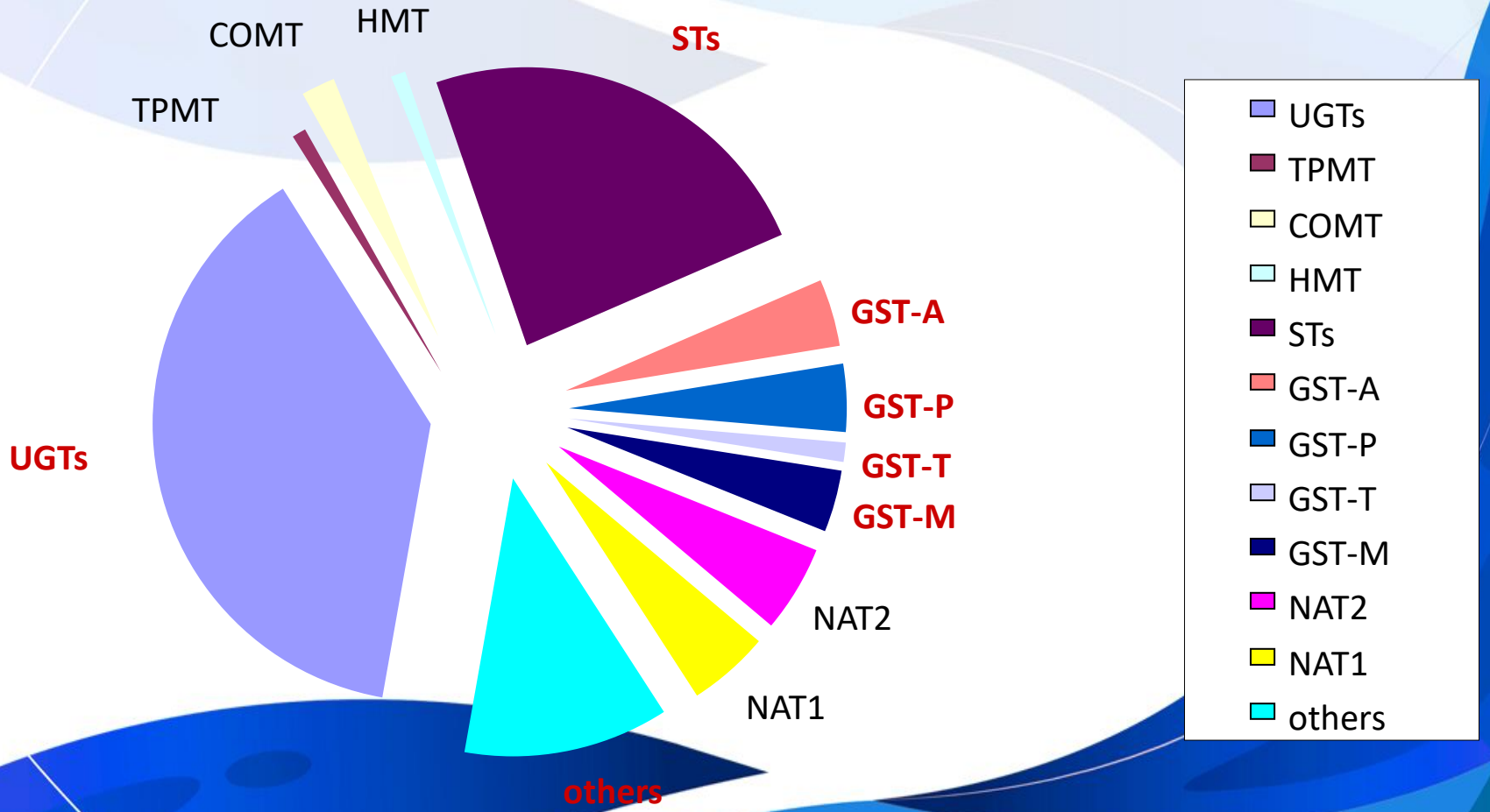
Human Phase I Enzymes of Drug Metabolism



CYP: cytochrome P450, NQ01: NADPH:quinone oxidoreductase (DT diaphorase); DPD: dihydropyrimidine dehydrogenase; ADH: alcohol dehydrogenase; ALDH: aldehyde dehydrogenase

Evans and Relling, Science (1999)

Human Phase II Enzymes of Drug Metabolism



HMT: histamine methyltransferase; TPMT: thiopurine methyltransferase; COMT: catechol O-methyltransferase; UGT: Uridine Glucuronosyl-S-Transferases; ST: Sulfotransferase; GST: Glutathione-S-Transferases

Evans and Relling, Science (1999)

Microsomal Enzyme
Cytochrome P450:
A superstar of xenobiotic metabolism

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 (>50%)**, **carcinogens**, **pesticides**, **petroleum products**, and **pollutants**
- 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 (cont'd)

- **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**

Properties of Human Cytochrome P450s (cont'd)

- **6 CYP specieses** are available (minimum)
- 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

Alcohol modulates P450 activity

Alcohol – induced toxicity of acetaminophen (Tylenol)

- Acetaminophen in small amounts is metabolized by **glucuronidation** and **sulfation**.
- Larger ingestions result in production of a toxic metabolite N-acetyl-p-benzoquinoneimine (**NAPQI**) by P450.
  - The NAPQI is normally detoxified by **glutathione**.
- However, **chronic alcoholism depletes glutathione, and also induces P450** to increase toxicity.
- Thus, previous chronic alcohol ingestion increases the risk for acetaminophen toxicity.

Smoking modulates P450 activity

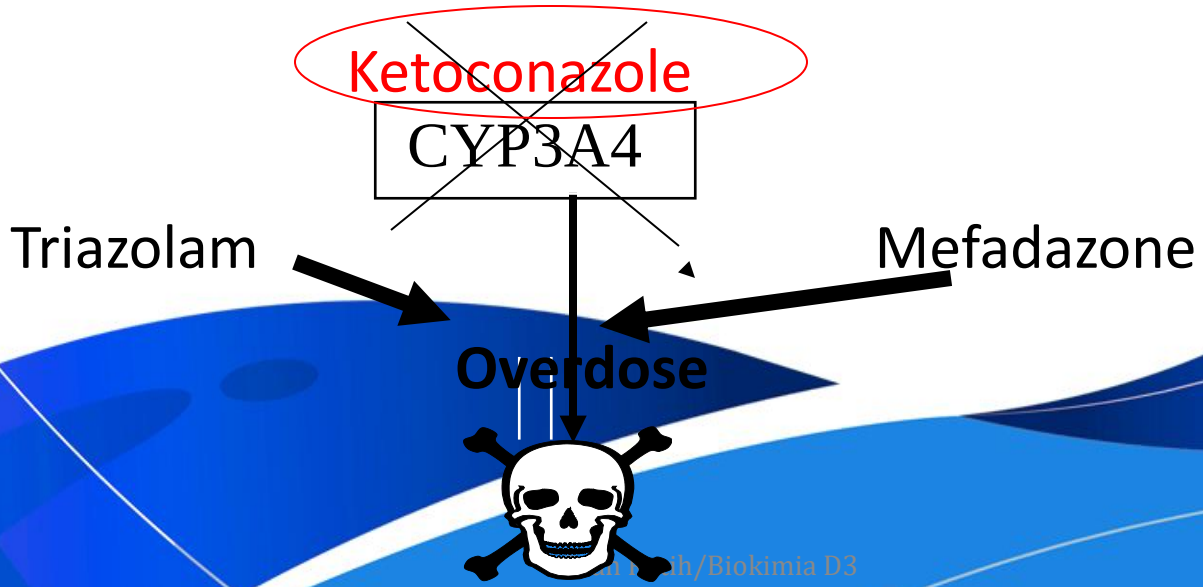
- **Polycyclic hydrocarbons** in cigarette smoke induce the metabolism of CYP1A substrates as a result of **CYP1A** induction
- The average content of CYP1A in the liver biopsies from smokers (16.3 pmol/mg of protein) was significantly higher than that from nonsmokers (4.7)
- **Theophylline**, **caffeine**, **fluvoxamine, clozapine**, and **olanzapine clearance** is significantly **increased** in smokers compared with nonsmokers

Herbal supplements modulate P450 activity

- **St. John's Wort (Hypericum perforatum) extracts**, preparations that are used in the treatment of **depression**, **inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4**
- **Ginseng (CYP1A1, 1A2, 1B1)-inhibition**
- **The Licorice Root** derived Isoflavan Glabridin **inhibits P450s 3A4, 2B6, and 2C9**

Multiple drug interactions modulate P450 activity

A 63 year old man receiving medication for major depression showed he boarded a plane in Toronto to fly to London. On arrival he was unrousable. In his Carry-on bag he had Mefadazone (for depression), Ketoconazole (for fungal infection) and Triazolam (an antipsychotic also used for insomnia). All three of these drugs bind to CYP3A4. Ketoconazole inhibits CYP3A4 and caused the other two drugs to become overdosed during 6 hr flight (sitting still is a factor).

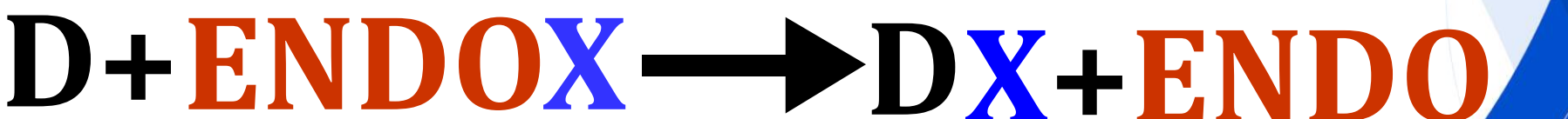


Non-CYP drug oxidations

- **Monoamine Oxidase (MAO), Diamine Oxidase (DAO) -** MAO (mitochondrial) **oxidatively deaminates endogenous substrates** including **neurotransmitters** (dopamine, serotonin, norepinephrine, epinephrine); drugs designed to inhibit MAO used to affect balance of CNS neurotransmitters (L-DOPA)
- **Alcohol & Aldehyde Dehydrogenase -** non-specific enzymes found in soluble fraction of liver; ethanol metabolism
- **Xanthine Oxidase -** converts **hypoxanthine** to **xanthine**, and then to **uric acid**. Drug substrates include theophylline, 6-mercaptopurine. Allopurinol is substrate and inhibitor of xanthine oxidase; delays metabolism of other substrates; effective for treatment of gout.

PHASE 2 METABOLISM

A molecule endogenous to the body donates a portion of itself to the foreign molecule



ENDO = UDP glukuronat, sulfat, glutathion, metil, asetil, asam amino glisin, dll

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)→ **UDP Glucuronic acid**

2 NAD^+ → **2 $NADH + 2H^+$**

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

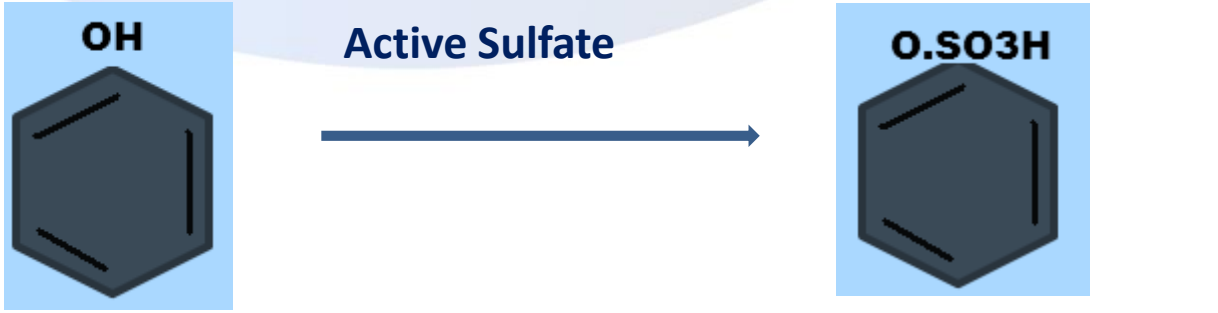
Most of the **bilirubin excreted** in the bile of mammals is in the form of **bilirubin diglucuronide**.

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



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

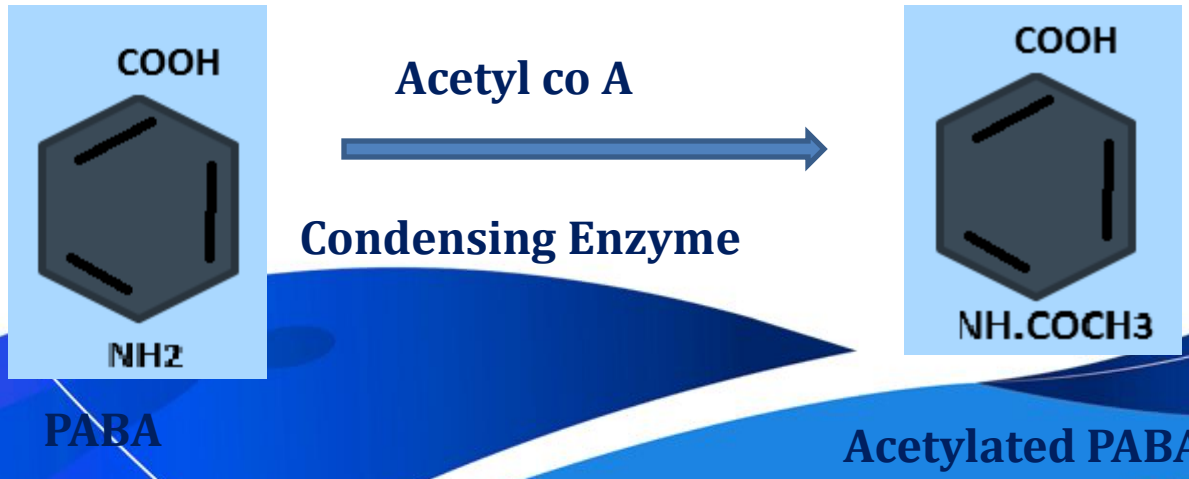
- Acetylation is represented by

- 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



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

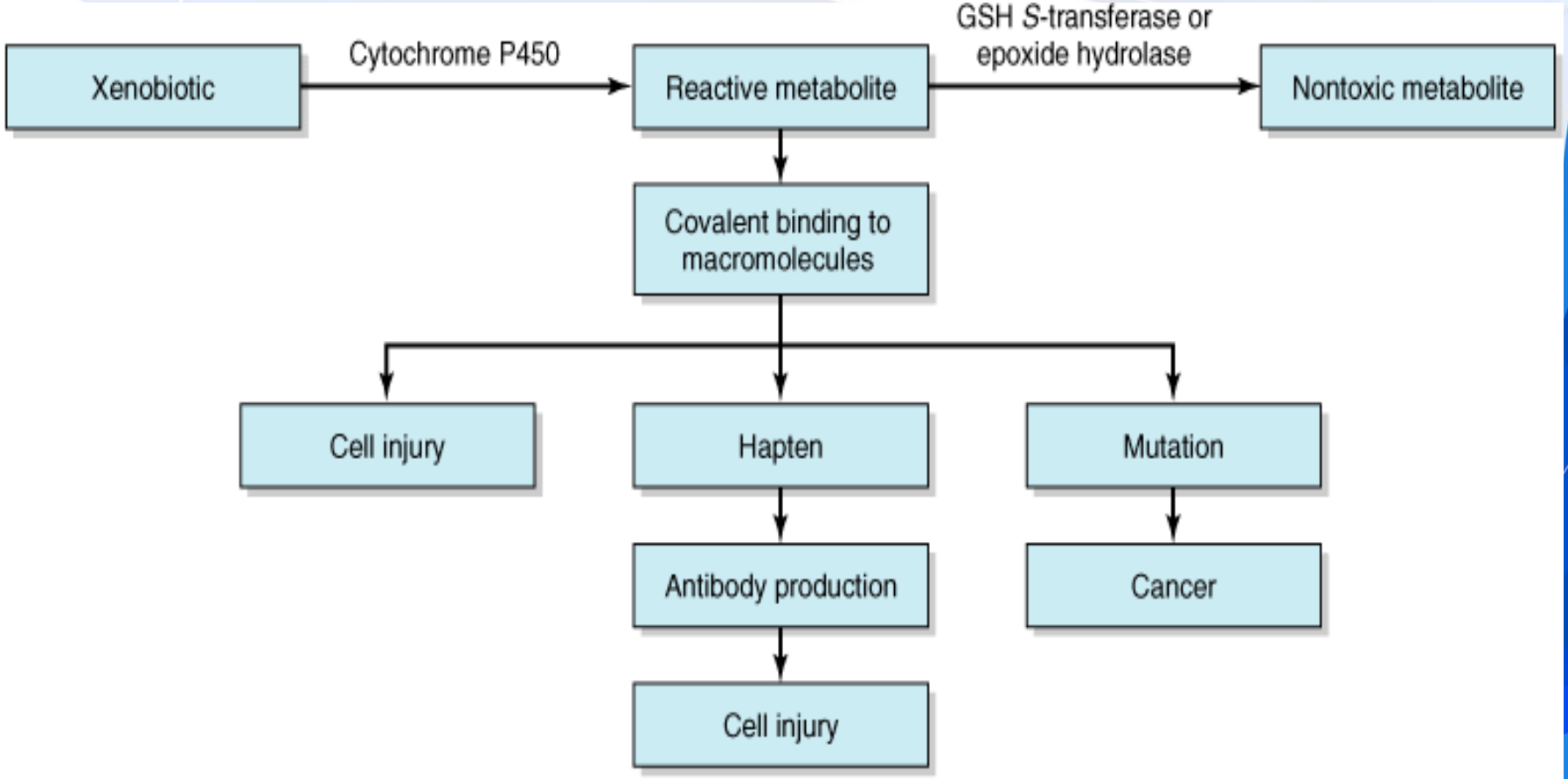
- Glutathione (ϒ-glutamyl-cysteinylglycine) is a **tripeptide** consisting of **glutamic acid**, **cysteine**, and **glycine**
- Glutathione is commonly **abbreviated GSH** (because of the sulfhydryl group of its cysteine, which is the business part of the molecule).
- A number of potentially toxic electrophilic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

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

- 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 P450 acts on these compounds, converting them into much less reactive dihydrodiols.

Summary

- Xenobiotics are **chemical compounds foreign** to **the body**
- 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.

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