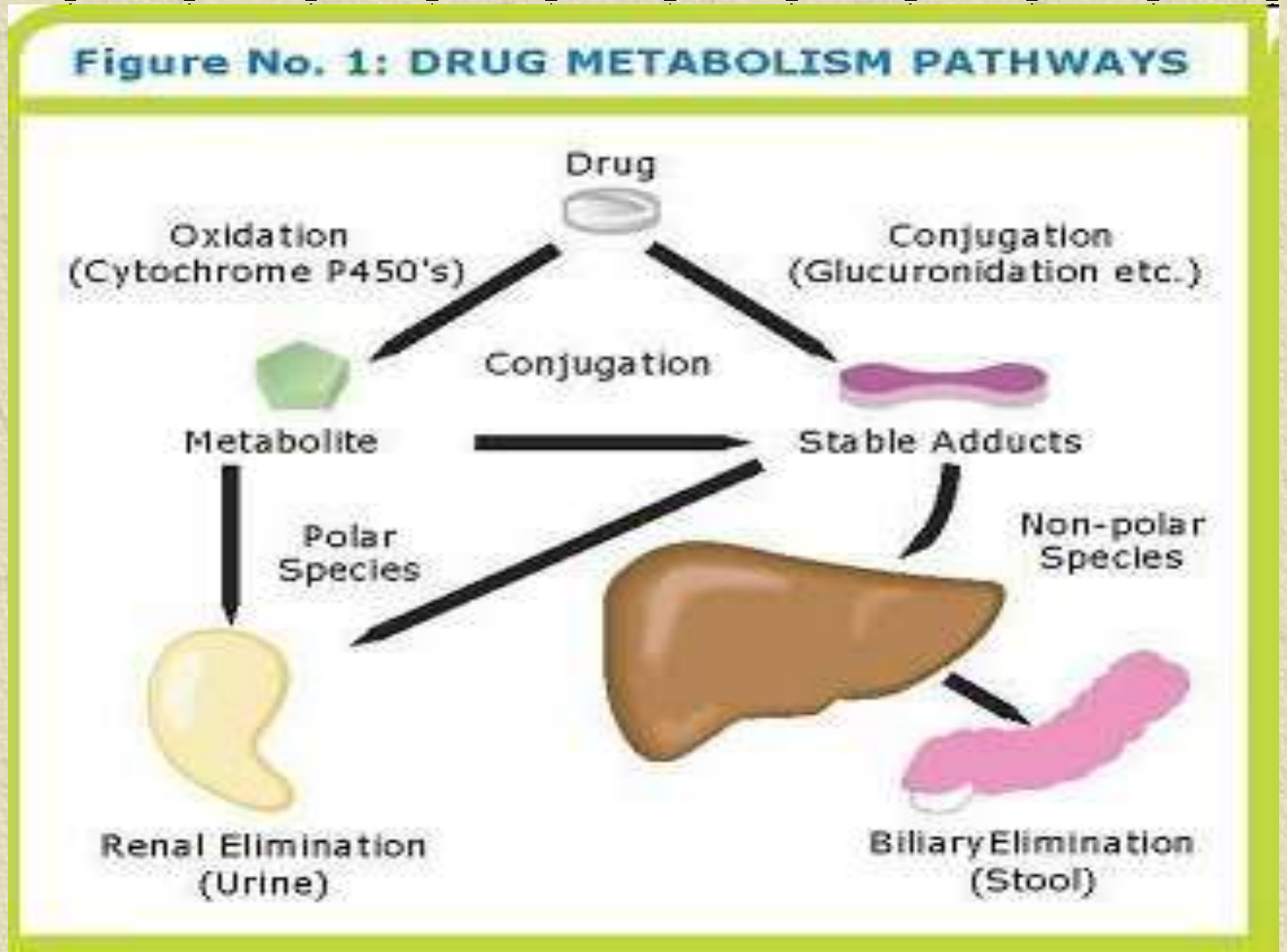
BIOTRANSFORMATION OF XENOBIOTICS



Poisons are xenobiotics, but not all xenobiotics are poisonous.

- ***Xenobiotic***: is a compound that is foreign to the body ; is a chemical which is found in an organism but which is not normally produced or expected to be present in body.
- **Endogenous:** Pigments , hormones
- **Nonendogenous**: Such as drugs, food additives, pollutants, toxin, etc
- Most of these compounds are subject to metabolism (**biotransformation**) in human body.

Definition of the biotransformation

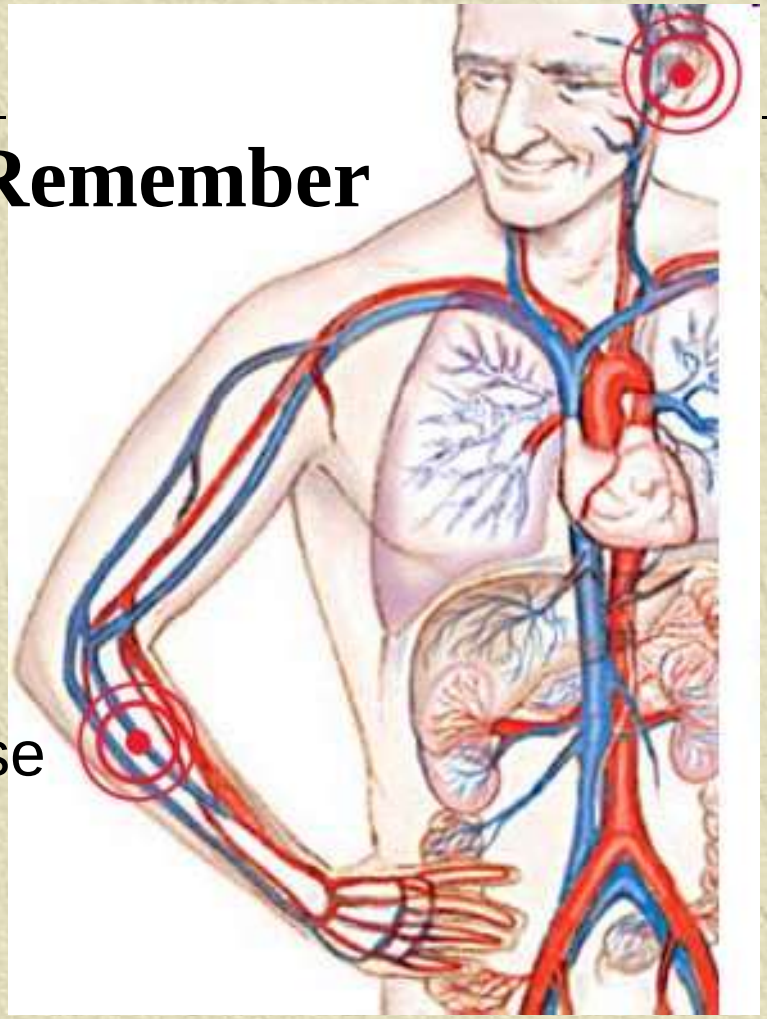
- Conversion of **lipophilic xenobiotics** to **water-soluble chemicals** by a process catalyzed by enzymes in the liver and other tissues.
- In most cases, biotransformation **lessens the toxicity** of xenobiotics, but many must undergo the process to exert their toxic effects.

BIOTRANSFORMATION OF XENOBIOTICS

- This process leads to rapid **excretion** and therefore **elimination** of the compound from the organism.
- However, the biotransformation may also change the chemical and biological activity of the substances.
- The **products of metabolism** are usually **more water-soluble** than the original compound.
- **Rarely** metabolism may actually **decrease** water-solubility and so **reduce excretion**.

Metabolism

Important Points to Remember

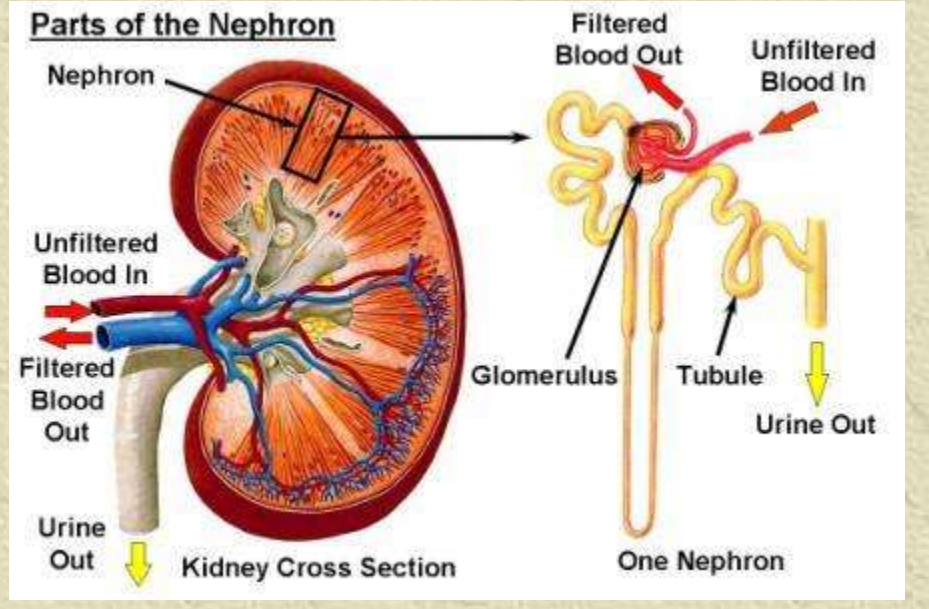


Most drugs entering the body are lipophilic

Drug molecules easily diffuse through the lipophilic membranes of the GIT

Metabolism - Important Points to Remember

Some of the Xenobiotics are NOT completely excreted in the urine due to the Reabsorption in the renal tubules



Product of Metabolism

The product of metabolism must become hydrophilic or converted to a water-soluble substance for elimination

ELIMINATION

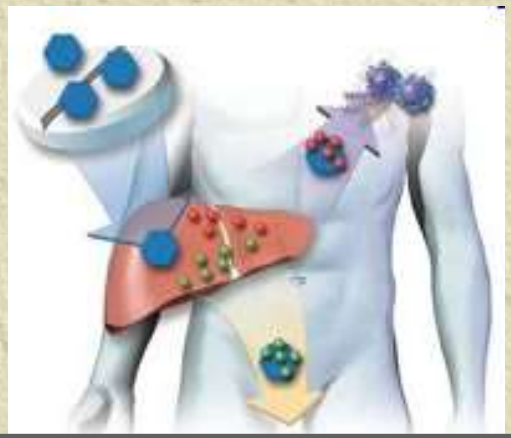
COMPLETE

Forms inactive and non-toxic substance

INCOMPLETE

Unwanted biological effect

Product of Metabolism

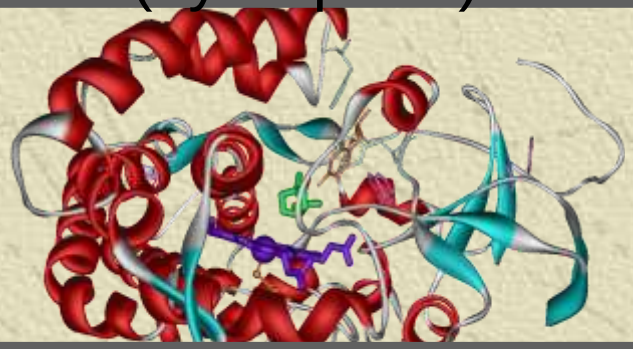


Xenobiotics

Must be converted to a water-soluble substance (hydrophilic)

Prodrugs/Metabolites

Are mostly lipophilic or lipid-soluble compounds



Metabolism

Is also called **DETOXIFICATION** or **DETOXICATION**

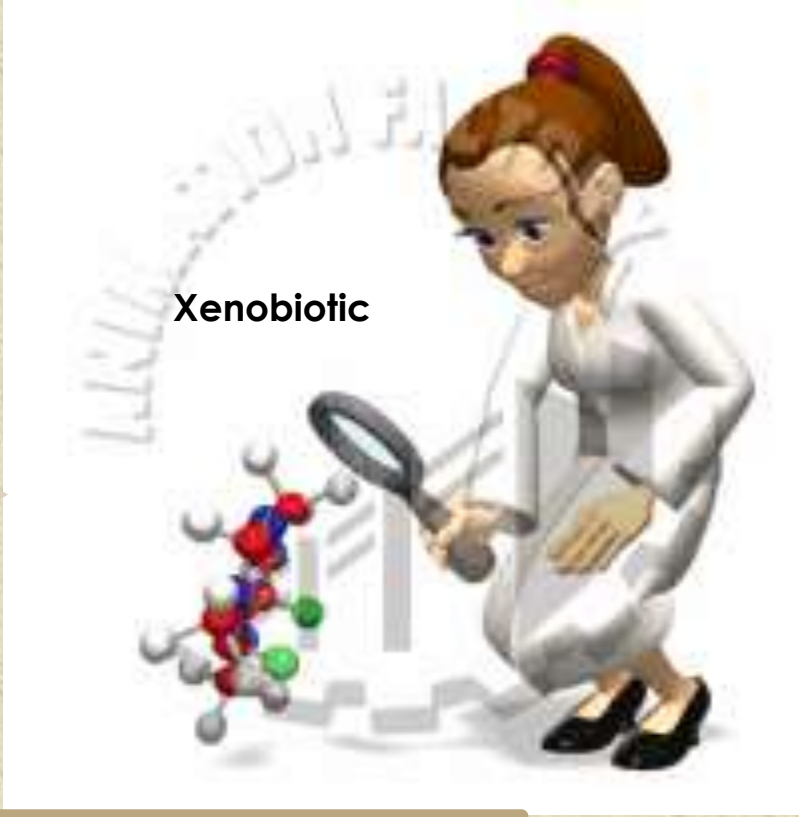
Purpose of Biotransformation

1. Facilitates excretion: converts lipophilic to hydrophilic compounds
2. Detoxification/inactivation:converts chemicals to less toxic forms
3. Metabolic activation: converts chemicals to more toxic active forms

BIOTRANSFORMATION OF XENOBIOTICS

- **The metabolism** of the xenobiotics can be divided into **two phases**: **phase 1 and phase 2.**
  - **- Phase I reactions** includes **alteration** of the original foreign molecule so as to add on a **functional group** which can be conjugated in phase.
  - - **Phase II** involves the **addition of a readily available, polar endogenous substance** to the foreign molecule.
  - - This polar moiety is conjugated either **to an existing group** or **to one added in a phase I reaction**.
  - -The polar moiety renders the foreign molecule more water-soluble and so more readily cleared from the body and less likely to exert a toxic effect.
- **For many compounds** there is an **initial Phase I** reaction to produce substances, which are conjugated by Phase II process.
  - **-** In other chemicals **only a Phase II process** may be utilized.

Introduction of Functional Polar Groups to Xenobiotics

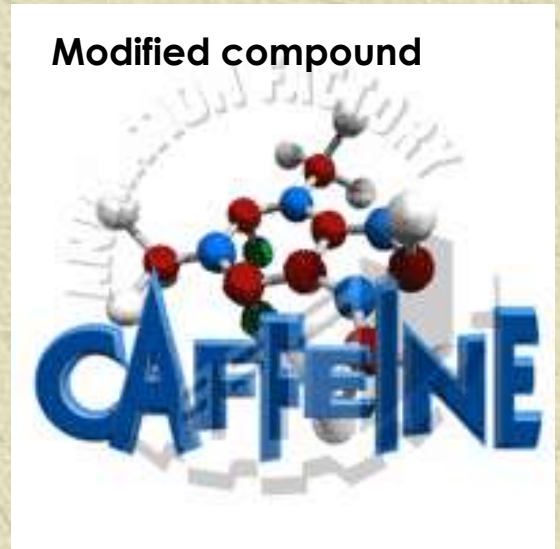


DIRECT INTRODUCTION

Introduction of Functional Polar Groups to Xenobiotics

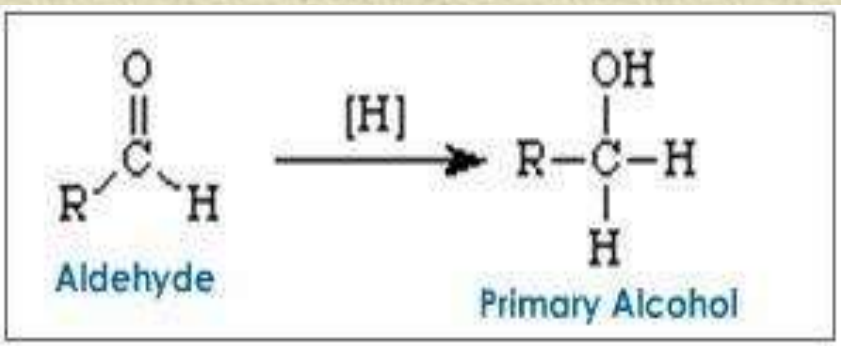
Original compound

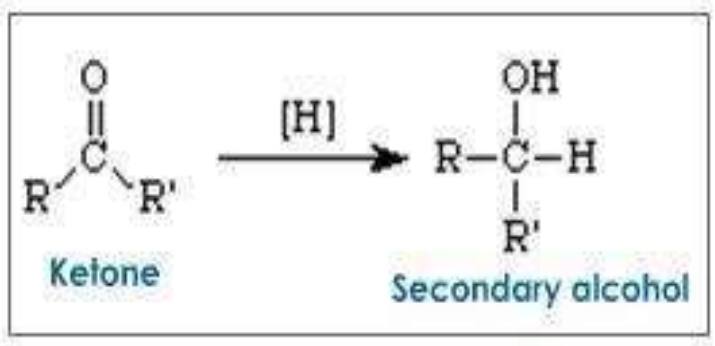
Modified compound



MODIFICATION

Introduction of Functional Polar Groups to Xenobiotics

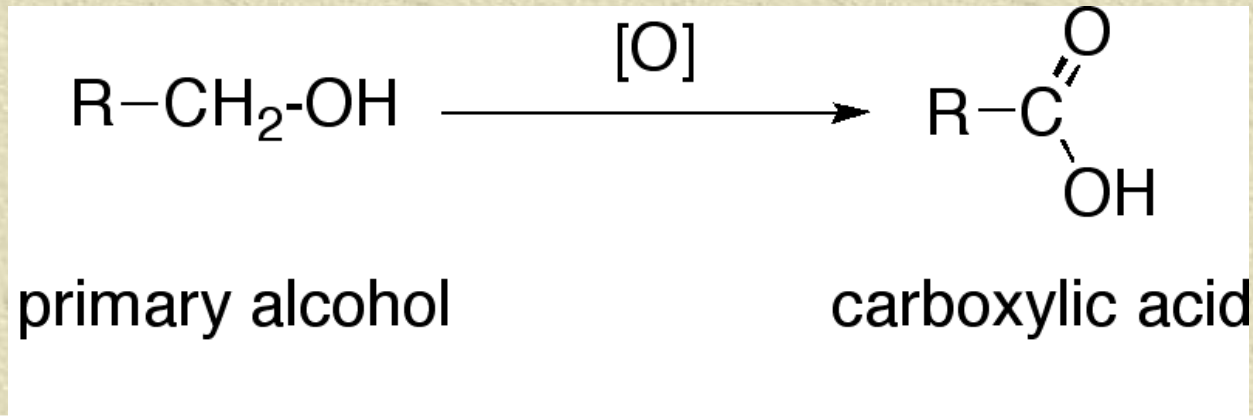




Reduction of Ketones & Aldehydes to Alcohol

UNMASKING of the EXISTING FUNCTIONALITY

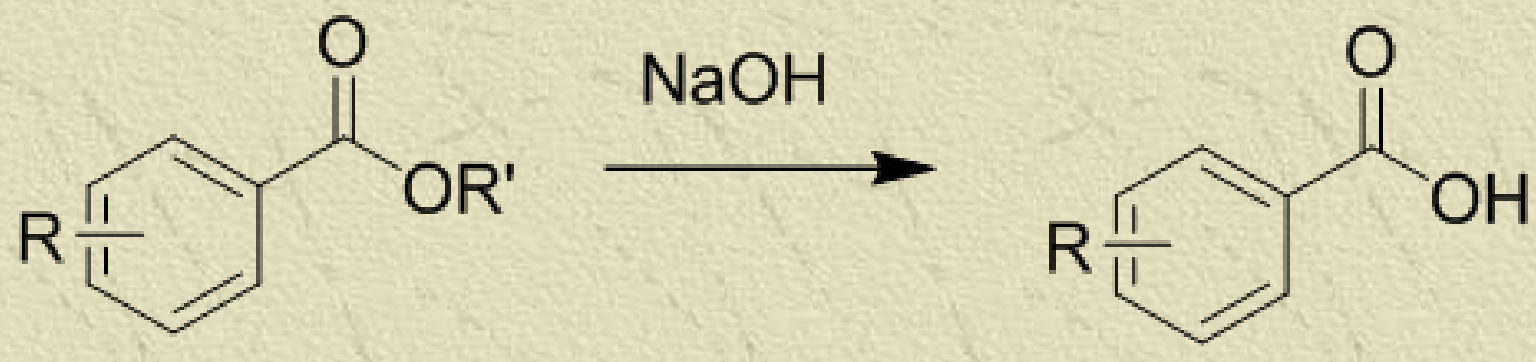
Introduction of Functional Polar Groups to Xenobiotics



Oxidation of Alcohol to Acid

UNMASKING of the EXISTING FUNCTIONALITY

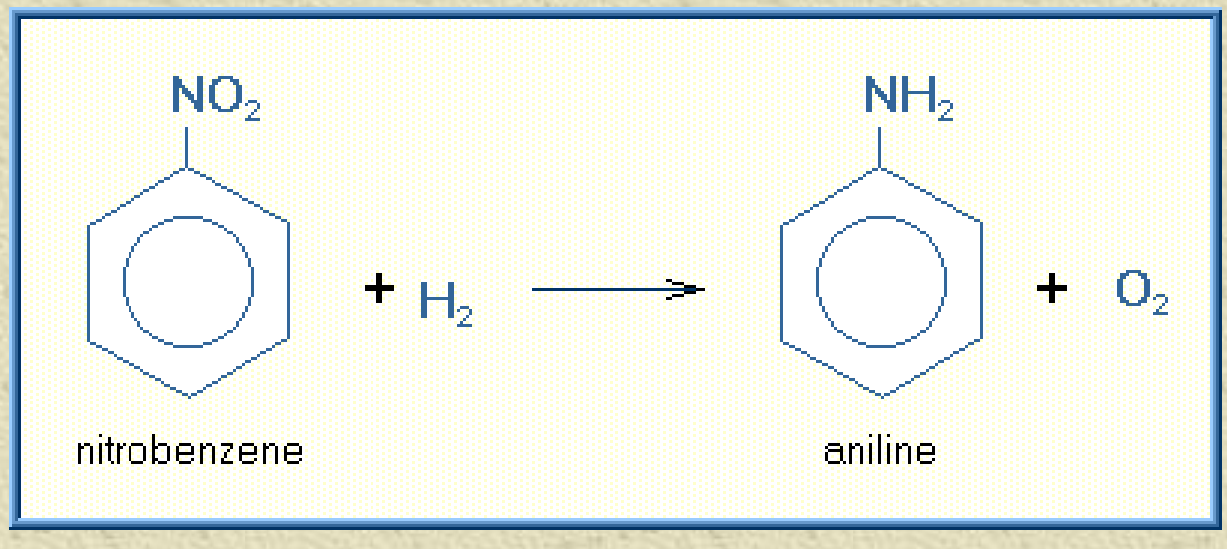
Introduction of Functional Polar Groups to Xenobiotics



Hydrolysis of Ester & Amide to Acid

UNMASKING of the EXISTING FUNCTIONALITY

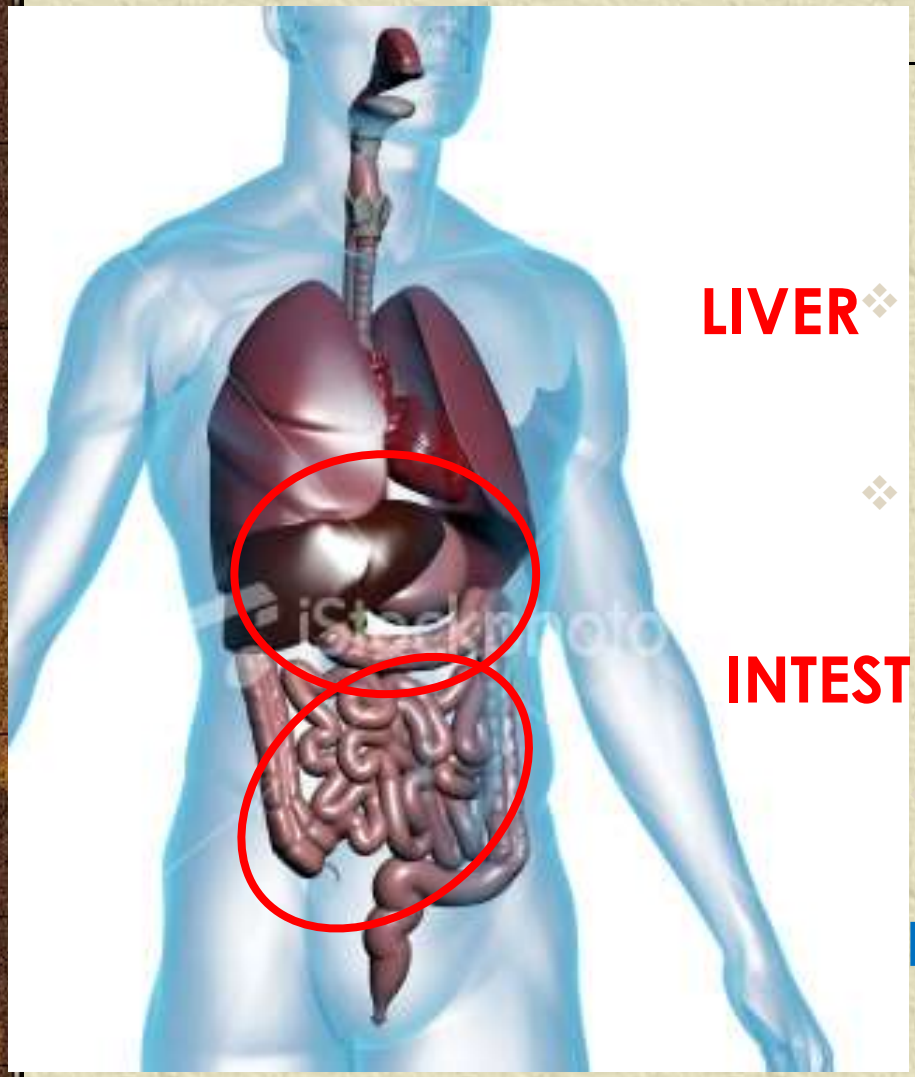
Introduction of Functional Polar Groups to Xenobiotics



Reduction of Nitro compounds to form NH_2 moiety

UNMASKING of the EXISTING FUNCTIONALITY

SITES of DRUG BIOTRANSFORMATION



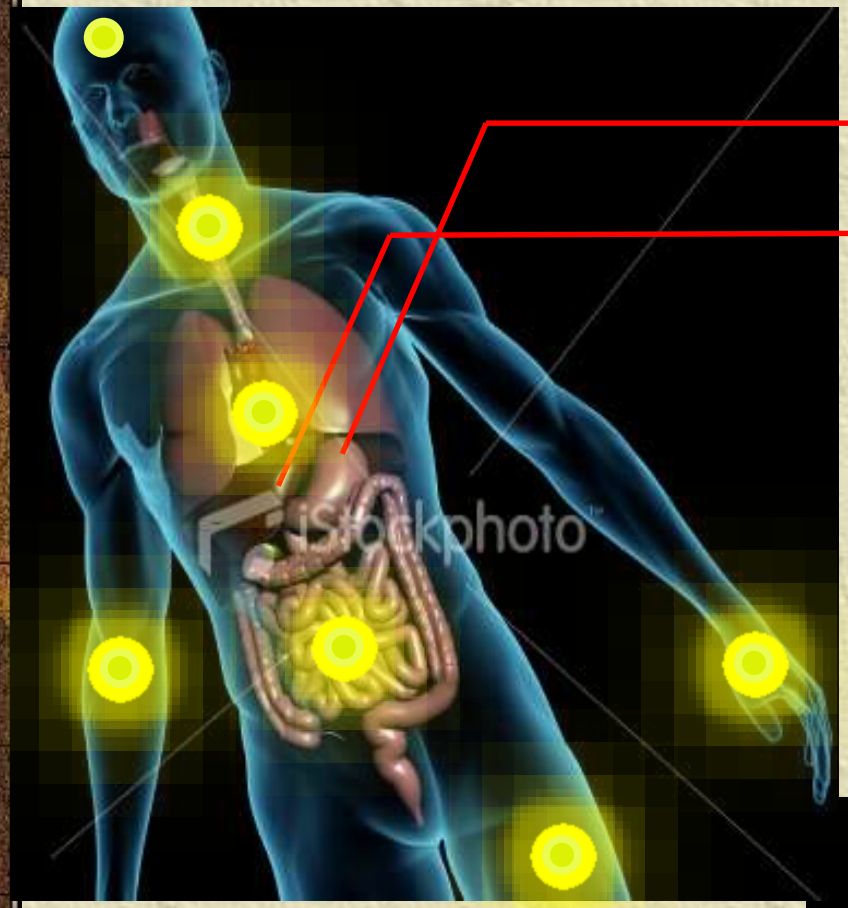
LIVER

- The most important organ in drug metabolism
- Contains almost all drug metabolizing enzymes

INTESTINAL MUCOSA

- Contains **CYP3A4** isoenzyme and **P-glycoprotein**

SITES of DRUG BIOTRANSFORMATION



Absorption site of oral drugs

pass

to bloodstream

through

liver

distributed

into

Body compartments

undergo

HEPATIC METABOLISM

First-Pass Effect

Sites of Biotransformation

- **Liver**
  - Primary site! Rich in enzymes
  - Acts on endogenous and exogenous compounds
- **Extrahepatic metabolism sites**
  - Intestinal wall
    - Sulfate conjugation
    - Esterase and lipases - important in prodrug metabolism

Lungs, kidney, placenta, brain, skin, adrenal glands

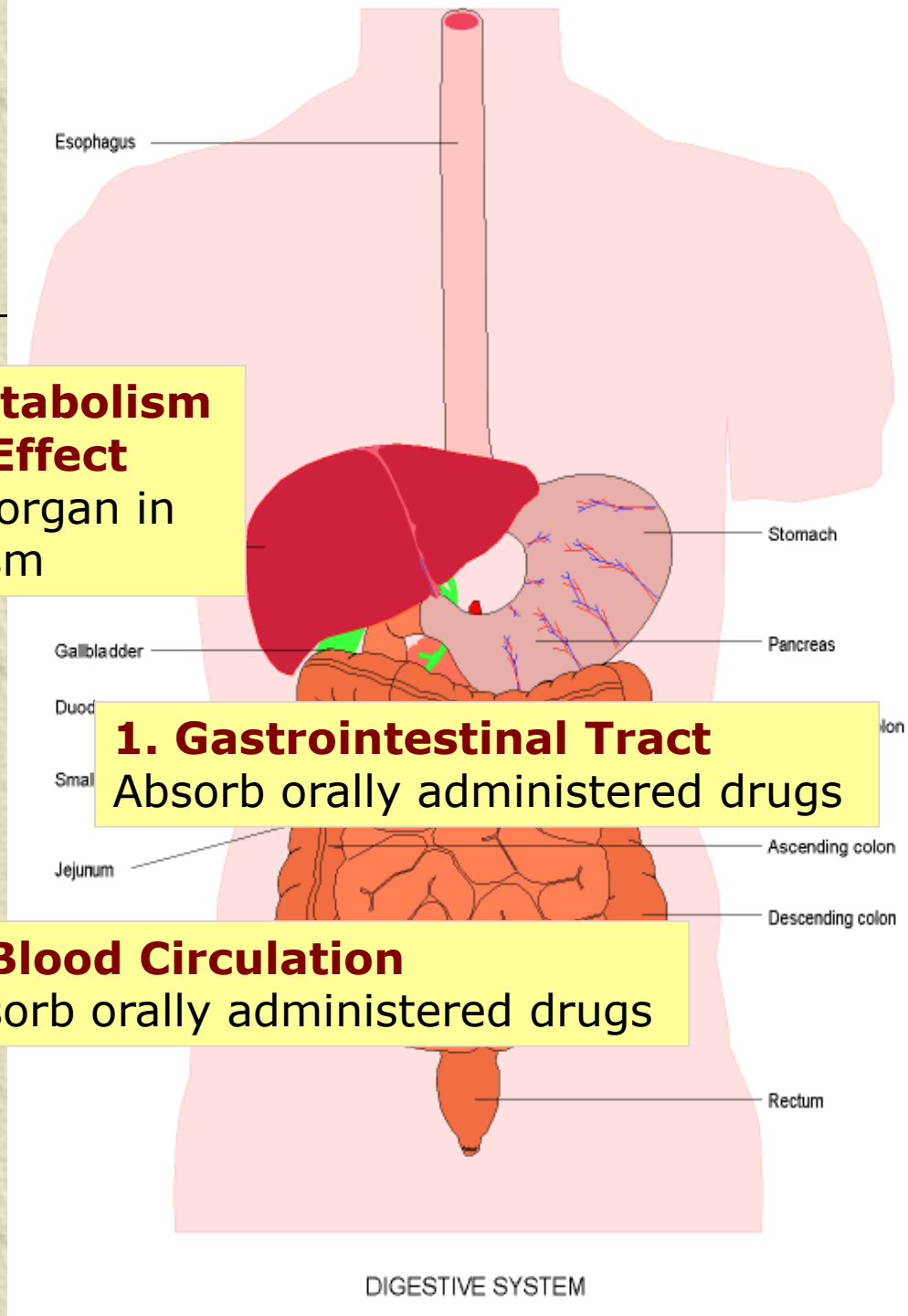
Sites of Drug Biotransformation

2. Liver (hepatic metabolism or First Pass Effect

The most important organ in drug metabolism

Some drugs may decrease oral bioavailability

Isoproterenol
Meperidine
Morphine
Nitroglycerin
Pentazocaine
Propoxyphene
Propranolol
salicylamide



1. Gastrointestinal Tract

Absorb orally administered drugs

3. Blood Circulation

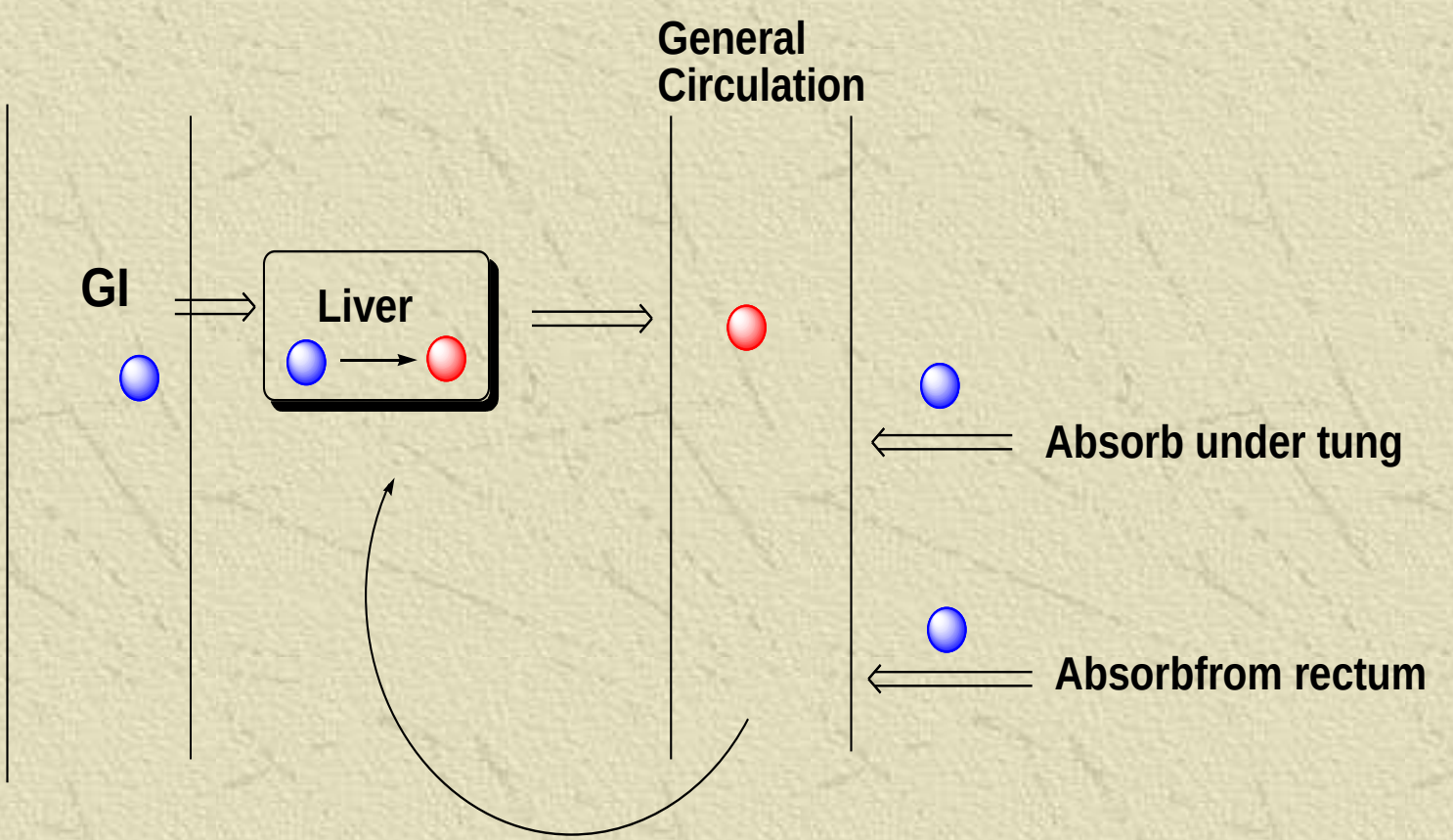
Absorb orally administered drugs

First-pass metabolism

Xenobiotic metabolized before reaching general circulation

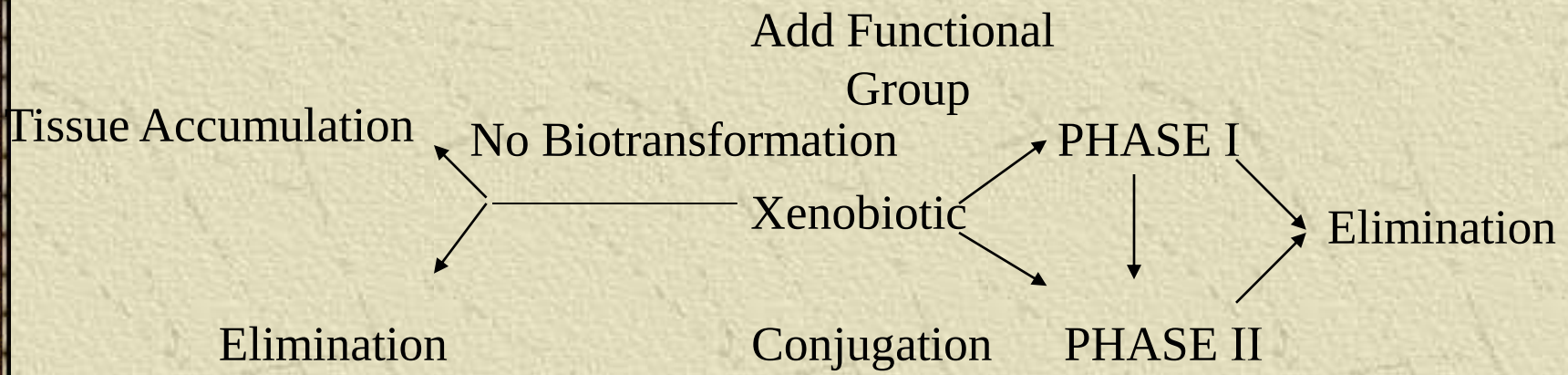
A) Lungs (inhaled substances), Intestinal mucosa, GI bacteria

B)



BIOTRANSFORMATION OF XENOBIOTICS

- The biotransformation is shown schematically as follow:



Phase I reactions

- **Oxidation**
- **Reduction**
- **Hydrolytic reactions (enzymatic hydrolysis)**
- **Dehalogenation**

Purpose

Introduction of polar functional groups in a molecule

- Increases a molecule's polarity
- Provide a functional group or handle on the molecule that can undergo Phase 2 reactions

Oxidation Reaction
PHASE 1 REACTIONS

- Do not produce suficiently hydrophilic or inactive metabolites

Phase II reactions (Conjugation reactions) include:

- Sulphation (sulphate conjugation)
- Glucuronidation (Glucoronic acid conjugation)
- Glutathione or Mercapturic acid conjugation
- Conjugation with Glycine, Glutamine and other Amino Acids
- Acetylation
- Methylation

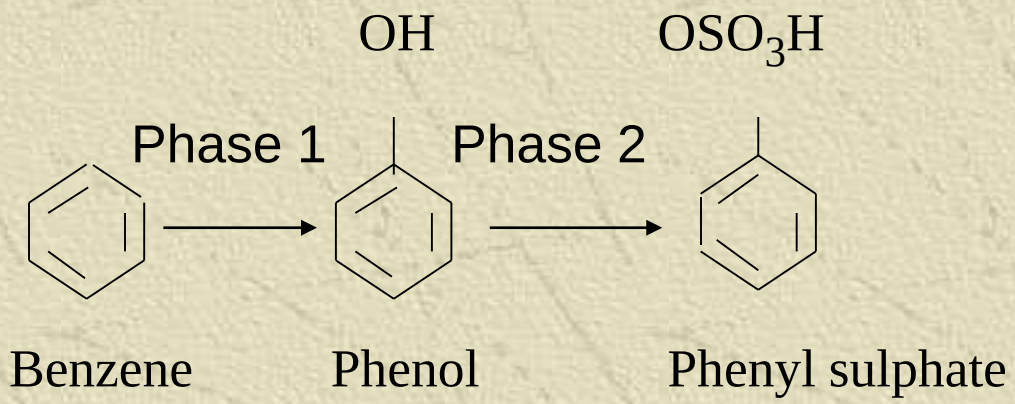
Phase II reactions

♣♣ Conjugation

- **Purpose**
  - **Introduce highly polar conjugates:**
    - ☻☻Glucuronic acid ☻☻ Sulfate
  - **Detoxification**
    - Glycine or other Amino Acids (some solubility), Acetyl , Methylations , Glutathione

BIOTRANSFORMATION OF XENOBIOTICS

Benzene metabolism



- Approximately 30 different enzymes catalyze reactions involved in xenobiotic metabolism. Enzymes involved in biotransformation are sometimes called “drug metabolizing enzymes”.

Phase Ⅰ: Oxidation

1. Hydroxylation

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

- Addition of an oxygen atom or bond
- Require NADH or NADPH and O_2 as cofactors

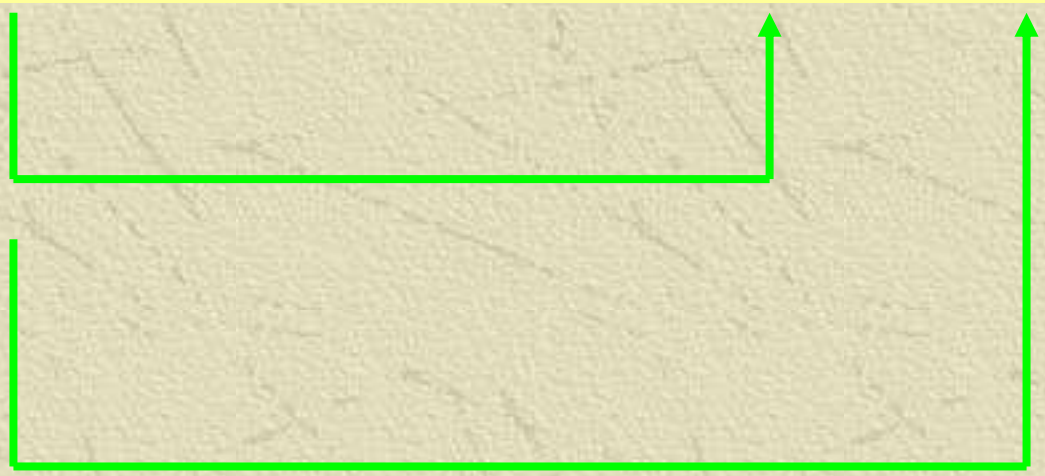
- *RH:* Xenobiotics *R-OH:* Metabolite
- *Enzymes:* The oxidative system is often known as the "mixed function oxidase system".

Cytochrome P450s-dependent monooxygenase

Hydroxylation：O_2

Uses molecular oxygen (O_2). One atom of oxygen is combined with hydrogen to form water, and the other atom of oxygen is introduced into the substrate molecule.

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



Anything that affects the activity of any oxidative enzyme can affect the way the body reacts to a given drug or other xenobiotic.

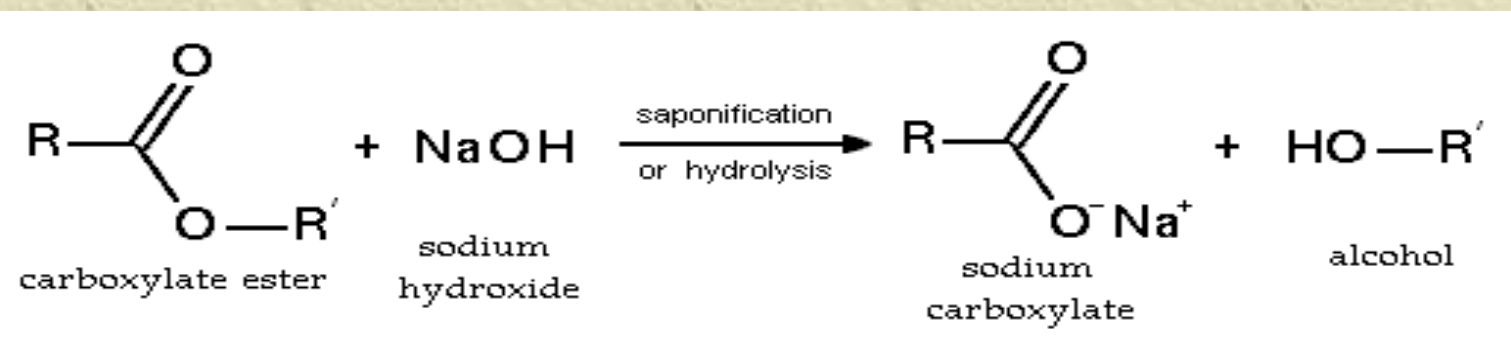
- **Deamination** – replacement of an amine group (NH_2) with an oxygen (O) atom
- **N-, O-, or S-Dealkylation** – replacement of an alkyl group (e.g., CH_3) with a hydrogen atom. Typically, the alkyl group in the parent molecule is bonded to a N, O, or S atom.
- **Aliphatic or aromatic hydroxylation** – addition of a hydroxyl group (OH) to a molecule
- **N-oxidation** – replacement of a hydrogen atom on an amine with an oxygen
- **S-oxidation** – addition of an oxygen atom to a sulfur atom
- **Conversion of a hydroxyl group (alcohol) to a carboxyl group** (acid)

Reduction

- Azo reduction – reduction of an azo bond (N=N) to two amines (NH_2)
- Nitro reduction – reduction of a nitro group (NO_2) to an amine

Hydrolysis

- Addition of water (H_2O) to an ester bond (CO-O-C) to form an alcohol (C-OH) and a carboxylic acid (COOH)



Role of Cytochrome P-450 Monooxygenases in Oxidative Biotransformation

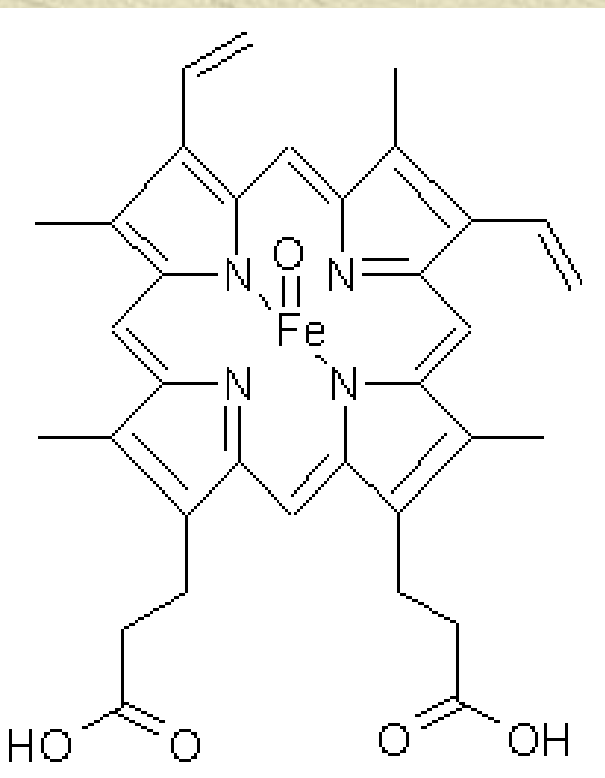
General Equation describing the oxidation of many xenobiotics (R-H) forming a metabolite (R-OH)

$$\underset{\text{substrate}}{R\text{-}H} + \underset{\text{Reducing agent}}{NADPH} + \underset{\text{Molecular O2}}{O_2} + H^+ \longrightarrow R\text{-}OH + NADP^+ + H_2O$$

- Mixed Function in the biotransformation with Monooxygenases
- Requires both molecular and a reducing agent
- Enzyme responsible for transferring an oxygen atom to the substrate is called **Cytochrome P-450**

What is Cytochrome P-450

structure



Important features:

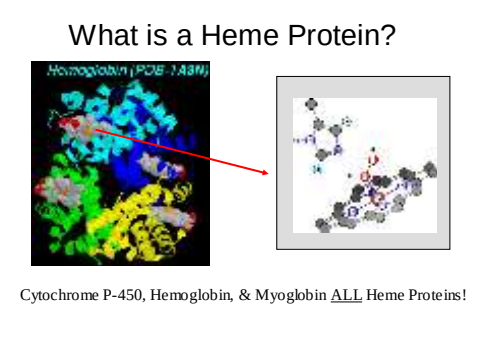
- plays a vital role in oxidation of lipophilic xenobiotics
- metabolize almost unlimited number of diverse substrates by a variety of oxidative transformations.
- **located in the endoplasmic reticulum**

Cytochrome P450s-dependent monooxygenase

CYP or Cytochrome P-450

★ Heme proteins

★ Iron containing porphyrin - binds O2



★ The name cytochrome P450 is derived from the spectral properties of this hemoprotein → in its reduced (ferrous, Fe2+) form, it binds CO to give a complex that absorbs light maximally at 450 nm

- After homogenization and fractionation of the cells, this enzyme system is isolated in the so-called **microsomal fraction** and very often they are named **microsomal enzymes** (enzymes isolated by disruption of the liver cells).
  - **The liver** (**Endoplasmic reticulum**) has the **highest concentration** of this enzyme (**cytochrome P-450**), although it can be found in other tissues.

Cytochrome P-450

- Endoplasmic reticulum - microsomes when disrupted
- Enzymes are **membrane bound**
- Explains why lipophilic drugs are processed
- Catalytic process → heme binds O_2

Microsomal drug oxidations require:

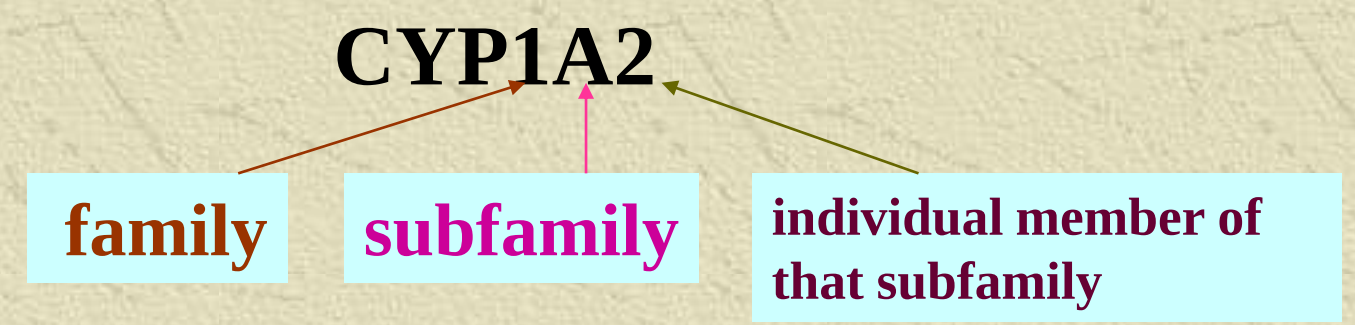
cytochrome P450

cytochrome P450 reductase

NADPH & O_2

Cytochrome P450: Isozymes

- **Isozymes** - multiple forms of an enzyme
- **Supergene family**
  - More than **8,000** P450 genes as of November/2007
  - More than **368** gene families, **814** subfamilies
  - Human: ***18 families, 43 subfamilies, 57 sequenced genes***
- **Nomenclature**



Cytochrome P450

- Approximately **50%** of the ingested drugs are metabolized by isoforms of **cytochrome P450**.
- These enzymes also act on various carcinogens and pollutants.
- One important feature of cytochrome P-450 is its **inducibility**. Thus, treatment of an animal with certain substance may lead to an increase in the synthesis of one or more isozymes of cytochrome P-450 (phenobarbital etc.).

CYP(gene family)(subfamily)(individual gene)
CYP1A2: metabolizes caffeine
CYP3A4: most abundant CYP with broad substrate-specificity
CYP2E1: metabolizes acetaminophen and ethanol

- Most CYPs are located in the liver ER (microsomes).
- CYPs are heme-containing proteins
- CYPs play key roles in biosynthesis or catabolism of steroid hormones, bile acids, fat-soluble vitamins, fatty acids and eicosanoids.

CYP1A Family

CYP1A1:

1. Organ: Lung/intestine
2. Substrates: polycyclic arylhydrocarbons (PAH), estradiol, prostaglandins

CYP1A2:

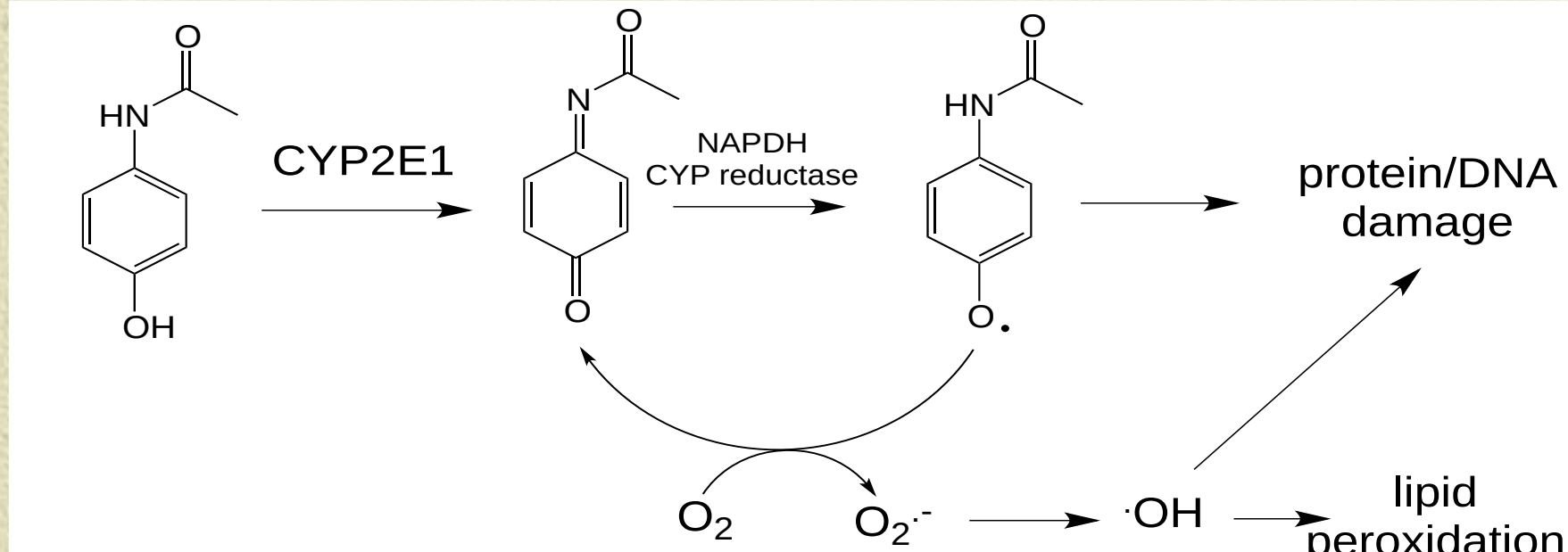
1. Organ: liver
2. Substrates: aromatic amines (e.g. caffeine)

CYP2E1

Organ: Liver

Substrates: **alcohol (ethanol), benzene, caffeine, Tylenol**

Inducers: ethanol



Leads to hepatocellular necrosis and liver damage

CYP3A4

Organ: Liver, small intestine

Substrates: aflatoxin, benzo(a)pyrene and other PAHs

CYP3A4 is the major CYP in human liver.

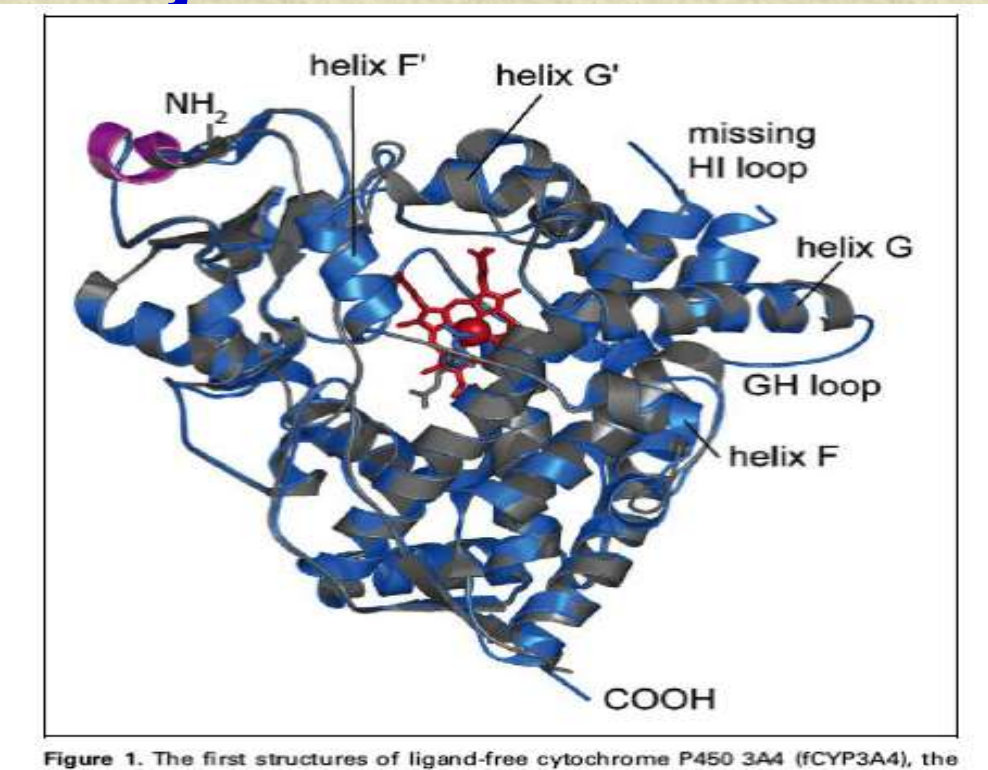


Figure 1. The first structures of ligand-free cytochrome P450 3A4 (fCYP3A4), the

Flavin-containing Monooxygenase (FMO)

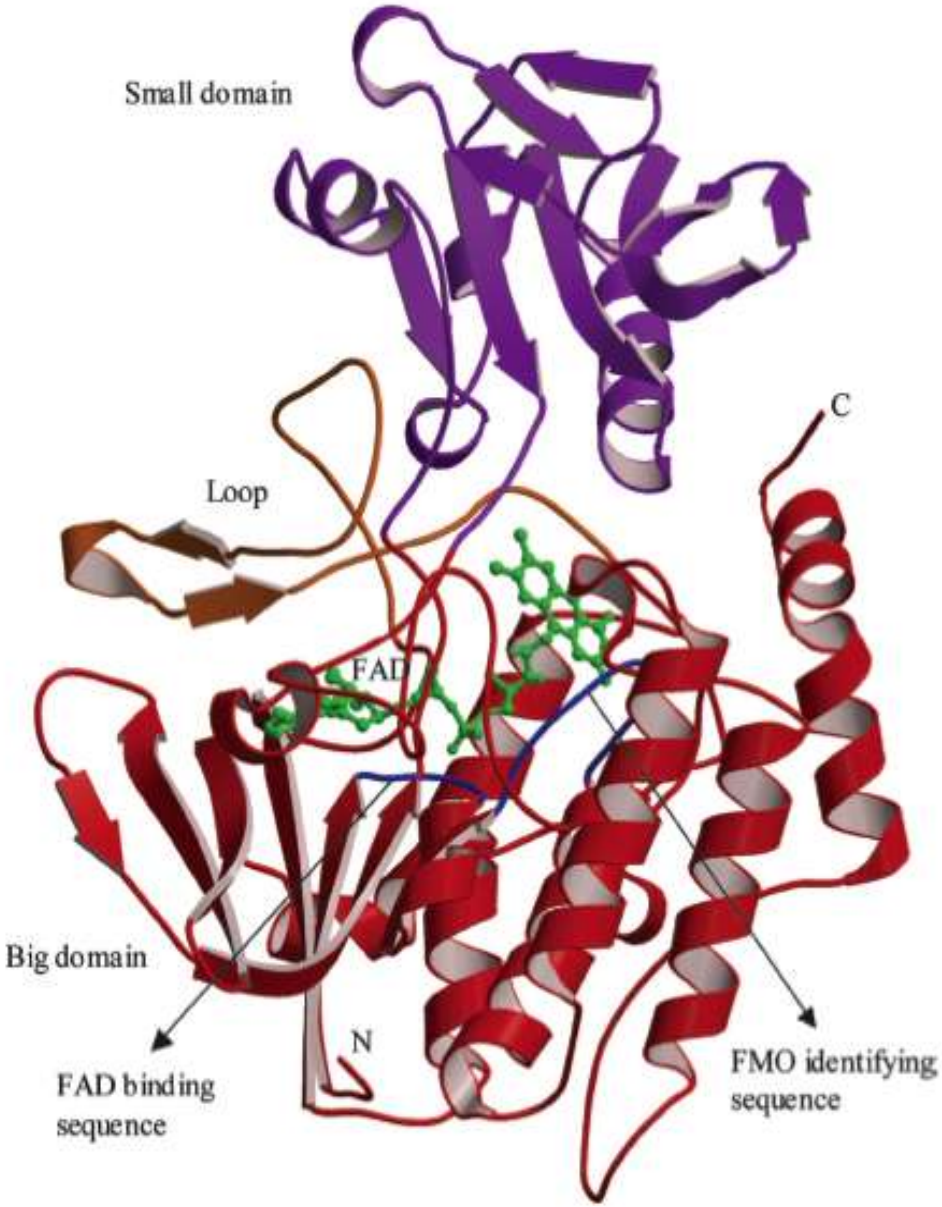
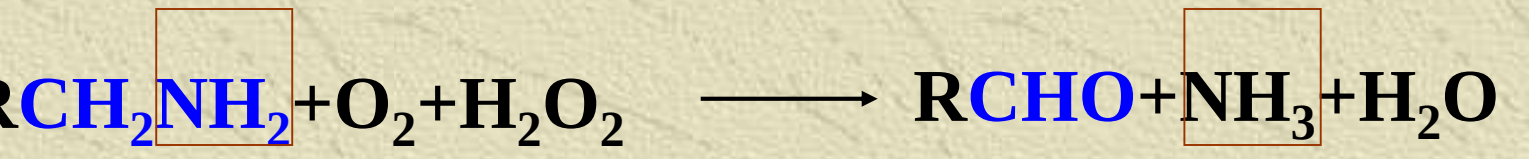


Fig. 2. Ribbon representation of the protein and ball-and-stick model of FAD. The strand–turn–helix motifs and the loop interlinking the two domains are labeled. FAD is in the large domain and has no interaction with the small domain.

- FMO's oxidize **nucleophilic nitrogen, sulfur and phosphorus heteroatoms** of a variety of xenobiotics.
- FMO's are **not** inducible and are constitutively expressed.
- Can be inhibited by other substrates.
- Located in microsomal fraction of liver, kidney, and lung.

Non-microsomal enzymes (Phase I)
Monoamine oxidase, MAO; Diamine oxidase, DAO



★ **MAO catalyze the oxidative deamination of monoamines.**

★ Oxygen is used to remove an amine group from a molecule, resulting in the corresponding aldehyde and ammonia.

★ MAO are found bound to the outer membrane of mitochondria in most cell types in the body. They belong to protein family of flavin containing amine oxidoreductases.

ADH and ALDH

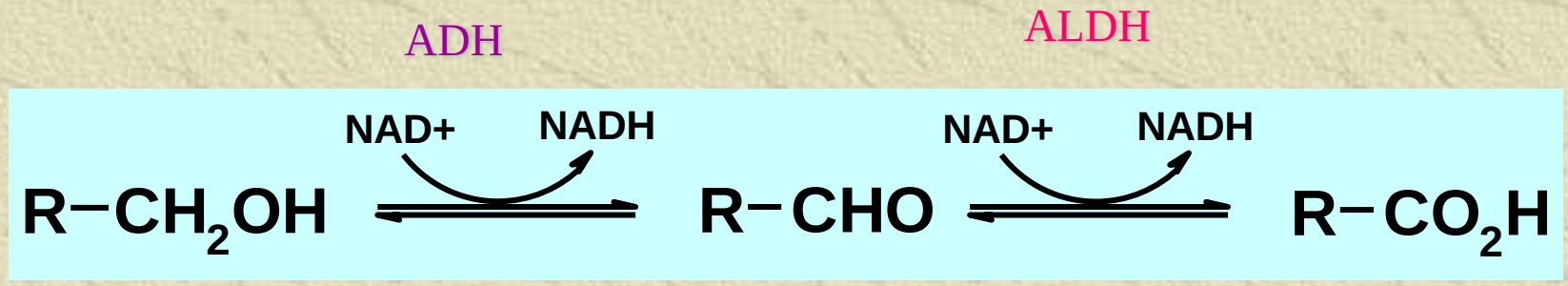
ADH Alcohol Dehydrogenase

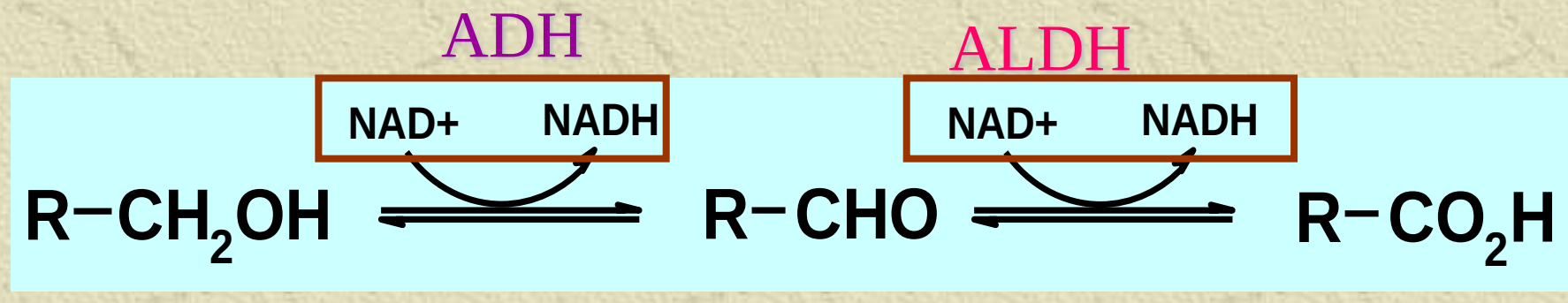
ALDH Aldehyde Dehydrogenase

- Alcohol Dehydrogenase belongs to the oxidoreductase family of enzymes.
- High concentrations within the liver and kidney.

Function

- The primary and most common role of **ADH** in humans is to detoxify incoming **ethanol** by converting it into **aldehyde**.
- The resulting **aldehyde**, a more toxic molecule than ethanol, is quickly converted into **acetate** by **aldehyde dehydrogenase** (**ALDH**) and other molecules easily utilized by the cell.





During this reaction, hydrogen is removed from the alcohol and transferred to a molecule called nicotinamide adenine dinucleotide (NAD), converting it to reduced NAD (NADH).

NADH participates in numerous other metabolic reactions, passing on the hydrogen to other compounds or electron transfer chain.

Absorption

- Soluble in water
- Small size - penetrates everywhere, easily crosses all bio membranes
- Rapidly absorbed from GI

80 %

small intestine

Blood until metabolized

- In people who consume alcohol at moderate levels and/or only occasionally, most of the alcohol is broken down by ADH and ALDH.

- after higher alcohol consumption, the **MEOS** plays a role in alcohol metabolism.

$$CH_3CH_2OH + NADPH + O_2 + H^+ \xrightarrow{MEOS} CH_3CHO + NADP^+ + 2H_2O$$

$$CH3CHO \xrightarrow{ALDH} \mathbf{CH3COOH}$$

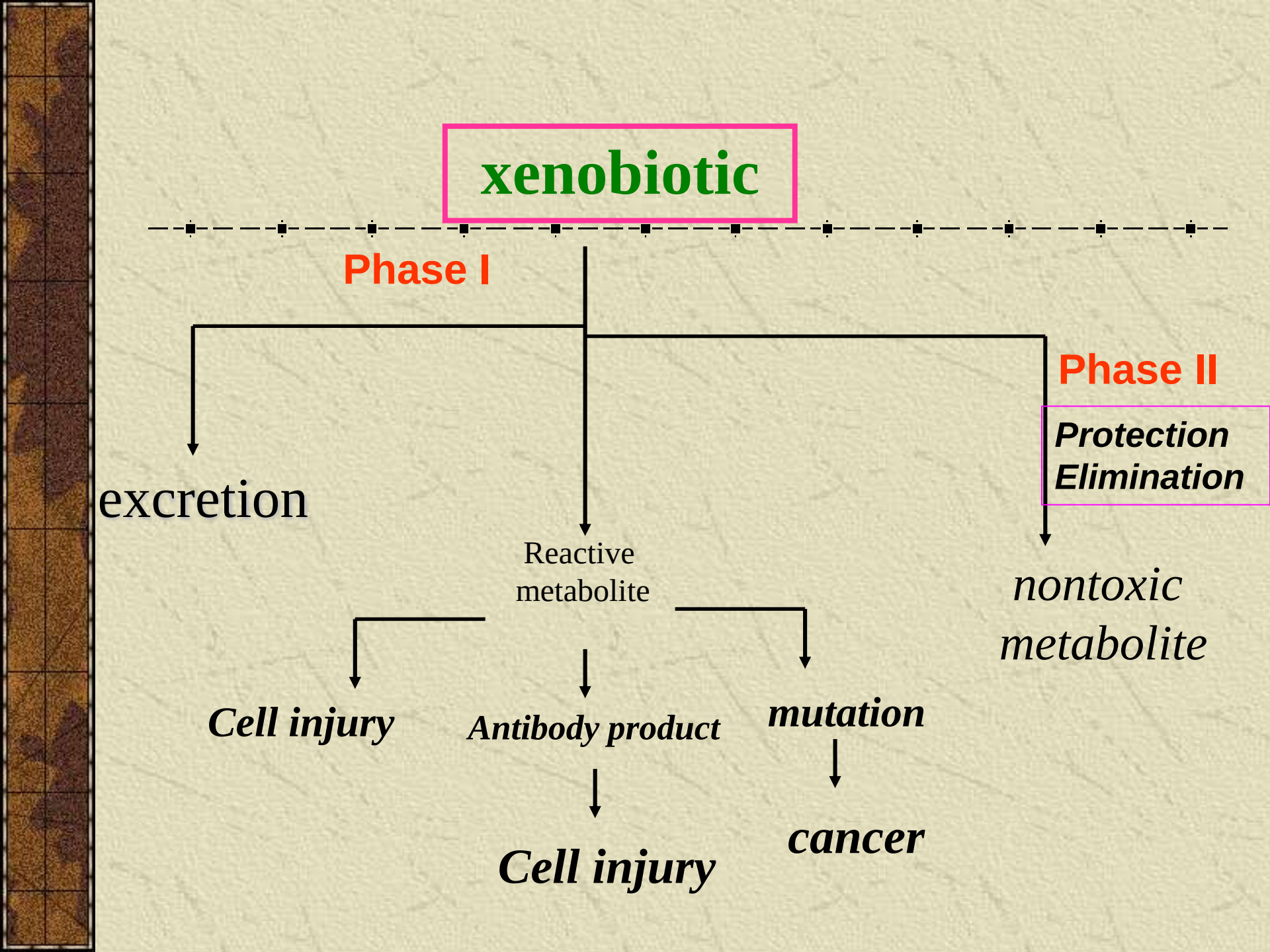
MEOS: Microsomal Ethanol-Oxidizing System, is also called Cytochrome P450-dependent Microsomal Ethanol Oxidizing System. Converts alcohol to acetaldehyde

MEOS metabolize not only alcohol but also other compounds (certain drugs). Enhanced MEOS activity resulting from high alcohol consumption also can alter the metabolism of those drugs.

This may contribute to harmful interactions between alcohol and those drugs or otherwise influence the activity of those medications.

Phase II: Conjugation

- In phase I reactions, xenobiotics are generally converted to more polar, hydroxylated derivatives.
- In phase II reactions, these derivatives are **conjugated with molecules** such as glucuronic acid, sulfate, or glutathione.
- This renders them even **more water-soluble**, and they are eventually **excreted in the urine or bile.**

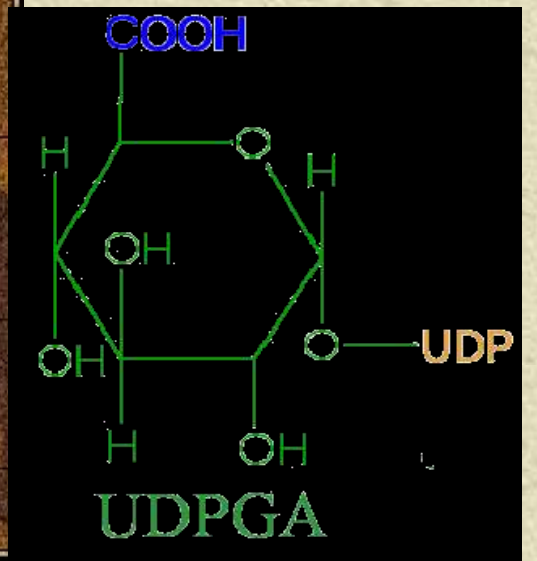

xenobiotic
Phase I
Phase II
Protection
Elimination
excretion
Reactive
metabolite
nontoxic
metabolite
Cell injury
Antibody product
mutation
cancer
Cell injury

Phase II reactions

- **Involve addition of a cofactor to a substrate to form a new product**. Therefore, the rate of these reactions can be limited by the availability of the cofactor.
- Phase II enzymes may be either **microsomal** or **cytosolic**. This is because the primary purpose of the Phase II reactions is not so much to increase the polarity of the parent compound (although that is part of what they accomplish). **The primary purpose is to increase the molecular weight of the parent compound to make it a better substrate for active transport mechanisms in the biliary tract**.

1. Glucuronidation

- One of the major Phase II enzymatic pathways. *Replacement of a hydrogen atom* with a glucuronic acid
- **UDP(Uridine diphosphate)-glucuronic acid (UDPGA)** is the glucuronyl donor
- UDP-glucuronyl transferases (UGT), present in both the endoplasmic reticulum(ER) and cytosol, are the catalysts.
  - Liver, lung, kidney, skin, brain and intestine

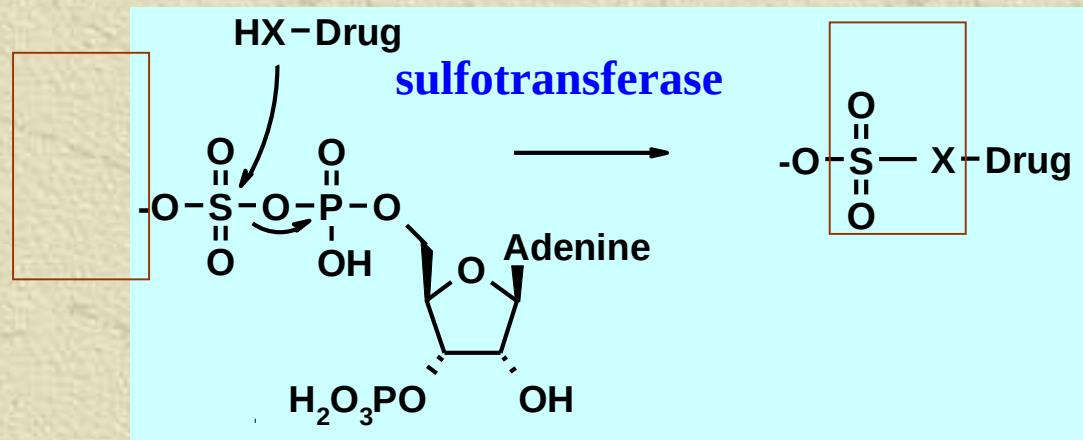


- Attachment sites are hydroxyls
- Alcohols, phenols, amines, enols, N-hydroxyls, sulfides, acids

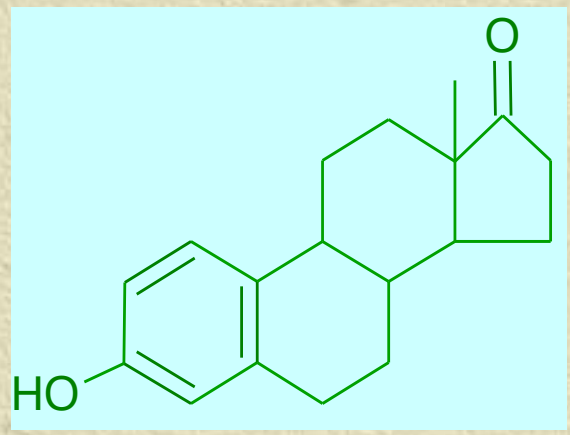
2. Sulfate Conjugation

- Some alcohols, arylamines, and phenols are sulfated.
- Catalyzed by **sulfotransferases**
  - liver, kidney and intestine
- **Sulfate donor:** adenosine 3'-phosphate-5'-phosphosulfate (PAPS); this compound is called "active sulfate."
- Leads to inactive water-soluble metabolites
- **Glucuronate conjugation often more competitive process**

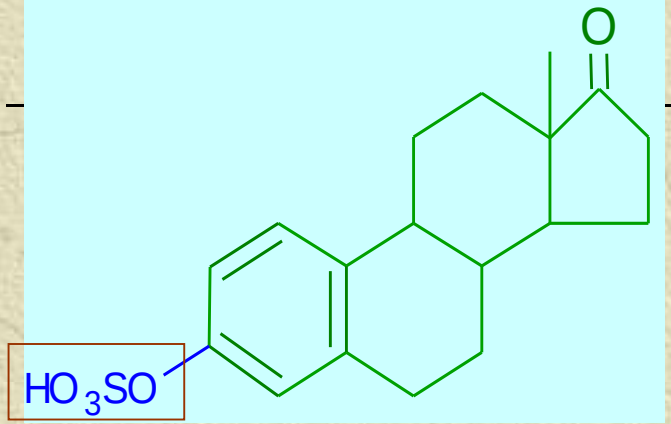
PAPS



Sulfate Conjugation



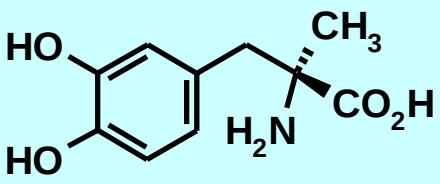
+PAPS



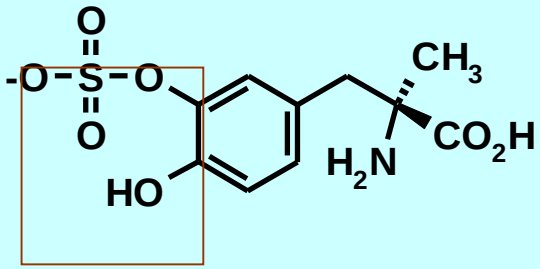
+PAP

estrone

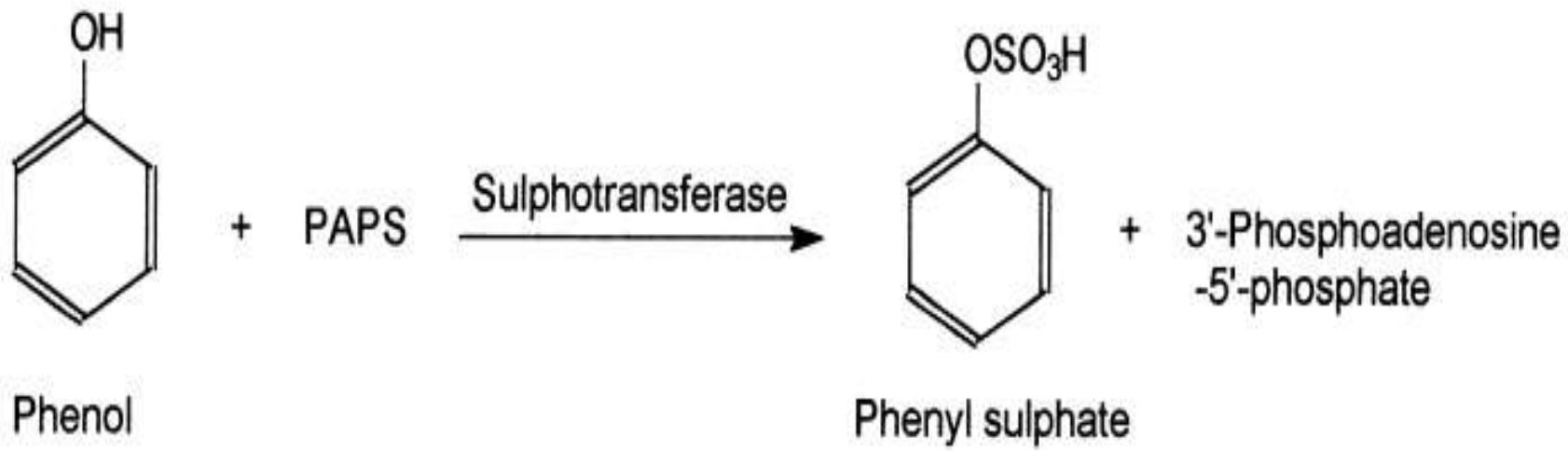
estrone sulfate



alpha-Methyldopa
Aldomet® - Merck
Antihypertensive



- ***Replacement of a hydrogen atom (H)*** with a **sulfonate (SO_3^-)**
- Produces a highly water-soluble sulfuric acid ester



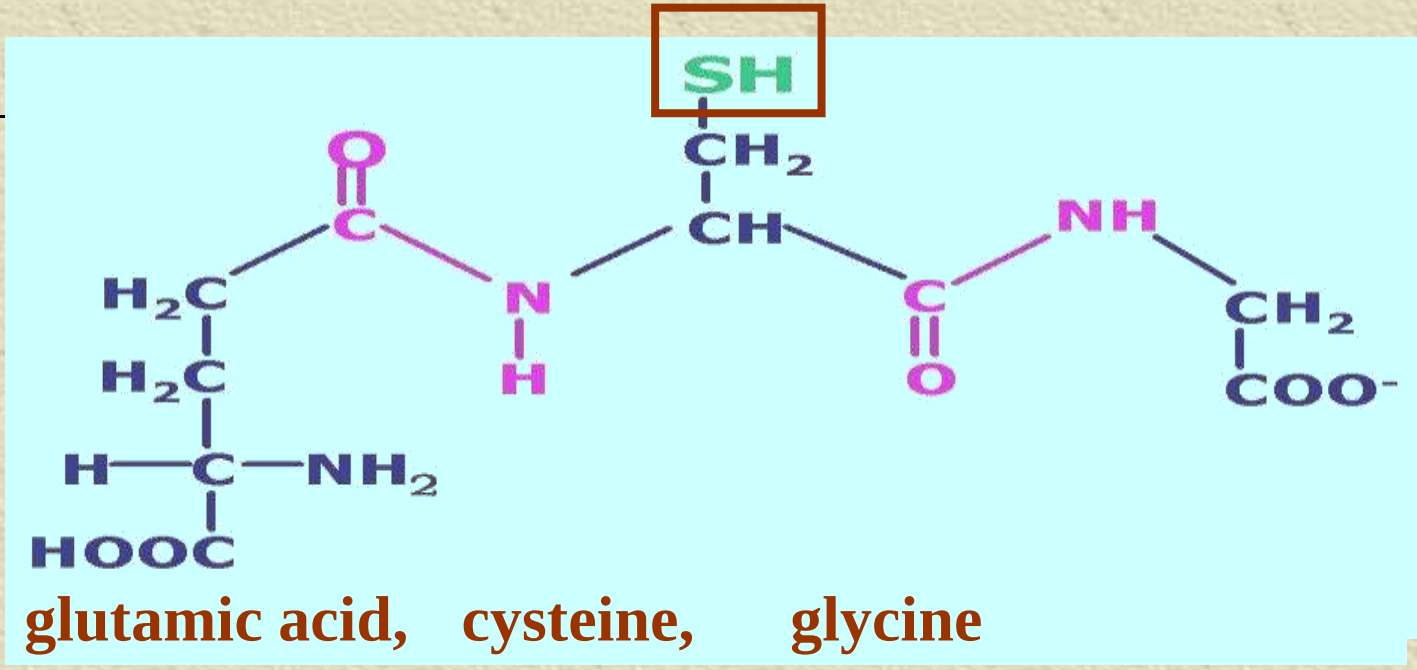
Conjugation of a phenol and an aliphatic alcohol with sulphate. PAPS is the sulphate donor, phosphoadenosinephosphosulphate.

- **Phase II reactions.** The addition of **sulfate moiety** to a hydroxyl group is **a major route of conjugation** for foreign compounds, and also **endogenous compounds**, such **as steroids**.

Glutathione conjugation

- Adds a **glutathione** molecule to the parent compound, either by direct addition or by replacement of an electrophilic substituent (e.g., a halogen atom)
- Uses the enzyme **glutathione transferase** (**GST**)
- Uses the cofactor called **glutathione** **One of the major Phase II enzymatic pathways**

3. Conjugation with glutathione



glutamic acid, cysteine, glycine

$$R + GSH \xrightarrow{GST} R\text{-}S\text{-}G$$

where R= an electrophilic xenobiotics

R: **epoxides and halogenides**

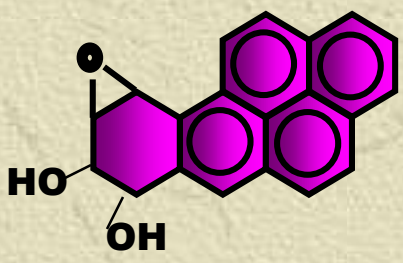
GST: **Glutathione S-Transferases (Liver and kidney)**

Glutathione (GSH) Conjugation

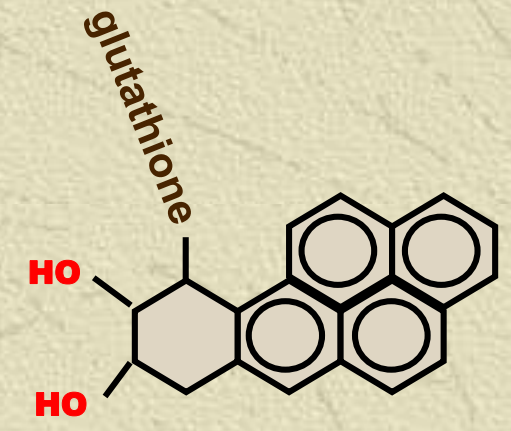
- **DETOXIFICATION** of **electrophiles!**
- Electrophilic chemicals cause:
  - Tissue necrosis
  - Carcinogenicity
  - Mutagenicity
  - Teratogenicity
- The thiol (SH group) ties up potent electrophiles

Glutathione S-transferase

(+)benzo[a]pyrene-
7,8-dihydrodiol-
9-10-epoxide



GST
+ glutathione



DNA reactive;
lung and skin
tumors

Inactive

DETOXIFICATION

4.Acetylation

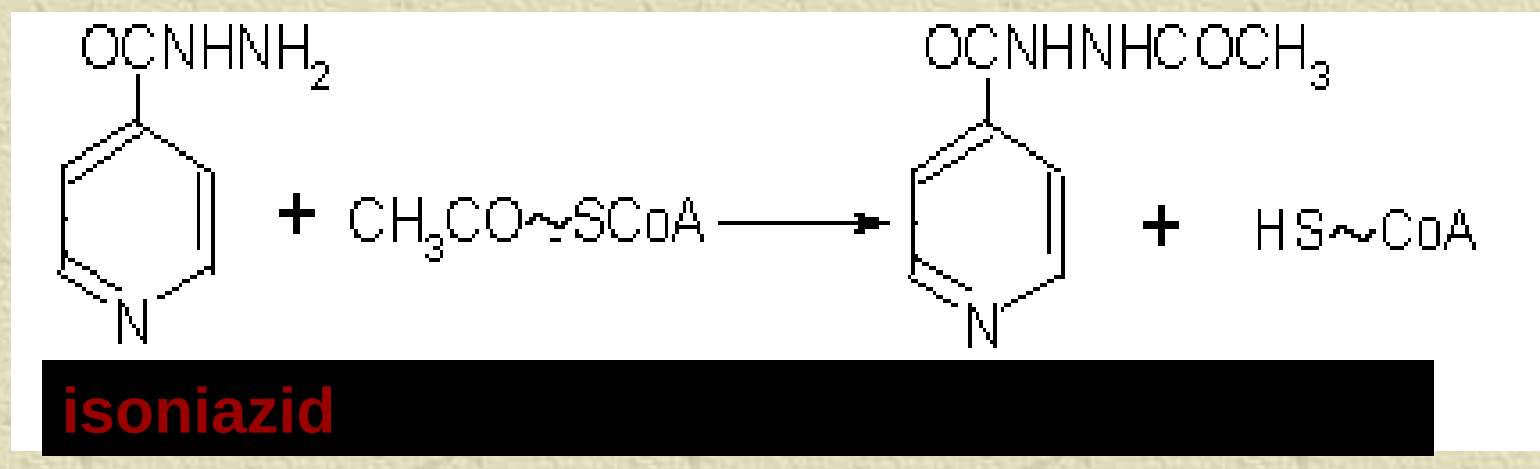
- ***Replacement of a hydrogen atom*** with an **acetyl group**
- Uses the enzyme **acetyltransferase**
- Uses the cofactor called **acetyl CoA** (acetyl coenzyme A)
- Sometimes results in a less water-soluble product

4. Acetylation

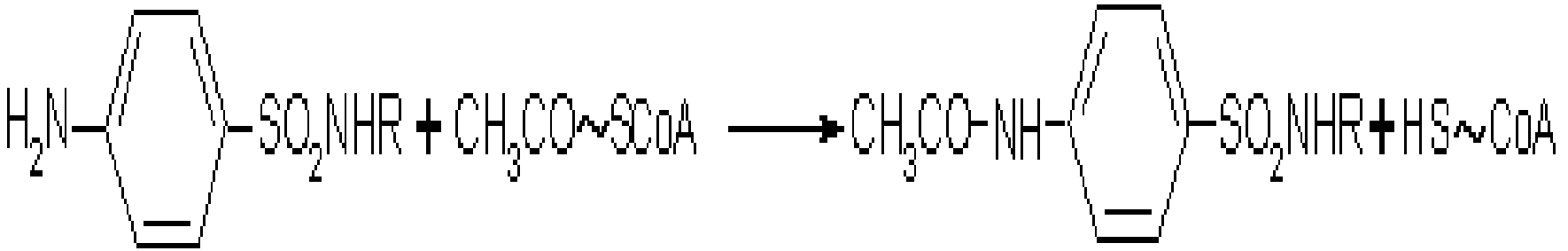
X + Acetyl-CoA - - - - >Acetyl-X + CoS

where X represents a xenobiotics.
(for: **aromatic amines)**

• Enzyme: acetyltransferases - cytosol of various tissues, particularly in liver.



isoniazid



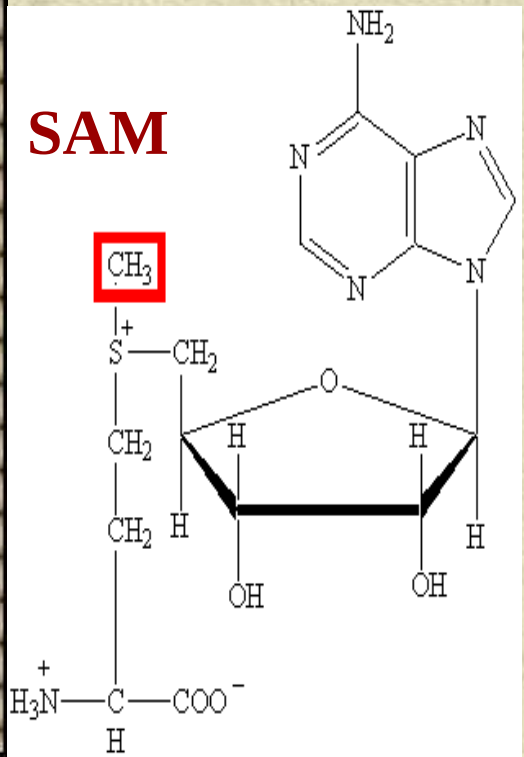
sulfanilamide

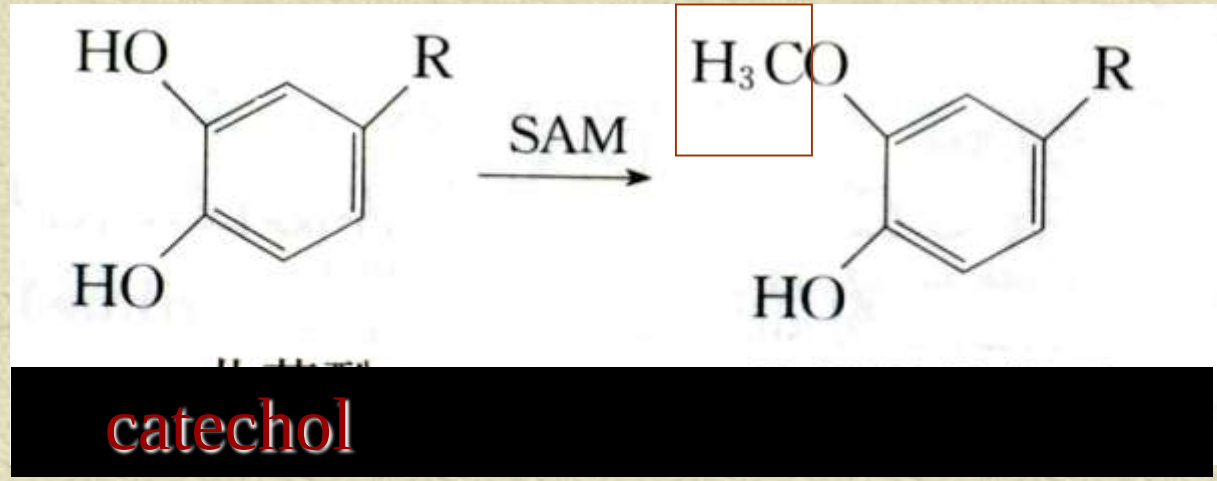
- **Important for drugs with primary amino groups**
- Generally, metabolites are nontoxic and inactive
- **Acetylation does NOT increase water solubility**
- Detoxification or termination of drug activity

5. Methylation

- ***Replacement of a hydrogen atom*** with a **methyl group**
- Uses the enzyme **methyltransferase**
- Uses the cofactor called **SAM** (**S-adenosyl methionine**)
- Common but relatively minor pathway

A few xenobiotics are subject to methylation.





Metabolism via Methylation

- **Important in the inactivation of physiologically active biogenic amines → neurotransmitters**
  - **norepinephrine, dopamine, serotonin, histamine**
- ***Minor*** pathway in the metabolism of drugs
- **Methylation does NOT increase water solubility**
- Most methylated products are inactive

- **Amino acid conjugation**
  - Adds an **amino acid** to the parent compound.
- **Mercapturic acid formation**
  - Formed by cleavage of the glycine and glutamic acid substituents from a glutathione conjugate, followed by N-acetylation of the resulting product

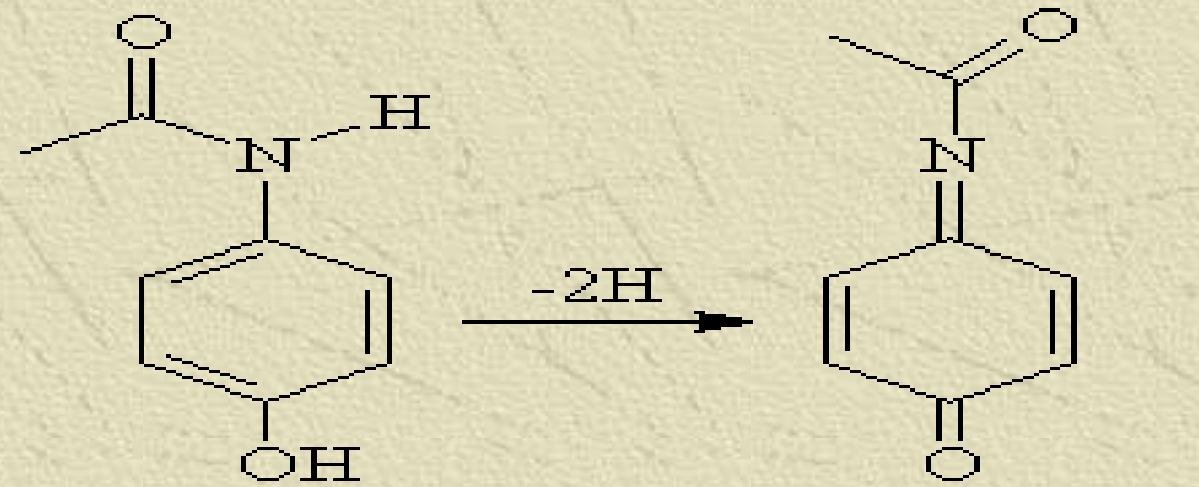
Significance of Biotransformation Reactions in Toxicology

- Biotransformation is a major part of the pathway for elimination of many xenobiotic compounds.
- Biotransformation can result in either a **decrease** ↓ or an **increase** ↑ (or no change) in toxicity.
- Biotransformation can result in the formation of reactive metabolites.

Example – metabolism of acetaminophen

- Acetaminophen is metabolized in the liver by **sulfation** and **glucuronidation** to form non-toxic conjugates
- These are low capacity pathways, in that the cofactors are available in only limited concentrations, so these are rate-limiting.
- As long as the amount of acetaminophen in the liver is relatively low, the Phase II pathways can handle the compound, and there is no toxicity.
- If the concentration of acetaminophen becomes high enough to overwhelm the capacity of the Phase II pathways, an alternate metabolic pathway, involving Phase I enzymes, becomes active.

- **The product of the Phase I reaction is a highly reactive quinoneimine**, which can bind covalently to cellular macromolecules, especially proteins.
- The binding of the reactive intermediate to cellular macromolecules destroys the activity of those molecules, and can lead to compromised cell function and, ultimately, cell death.



Another good example – metabolism of **carbon tetrachloride**

- Carbon tetrachloride is metabolized by the cytochrome P-450 system in the liver by abstraction of one of the four chlorine atoms.
- This results in formation of a highly reactive **trichloromethane radical**, which initiates a cascade of **lipid peroxidation** by removing a hydrogen atom from membrane phospholipids.
- Damage to the cell membrane causes loss of osmotic integrity, cell swelling and death.