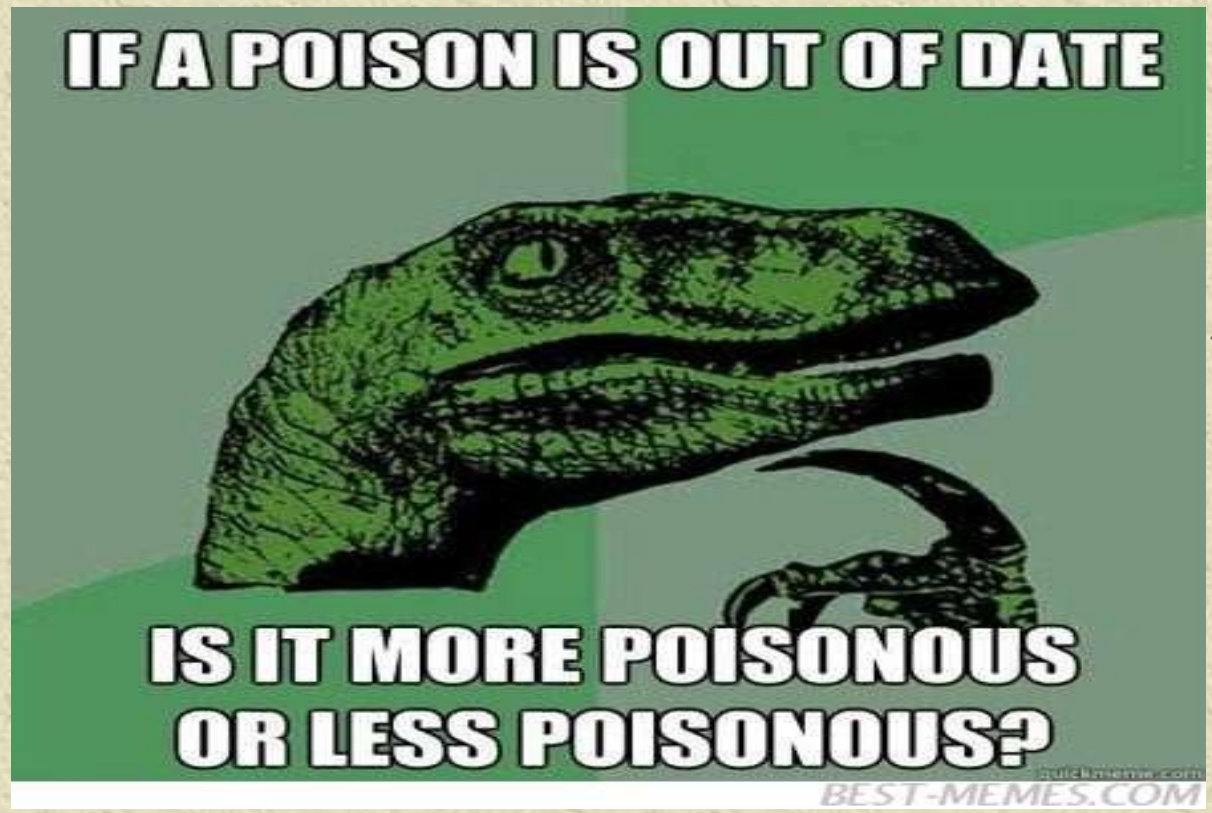
GENERAL



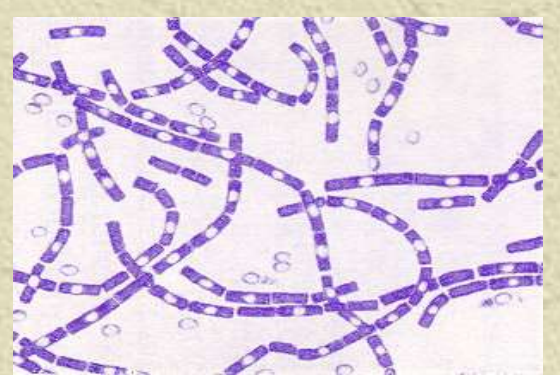
Лектор: доц. д-р В. Данчева, дм

Toxicology is a scientific and medical discipline for the adverse effects of the poisons.

Phytotoxins

Zootoxins

Bacteriotoxins



BASIC CONCEPTS

- **Poison is any solid, liquid or gas that through either oral or topical routes can interfere with life processes in the organism**.
  - So, as poison could be defined any agent capable to produce a noxious response in a biologic system, seriously injuring function or producing death.
  - In other hand, every known chemical has the potential to produce injury or death if present in a sufficient amount.

Toxicology Terminology

- **Toxicants** – substances that produce adverse biological effects of any nature
  - ✓ May be chemical or physical in nature
  - ✓ Effects may be of various types (acute, chronic, etc.)
- **Toxins** – specific proteins produced by living organisms (mushroom toxin or tetanus toxin)
  - ✓ Most exhibit immediate effects
- **Poisons** – toxicants that cause immediate death or illness when experienced in very small amounts

Paracelsus (1493-1541) '**Grandfather of Toxicology**'

"All substances are poisons;

there is none which is not a poison.

The right dose differentiates a poison from a remedy."

"**The dose makes the poison**"

Organic toxins	❖Substances that were originally derived from living organisms ❖Contain carbon and often are large molecules ❖Can be synthesized (that is man-made) as well as be obtained from natural sources
Inorganic toxins	❖Specific chemicals that are not derived from living organisms (minerals) ❖Generally small molecules consisting of only a few atoms (NO_2)

Basic concepts

- **Toxicity is potential for a compound to produce injury in biological system**.
  - Usually, the word **"toxicity"** is used to describe the nature of adverse effects.
  - The toxicity is **usually** expressed **as milligrams (mg) of the substance per kilogram (kg)** of body weight that will produce defined biologic effects.

BASIC CONCEPTS

- **The dose** is the total amount of poison received per organism (person or animal).
- **The lethal dose (LD)** is the lowest dose that causes death in any animal during the period of observation (usually 14 days). Various percentages can be attached to the LD value to indicate doses required to kill **1% (LD_1), 50% (LD_{50})** or **100% (LD_{100})** of test animals.
- **Median lethal dose (LD_{50})** or (MLD) is a commonly used measure of toxicity.
- **The lethal concentration (LC)** is the **lowest concentration** of compound in the air that causes death.
  - It is **expressed as milligrams of compound per meter cubic** of the air.

Types of doses in Toxicology

- **Exposure dose** – the amount of a xenobiotic encountered in the environment
- **Absorbed dose** – the actual amount of the exposed dose that enters the body
- **Administered dose** – the quantity administered usually orally or by injection
- **Total dose** – the sum of all individual doses

Effective dose

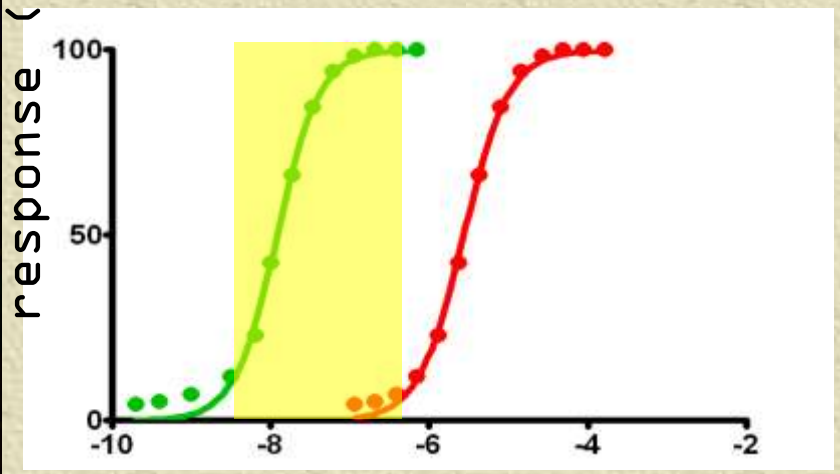
- **Effective doses** (EDs) are used to indicate the **effectiveness** of a substance. Normally, effective dose refers to a **beneficial effect** (**relief of pain**). It might also stand for a **harmful** effect. Thus the specific endpoint must be indicated.

Therapeutic Index/Ratio (TI)

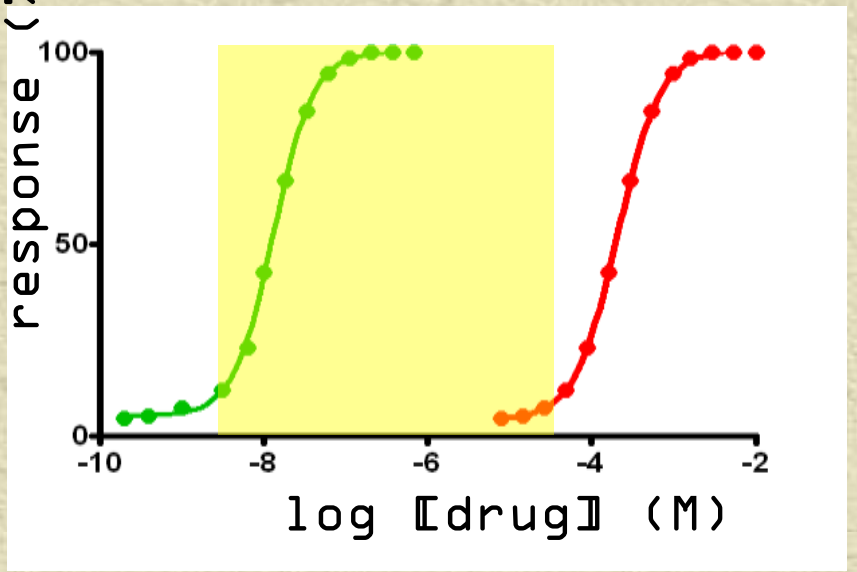
- TI (or Window) measures "**how safe a drug is**" or "**Margin of Safety**".
- **High Therapeutic Index = safe**
- **Low Therapeutic Index = not so safe**
- **The larger the ratio, the safer the drug**

Therapeutic Index (TI)

The ratio of the dose of the drug that produces an unwanted (toxic) effect to that producing a wanted (therapeutic) effect.



Small TI: e.g. warfarin



Large TI: e.g. penicillin, aspirin

Toxicity of compounds

Classification	Toxicity
Extremely toxic	< 1 mg/kg
Highly toxic	1 - 50 mg/kg
Moderately toxic	50 - 500 mg/kg
Slightly toxic	0.5 - 5 g/kg
Practically nontoxic	5 - 15 g/kg
Relatively harmless	> 15 g/kg

BASIC CONCEPTS

- **Acute poisoning** is a term that describes the biologic effects of a **single high dose** of the poisons or multiple doses during 24-hour period.
- **Sub-acute poisoning –** 1 month repeated doses
- **Fulminant poisoning**
- **Sub-chronic poisoning** – 1-3 months repeated doses
- **Chronic poisoning** (>3 months) - **repeated** (**prolonged**) **exposure** to relatively low doses of the poisons. The ratio of the acute to chronic LD_{50} doses is the **chronicity factor.**

$$Chronicity\ factor = \frac{Acute\ LD_{50}}{90\ day\ LD_{50}}$$

Systemic and organ toxins

- **A systemic toxin** is one that affects the entire body or many organs rather than a specific site
- **An organ toxin** is one that affects only specific tissues or organs

Adverse Drugs Reactions (ADRs)

ADRs are noxious or unintended responses occurring at **therapeutic** doses (WHO definition) ~ 5% of all acute hospital admissions

Type A (**augmented**) ADRs	Effects are: · related to known pharmacology, but undesirable · common, dose-related · predictable	Examples · haemorrhage with anticoagulants · respiratory depression with opioids · sedation with older antihistamine drugs
Type B (**bizarre**) ADRs	Effects are: · unrelated to known pharmacology · rare · unpredictable · often idiosyncratic	Examples · anaphylaxis with penicillin · allergic liver damage by halothane · bone marrow suppression by chloramphenicol · individual allergy/genetic basis

Factors determining adverse effects

- Intrinsic toxicity
- Dose
- Exposure conditions
- Response of host

Intrinsic toxicity

- **Chemical properties**
  - Molecular structure & functional groups
  - Solubility – Insolubility
  - Volatility
  - Stability (light, water, acids, enzymes….)
  - Reactivity
- **Physical properties**
  - Gas (density….)
  - Liquid (vapour pressure….)
  - Solid (crystal structure, size, shape….)

Routes of Exposure

gastrointestinal tract (ingestion)

lung (inhalation)

skin (topical, percutaneous or dermal)

Injection (s.c., i.v., i.m., i.p. bite, puncture, cut)

- The **nature and magnitude** of **toxic effects** depend **on many factors,** among which are:
  - **Physicochemical properties of the substance**
  - **Biotransformation**
  - **Condition of exposure** (time, temperature etc.)
  - **Presence of bioprotective mechanism** (antioxidant systems etc.)

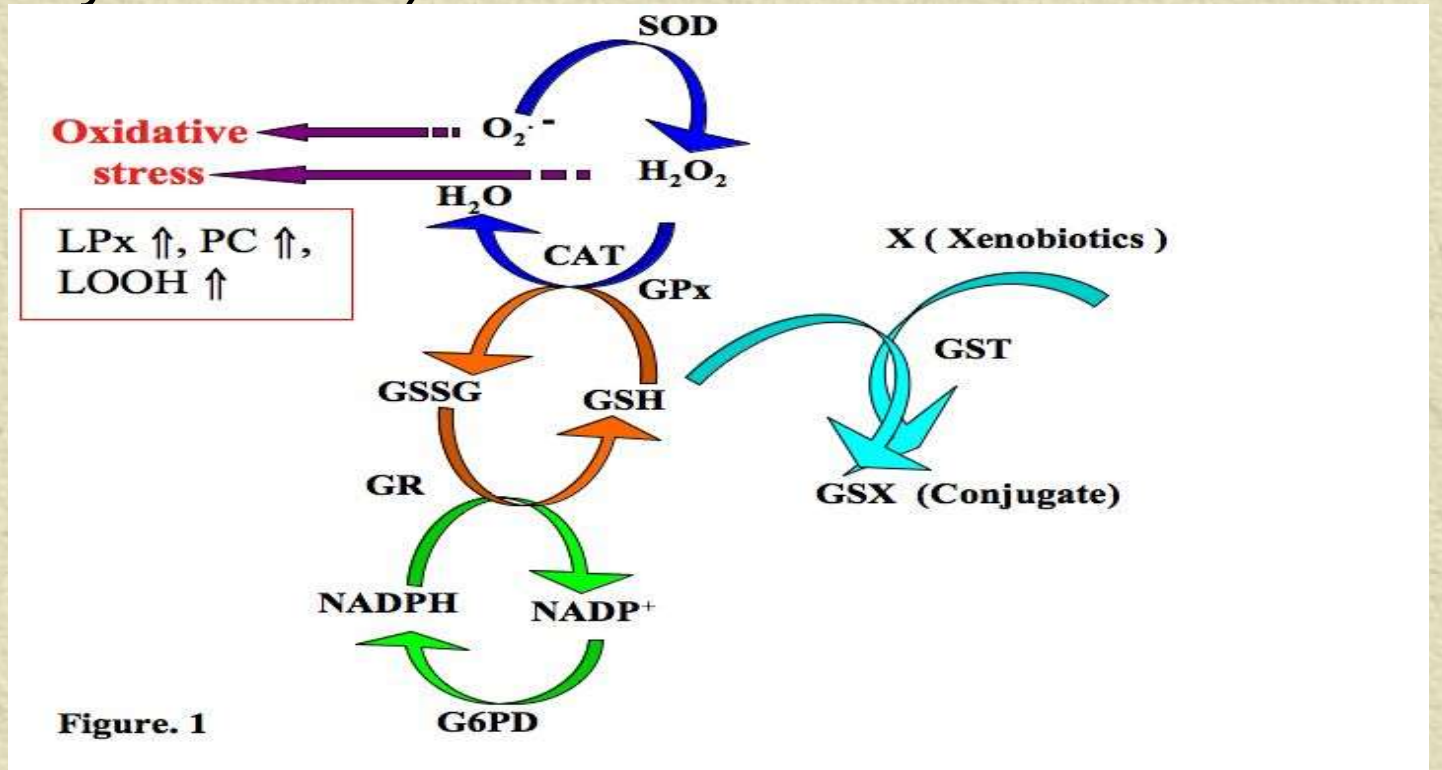
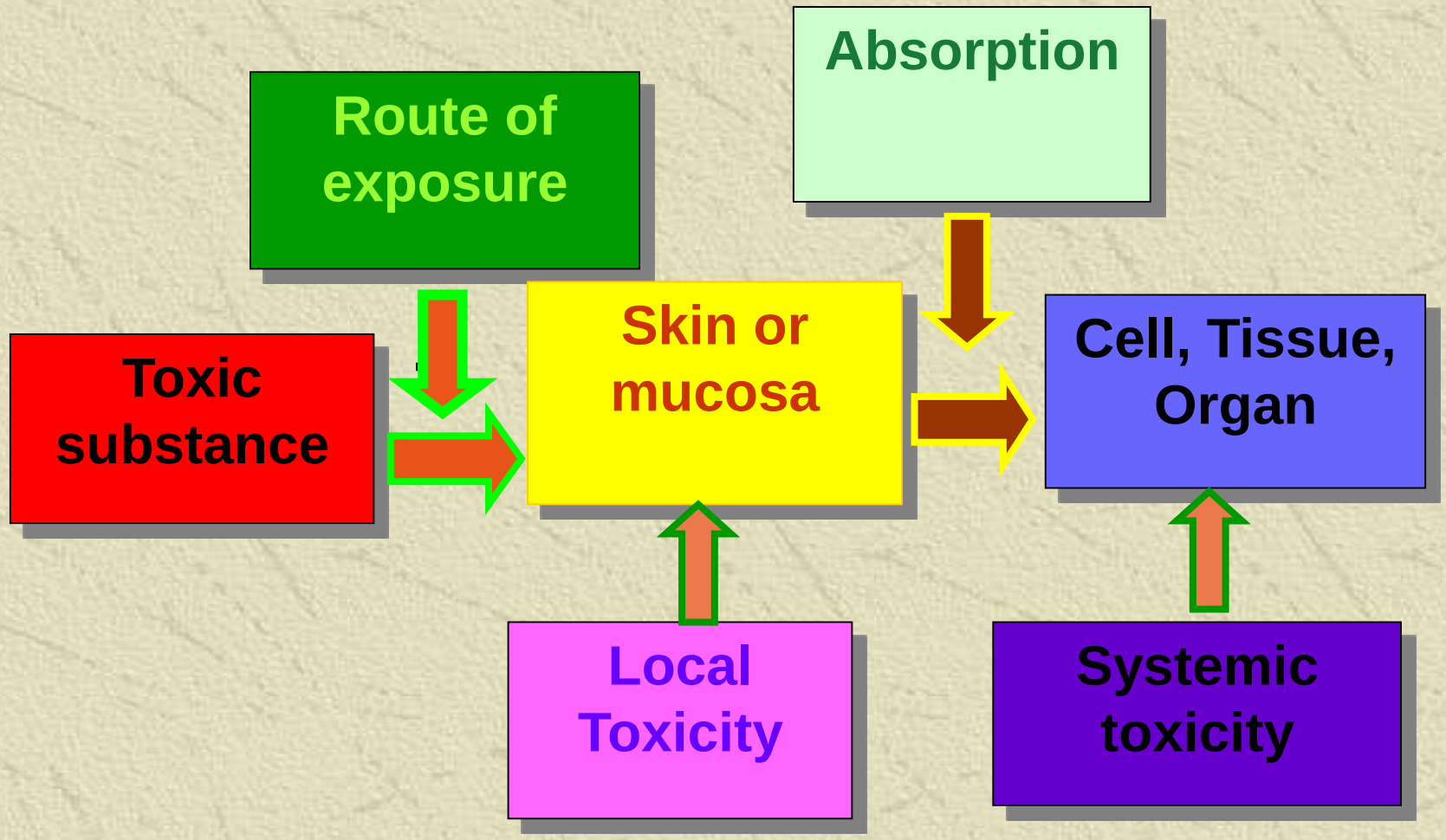


Figure. 1

- Prolonged exposure to a compound may allow people to develop **tolerance** to the poison. In this case the size of the dose producing lethality upon repeated exposure increases.



The induction of toxic effects largely depends on the disposition of the substances concerned.

Interaction of a substance with a living organism

Kinetic Phase

absorption, distribution, **metabolism,** and **excretion** → the fate of substance in the body

the body has a number of defense mechanisms at various levels of the kinetic phase, metabolism & excretion

Dynamic Phase

interactions of the toxicant within the organism and describes processes at organ, tissue, cellular, and molecular levels

BASIC CONCEPTS

- **Toxicokinetics is the movement and disposition of poisons in the organism (ADME)**
  - **A**bsorption;
  - **D**istribution of chemical within the body;
  - **M**etabolism (Biotransformation);
  - **E**xcretion;
- A chemical absorbed into the bloodstream is distributed throughout the body, including the site where it produces damage.
  - This site is usually the **target organ** or **target tissue**.
  - A chemical may have **one or several target organs**, and in turn, **several chemicals** may have the **same target structure**.

NATURE OF TOXIC EFFECTS

- The major mechanisms of action of drugs and chemicals are:

1. **Inflammation** - frequently local response to irritant chemicals or components of systemic tissue injury. - The inflammatory response may be **acute** or **chronic**.
2. **Necrosis** - this is death of tissue or cells, resulting from a variety of pathological processes: **corrosion**, **severe hypoxia**, **membrane damage**, **reactive metabolite binding**, **inhibition of protein synthesis** and **chromosome injury**.
3. **Enzyme inhibition** by chemical, which may **inhibit biologically vital pathway**.

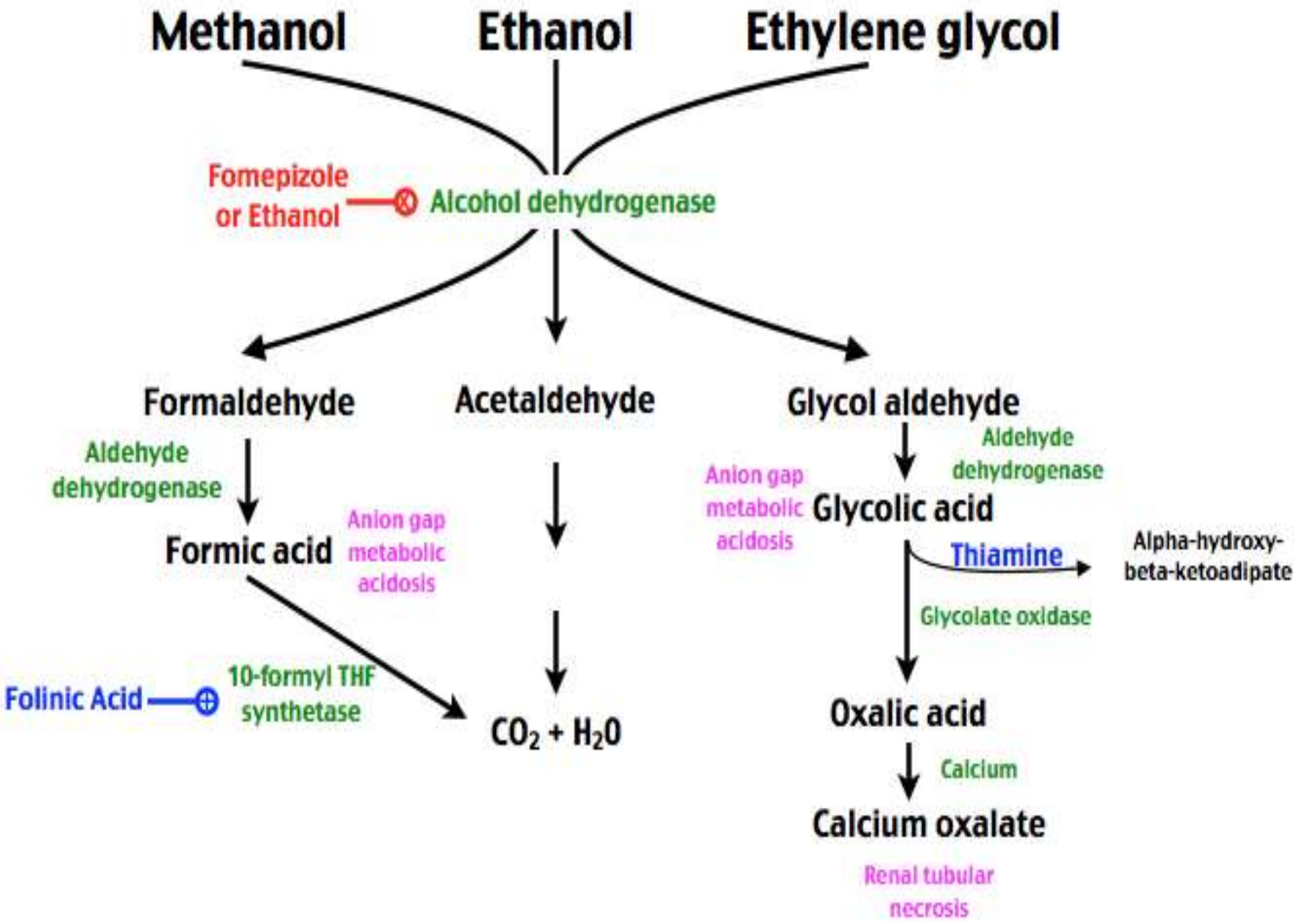
NATURE OF TOXIC EFFECTS

4.Biochemical uncoupling of the synthesis of **high-energy phosphate molecules**.

- In this case the electron transport continues and results **in excess liberation of energy as heat**.

5.Lethal synthesis occurs when foreign substances of close structural similarity to normal biological substances **metabolize** to a **toxic product**.

Toxic Alcohol Metabolism



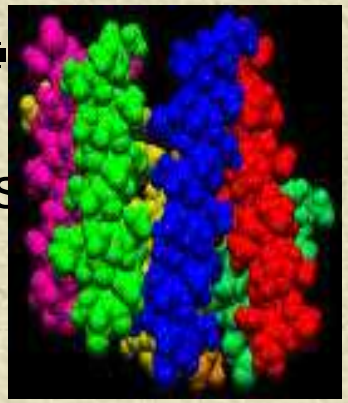
6. **Lipid peroxidation** in biological membrane by free radicals starts a chain of events causing cellular dysfunction and death.

7.**Covalent binding** of electrophylic reactive metabolite to **nucleophylic macromolecules** (e.g., S, O, and N atoms in cysteine, tyrosine, and histidine, respectively) and nucleic acids (e.g., N and O atoms in purine or pyrimidine).

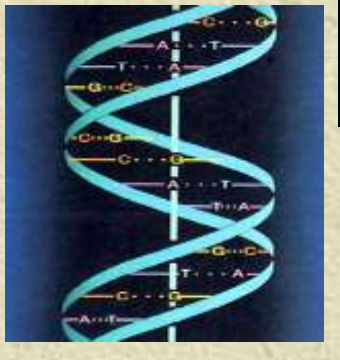
8.**Receptor interaction** at a cellular or macromolecular level with specific chemical structures.

- It may **modulate the normal biologic effects**, mediated by the receptor.

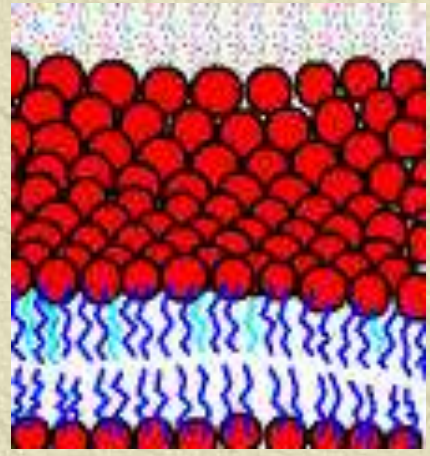
Some xenobiotics cause toxicity by disrupting normal cell functions:



- Bind and damage **proteins** (structural, enzymes



- Bind and damage **DNA** (mutations)
- Bind and damage **lipids**
- React in the cell with **oxygen** to form "**free radicals**" which damage lipid, protein, and DNA



NATURE OF TOXIC EFFECTS

9.Immune-mediated hypersensitivity reaction by antigenic materials, resulting respectively in **allergic contact dermatitis** and **asthma**.

- **Type I hypersensitivity reaction –** IgE-mediated mast cell degranulation
- **Type II antibody-mediated cytotoxic hypersensitivity-** involve haematological reactions i.e. those pertaining to the blood cells and blood-forming organs
- **Type III immune complex-mediated** hypersensitivity
- **Type IV delayed-type** hypersensitivity

10. **Immunosuppression** by chemicals.

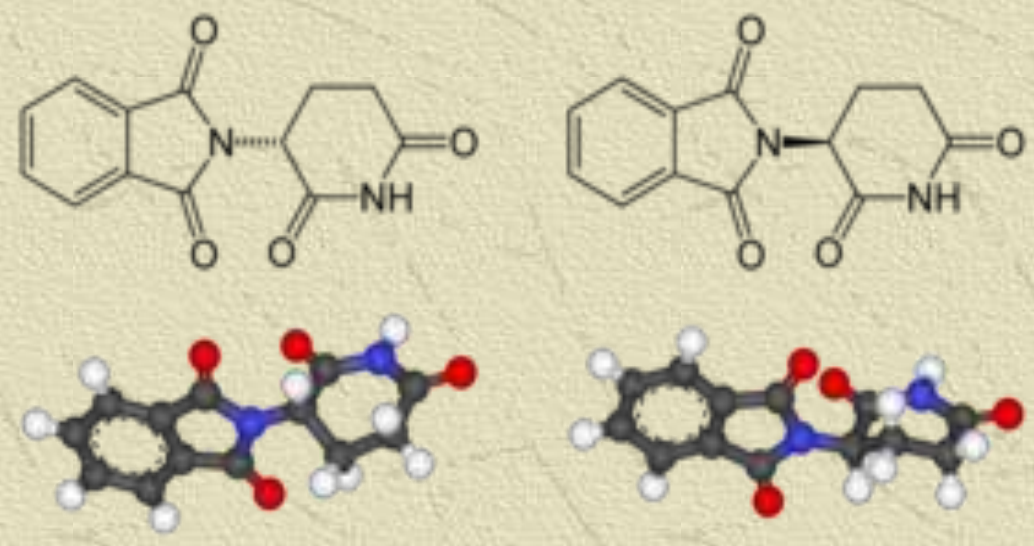
- The adverse effect is manifested as increased susceptibility to ineffective agents.

11. **Neoplasia,** resulting from aberration of tissue growth and control mechanisms of cell division and leading to abnormal proliferation.

12. **Genotoxicity** caused by chemicals, which interact **with DNA** and possibly, lead to **heritable changes**.

Teratogenesis - the creation of birth defects during fetal development

Teratogens: substances that induce birth defects.



Thalidomide
(R)-enantiomer
sedative

Thalidomide
(S)-enantiomer
teratogen

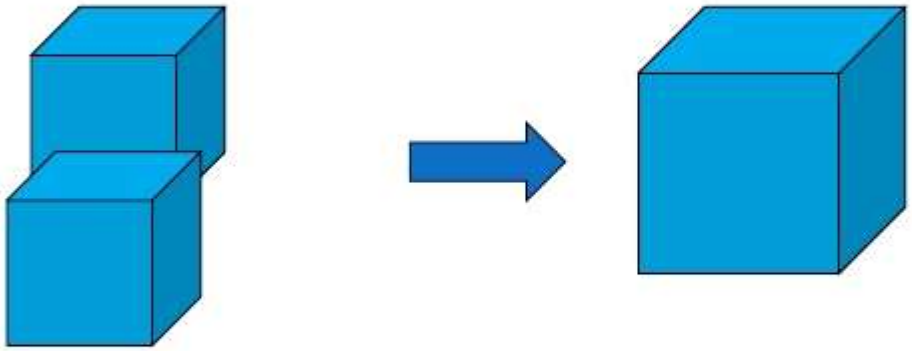
Interactions

- Additive
- Antagonistic
- Synergistic
- Potentiation

Interactions - Independent

No interaction

Interactions - Additive

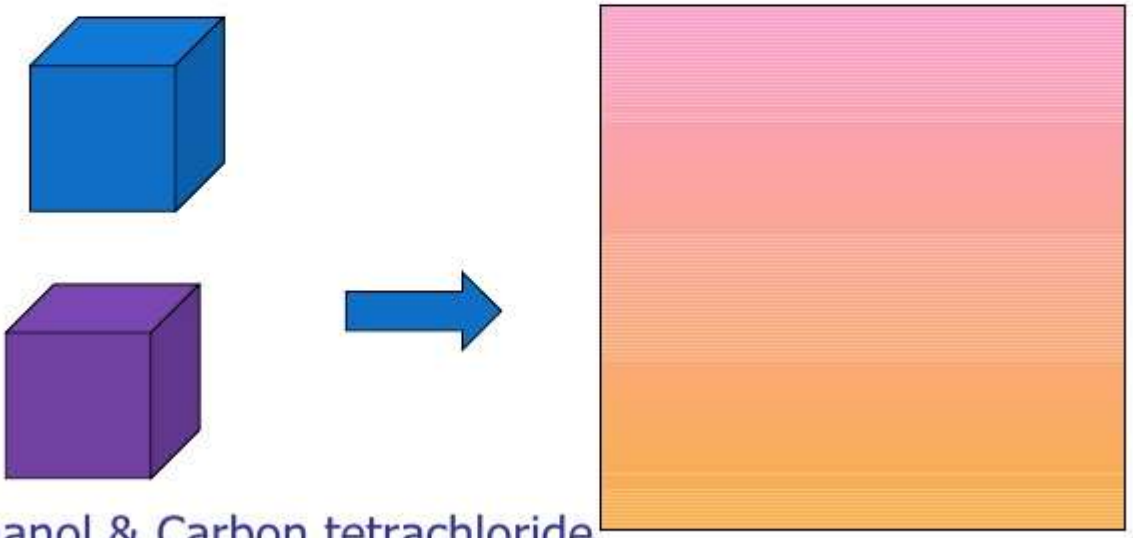


The combined effect is equal to the individual sum of the effects

Example – Narcotics, usually same target organ same mechanism

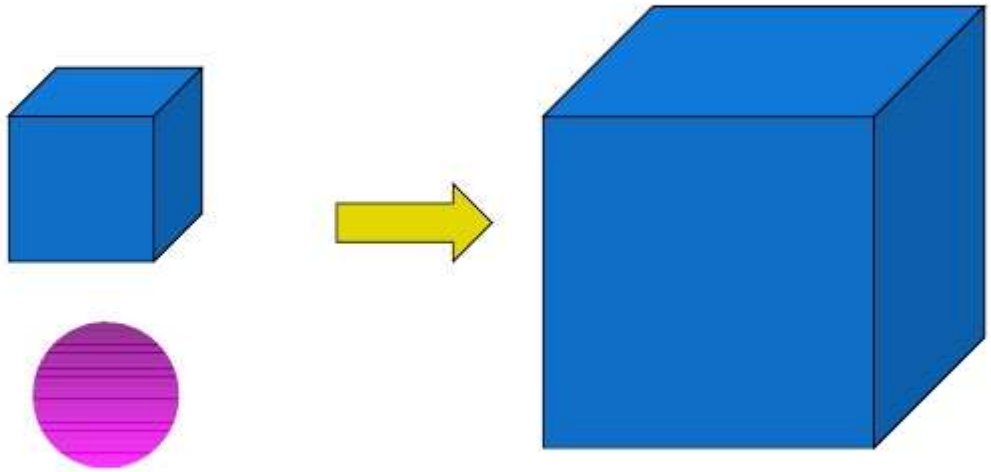
Interactions - Synergistic

Combined effect is greater than sum of individuals



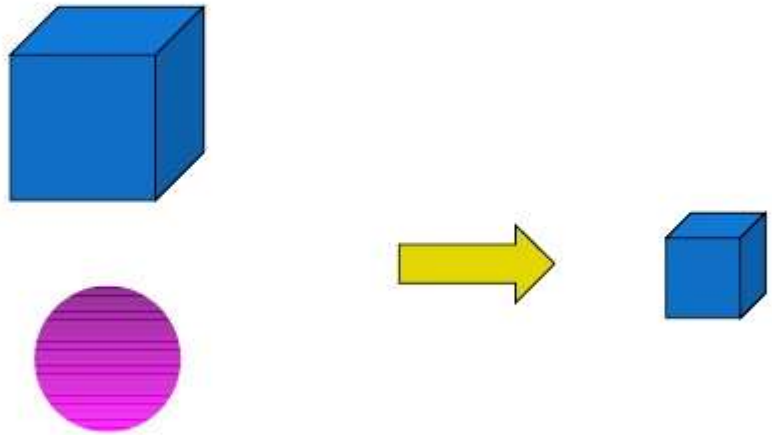
e.g. Ethanol & Carbon tetrachloride

Interactions - Potentiation



Substance increases the effect of a hazardous substance
e.g. Isopropanol & carbon tetrachloride,
barbiturates and solvents

Interactions - Antagonistic



Substance reduces effect of another substance
Cd & Zn – less kidney damage

CLASSIFICATION OF TOXIC AGENTS

- The toxic agents are classified in a variety of ways, depending on their **target organ** (liver, kidney, etc.), their **use** (pesticides, solvents, etc.), their **origin** (animal and plant toxins) etc. In our classification the poisons, which cause intoxications in disaster situations are divided **into five groups, according to their mechanism** of the toxic effect:

1. **Anticholinesterase compounds** - organophosphorus esters, carbamate esters.
2. **Cellular asphyxia** - inducing compounds: carbon monoxide, cyanide etc.
3. **Pulmonary edema-** inducing compounds: phosgene, ammonia, chlorine, nitrogen oxides, etc..
4. **Sensory irritant compounds**: mineral acids, modern riot control compounds, etc.
5. **CNS depression** (narcosis) - inducing compounds: aliphatic hydrocarbons, aromatic hydrocarbons, halogenated hydrocarbons, carbon disulfide, etc.

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**.

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.

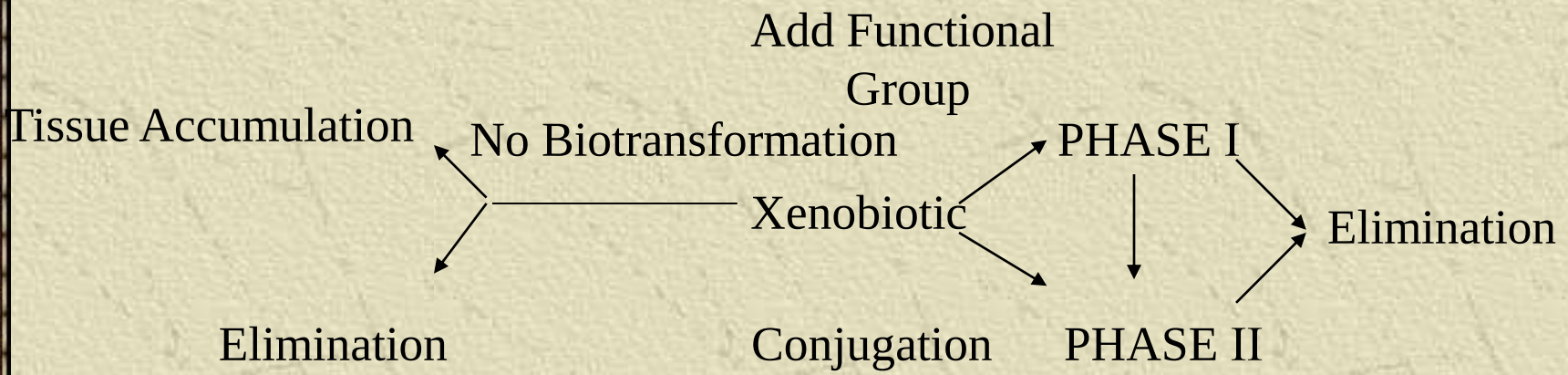
Sites of Biotransformation

- **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

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

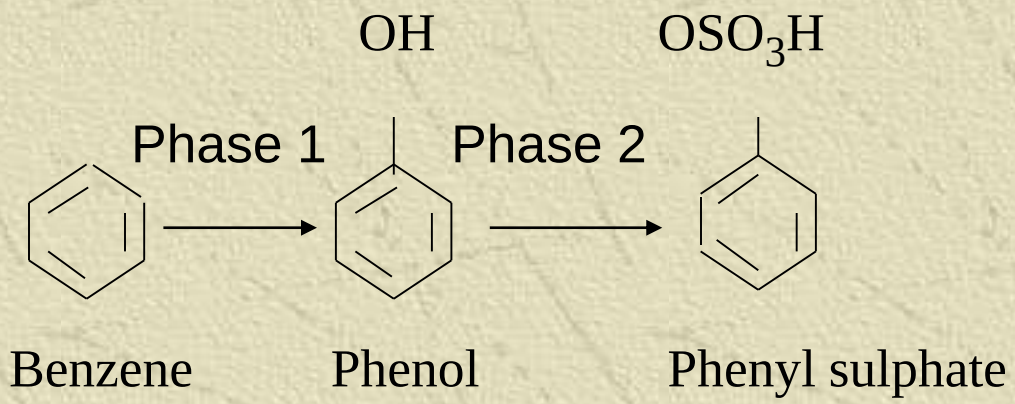
- **Increases a molecule's polarity**
- **Provide a functional group or handle on the molecule that can undergo Phase 2 reactions**

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**

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

Role of Cytochrome P-450 Monooxygenases in Oxidative Biotransformation

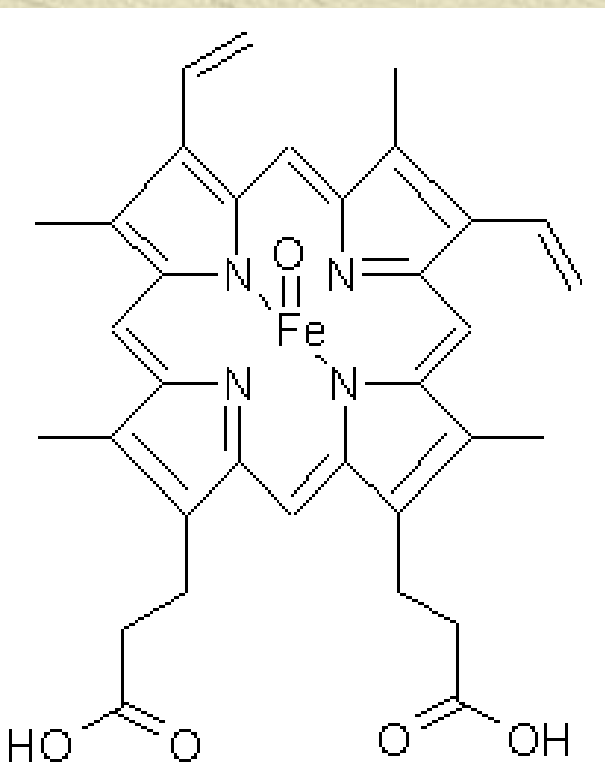
General Equation describing the oxidation of many xenobiotics (R-H) forming a metabolite (R-OH)

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

- 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



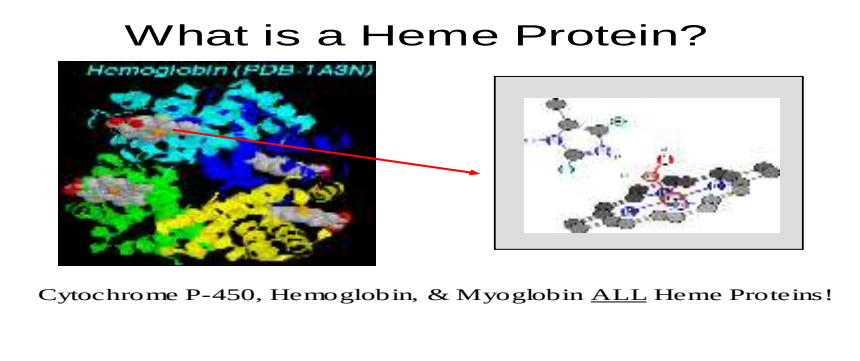
Important features:

- 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

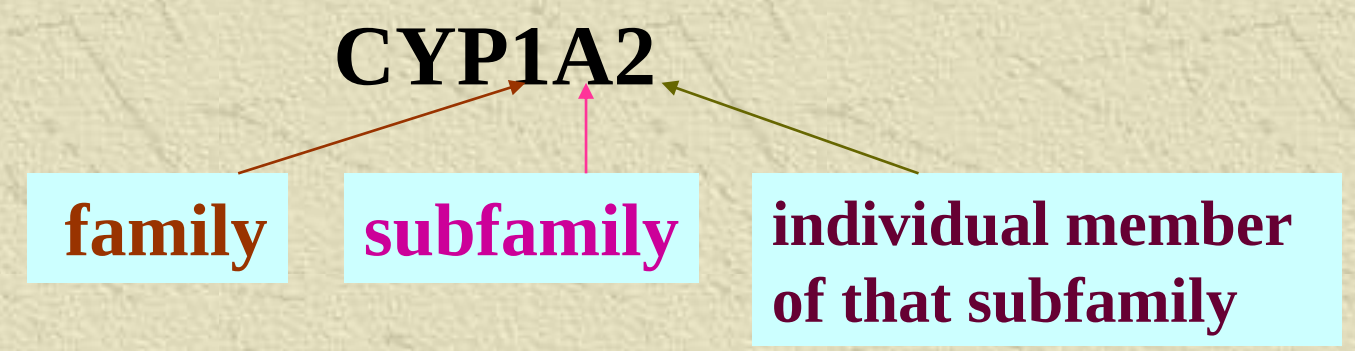
cytochrome P450 reductase

NADPH & O_2

The actual reaction mechanism is as follows:

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:

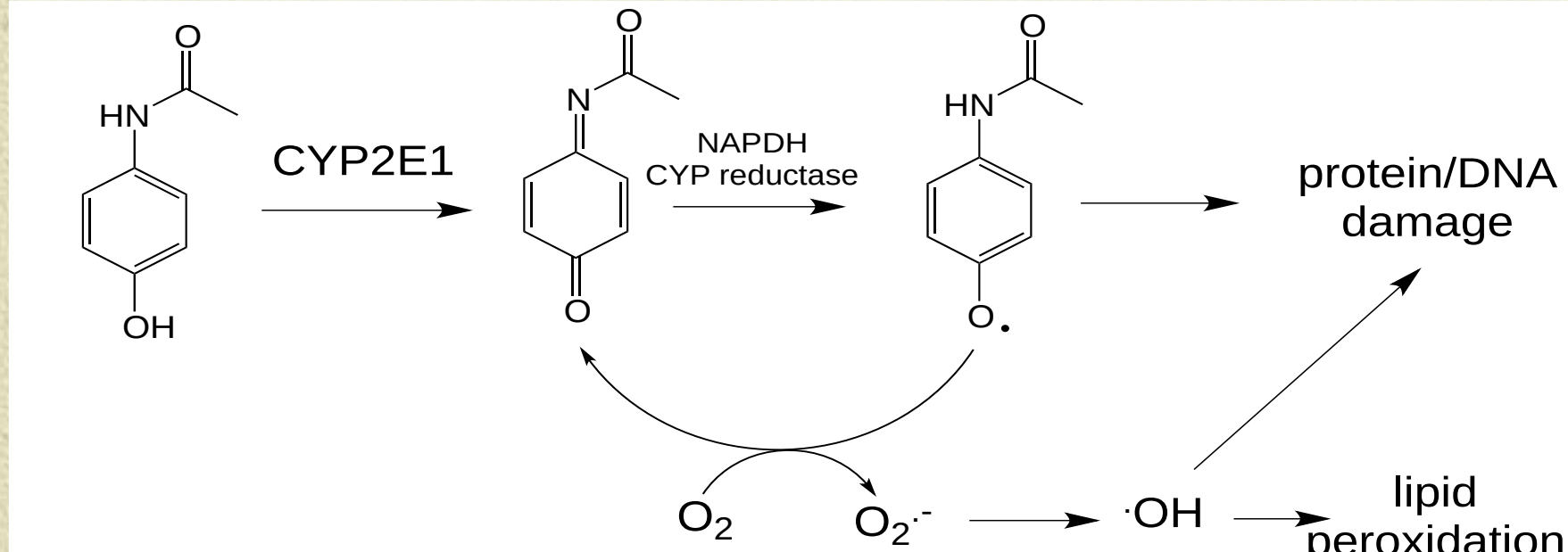
1. Organ: liver
2. Substrates: aromatic amines (e.g. caffeine)

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.

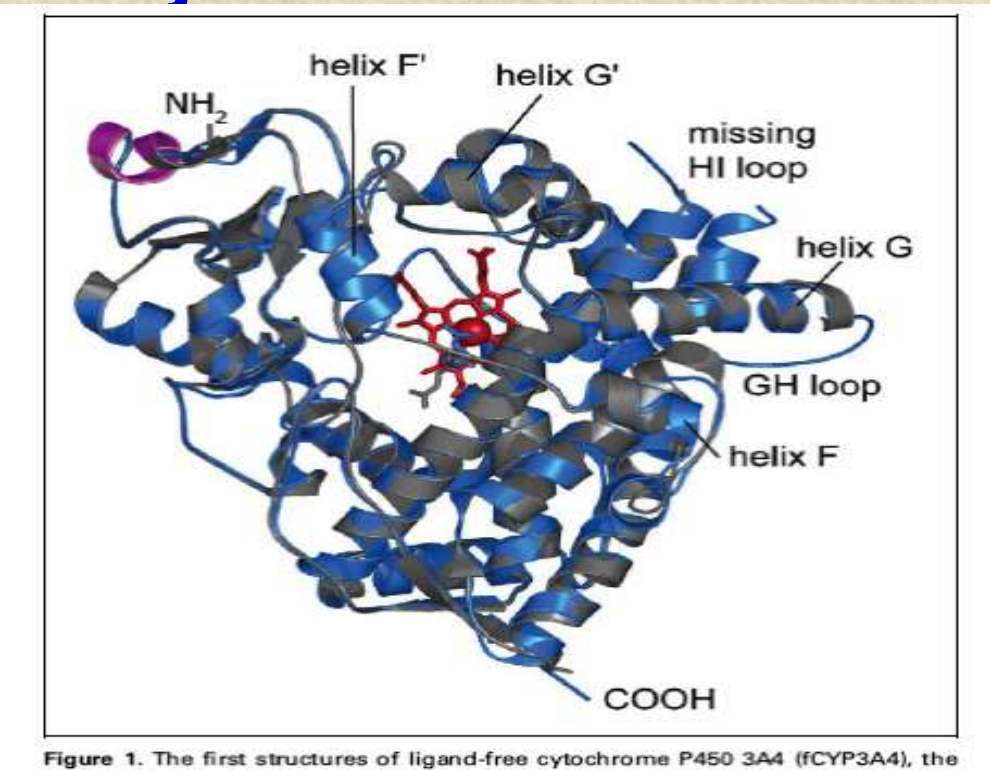


Figure 1. The first structures of ligand-free cytochrome P450 3A4 (fCYP3A4), the

Flavin-containing Monooxygenase (FMO)

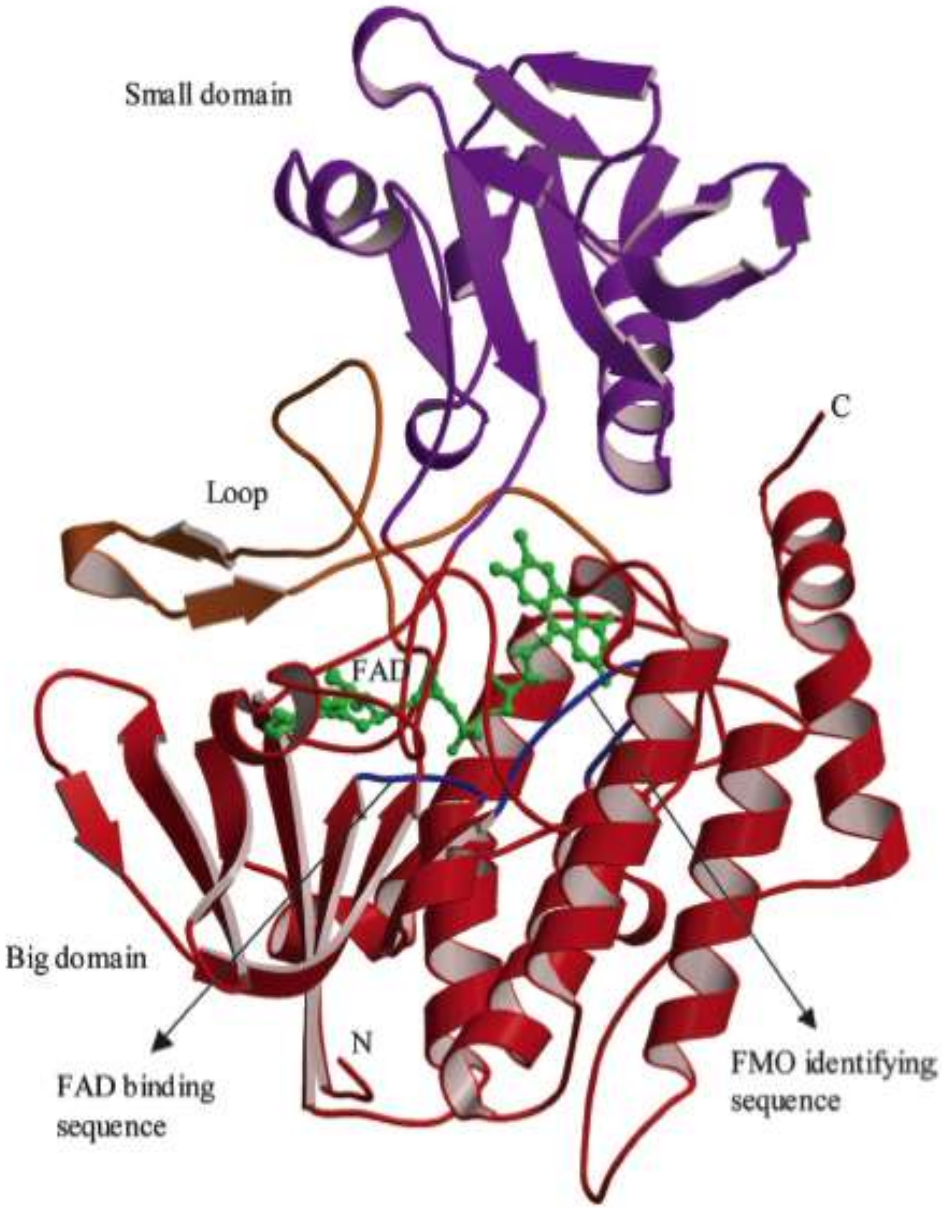


Fig. 2. Ribbon representation of the protein and ball-and-stick model of FAD. The strand–turn–helix motifs and the loop interlinking the two domains are labeled. FAD is in the large domain and has no interaction with the small domain.

- 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

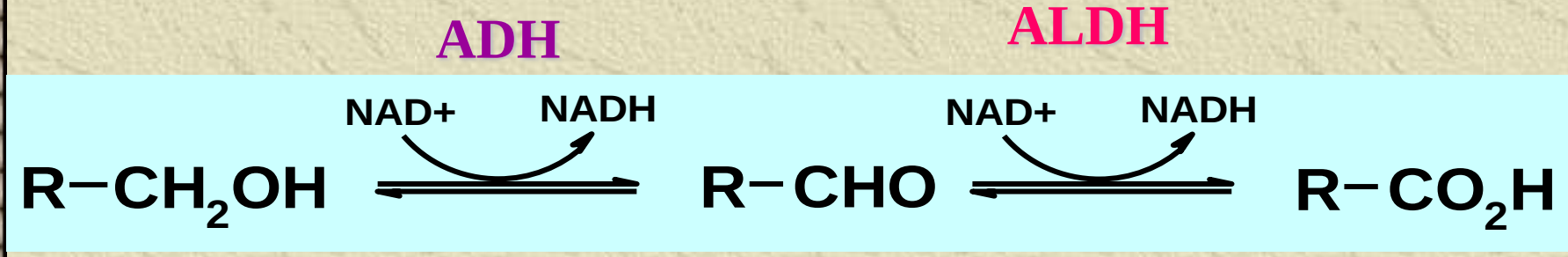
ADH **Alcohol Dehydrogenase**

ALDH **Aldehyde Dehydrogenase**

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

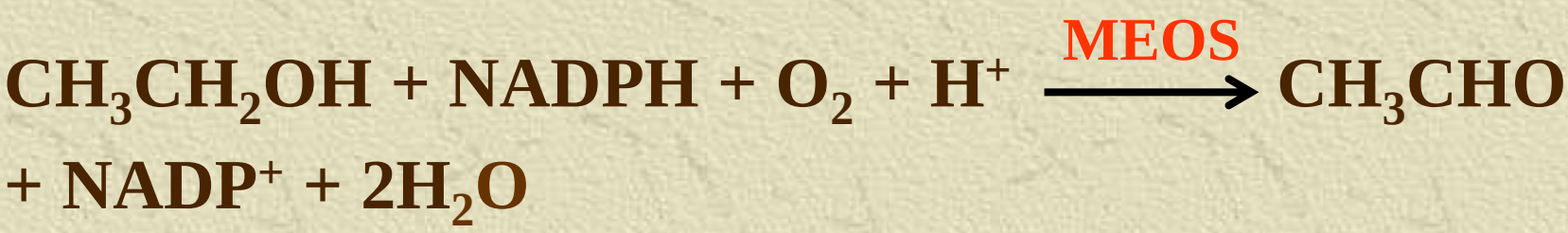
Function

- 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.



- 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.



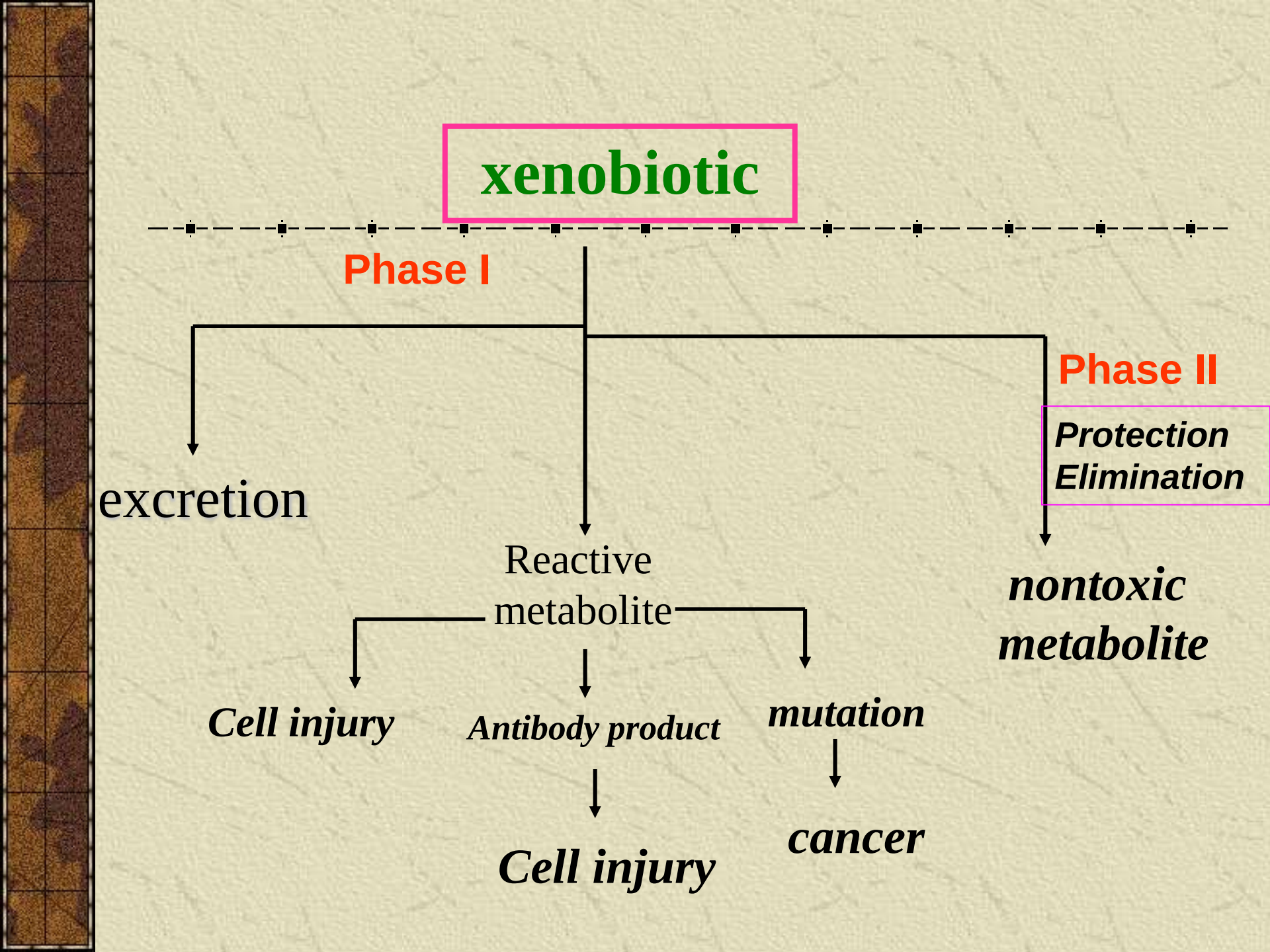
MEOS: **Microsomal Ethanol-Oxidizing System**, is also called **Cytochrome P450-dependent Microsomal Ethanol Oxidizing System**. Converts alcohol to acetaldehyde

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.**

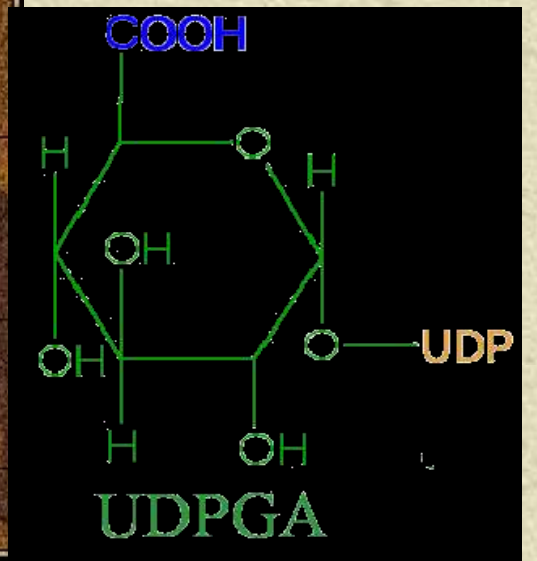

xenobiotic
Phase I
Phase II
Protection
Elimination
excretion
Reactive
metabolite
nontoxic
metabolite
Cell injury
Antibody product
mutation
Cell injury
cancer

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

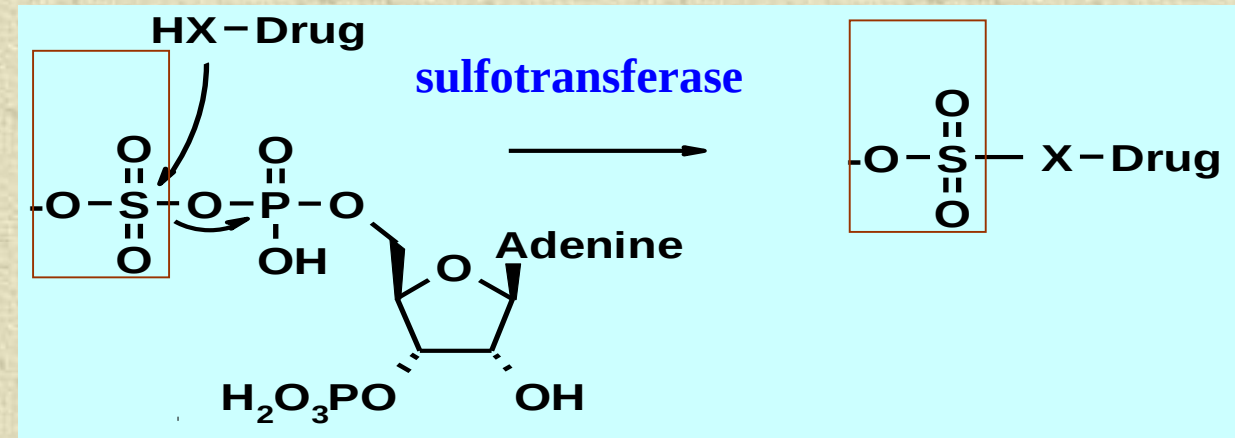


- Attachment sites are hydroxyls
- **Alcohols, phenols, amines, enols, N-hydroxyls, sulfides, acids**

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



- **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

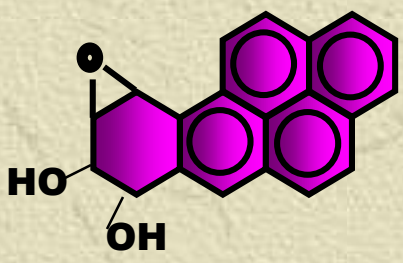
- Adds a **glutathione** molecule to the parent compound, either by direct addition or by replacement of an electrophilic substituent (e.g., a halogen atom)
- Uses the enzyme **glutathione transferase** (**GST**)
- Uses the cofactor called **glutathione** **One of the major Phase II enzymatic pathways**

Glutathione (GSH) Conjugation

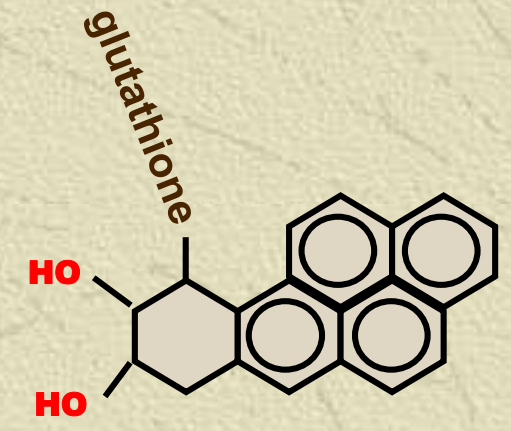
- **DETOXIFICATION** of **electrophiles!**
- Electrophilic chemicals cause:
  - Tissue necrosis
  - Carcinogenicity
  - Mutagenicity
  - Teratogenicity
- The thiol (SH group) ties up potent electrophiles

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

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

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

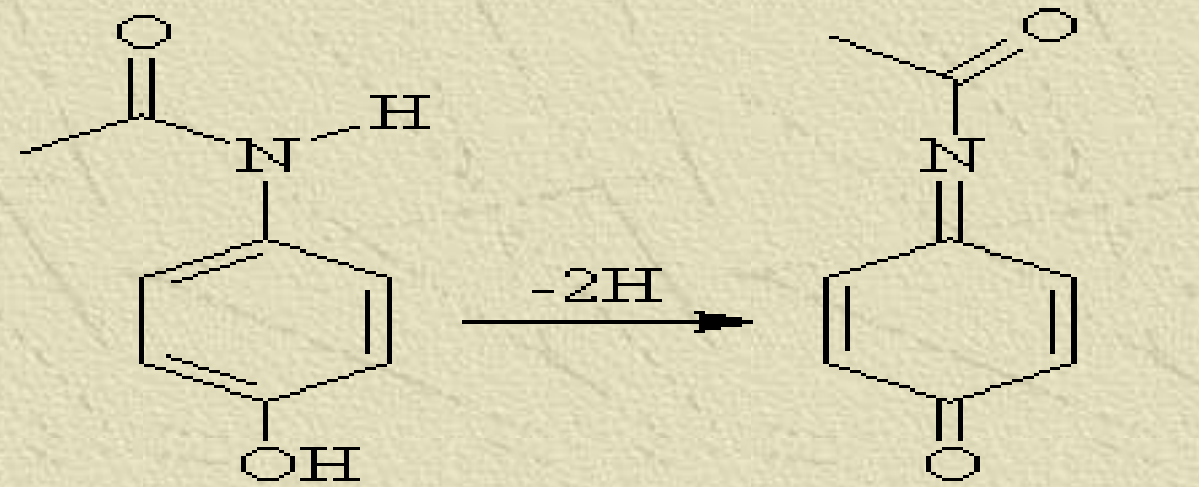
Significance of Biotransformation Reactions in Toxicology

- 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.

Factors affecting metabolism

- **Age** The metabolizing enzymes in neonates are not fully developed, therefore those cannot efficiently metabolize drugs. Also in the elderly, enzymatic systems may not function well leading to same conclusion.
- **Sex** (activity is generally higher in males than in females). Linked to hormonal differences. **Genetics** - Genetic differences can influence amount and efficiency of metabolic enzymes
- **Organ** (activity of many enzymes is highest in the liver)
- **General health status** (e.g., hepatic injury decreases metabolic activity in the liver)

- **Pharmacogenetic factors** Some individuals may be deficient in some enzymes, regardless of sexMales who are deficient in **glucose -6-phosphate dehydrogenase** are more prone to **hemolysis** when subjected to some drugs like **sulfonamides**
- **Pregnancy** Hepatic metabolism of drugs is decreased in pregnancy.
- **Nutritional status** (liver dysfunction) Malnutrition can cause a decreased level of some enzyme system and liver dysfunction can lead to decreased metabolism

- **Bioactivation** Some drugs may be transformed to more toxic metabolites
- **Enzyme induction/inhibition** A result of this is either an increase in the metabolism or a decrease in the drug metabolism
- **Changes in the kinetic mechanism**: depending on whether the concentration of drug is in the therapeutic or overdose range

Organ-directed toxicity
The main target organs for the systemic toxicity of xenobiotics are:

- Skin, mucous membrane
- Lungs
- Liver, kidney
- Bone marrow
- Immune system
- Nervous system (central & peripheral)
- Cardiovascular system
- Reproductive system
- Muscle and bones

Organs particularly susceptible to toxin damage are the liver and kidney

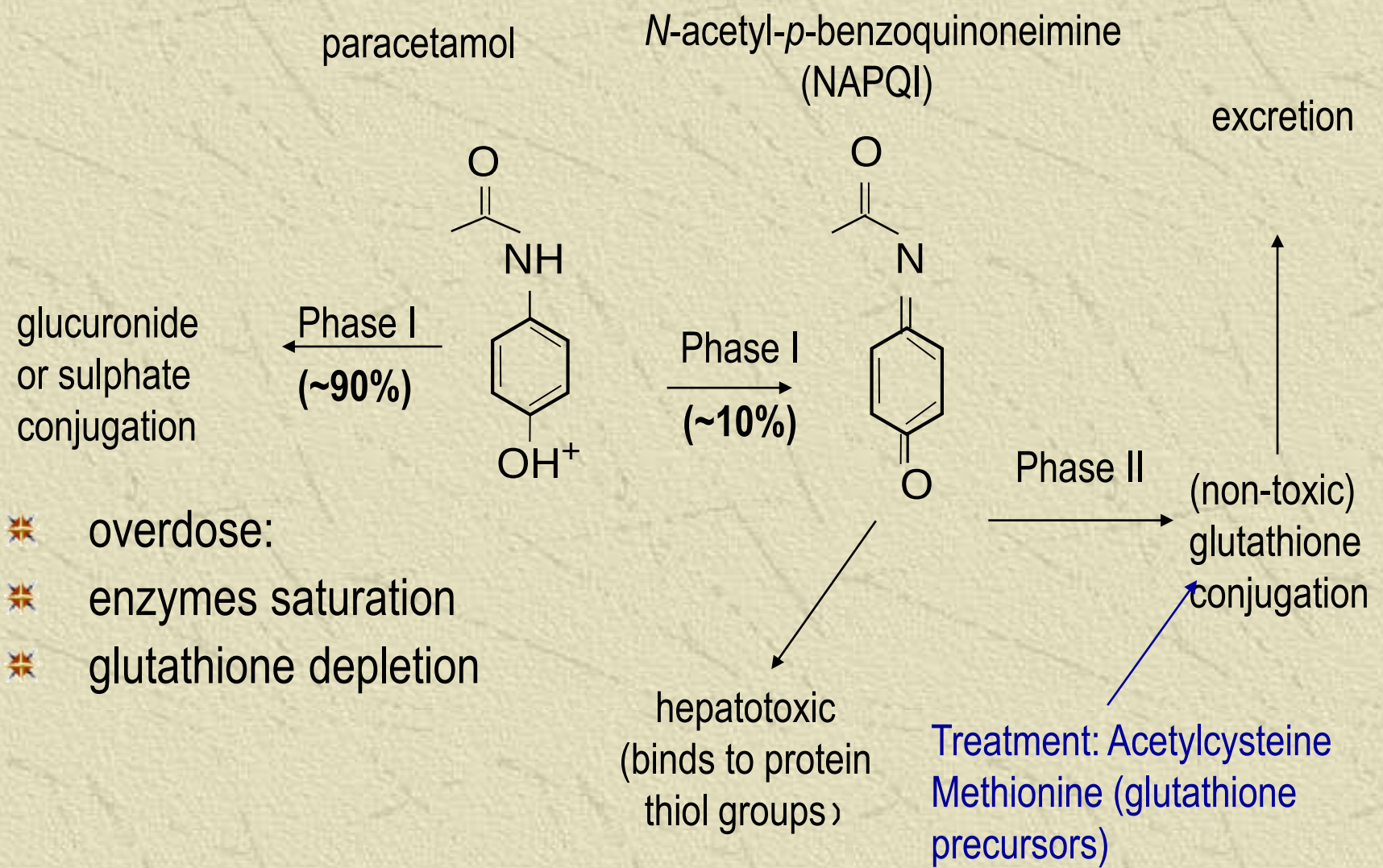
Hepatotoxicity

(i) hepatic necrosis
paracetamol poisoning

(ii) hepatic inflammation (hepatitis)
halothane can covalently bind to liver proteins to trigger an autoimmune reaction

(iii) chronic liver damage (cirrhosis)
long-term ethanol abuse causes cellular toxicity and inflammation and malnutrition as ethanol becomes a food source

Paracetamol is a prominent cause of hepatic poisoning 48 % of all poison admissions and >200 deaths/year)



- overdose:
- enzymes saturation
- glutathione depletion

Nephrotoxicity

(i) changes in glomerular filration rate (GFR)

Largely due to drugs that alter blood flow :

NSAIDs (eg. aspirin) reduce prostaglandins which in turn reduces blood flow/GFR

ACE (angiotensin-converting-enzyme – RR↑)inhibitors (eg. ramipril) increase blood flow/GFR

(ii) allergic nephritis

allergic reaction to NSAIDs (eg. fenoprofen) and antibiotics (eg. metacillin)

(iii) chronic nephritis

long-term NSAID and paracetamol use

Neurotoxicity

- Compounds that have a toxic effect on the nervous system:
  - Toxicants of the central nervous system (CNS)
  - Toxicants of the peripheral nervous system (PNS)
  - Toxicants of a combined effect

- **CNS inhibitors:**
  - Chlorinated hydrocarbons, benzene, aceton, dietyl eter
- **Psychomimetics:**
  - They can disturb psychical activities
  - Mescalin, phenylethylamine derivatives, indole derivaties
- **Compounds that inhibiting the respiration center**
  - Narcotics, hydrocarbons

- **Convulsion toxicants**
  - Convulsion in central origin
  - Organophosphorus pesticide
- **Toxicants, paralyzing transmission of nerve impulses to the muscle**
  - Botulinum
- **Toxicants, paralyzing transmission of nerve impulses in the nerve**
  - Tetrodotoxin

- **Neuroparalytic poisons:**
  - anticholinesteratic
- **Toxicants, acting with mediators or synaptic poisons:**
  - Adrenaline, ephedrine, hydrazines, etc.

According to the final result, toxic responses can be grouped as:

- Direct injury of cell or tissue
- Biochemical damage
- **Neurotoxicity**
- **Immunotoxicity**
- Teratogenicity
- Genetic toxicity
- Carcinogenicity
- **Endocrine disruption**

PRINCIPLES OF THE THERAPY OF INTOXICATIONS

I. **Reduction and elimination** of the poisons in **the site of entry**.

II. **Enhance of the elimination** of the poisons, which are absorbed **in the blood and the tissues.**

III. **Antidotal therapy**

GI Decontamination

- **Removing** poison **from the stomach** is most effective in the **first 2 hours** after ingestion and is of **limited benefit** more **4 hours after ingestion**.
- For removing of the poison from the stomach it is necessary:
  - **to induce vomiting** or
  - **to perform gastric lavage**

GI Decontamination

1. Vomiting. For inducing of **vomiting** first have to give **0,5 – 1 L water** to drink, followed by **30 ml** (10 - 15 ml per children) **ipecac syrup orally** (from the root of **Cephalus Ipecachuana**: emetine & cephaline).

- For inducing of vomiting can also use **apomorphine**, which produces a more rapid onset of action.

Contraindications

- Convulsions
- Corrosives
- Sharp objects (e.g. needles)
- Coma or impending coma
- Decreased gag reflex
- Severe CVS disease or respiratory distress or emphysema
- Recent surgical intervention
- Hemorrhagic tendencies (varices, active peptic ulcer, Trombocytopenia)
- Previous significant vomiting (spontaneously)
- Less than 6 m of age (not well developed gag reflex)

GI Decontamination

2. Gastric lavage.

- It can be performed when:
  - **if there is not vomiting** after receiving syrup of ipecac;
  - if the patient is **in coma**;
  - if ingested **amount** of the toxic substance is large;
  - if there is a **central nervous system depression** with an inadequate gag reflex;

GI Decontamination

- It must not perform gastric lavage if the patient have been ingested **a caustic substance** (the tube may perforate his esophagus).
- **Gastric lavage** begins by injecting about 30 ml of air through the tube and aspirating of the stomach content with 50 ml syringe.
- ✓ The aspirate has to be sent to **the laboratory for analysis**.
- ✓ Then instill up to **200 ml** of solution for lavage and **massage** the patient's stomach **to mix** the content. After those unclamp the outflow tube and clamp the inflow tube (record the out flow amount).

These procedures are repeated **using 5 to 10 liters** of fluid for lavage.

The induced vomiting and gastric lavage will remove only **50% to 60%** of the gastric contents.

- ✓ For absorption of the remaining poison have to give **slurry of activated charcoal and water or saline solution**.

Complications:

1. Bradycardia, especially in cases of OP or digitalis toxicity
2. Laryngospasm and cyanosis
3. Vomiting & aspiration pneumonia
4. Stress reaction – hypertension, tachycardia
5. Mechanical gut injury
6. Faulty introduction of the tube in the trachea

Contraindications:

- **Absolute contraindications:**

1. Corrosives
2. Froth producing substances as shampoo or liquid soap
3. Oesophageal varices or peptic ulcer

- **Relative contraindications:**

1. Coma
2. Convulsions

GI Decontamination

3. Absorption therapy with **activated charcoal** is the **physical binding of a poison** to an **unabsorbable carrier**, which is eliminated in the feces.

- Activated charcoal is most effective for large, nonpolar molecule. Ionized agents are less strongly absorbed than neutral compounds.

GI Decontamination

Activated charcoal

- Limits drug absorption in the GI tract
- Within 60 minutes of ingestion
- Patient must be awake or intubated
- 1 gram/kg PO or GT

GI Decontamination

- **Not** good (not absorbed by) for:
  - **Metals (Lithium, Iron, Lead, Mercury….)**
  - **Alcohols**
  - **Hydrocarbons, Petroleum distillates**
  - **Oils**
  - **Glycols**
  - **Caustics (Corrosives)**
  - **Sodium chloride, Sodium hypochlorite bleach**
  - **Cyanide**

Contraindications

- Coma
- Intestinal obstruction or ileus with distention
- Corrosives
- If an oral antidote is given

GI Decontamination

4. Cathartics (Laxatives)

There are substances that enhance the passage of material through GIT and decrease the time of contact between the poison and the absorptive surfaces of the stomach and intestine.

a) **Osmotic Cathartics**: increase osmotic pressure in the lumen, as **Mg sulfate**.

b) **Irritant Cathartics**: act by increasing motility, such as **caster oil.**

Preferred agents are the **saline cathartics:**

sodium sulfate, magnesium sulfate, citrate or (phosphate) and **sorbitol**, which have a relatively **prompt onset** of action.

They have lower toxicity, than the oil-based cathartics (**oleum ricini**), which present **aspiration risk**.

GI Decontamination

Contraindications:

1. GIT hemorrhage
2. Recent bowel surgery
3. Intestinal obstruction
4. Renal failure for magnesium salts

GI Decontamination

5. Whole bowel Irrigation

The goal of WBI is to clean GIT from unabsorbed bed ingested toxins. Polyethylene glycol electrolyte solutions are used.

Indications:

- **Ingestion of a toxin that is known to be poorly absorbed by AC**
- **Ingestion of massive amounts of drugs/impractical AC**
- **Ingestion of sustained-release or enteric-coated preparations (e.g. aspirin)**
- **Ingestion of large amount of drugs that may form concretions or bezoars (e.g. salisylates, barbiturates, carbamazepine)**
- **Removal of ingested packets of illegal drugs (body packers, cocaine)**

Inhalation exposures

1. Immediate, cautious removal of the patient from the hazardous environment.
2. Observe for airway obstruction
3. Administration of 100% humidified O_2, assisted ventilation, and bronchodilators.
4. Observe for edema of the respiratory tract and later non-cardiogenic pulmonary edema.
5. Intubate as necessary
6. Treatment should not await laboratory results.

Decontamination

Skin

- Protect yourself and other workers
- Remove clothing
- Flush with water for at least 30 min. or use a normal saline
- Use soap and water if oily substance
- Chemical neutralization can potentiate injury
- Corrosive agents injure skin and can have systemic effects
- Toxic substances such as OP compounds, metal compounds, phenol, may penetrate the intact skin and must be handled with proper protective equipment.

Ocular exposures

1.Remove contact lens

2.Ocular decontamination consists of at least 15 minutes of immediate irrigation of eyes with normal saline or water.

3.Use local anesthetic drops

4.Alkaline or acid irrigating solutions should be avoided.

5.Continue irrigation until pH is normal

6. Alkaline corneal burns are requiring ophthalmic consultation.

II. Measures to enhance elimination of the poisons

1. Diuresis

The **basic principle** of diuresis is **ion trapping**. **Increasing urinary flow** to **two-three times** normal was carried out in the past, but this has been **replaced with adjustment of urine pH** and maintenance of **normal urine flow**.

Alteration of the pH of the urine in the **renal tubules** (after glomerular filtration) can **ionize** and "**trap**" the agent. Once the **toxin is ionized**, then **reabsorption** from the renal tubules is **impaired** and the result is that more of the drug **is excreted** in the urine.

Diuresis

Forced diuresis

Simple method for some poisons.

It is efficient only in poisons with the following properties:

- Substances excreted mainly by kidneys
- Substances with low volume distribution
- Substances with low protein binding

Types:

1. **Fluid diuresis**
2. **Osmotic diuresis**: **manithol 10%, furosemide** which are excreted by renal tubules leading to increase in its osmotic pressure.

Manipulation of Urine pH - Forced alkaline diuresis

Forced alkaline diuresis is indicated in the treatment of poisonings (**salicylate, phenobarbital**, antihistamine, cocaine, tricyclic antidepressants, sulfonamides, etc) except if the patient is: **in shock**, in **heart failure**, has **impaired renal function**. Urinary pH should be maintained above **7.5**, ideally **8.0 -8.5**.

Increasing the pH of the urine from 7 to 8 results in a ten fold increase in the concentration of aspirin in the urine. The volume of the diuresis need not be **more than 500 ml per hour**. In the first hour infuse:

- 500 ml 5% dextrose
- 500 ml **bicarbonate 1.4% ($NaHCO_3$)**
- 500 ml dextrose 5%

Potassium chloride should be added to keep serum potassium above 3.5 mmol/l.

Acid Diuresis

It is uncommonly used method for certain substances such as amphetamines, phencyclidine, quinine. It is a dangerous method because of the risk of myoglobin precipitation in renal tubules.

Infuse:

500 ml 5% dextrose + 500 ml 5% dextrose

Arginine/lysine or **Ammonium chloride**

Mechanisms of Diuresis

- Enhances urinary excretion of weak acids
- Traps weak acids in ionized state (ion trapping)
- Prevents reabsorption by renal tubules

Extracorporeal techniques

2. Dialysis

By allowing toxic substances to pass through semi permeable membrane depending on the concentration gradient. It is beneficial when renal function is impaired. For good results substances must have:

- **Low volume distribution**
- **Low molecular weight**
- **Low protein binding**

Examples for dialyzable substances: alcohols, barbiturates, salicylates

Examples for non-dialyzable substances: opiates, atropine, antidepressants.

Peritoneal dialysis (PD)

Diffusion of toxins from mesenteric capillaries across the peritoneal membrane into dialysate dwelling in the peritoneal cavity. Acts by considering peritoneum as semi permeable membrane. Has limited value for the management of some poisonings.

This method is most **useful in situation of renal failure** or **anurea.** PD involves ion-trapping principles. Dialysis fluids may need **frequent change** and attention to **prevent infection** is also necessary**.**

Complications:

- **Intra-abdominal bleeding**
- **Perforation of abdominal organs**
- **Peritonitis**
- **Dehydration or over hydration**

Contraindications:

- **Pregnancy**
- **Abdominal hernia**
- **Respiratory distress**

Hemodialysis

Two catheters are inserted. Blood is pumped from one catheter through the dialysis unit (a cellophane bag) and returned through the other catheter. The **haemodialysis** relies on passage to the toxic agent through a **semi permeable dialysis membrane** so it can equilibrate with the dialysate and subsequently **be removed**. This is in part dependent on the **molecular weight** of the compound.

Some drugs such as **phenobarbital** can readily cross these membranes and go from high concentrations in plasma to a lower concentration in the dialysate.

Complications:

hypotension, bleeding tendency (due to heparin), cross infections, muscle cramps, air embolism.

Hemoperfusion

Haemoperfusion is passing of blood through a **column of charcoal** or **absorbent resin**. It is an important technique of **extracorporeal drug/toxic removal**.Using equipment and vascular access similar to that for HD. Systemic anticoagulation is required, often in higher doses than for HD, and **trombocytopenia** is a common complication.

- Because the drug or toxin is in direct contact with the absorbent material, drug size, water solubility, and protein binding are less important limiting factors
- For most drugs, hemoperfusion can achieve greater clearance rates than HD. For example, the **HD** clearance for **phenobarbital** is **60-80** mL/min, whereas the **hemoperfusion** clearance is **200-300** mL/min.

Contraindications:

Patients with **coagulopathy**
Patients with uncorrected hypotension

Complications:

Thrombocytopenia
Hypocalcemia
Hypoglycemia
Hypotension
Adsorption of therapeutic drugs

Hemofiltration (similar to hemodialysis, except that the blood is pumped through a hemifilter, where waste products and water are removed by hydrostatic pressure. Replacement fluid is added and the blood is returned to the patient).

Plasmapheresis and Plasma exchange (separation of cellular blood components from plasma, then cells are resuspended in fresh frozen plasma, and reinfused again).

Exchange transfusion (removal of the patient's blood, replacement with fresh whole blood).

Plasma perfusion (combination of plasmapheresis and hemoperfusion).