Mechanisms of Toxicity
Lecture: 2

General Toxicology Theory
Fourth Year Students
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Lecture Topics

STEP 1—Delivery: From the Site of Exposure to the Target:
Absorption versus Pre-systemic Elimination
Distribution to and Away from the Target
Mechanisms Facilitating Distribution to a Target,& Mechanisms Opposing Distribution to a Target
Excretion versus Reabsorption
Toxication versus Detoxication
STEP 2—reaction Of the Ultimate Toxicant With The Target Molecule
Attributes of Target Molecules Types of Reactions
Non-covalent Binding, Covalent Binding, Hydrogen Abstraction, Electron Transfer, Enzymatic Reactions
Effects of Toxicants on Target Molecules
Dysfunction of Target Molecules, Destruction of Target Molecules, Neoantigen Formation
-Toxicity Not Initiated by Reaction with Target Molecules

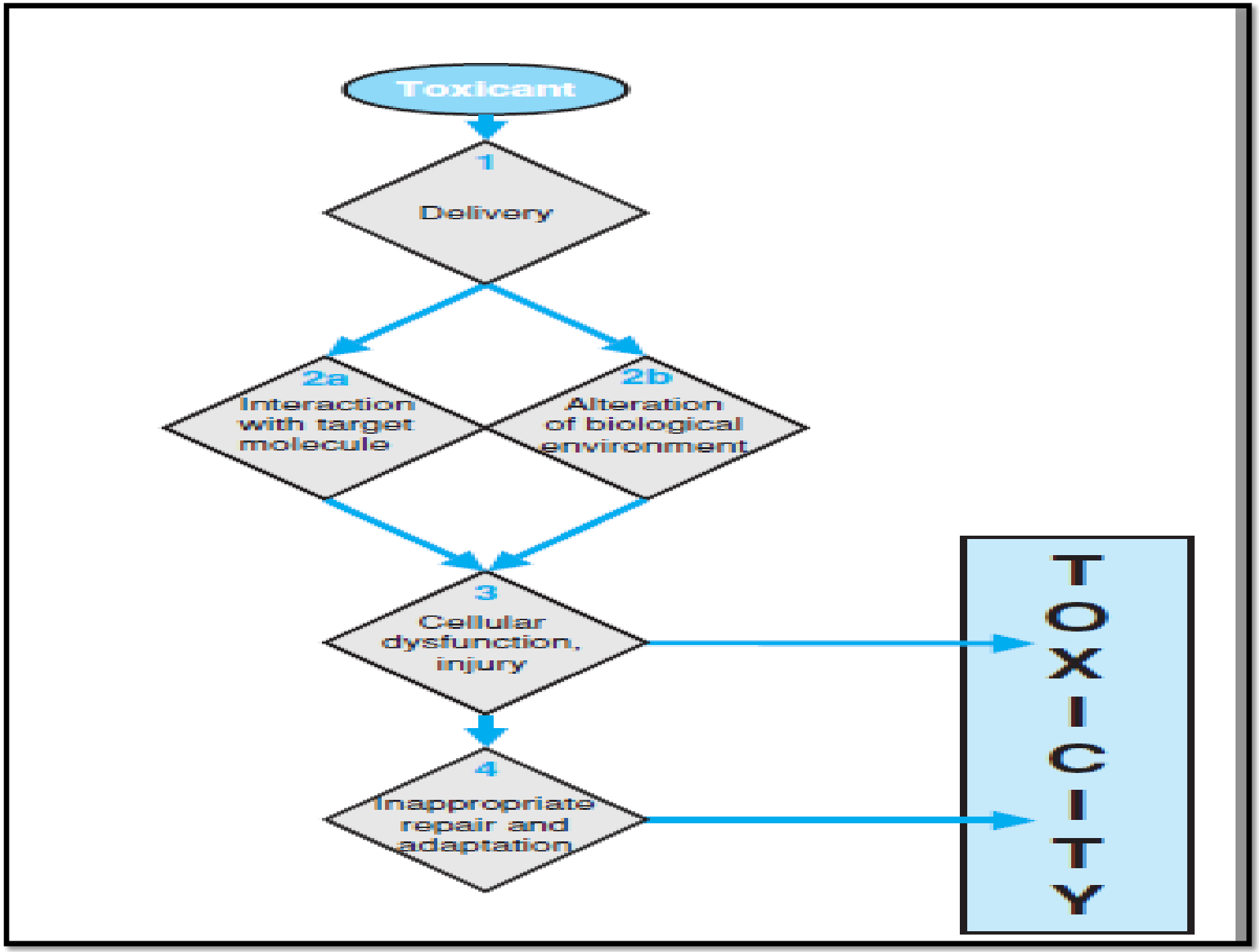
-A common course is when a **toxicant delivered to its target reacts with it, and the resultant cellular dysfunction** manifests itself in toxicity.

: An example of this route to toxicity is that taken by the **puffer fish poison, tetrodotoxin**. After ingestion, this poison reaches the voltage-gated Na+ channels of motor neurons (**step 1**). Interaction of **tetrodotoxin** with this target (**step 2a**) results in **blockade of Na+ channels**, inhibition of the activity of motor neurons (**step 3**), and ultimately skeletal muscle paralysis. **No repair mechanisms can prevent the onset of such toxicity**.

-Sometimes a xenobiotic does not react with a specific target molecule but rather adversely **influences the biological (micro) environment**, causing molecular, organellar, cellular, or organ dysfunction leading to deleterious effects. For example, **2,4-dinitrophenol,** after entering the mitochondrial matrix space (step 1), collapses the outwardly directed proton gradient across the inner membrane by its mere presence there (step 2b), causing mitochondrial dysfunction (step 3), which is manifest in toxic effects such as **hyperthermia and seizures.**

- Chemicals that precipitate in renal tubules and block urine formation represent another example for such a course (**step 2b**).

- The most complex path to toxicity involves more steps (Fig. 1). First, the toxicant is delivered to its target or targets (step 1), after which the **ultimate toxicant** interacts with endogenous target molecules (step 2a), triggering perturbations (**disturbances**) in cell function and/or structure (step 3), which initiate repair mechanisms at the molecular, cellular, and/or tissue levels as well as adaptive mechanisms to diminish delivery, boost repair capacity and/or compensate for dysfunction (step 4). When the perturbations induced by the toxicant exceed repair and adaptive capacity or when repair and adaptation becomes malfunctional, toxicity occurs. **Tissue necrosis, cancer, and fibrosis** are examples of chemically induced toxicities whose development follow this four-step course.


Toxicant
1
Delivery
2a
Interaction with target molecule
2b
Alteration of biological environment
3
Cellular dysfunction, injury
4
Inappropriate repair and adaptation
TOXICITY

STEP 1—DELIVERY: (from the site of exposure to the target): Theoretically, the intensity of a toxic effect depends primarily on the **concentration** and **persistence** of the ultimate toxicant at its site of action.

-The **ultimate toxicant** is the chemical species that reacts with the endogenous target molecule (e.g., receptor, enzyme, DNA, microfilamental protein, lipid) or critically alters the biological (micro) environment, initiating structural and/or functional alterations that result is toxicity.

- Often the **ultimate toxicant*** ** is the **original chemical** to which the organism is exposed (**parent compound**). In other cases, the ultimate toxicant is a **metabolite of the parent compound** or a **reactive oxygen or nitrogen species (ROS or RNS)** generated during the biotransformation of the toxicant. Occasionally, the ultimate toxicant is **an unchanged or altered endogenous** molecule (examples in the next slides).

- Types of Ultimate Toxicants and Their Sources

1- Parent xenobiotics as ultimate toxicants:

Pb ions

Tetrodotoxin

CO

2- Xenobiotic metabolites as ultimate toxicants:

Amygdalin → HCN

Ethylene glycol → Oxalic acid

Acetaminophen → *N*-Acetyl-*p*-benzoquinoneimine

CCl_4 → $CCl_3OO\bullet$

3- Reactive oxygen or nitrogen species as ultimate toxicants:

Hydrogen peroxide, Diquat, doxorubicin, nitrofurantoin → **Hydroxyl radical (HO•)**

4-Endogenous compounds as ultimate toxicants:

Sulfonamides→ albumin-bound bilirubin → Bilirubin

$CCl_3OO\bullet$ →unsaturated fatty acids → Lipid peroxyl radicals

$CCl_3OO\bullet$ →unsaturated fatty acids → Lipid alkoxyl radicals

-The concentration of the ultimate toxicant at the target molecule depends on the relative effectiveness of the processes that increase or decrease it's concentration at the target site .

-The accumulation of the ultimate toxicant at its target is facilitated by its absorption, distribution to the site of action, reabsorption, and toxication (metabolic activation).

-Conversely, presystemic elimination, distribution away from the site of action, excretion, and detoxication **oppose these processes and work against the accumulation of the ultimate toxicant at the target molecule.**

- Whereas transporters may contribute to the gastrointestinal absorption of some chemicals (e.g., salicylate and valproate by monocarboxylate transporters, some *β*-lactam antibiotics and ACE inhibitor drugs by peptide transporters, Fe2+, Cd2+, as well as some other divalent metal ions by the divalent metal-ion transporter ,the vast majority of toxicants traverse epithelial barriers and reach the blood capillaries by diffusing through cells.

-**General Factors Affecting Absorption of Toxicants are include:**

The rate of absorption is related to the **'concentration of the chemical at the absorbing surface**, which depends on the rate of exposure and the dissolution of the chemical. It is also related to the **'area of the exposed site**', the characteristics of the epithelial layer through which absorption takes place (e.g., the thickness of the stratum corneum in the skin), the intensity of the subepithelial microcirculation, and the physicochemical properties of the toxicant. **'Lipid solubility'** is usually the most important property influencing absorption.

-In general, lipid-soluble chemicals are absorbed more readily than are water-soluble substances.

- **Presystemic Elimination:** During transfer from the site of exposure to the systemic circulation, **toxicants may be eliminated*****. This is not unusual for chemicals absorbed from the gastrointestinal (GI) tract because they must first pass through the GI mucosal cells, liver, and lung before being distributed to the rest of the body by the systemic circulation. The **GI mucosa and the liver** may eliminate a significant fraction of a toxicant during its passage through these tissues, decreasing its systemic availability.
- For example, ethanol is oxidized by alcohol dehydrogenase in the gastric mucosa, **morphine** is glucuronidated in intestinal mucosa and liver, and manganese is taken up from the portal blood into liver and excreted into bile.

- Thus, **presystemic or first-pass elimination reduces the toxic effects** of chemicals that reach their target sites by way of the systemic circulation. In contrast, the processes involved in **presystemic** elimination may contribute to injury of the digestive mucosa, liver, and lungs by chemicals such as **ethanol, iron salts, α-amanitin, and paraquat because these processes promote their delivery to those sites.**

-**Distribution to and Away from the Target**

-Toxicants exit the blood during the distribution phase, enter the extracellular space, and may penetrate into cells.

-Chemicals dissolved in plasma water may diffuse through the capillary endothelium via aqueous intercellular spaces and transcellular pores called **fenestrae and/or across the cell membrane**.

-Lipid-soluble compounds move readily into cells by diffusion.

-In contrast, highly ionized and hydrophilic xenobiotics (e.g., tubocurarine and aminoglycosides) are largely restricted to the **extracellular space** unless specialized membrane carrier systems are available to transport them.

-Some **mechanisms facilitate** whereas others **delay** the distribution of toxicants to their targets.

A- Mechanisms Facilitating Distribution to a Target sites may be enhanced by

(1) the porosity of the capillary endothelium.

(2) specialized membrane transport.

(3) accumulation in cell organelles.

(4) reversible intracellular binding.

B- **Mechanisms Opposing Distribution to a Target:** Distribution of toxicants to specific sites may be hindered by several processes. The processes include:

(1) binding to plasma proteins.

(2) specialized barriers.

(3) distribution to storage sites such as adipose tissue.

(4) association with intracellular binding proteins.

(5) export from cells.

-Excretion versus Reabsorption

Excretion is a physical mechanism whereas biotransformation is a chemical mechanism for eliminating the toxicant. The major excretory organs (**kidney and liver**) can efficiently remove only **highly hydrophilic**, usually **ionized chemicals such as organic acids and bases**. The reasons for this are as follows:

(1) in the renal glomeruli, only compounds dissolved in plasma water can be filtered.

(2) transporters in hepatocytes and renal proximal tubular cells are specialized for secretion of highly hydrophilic organic acids and bases.

(3) only hydrophilic chemicals are freely soluble in the aqueous urine and bile.

(4) lipid-soluble compounds are readily reabsorbed by transcellular diffusion. ‘’**Therefore, The route and speed of excretion depend largely on the physicochemical properties of the toxicant**”.

- **There are no efficient elimination mechanisms for nonvolatile, highly lipophilic** chemicals such as **polyhalogenated biphenyls** and **chlorinated hydrocarbon insecticides**. If they are resistant to biotransformation, such chemicals are eliminated very slowly and tend to accumulate in the body upon repeated exposure.

-Three rather inefficient processes are available for the elimination of such chemicals:

(1) excretion by the mammary gland after the chemical is dissolved in the milk lipids.

(2) excretion in bile in association with biliary micelles and/or phospholipid vesicles.

(3) intestinal excretion, an incompletely understood transport from blood into the intestinal lumen. **Volatile, nonreactive** toxicants such as gases and volatile liquids diffuse from pulmonary capillaries into the alveoli and are exhaled.

-Reabsorption:

1- From the kidney: Toxicants delivered into the **renal** tubules may diffuse back across the tubular cells into the peritubular capillaries. This process is facilitated by tubular fluid reabsorption, which increases the intratubular concentration as well as the residence time of the chemical by slowing urine flow.

Reabsorption by diffusion is dependent on the **lipid solubility** of the chemical. For organic acids and bases, diffusion is inversely related to the extent of ionization, because the nonionized molecule is more lipid-soluble. The ionization of weak organic acids, such as salicylic acid and phenobarbital, and bases, such as amphetamine, procainamide, and quinidine, is strongly pH-dependent in the physiologic range. **Therefore their reabsorption is influenced significantly by the pH of the tubular fluid.(applied in treatment of toxicity***).**

2- By G.I.T: Toxicants delivered to the GI tract by biliary, gastric, and intestinal excretion and secretion by salivary glands and the exocrine pancreas may be reabsorbed by diffusion across the intestinal mucosa. Because compounds secreted into bile are usually **organic acids**, their reabsorption is possible only if they are **sufficiently lipophilic** or are converted to more **lipid-soluble** forms in the intestinal lumen. For example, glucuronides of toxicants such as diethylstilbestrol, and glucuronides of the hydroxylated metabolites of polycyclic aromatic hydrocarbons are hydrolyzed by **the *β*-glucuronidase of intestinal microorganisms**, and the released aglycones are reabsorbed .

-Glutathione conjugates of trichloroethylene are hydrolyzed by intestinal and pancreatic peptidases, yielding the cysteine conjugates, which are reabsorbed and serve as precursors of some **nephrotoxic** metabolites.

Toxication versus Detoxication

-Toxication: A number of xenobiotics (e.g., nicotine, aminoglycosides, heavy-metal ions, HCN, CO) are **directly toxic**, whereas the toxicity of others is due largely to **metabolites**. Biotransformation to harmful products is called ***toxication* or *metabolic activation.***

- With some xenobiotics, toxication confers physicochemical properties that adversely alter the microenvironment of biological processes or structures. For example, oxalic acid formed from ethylene glycol may cause acidosis and hypocalcaemia as well as obstruction of renal tubules by precipitation as calcium oxalate.

-Occasionally, chemicals **acquire structural features** and reactivity by biotransformation that allows for a more efficient interaction with specific receptors or enzymes:
e.g.1: the general anesthetic methoxyflurane releases fluoride ion which inhibits several enzymes (including enolase in the glycolytic pathway) and which contributes to **renal injury** after prolonged anesthesia.
e.g. 2: some cephalosporin antibiotics (e.g., cephoperazone) may cause hemorrhage because they undergo biotransformation with release of 1-methyltetrazole-5-thiol that inhibits vitamin K epoxide reductase and thus impairs activation clotting factors.

-Detoxication: biotransformation that eliminates an ultimate toxicant or prevents its formation. It can take several pathways, depending on the chemical nature of the toxic substance :

1. Detoxication of toxicants with No functional groups:

In general, chemicals without functional groups, such as benzene and toluene, are detoxicated **in two phases**. Initially, a functional group such as hydroxyl or carboxyl is introduced into the molecule, most often by cytochrome-P450 enzymes. Subsequently, an endogenous acid, such as glucuronic acid, sulfuric acid, or an amino acid, is added to the functional group by a transferase. With some exceptions, the final products are inactive, highly hydrophilic organic acids that are readily excreted.

2. Detoxication of Free Radicals Because $O^{-}\bullet 2$ can be converted into much more reactive compounds , its elimination is an important detoxication mechanism. This is carried out by **superoxide dismutases (SOD)**, high-capacity enzymes located in the cytosol (Cu, Zn-SOD) and the mitochondria (Mn-SOD), which convert $O^{-}\bullet 2$ to HOOH . Subsequently, HOOH is reduced **to water** by **catalase** in the peroxisomes (also in the mitochondria in cardiac muscle), by the selenocysteine-containing glutathione **peroxidases** in the cytosol and mitochondria, and by **peroxiredoxins** in the cytosol, mitochondria and endoplasmic reticulum

Lecture Topics

- STEP 2-Reaction of The Ultimate Toxicant With the Target Molecule

I-Attributes of Target Molecules &Types of Reactions

II-Effects of Toxicants on Target Molecules

-STEP 3-Cellular Dysfunction and Resultant Toxicities:

-STEP 4-Inappropriate Repair and Adaptation

I- Attributes of Target Molecules:

Practically all endogenous compounds are potential targets for toxicants, but the most prevalent and toxicologically relevant targets are **macromolecules** such as **nucleic acids** (**especially DNA**) and proteins. Among the **small molecules**, membrane lipids are frequently involved, whereas cofactors such as **coenzyme A** and **pyridoxal** rarely are involved.

:- **To be a target, an endogenous molecule must possess the** appropriate reactivity and/or steric configuration to allow the ultimate toxicant to enter into covalent or non-covalent reactions. For these reactions to occur, the target molecule must be accessible to a sufficiently high concentration of the ultimate toxicant. Thus, endogenous molecules that are exposed to reactive chemicals or are adjacent to sites where reactive metabolites are formed are frequently targets.

-For example, **thyroperoxidase,** the enzyme involved in thyroid hormone synthesis, converts some nucleophilic xenobiotics (such as methimazole) into reactive free radicals that **inactivate thyroperoxidase** .This is the basis for the **anti-thyroid** as well as the thyroid tumor–inducing effect of these chemicals. **Carbon tetrachloride(CCl4)**, which is activated by cytochrome P450, destroys this enzyme as well as the neighboring microsomal membranes.

2-Types of Reactions: the ultimate toxicant may bind to the target molecules through:

a- Non-covalent Binding: This type of binding can be due to apolar interactions or the formation of hydrogen and ionic bonds and is typically involved in the interaction of toxicants with targets such as membrane receptors, intracellular receptors, ion channels, and some enzymes. For example, such interactions are responsible for the binding of strychnine to the glycine receptor on motor neurons in the spinal cord, and warfarin to vitamin K 2,3-epoxide reductase.

b- Covalent Binding: Being practically irreversible, covalent binding is of great toxicologic importance because it permanently alters endogenous molecules . **Covalent adduct** formation is common with electrophilic toxicants such as nonionic and cationic electrophiles and radical cations. These toxicants react with nucleophilic atoms that are abundant in biological macromolecules, such as proteins and nucleic acids. Metal ions such as silver and mercury , lithium, calcium, and barium, chromium, zinc, and lead are examples.

C- Hydrogen Abstraction: Neutral free radicals, such as those generated in reactions such as $NO^{\cdot}$,$OH^{\cdot}$, can readily abstract H atoms from endogenous compounds, converting those compounds into radicals. Radicals can remove hydrogen from CH2 groups of free **amino acids or from amino acid** residues in proteins and convert them to carbonyls. These carbonyls react covalently with amines, forming cross-links with **DNA or other proteins**.

d- Electron Transfer: Chemicals can oxidize Fe(II) in hemoglobin to Fe(III), producing methemoglobinemia. Nitrite can oxidize hemoglobin, whereas dapsone, and 5-hydroxy primaquine, are co-oxidized with oxyhemoglobin, forming methemoglobin and hydrogen peroxide.

e- Enzymatic Reactions : A few toxins act enzymatically on specific target proteins. For example:

-Botulinum toxin acts as a Zn-protease; it hydrolyses the fusion proteins that assist in exocytosis of the neurotransmitter acetylcholine in cholinergic neurons, most importantly motor neurons, causing paralysis.

II- Effects of Toxicants on Target Molecules

Reaction of the ultimate toxicant with endogenous molecules may cause :

1- **Dysfunction of Target Molecules:** Some toxicants activate protein target molecules, mimicking endogenous ligands. For example, morphine activates opioid receptors, clofibrate is an agonist on the peroxisome proliferator–activated receptor. More commonly, chemicals inhibit the function of target molecules. Several xenobiotics—such as atropine, curare—block neurotransmitter receptors by attaching to the ligand-binding sites .

2-Destruction of Target Molecules: In addition to **adduct formation**, toxicants alter the primary structure of endogenous molecules by means of **cross-linking** and **fragmentation** .

-Some target molecules are susceptible to spontaneous degradation after chemical attack. Free radicals such as Cl3COO• and HO• can initiate peroxidative degradation of lipids by hydrogen abstraction from fatty acids . The lipid radical (L•) formed is converted successively to lipid peroxyl radical (LOO•) by oxygen fixation, lipid hydroperoxide (LOOH) by hydrogen abstraction, and lipid alkoxyl radical (LO•).Subsequent fragmentation gives rise to hydrocarbons such as **ethane** and reactive aldehydes such as 4- hydroxynon-2-enal (**4-HNE**)and malondialdehyde (**MDA**) . Thus, lipid peroxidation not only destroys lipids in cellular membranes but also generates endogenous toxicants, both free radicals (e.g., **LOO•, LO•)** and electrophiles (e.g., 4-oxonon-2-enal, 4-hydroxynon-2- enal). These substances can readily react with adjacent molecules, such as membrane proteins, or diffuse to more distant molecules such as DNA.

-Toxicity Not Initiated by Reaction with Target Molecules:

Some xenobiotics **do not only interact with a specific endogenous** target molecule to induce toxicity, but instead **alter the biological microenvironment** .Included here are:

(**1) Chemicals that alter H+ ion concentrations in the aqueous biophase**, such as acids and substances biotransformed to acids, such as methanol and ethylene glycol, as well as protonophoric uncouplers such as **2,4 dinitrophenol and pentachlorophenol**, which dissociate their phenolic protons in the mitochondrial matrix, thus dissipating the proton gradient that drives ATP synthesis.

(2) Solvents and detergents that physicochemically alter the lipid phase of cell membranes and destroy transmembrane solute gradients that are essential to cell functions.

(3) Other xenobiotics that cause harm merely **by occupying a site or space**. For example, some chemicals (e.g., **ethylene glycol, methotrexate, acyclovir**) form water-insoluble precipitates in the renal tubules. By occupying bilirubin binding sites on **albumin**, compounds such as the sulfonamides induce bilirubin toxicity (kernicterus) in neonates. **Carbon dioxide** displaces oxygen in the pulmonary alveolar space and causes **asphyxiation**.

-STEP 3-Cellular Dysfunction and Resultant Toxicities:

The reaction of toxicants with a target molecule may result in impaired cellular function as the third step in the development of toxicity (Fig. 1).

-Each cell in a multicellular organism carries out defined programs. Certain programs determine the **destiny of cells**—that is, whether they undergo division, differentiation (i.e., express proteins for specialized functions), or apoptosis.

- Other programs control the ongoing (momentary) activity of differentiated cells, determining whether they secrete more or less of a substance, whether they contract or relax, and whether they transport and metabolize nutrients at higher or lower rates. **For regulations of these cellular programs, cells possess signaling networks that can be activated and inactivated by external signaling molecules.**

- Dysregulation of Transcription by Chemicals Acting Through Ligand-Activated Transcription Factors:

Several **endogenous compounds**, such as hormones (e.g., steroids, thyroid hormones) and vitamins (retinoids and vitamin D), influence gene expression by binding to and activating TFs which may lead to teratogenesis (craniofacial, cardiac, thymic malformations).

-Xenobiotics may mimic the natural ligands. For example, fibric acid–type lipid lowering drugs and phthalate esters substitute for polyunsaturated fatty acids as ligands for the peroxisome proliferator-activated receptor (PPARα) ,and **xeno-estrogens** (e.g., diethylstilbestrol) substitute estradiol, an endogenous ligand for estrogen receptors that may lead to mammary and hepatic carcinogenesis.

- Dysregulation of Transcription by Chemicals Altering the Regulatory Region of Genes:

Xenobiotics may **dysregulate transcription** by also altering the regulatory gene regions through direct chemical interaction or by changing their **methylation pattern**.

- It has been hypothesized that **thalidomide** (or its hydrolysis product) exerts **teratogenic** effect in the embryo by intercalating to GGGCGG sequences (**also called GC boxes**) that are binding sites for Sp1, a transcription activator for RNA polymerase II .Thalidomide would thus impair insulin-like growth factor-1 (**IGF-2**) and fibroblast growth factor-2 (**FGF-2**) signaling pathways necessary for **angiogenesis and limb formation**, because both pathways encompass multiple proteins whose genes contain GC boxes in the regulatory region .

-Dysregulation of Electrically Excitable Cells: Many xenobiotics influence cellular activity in excitable cells, such as neurons, skeletal, cardiac, and smooth muscle cells.

-Altered regulation of neural and/or muscle activity is the basic mechanism of action of many drugs and is responsible for toxicities associated with drug overdosage, pesticides, and microbial, plant, and animal toxins .

E.g. tetrodotoxin, which blocks voltage-gated Na+ channels in motor neurons, causes skeletal muscle paralysis. In contrast, **cyclodiene** insecticides, which block GABA receptors in the central nervous system, induce neuronal excitation and convulsions.

B-Impairment of External Cellular Maintenance:
Hepatocytes produce and release into the circulation a number of proteins and nutrients. They remove **cholesterol and bilirubin** from the circulation, converting them into bile acids and bilirubin glucuronides, respectively, for subsequent excretion into bile. Interruption of these processes may be harmful to the organism, the liver, or both. For example, inhibition of hepatic synthesis of coagulation factors **by coumarins does not harm the liver, but may cause death by hemorrhage**.

-STEP 4-Inappropriate Repair and Adaptation:

-For example (**Protein repair mechanism**), **CYP2E1**, in which the heme becomes cross-linked to the protein as a consequence of reductive dechlorination of CCl4 to the trichloromethyl free radical by this enzyme, is eliminated by **proteosomal degradation**. Damaged proteins are first conjugated with **ubiquitin** in an ATP-dependent manner, allowing their recognition by the **proteosome** that degrades them. Damaged proteins can be eliminated also by proteolysis in lysosomes.

- Repair of Lipids: Peroxidized lipids are repaired by a complex process that operates in concert with a series of reductants as well as with **glutathione peroxidase** and **reductase** .

-Phospholipids containing fatty acid hydroperoxides are preferentially hydrolyzed by phospholipase A2, with the peroxidized fatty acids replaced by normal fatty acids .Again, NADPH is needed to “repair” the reductants that are oxidized in the process.

-The fourth step in the development of toxicity is inappropriate repair and adaptation (Fig. 1-Part I). As noted previously, many toxicants alter macromolecules, which eventually cause damage at higher levels of the biological **hierarchy** in the organism. Progression of toxic lesions can be intercepted by repair mechanisms operating at molecular, cellular, and tissue levels .

-**Another strategy whereby the organism can resist the noxious chemical is by increasing its own readiness to cope with it and with its harmful effects**. Such phenomenon is **called adaptation**. The capacity of the organism to repair itself and adapt to the toxic exposure and effects is so important in determining the outcome of chemical exposure, e.g. on mechanisms of repair and adaptation will include the followings: -

- **Molecular Repair**:

Damaged molecules may be repaired in different ways. Some chemical alterations, such as oxidation of protein thiols and methylation of DNA, are simply reversed.

***How?? **Hydrolytic removal** of the molecule's damaged unit or units and **insertion of a newly** synthesized unit or units often occur with chemically altered DNA and peroxidized lipids.

-In some instances, the damaged molecule is totally **degraded and resynthesized**. This process is time consuming but unavoidable in cases such **as the regeneration of cholinesterase after organophosphate intoxication**.

1- Repair of Proteins: (Thiol groups are the target)

Thiol groups are essential for the function of numerous **proteins**, such as receptors, enzymes, cytoskeletal proteins, and TFs. **Oxidation of protein thiols** (Prot-SHs) to protein **disulfides** (Prot-SS, Prot1-SS-Prot2), protein-glutathione mixed disulfides (Prot-SSG), and protein sulfenic acids (Prot-SOH) as well as oxidation of methionine in proteins to methionine sulfoxide can be **reversed by enzymatic reduction** .The endogenous reductants are **thioredoxins and glutaredoxins**, small, ubiquitous proteins with two redox-active cysteines in their active centers .These proteins as well as thioredoxin-reductase have two isoenzymes; those labeled **1** are located in the **cytosol**, whereas **2** are **mitochondrial**.

- Because the catalytic thiol groups in these proteins become **oxidized**, they are reduced by **NADPH**, which is generated by NADP+-dependent isocitrate dehydrogenase localized in various cell compartments (**cytosol, mitochondria, peroxysomes**), as well as by the cytosolic glucose-6-phosphate dehydrogenase and 6 phosphogluconate dehydrogenase in the pentose phosphate pathway.

***2*- Repair of Lipids:** **peroxidized lipids** are repaired by a complex process **??..**that operates in concert with a series of reductants as well as with glutathione peroxidase and reductase .Phospholipids containing fatty acid hydroperoxides are preferentially hydrolyzed by phospholipase A2, with the peroxidized fatty acids replaced by normal fatty acids .Again, NADPH is needed to **"repair"** the reductants that are oxidized in the process.

3- Repair of DNA*:*

Despite its high reactivity with electrophiles and free radicals, **nuclear DNA** is remarkably stable, in part because it is packaged in chromatin and because several repair mechanisms are available to correct alterations.

-The **mitochondrial DNA**, however, lacks histones and efficient repair mechanisms and therefore is more prone to damage. Different types of damages are corrected by specialized mechanisms, each employing a different set of repair proteins.

E.g. on Direct DNA Repair: Certain covalent DNA modifications are directly reversed by **enzymes** such **DNA photolyase**, which cleaves adjacent pyrimidines dimerized by UV light. In as much as this chromophore-equipped enzyme uses the energy of visible light to correct damage, its use is restricted to light-exposed cells.

Cellular Repair: "A Strategy in Peripheral Neurons"

: Repair of damaged cells is not a widely applied strategy in overcoming cellular injuries??why

:In most tissues, injured cells die, with the survivors dividing to replace the lost cells. A notable exception is **nerve tissue**, because mature neurons have lost their ability to multiply.

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-In **peripheral neurons** with axonal damage, repair does occur and requires macrophages and Schwann cells. **Macrophages** remove debris by phagocytosis and produce cytokines and growth which activate Schwann cells to proliferate and transdifferentiate from myelinating operation mode into a growth-supporting mode. **Schwann cells** play an indispensable role in promoting axonal regeneration by increasing their synthesis of cell adhesion molecules (e.g., N-CAM), by elaborating extracellular matrix proteins for base membrane construction, and by producing an array of neurotrophic factors (e.g., nerve growth factor, glial–cell line–derived growth factor) and their receptors .While comigrating with the regrowing axon, **Schwann cells** physically guide as well as chemically **lure** the axon to reinnervate the target cell

- **Tissue Repair:** In tissues with cells capable of multiplying, damage is reversed by deletion of the injured cells and regeneration of the tissue by proliferation. The damaged cells are eliminated by either apoptosis or necrosis.

- Importance (significance)of apoptosis according to cell types:

-apoptosis of damaged cells has a full value as a tissue repair process only for tissues that are made up of **constantly renewing cells** (e.g., the bone marrow, the respiratory and gastrointestinal epithelium, and the epidermis of the skin), or of **conditionally dividing cells** (e.g., hepatic and renal parenchymal cells), because in these tissues the apoptotic cells are readily replaced. The value of apoptosis as a tissue repair strategy is markedly **lessened in organs containing nonreplicating and nonreplaceable cells**, such as the neurons, cardiac muscle cells, and female germ cells, because deletion of such cells, if extensive, can cause a deficit in the organ's function.

-Apoptosis in the **pulmonary alveolar epithelium**, an extremely tight barrier, could cause flooding of the alveolar space with interstitial fluid, a potentially lethal outcome**.??**

Mechanisms of Adaptation

Adaptation may be defined as a **noxa**-induced capability of the organism for increased tolerance to the noxa itself.
It involves responses acting to preserve or regain the biological homeostasis in the face of increased harm. Theoretically, adaptation to toxicity may result from biological changes causing :

(1) diminished delivery of the causative chemical(s) to the target.

(2) decreased size or susceptibility of the target.

(3) increased capacity of the organism to repair itself.

(4) strengthened mechanisms to compensate the toxicant inflicted dysfunction. **Mechanistically, adaptation involves sensing the noxious chemical and/or the initial damage or dysfunction, and a response that typically occurs through altered gene expression**.

Adaptation by Decreasing Delivery to the Target

The first step in the development of toxicity is delivery of the ultimate toxicant (**axenobiotic, its metabolite or xenobiotic-generated ROS and RNS*****) to the target .Certain chemicals induce adaptive changes that lessen their delivery by diminishing the absorption, increasing their sequestration by intracellular binding proteins, enhancing their detoxication, or promoting their cellular export. E.g. ***Repression of Iron Absorption*** an adaptive mechanism affecting absorption is induced by iron whose uptake from the intestinal lumen into the enterocyte is mediated by the divalent metal transporter 1 (DMT1). High iron intake diminishes the expression of DMT1 in the apical membrane of enterocytes, whereas low intake has the opposite effect.

-Adaptation by Decreasing the Target Density or Responsiveness

Decreasing the **density and sensitivity** of the xenobiotic target is an adaptation mechanism for several **cell surface receptors**. Such alterations underlie the tolerance **induced by opioids**.

Induction of Opioid Tolerance the main target of opioids (e.g., morphine, heroine, methadone) is the ***μ*-opioid receptor**. Stimulation of this G*i* -protein-coupled inhibitory receptor by an agonist results in adenyl-cyclase inhibition (causing decline in cyclic AMP levels and PKA activity) and K+ channel opening (causing hyperpolarization) in neurons with opioid receptors. **Even brief stimulation in**duces adaptive alterations: the **receptor is desensitized** by G-protein receptor kinase-mediated phosphorylation and *β*-arrestin binding, then becomes **uncoupled from the G protein** and **internalized** .Whereas some receptors are **recycled to the cell membrane**, others are **degraded in the lysosomes**, causing receptor **down regulation**. **Upon prolonged stimulation**, adenyl-cyclase signaling undergoes a compensatory increase**???(upregualtion in** adenyl-cyclase signaling) **.**

Thanks for Listening