Unit 5

MECHANISM OF TOXICITY

Structure

5.1 INTRODUCTION

To show systemic toxicity, toxicants must be absorbed in the blood and delivered (or distributed) to the target organ. Once toxicants have entered the body, they are enzymatically modified (metabolized) and excreted. The efficiency of absorption, distribution, metabolism and excretion (ADME) affects attainment of toxic concentration and duration of stay of toxicants in the body. Each of these processes is affected by several factors including physicochemical properties of toxicants, anatomical features of the body, genetic and physiological factors, and environmental factors. In this unit, we will discuss the process, parameters to measure the efficiency, and factors affecting ADME.

Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ understand the process of ADME orhow toxicants enter the body, move to various organs, areenzymatically modified and removed from the body,
- ❖ know factors affecting the ADME,
- ❖ know role of organs such as liver, gastrointestinal tract, lungs and skin in disposal and activation or toxicants and,
- ❖ learn basics of how to quantify or measure the efficiency of ADME processes.

5.2 INTRODUCTION TO ADME—ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION OF TOXICANTS

We have already discussed in the previous units that systemic toxicity happens when:

1. a toxicant is 'absorbed' from the site-of-exposure (such portion of skin, gastrointestinal tract, lungs, etc.) and reaches systemic circulation,
2. followed by its 'distribution' via blood to the site-of-toxicity or target organ,
3. its interaction with the target molecule in the target organ resulting in alteration of the function of the former,
4. which in turn causes alteration in function of cell/irreparable injury to cells, followed by abnormality in the function of organ and manifestation of toxicity,

The above process usually happens in a step wise manner. However, while toxicants are being absorbed and distributed, these simultaneously undergo 'metabolism' (also known as biotransformation) and 'excretion'. The extent and rate of Absorption, Distribution, Metabolism and Excretion (ADME) thus determines the 'concentration' and 'duration of stay' of toxicants in the body (Unit 3). The entire process can be diagrammatically summarized as given in Fig. 5.1.

The quantitative study (i.e. rate and extent) of ADME of toxicants is known as 'toxicokinetics'. In this section, we will study the qualitative aspects i.e. mechanism and factors affecting absorption, distribution, metabolism and excretion.

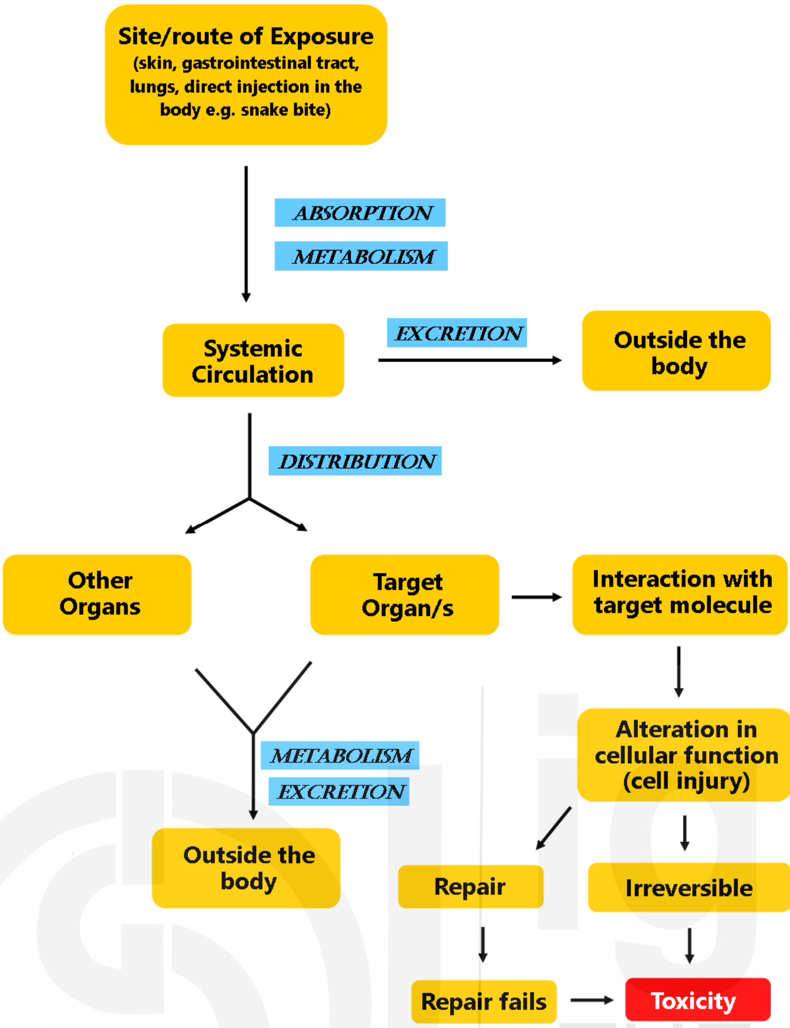

Fig. 5.1: Overview of the events leading to toxicity.

5.2.1 Absorption

Absorption is the process by which toxicants cross various tissues (epithelial layer, lamina, sub-mucosa, dermis, etc.) at the site-of-exposure and enter systemic circulation.To absorb and reach bloodstream from the site-of-exposure, toxicants have to cross cell membranes, move through intercellular space in the tissues, and again cross a cell membrane to enter another tissue or blood vessels. Poor absorption of toxicants may not initiate a toxic response (see Unit 3).

The extent and rate of absorption depends on the physicochemical properties of toxicants (lipid solubility, size, etc.) and attributes of the site where absorption takes place: i.e. surface area, properties of epithelium (presence of transporters, thickness of epithelium, etc.) and blood supply in the underlying tissue. In general, absorption readily takes place if the toxicant is lipid-soluble, and the site of absorption has a large surface area and good supply of blood.

The efficiency of absorption is expressed by the term ‘bioavailability’ which is defined as ‘fraction of administered chemical reaching the systemic circulation’. We will discuss more about bioavailability at the end of this section.

The common routes through which humans are exposed to most of the toxicants are oral (ingestion), lungs (inhalation), and skin surface. Below, we will discuss absorption of toxicants via these routes and factors affecting rate and extent of absorption.

Presystemic Elimination

Presystemic elimination is the removal or inactivation of the absorbed toxicant before it reaches systemic circulation for distribution to the target site/s. Two processes are mainly responsible for presystemic elimination, viz. i) inactivation of absorbed toxicant due to biotransformation in liver, enterocytes (epithelial cells of gastrointestinal tract), and lungs; 2) excretion oftoxicants without or after biotransformation into bile by liver. The overall effect of presystemic elimination is to reduce the total amount of toxicant absorbed from the site-of-exposure before it reaches systemic circulation (thus decreasing the bioavailability). Toxicants absorbed in gastrointestinal tract undergo significant presystemic elimination. We will see more about biotransformation in subsection 5.2.3.

Absorption via oral route: Toxicants entering via oral route are absorbed in gastrointestinal tract. Though some amounts of toxicants can be absorbed in the buccal cavity, most of it is absorbed in the stomach and intestine. After being absorbed in gastrointestinal tract, toxicants are carried first to the liver (Fig. 5.2). Here, toxicants may become substrates for 'xenobiotic metabolizing enzymes' and undergo enzymatic reactions resulting in modification of their chemical structure–a process known as 'biotransformation' or 'xenobiotics metabolism' (see below). Biotransformation may inactivate a fraction of absorbed toxicants and promote their excretion before they reach systemic circulation–'Presystemic elimination'.

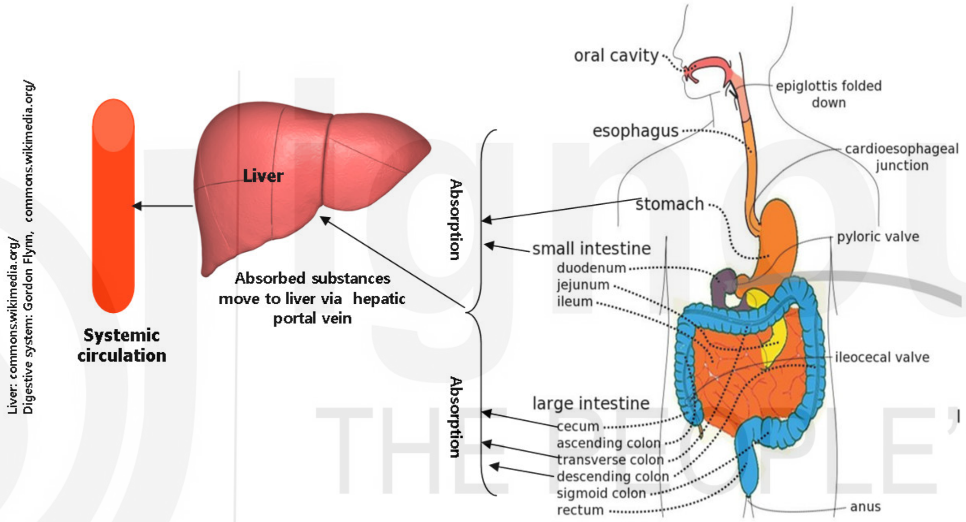

Fig. 5.2: Absorption and movement of toxicants exposed via oral route.

Lipid-soluble toxicants can cross cell membrane by *simple diffusion,* and thus, are absorbed efficiently. Toxicants (such as those with weak acidic groups) that remain predominantly in uncharged form at acidic pH (thus more lipid-soluble than the charged form) are absorbed well in the stomach. Conversely, organic bases are predominantly in charged form in stomach and are poorly absorbed here. However, a more basic environment of intestine causes molecules of organic bases to lose charge (thus more lipid-soluble) and are absorbed efficiently. For example, salicylic acid (pKa=2.19) remains uncharged in low pH conditions of stomach (pH=2) and is well absorbed, whereas aniline (pKa 4.6) is uncharged in more basic pH of intestine (pH=6–7.4) and is absorbed well here. From above discussion, we learnt how pH of the surroundings can affect absorption of toxicants. Small water-soluble toxicants are not able to cross cell membrane by simple diffusion. They get access to the body by *facilitated diffusion* and *active transport* using membrane transporters, or diffuse via *aqueous pores* between two adjacent cells. Large particulates are absorbed via the process of *pinocytosis* (Fig. 5.3).

Absorption of toxicants via gastrointestinal tract is affected by the presence of food, certain components of food and motility of the intestine. Let us see few examples of these: i) citrate is widely used as a flavouring agent. It causes widening the paracellular space by disrupting the tight junctions between adjacent cells of intestinal epithelium. Citrate chelates calcium ions involved in stabilization of tight junctions. Citrate therefore, increases absorption of substances including aluminium, lead, etc, ii) inorganic mercury may react with the thiol group (–SH) containing substances in food (such as cysteine) and the resultant complex gets absorbed by using amino acid transporters, iii) laxatives decrease the absorption of toxicants by increase the motility of intestine (condition similar to diarrhoea), thus reducing the total time for which toxicants stay in the intestine.

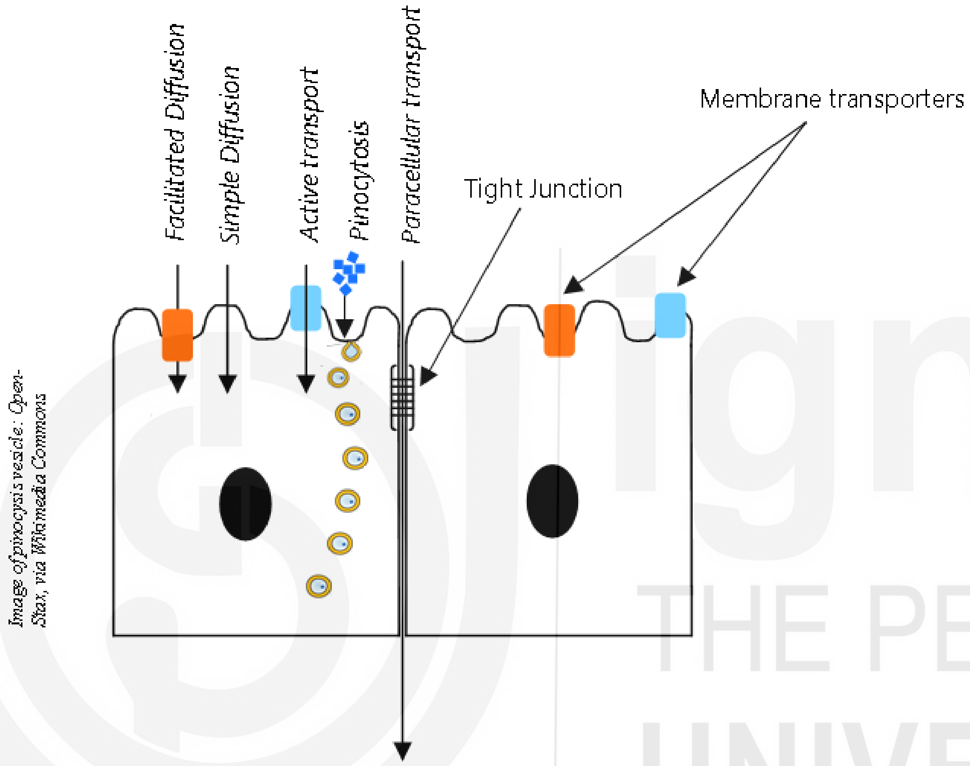

Fig. 5.3: Various ways in which toxicants can cross cell membrane.

Absorption via lungs: Inhaled toxicants (gases, vapours of volatile liquids, aerosols and particles) are mainly absorbed in the lungs. The principles of absorption of gases and vapours and, aerosols and that of particles are different, so we will discuss them separately.

Inhaled molecules of gases and vapours fill the alveolar space in the lungs from where these diffuse across the thin alveolar epithelium and enter the blood. Toxicants dissolved in blood are then quickly carried to other parts of the body. The inspired gas keeps dissolving in the blood from alveolar space till an equilibrium is established at which the ratio of concentration of gas in blood and alveolar sac is constant. This ratio is called ‘blood-to-gas’ partition coefficient and indicates solubility of gas in blood with respect to alveolar air. The greater the blood-to-gas partition coefficient, greater is the solubility and faster it will dissolve in blood with each inhalation. However, a more soluble gas slowly reaches equilibrium and more cycles of inhalations (or time) are required to equilibrate with the blood. Anaesthetic gases with high blood-to-gas partition coefficient dissolve in higher concentration in blood, however, have slower rate of induction of anaesthesia.

The principal governing factors for the absorption of aerosol and particles are the size of aerosols and water solubility. Coarser particles (5 micron or larger) are mainly deposited in the nasopharyngeal region. Particles soluble in mucus lining of the nasal passage may get absorbed, or removed by sneezing or nasal wiping. Fine particles (about 2.5 micron) reach deeper in the respirator tree, i.e. tracheobronchiolar region. These may be displaced from here together with mucus towards mouth, and swallowed or spited out. In the former situation, the swallowed toxicant may be absorbed in gastrointestinal tract. Ultrafine particles (1micron or less) can penetrate further deeper into the alveolar sac. These may get absorbed in blood or reach lymphatic system after being phagocytosed by alveolar macrophages.

Absorption via skin: Our skin comes in contact with several chemicals including potent toxicants (e.g. farmers during formulation and application of pesticides). Skin acts as a barrier and prevents or reduces the amount of several toxicants from reaching the blood. Skin, however, is not an absolute barrier and many toxicants can penetrate through it to access blood.

Skin is comprised of three layers; the outer 'epidermis', below which lies 'dermis', and the innermost fatty layer 'hypodermis'. Epidermis is a multilayered structure, and the outermost layer of epidermis the 'stratum corneum' or horny layer is composed of densely packed flat cells lacking nuclei, and embedded in a lipid rich extracellular matrix. Stratum corneum represents an important barrier to absorption of toxicants. The second layer– dermis, is a connective tissue layer rich in blood supply. Dermis is interrupted by several tubular structures i.e. sweat glands, oil glands and hair follicles (collectively called 'dermal appendages'). Dermal appendages open at the surface of skin by traversing stratum corneum (Fig. 5.4). Significant absorption of toxicants may take place through dermal appendages.

To get absorbed in blood, a toxicant must reach dermis and move into the blood vessels.

In general: i) stratum corneum poses a barrier to large water-soluble chemicals,however, small fat-soluble toxicants can cross this layer and reach dermis ii) water-soluble chemicals can penetrate skin and reach dermis via dermal appendages.

Several factors can affect absorption of toxicants via skin, such as– size of toxicant molecules, temperature of skin, solvent in which toxicant is dissolved, and moisture level of stratum corneum. Chemicals with molecular weight of >500 Daltons are poorly absorbed via skin, solvents (such as acetone) and excessive application of soaps can disrupt the integrity of stratum corneum, making skin more permeable for toxicants. Hydration of skin such as during sweating increases permeability of skin for certain toxicants. High temperature increases blood flow in dermis thereby removing absorbed toxicants at a faster rate; and maintains a concentration gradient for further absorption of more toxicant. Sweating and high temperature together can cause significant absorption of toxicants in people handling hazardous substances at their workplace (factory workers, farmers) in tropical climatic conditions.

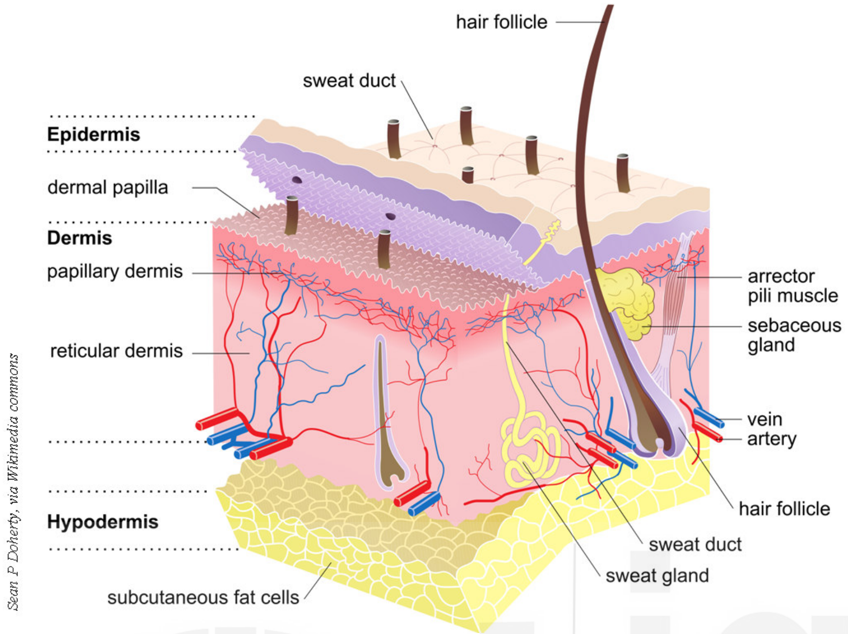

Fig. 5.4: Illustration of skin showing anatomical features. (Source: Image credits Sean P Doherty, CC BY-SA 4.0 httpscreativecommons.orglicensesby-sa4.0, via Wikimedia Commons)

5.2.2 Distribution

After a chemical enters systemic circulation, it travels to various organs of the body including its target organ via blood–a process known as distribution. After reaching the target organ, toxicants move out of the blood capillaries and enter various cells of the organ. While inside an organ, toxicants may encounter their target molecules (if found in that organ) and interact with the latter and show toxic response. Students may note that toxicants are not homogeneously distributed in all parts of the body i.e. their concentrations are not same throughout the body after distribution. There are several factors responsible for this heterogeneous distribution, of which we will discuss a few major factors here:

To enter various organs and cells of each organ, toxicants must cross cell membranes of capillary endothelial cells and those of cells of the organs. Recall, in the absorption section we learnt that–'whether a chemical will be able to cross cell membrane depends on its physicochemical properties and properties of cell membrane'. Thus, lipophilic (fat-soluble) toxicants are widely distributed in the body, whereas, lipophobic (water-soluble) toxicants have restricted distribution. For example, the blood-brain barrier prevents many water-soluble chemicals from entering brain, whereas fat-soluble chemicals can easily enter brain by simple diffusion.

In addition to the above-mentioned properties, toxicants that are extensively bound tothe plasma proteins (such as albumin) are mainly restricted to blood vascular system, because it is only the free or unbound form that can move out of the blood vesselsby crossing capillary endothelial cells. Further, certain toxicants tend to accumulate in a particular tissue of the body, and these tissues are known as 'storage depot'. For example, fat-soluble toxicants such as DDT, TCDD, etc., accumulate in fatty tissues (adipose tissue), cadmium is stored in liver in conjugation with a protein named 'metallothionein', and most of the lead and strontium to which humans are exposed are found in the matrix of bones in conjugation with hydroxyapatite crystals. Toxicants accumulated in the storage depots may remain there for several years. The concentration of such toxicants is usually too low in the blood to show a toxic response. However, during periods of quick mobilization of toxicants from the storage depot, a toxic concentration is attained in the blood that may result in toxic response. For example, DDT stored in adipose tissue is mobilized into blood at a faster rate during starvation and weight loss. This causes increase in exposure of internal organs to high concentrations of DDT and may cause toxicity. Let us see another example: about 80–90% of the lead we absorb is stored in bones. Depending on the extent of exposure, over the years, a high amount of lead can get accumulated in the bones. In postmenopausal women, lead can be mobilized from the bones due to age related osteoporosis, with its resultant increase in blood leadingtotoxic levels.

The extent of distribution of toxicants (or xenobiotics) in the body is measured by 'Volume of Distribution' (V). It is defined as 'virtual volume of the body in which toxicants appear to distribute'.

$$\text{Volume of distribution (V)} \quad \frac{\text{Amount of toxicant administered (mg)}}{\text{Concentration of toxicant in blood (mg/L)}}$$

Suppose,

Amount of toxicant administered= 200 mg; concentration of toxicant in blood= 4 mg/L

V= 200/4= **50 L**

So, a toxicant with lower value of 'V' is expected to remain in the blood vascular system or have limited distribution, whereas a toxicant with higher 'V' (such as lipid-soluble substances) is expected to leave blood vascular system and enter various organs of the body.

5.2.3 Metabolism

Metabolism of xenobiotics is also known as biotransformation. It is aprocess during which certain enzymes of our body (called xenobiotics metabolizing enzymes) modify the chemical structure of parent chemicals (original form in which a chemical was exposed). There are several fates of a chemical after it undergoes biotransformation: i) it may be converted into an inactive metabolite (detoxification or bio-inactivation), ii) an otherwise non-toxic chemical is converted into a potent toxicant (bio-activation or toxication), iii) a lesser toxic chemical is converted to more a potent toxic metabolite (bio-activation).

Though xenobiotics metabolizing enzymes are found in many tissues of many organs in the body, their highest concentration is found in liver and enterocytes (epithelial cells of gastrointestinal tract).Thus, these two organs are usually responsible for the biotransformation of most of xenobiotics to which humans and animals are exposed.

Humans (and animals) have limited number of xenobiotic metabolizing enzymes, and these havebroad and sometimes overlapping substrate specificities. For example, humans have 57 cytochrome P450 monooxygenase (CYP 450) enzymes and one of the isoforms- CYP 3A4 is known to metabolize more than 30% of the drugs. In many cases, a toxicant can be metabolized by more than one enzyme, for example, ethanol can be metabolized by three enzymes, viz. CYP450, alcohol dehydrogenase and catalase.

Biotransformation reactions can be grouped into two categories:

Phase I reactions: includes oxidation, reduction, and hydrolysis reactions,

Phase II reactions: conjugation reactions: attachment of hydrophilic endogenous molecules (glucuronic acid, glycine, glutathione and sulphate) to phase I metabolites or parent toxicants.

The significance of biotransformation reactions is that, they convert fat-soluble toxic substances into their more water-soluble metabolites that can be quickly excreted from the body (via urine, bile, sweat, etc.). This reduces the total duration for which toxic compounds stay in the body. For example, 'benzene' entering the body is converted to 'phenol'in phase I reaction. Phenol is then conjugated with sulphate in phase II reactions which not only detoxifies it, but also makes it more water soluble and easily excretable.We will discuss main factors which affect the metabolism of xenobiotics in section 5.4. Fig. 5.5, depicts the events contributing to elimination of toxicants from the body.

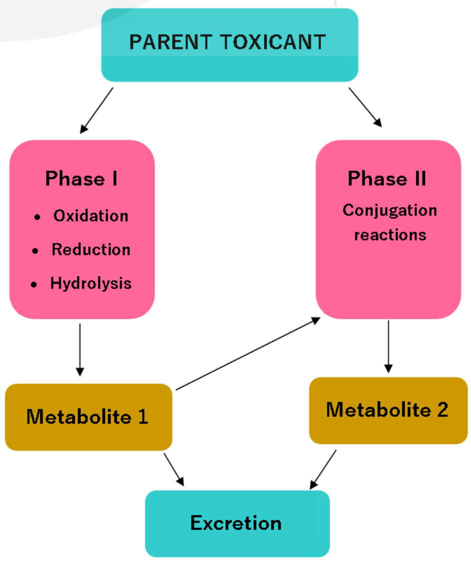

Fig. 5.5: Schematic of elimination of toxicants form the body.

5.2.4 Excretion

Excretion is removal of a substance (or toxicants) from blood to the external environment. The major routes through which various substances are excreted include urine, faeces, exhalation via lungs. Toxicants may also be removed from the body via minor routes including sweat, saliva and milk.

Urine: Excretion of toxicant via urine is the most important route; usually water-soluble substances are efficiently excreted in urine. Lipid-soluble substance sare reabsorbed efficiently from the kidney tubular fluid, thus, their residence time in blood is more. Since, pH of the medium affects the ionization of weak acid and bases (and thus the lipid-solubility, see above), the pH of urine can affect the rate of excretion of toxicants. This principle is used for speedy removal of salicylate (a weakly acidic chemical) from the body in cases of poisoning. By administering sodium bicarbonate, the pH of the urine is raised, and a high pH converts more salicylate molecules into ionized form (more water-soluble), and are removed by the kidneys at a faster rate.

Faeces: itis the second important route of excretion. Toxicants excreted in faeces originate from: 1) unabsorbed toxicants in gastrointestinal tract, 2) toxicants (or metabolites) secreted from blood into intestinal lumen and, 3) toxicants entering gastrointestinal tract via bile. The presence of toxicants in bile is due to a process whereby certain membrane transporters move toxicants (or conjugate formed in phase II reactions) from hepatocyte into bile canaliculi and ducts. Sometimes conjugates of toxicants carried via bile inthe intestinal lumenare deconjugated by intestinal or microbial enzymes (recall gut microflora), and the resulting parent toxicants are reabsorbed; and again, brought to the liver. This cycling of toxicants from the liver to intestine and back is called 'entero-hepatic cycling'. Repeated entero-hepatic cycling increases the residence time of toxicants in the body and thus requires interruption of the cycle. For example, victims/patients of poisoning are given activated charcoal via oral route, conjugates of toxicant entering intestine via bile are adsorbedto the charcoal and thus, are prevented from reabsorption.

Lungs: Gases and volatile liquids dissolved in blood are excreted by exhalation via lungs. The toxicants diffuse from blood to alveolar sac and are then exhaled. Substances with higher solubility in the blood (chloroform) are removed at slower rate than those with relatively low solubility (ethylene).

Milk: Lipid soluble toxicants get dissolved in the fat portion of milk (during synthesis in mammary gland) and are excrete from the body. Several toxicants including DDT, PCBs, aldrin, have been found in human milk. The secretion of toxicants in milk is of concern because it may pass from the mother to suckling, and to the general population via milk and milk products prepared from farm animals.

5.2.5 Bioavailability

We defined bioavailability above as–fraction (or percentage) of administered (or exposed) toxicant that reaches the systemic circulation intact or in original form. Bioavailability is a measure of the amount of a chemical that is absorbed from the site-of-exposure and has escaped presystemic elimination. Chemicals directly introduced into veins are considered to have 100% bioavailability. Chemicals administered (or exposed) via other routes have lower bioavailability due to incomplete absorption and, presystemic elimination. Therefore, only a fraction of administered amount reaches systemic circulation, and is available for distribution to the target site. Toxicants exposed via oral route usually undergo considerable presystemic elimination in enterocytes and liver, thus, have significantly low bioavailability. It is therefore possible that oral LD50 value of a toxicant may be higher than the LD50 value measured via other routes of exposure. For example, LD50 value for carbaryl (an insecticide) in rats via oral, intraperitoneal and intravenous routes are 230 mg/kg, 64 mg/kg, and 18 mg/kg respectively.

Within a population, there may exist a variation in bioavailability among individuals (exposedto a given chemical via same route). This is due to biological variations (genetic differences in the xenobiotics metabolizing enzymes, transporters, etc.), concurrent exposure to another chemical, age, gender, etc. (see below)

To estimate Bioavailability (F), the curve between 'plasma toxicant concentration' verses 'time' is plotted following exposure of animals via oral (or other test routes), and intravenous route. From these curves, the area under curve (AUC) is determined and 'F' can be estimated by the following formula:

$$\text{Bioavailability (F)} = \frac{\text{AUC via test route}}{\text{AUC intravenous route}} \quad \text{X } 100$$

5.2.6 Clearance

Clearance (CL) is a parameter used to quantify elimination of xenobiotics. It is defined as 'volume of plasma from which a substance is completely removed per unit time'. The unit of clearance is 'millilitres/minute' or 'litres/hour'. Clearance is thus indicative of the rate at which a substance is removed from the body–high clearance value means rapid removal of a substance from the blood or body.Since most substances are excreted or removed by the kidneys, the term 'renal clearance' (CL_R) is used frequently instead. Further, substances may be excreted via several organs viz. kidneys, bile, lungs, etc. In these cases, clearance is the sum of clearance via each of these organs thus, the 'total body clearance' or 'overall clearance' $=CL_R+CL_B+CL_L$.

$$\text{Total Clearance } (CL_{TOT}) = \frac{\text{Rate of elimination of xenobiotics}}{\text{Plasma concentration of toxicant}}$$

5.2.7 Elimination Half-life

Elimination half-life ($t_{1/2}$) is the time taken to reduce the concentration of axenobiotic in the blood by half. Thus, after one half-life, the concentration of a xenobiotic will be half of the initial concentration. Elimination half-life indicates the rapidity with which a substance is removed from the blood or the duration for which it would stay in the blood. Elimination '$t_{1/2}$' depends on clearance (CL) and volume of distribution (V):

$$t_{1/2} = \frac{0.693 \times V}{CL}$$

It can be deduced from above formula that large 'V' and low 'CL' increase '$t_{1/2}$'. Thus, lipid-soluble chemicals which are widely distributed stay for longer duration in the body (larger $t_{1/2}$ value).

5.2.8 Total body Burden

Total body burden refers to the amount of xenobiotics in the body at a given period of time.

Throughout our life, we are exposed to millions of xenobiotics, some of which stay in the body only for a short period of time (arsenic $t_{1/2=}$10 hours), whereas others stay for a longer duration or accumulate in the body (DDT $t_{1/2=}$ 3-6 years). However, a continuous exposure to chemicals with shorter $t_{1/2}$ can cause their persistent presence in the body. For example, continuous exposure to arsenic by regularly consuming contaminated food and water results in its persistent presence in the body. According to an estimate, humans may be carrying over 200 different xenobiotics at a time in the body. The presence of a toxic substance in the body does not necessarily indicate a state of toxicity, for which the chemical must be present above a threshold concentration levelin the body. However, measurement of total body burden gives information about the past and current exposure to chemicals, and a more realistic exposure assessment of chemicals (concentration to which organs are actually exposed) than monitoring concentration of chemicals in the environment. To estimate total body burden, samples of blood, urine, milk, hair, etc. are collected from a population and quantitatively analyzed–a process known as bio-monitoring.Such monitoring may indicate the overall state of toxic substances in environment and public health.

SAQ 1

a) State whether the following statements are true or false:

i) Toxicants absorbed via oral route usually have a high bioavailability. [True/False]

ii) Stratum corneum of skin is highly permeable to hydrophilic chemicals. [True/False]

iii) Liver is the main organ for the biotransformation of xenobiotics. [True/False]

iv) Toxicants accumulated in storage depots may show toxicity sometimes at the later stage of life. [True/False]

v) Entero-hepatic cycling of toxicants increases the elimination half life. True/False]

vi) The extent and rate of ADME is the same in all individuals of a species. [True/False]

b) Fill in the blanks with appropriate words:

i) The unit of clearance (CL) is......................

ii) Hydrophilic chemicals in intestine are absorbed by transport mechanisms such as ....

iii) The amount of xenobiotics in the body at a given point of time is called...........

5.3 ROLE OF LIVER, LUNGS, GASTROINTESTINAL TRACT AND SKIN IN ACTIVATION AND DETOXIFICATION OF TOXICANTS

We have seen above that the main routes through which toxicants enter our body are gastrointestinal tract (ingestion), lungs (inhales) and skin (contact with skin). Toxicants entering via these routes undergo biotransformation before they advance to systemic circulation. Liver and enterocytes of intestine, limit the amount of orally ingested toxicants by detoxification before these toxicants reach systemic circulation. Similarly, lung sand skin though less important than liver in xenobiotics metabolism, also detoxify certain toxicants entering via inhalation and via skin surface. Significant detoxification can reduce bioavailability of toxicants and prevent occurrence of a toxic response. In this regard, biotransformation reactions protect the body from toxic hazards. However, in many cases, biotransformation reactions convert an inactive parent toxicant into a potent toxicant– a process known as ‘toxication’ or bioactivation. For example, inhaled hexane is metabolized in liver intoan active neurotoxic metabolite by CYP450. In another example, biotransformation of ingested paracetamol produces certain hepatotoxic metabolites in phase I reactions, these, however, are quickly detoxified by phase II reaction and no toxicity is observed. Thus, it is the balance between the activation and detoxification process that actually determines occurrence of toxicity.

SAQ 2

State whether the following statements are true or false:

i) Sometimes a chemical may be activated to a potent toxicant in liver. [True/False]

ii) Biotransformation reactions protect body from toxic insult. [True/False]

5.4 FACTORS AFFECTING METABOLISM OF XENOBIOTICS

There are variations in metabolism of xenobiotics amongst individuals of different species (interspecific) and individuals of same species (intraspecific). Pesticides selectively harming target species and variation in response to drugs within human population can be partly explained by the differences in xenobiotics metabolism. There are several factors responsible for the variation in xenobiotics metabolism; we will discuss a few key factors in the following section:

5.4.1 Species

Differences in xenobiotics metabolism amongst various animal species can be due to: absence of the entire pathway of metabolism of xenobiotics in some species; presence of a low efficiency pathway in some species as compared to others and; presence of alternate pathways for metabolism of a xenobiotic in some species. These differences cause: some species to form toxic metabolites (or at a faster rate) of xenobiotics while others do not;some species are unable to detoxify xenobiotics at faster rate or; metabolize toxicants through multiple pathways thereby preventing accumulation of activated metabolite produced in one of the alternate pathways. Let us see few examples to explain the above situations:

1. Cats have lesser ability to conjugate xenobiotics with glucuronic acid in phase II biotransformation reactions, required for its rapid clearance from the blood. Thus, in cats, half-life of some xenobiotics is prolonged, andexplains the susceptibility of cats to the toxic effects of paracetamol. Similarly, the enzyme N-acetyltransferase responsiblefor acetylation of xenobioticsin phase II reactionsis absent in dogs, due to which the half-life of xenobiotics (e.g. hydralazines) is increased and results in toxicity.

2. Malathion, an insecticide is converted to malaoxon (the active form) in both humans and insects. However, humans rapidly detoxify malaoxon due to greater carboxylesterase activitythan insects. Insects thus accumulate malaoxon in their body, which explains species selective toxicity of malathion, towards insects.

3. Rats metabolize ‘coumarins’ into a toxic intermediate by epoxidation reaction.Humans however, use a different enzyme to metabolize coumarins and produce a metabolite which is non-toxic.

4. Tamoxifen, is used to treat breast cancer. It is carcinogenic in rats but not in humans. This difference is due to the fact thatthe rate of formation of toxic metabolite of the drug is slower, whereas detoxification pathway is more efficient in humans than rats. Thus, rats can accumulate several times higher amounts of the genotoxic metabolite in the body than humans.

From the above discussion, it can be deduced that significant differences in the ability to metabolize xenobiotics exists among various species; and is one of the important reasons for species selective toxicity. It also explains why certain pesticides do not harm humans or are toxic if taken at higher amounts.

5.4.2 Genetic Factors

Polymorphism in drug metabolizing enzymes causes inter-individual variations in the xenobiotics metabolism in a population. Several alleles of one or more xenobiotics metabolizing enzymes exist in a population, which produce enzymes that catalyze reactions at different rates. Thus, it is possible thatsome individuals in a population harboura particular allele which makes them metabolize a xenobiotic faster than other individuals. Such individuals are denoted as 'rapid metabolizers', and those metabolizing at slower rate as 'poor metabolizers'. Similarly, individuals with intermediate metabolizing capacity are called 'intermediate metabolizers'. Let us take the example of 'succinylcholine', a drug used to cause muscle relaxation required duringthe intubation of patients. This drug is rapidly metabolized by 'pseudocholinesterase' enzyme, which leads to the termination of drug action. In individuals with the normal allele, muscle relaxation lasts for about 8-15 minutes. However, individuals with abnormal allele show prolonged muscle relaxation which lasts for a few hours accompanied by breathlessness. Genetic polymorphism in several isoforms of CYP450 makes certain individuals of the population more susceptible totoxicants. For example, the isoform CYP2A6 metabolizes 'nicotine' and activates tobacco-specific mutagens. Individuals with abnormal allele poorly metabolize and activate tobacco-specific mutagens, thus, reducing the possibility of lung cancer.

From above discussion students may have understood that how genetic differences in xenobiotics metabolizing enzymes in a population can cause variation inxenobiotics metabolism and thus outcome of the toxic response.

5.4.3 Physiological Factors

Sex: There are differences in the xenobiotics metabolism between the two sexes of the same species. This is however not as pronounced in humans and other species as in the laboratory rats, in which male can metabolize several compounds at higher rates than the females. This is due to the dissimilarity in the expression of metabolizing enzymes between the two sexes. Thus, the activation or detoxification of xenobiotics between the two sexes may also differ. Lets us explain the above by using a few examples:

- Men have higher activity of CYP1A2 than women, due to which men can metabolize caffeine at a faster rate. Also, females require lower dose of hypnotic zolpidem (also metabolized by CYP1A2) due to slower rate of metabolism than males.

- After consumption of alcohol, females show higher alcohol levels in blood than males. This is because women have lower activity of gastric alcohol dehydrogenase, an enzyme responsible for metabolizing or degrading alcohol before it reaches systemic circulation. Thismakes women more sensitive to alcoholtoxicity than men.

Age: The ability to metabolize xenobiotics decreases with *age,* which is due to: the reduction in volume of liver, blood flow, and decrease in the activity of some of the metabolizing enzymes. Therefore, the bioavailability of certain xenobiotics is increased in elderly persons, making them susceptible to toxic insult. For example, the bioavailability of propranolol, a drug used in heart patients, is higher in elderly patients. Neonates have lower xenobiotics metabolism capacity, and an adult-like capacity is developed during first 6-8 months of life.

Disease: Liver is the most important site for xenobiotic metabolism, and any disease that decreases the capacity of liver can affect the metabolism of xenobiotics. Patients of hepatitis or cirrhosis have impaired ability to metabolize xenobiotics. These patients thus, have a higher concentration of xenobiotics in blood and making themsusceptible to toxic insult.

5.4.4 Nutritional Factors

Xenobiotic metabolism requires enzymes and cofactors, derived from both macro (protein, carbohydrate, and fats) and micronutrients (vitamins and minerals). Many co-factors such as FAD, NADH, acetyl CoA, glutathione, etc. used by enzymes are derived from vitamins and essential amino acids. Further, many enzymes require metals (such as iron, copper, zinc, nickel, etc.) for catalysis, binding with substrate or for stability of structure. Therefore, deficiencies of nutrients can affect xenobiotics metabolism. In general, severe protein and vitamin deficiency, and starvation can affect xenobiotic metabolism. For example: the toxic metabolites of paracetamol formed in the liver are detoxified by conjugation with glutathione and glucuronic acid. Both these co-substrates are depleted or not synthesized in the required quantity, in case of prolonged starvation.This results in accumulation of toxic metabolite of paracetamol, resulting in hepatotoxicity.

5.4.5 Food Components and Presence of Other Xenobiotics in the Body

Many xenobiotics metabolizing enzymes can be inhibited or induced (i.e. concentration of enzyme can be increased in cells by producing new enzyme molecules by increasing gene expression or translation) by drugs, herbal products, and food components. This leads to reduced or excessive metabolism of other concurrently exposed xenobiotics, and, is one of the reasons of ‘xenobiotic-food’ and ‘xenobiotic-xenobiotic’ interaction (also denoted as ‘drug-food’ and‘drug-drug’ interactions in the field of medicine). Let us see few examples of this phenomenon:

- Certain components in grapefruit juice inhibit intestinal CYP 450 isoform 'CYP3A4' and thus, can increase the bioavailability of many drugs such as felodipine, a drug used to treat hypertension. Similarly, blueberry juice and pomegranate juice can inhibit CYP3A4 and CYP2C9. Also, cruciferous vegetables (cabbage, broccoli, etc.) can induce CYP1A2.These are examples of xenobiotic-food interaction.

- CCl_4 is metabolized to toxic metabolite peroxyl radical ($CCl3OO^{\bullet}$) in liver by an isoform of CYP 450 'CYP2E1'. This isoform is induced by ethanol. Therefore, alcoholics are more vulnerable to liver injury caused by CCl_4. This is an example of xenobiotic-xenobiotic interaction.

5.4.6 Environmental Factors

Internal body *temperature* and that of surroundings may affect xenobiotics metabolism. At lower body temperature, the activity of metabolic enzymes (or affinity with the substrate) decreases resulting in accumulation and a high concentration of xenobiotics in the body. For example: the concentration of midazolam, a sedative drug increases several folds in plasma under low body temperature, which is due to lowering of CYP3A4 activity due to low temperature.Temperature of surroundings also affects the metabolism of xenobiotics; for example, under low surrounding temperature, the concentration of metabolic enzymes has been found to increase in laboratory rats.

The metabolism of several drugs is altered at *high altitude*due to hypoxic conditions. Under hypoxic conditions at high altitudes, the activity and expression of a few isoforms of CYP 450 increases whereas those of others decreases. For example: under high altitude hypoxic conditions, the metabolism of lidocaine, a local anaesthetic, is decreased thereby increasing its half-life inblood.

The changing *light-dark period* causes diurnal rhythmic changes in physiology, cellular metabolism, hormonal release, sleep cycle (circadian rhythm), etc. The circadian rhythm also affects xenobiotics metabolism in a rhythmic manner.The transcription of many isoforms of CYP 450 changes rhythmically and thus, affects the metabolism of xenobiotics. For example, toxicity of aconitine is higher when it is exposed to laboratory mice during daytime, because the activity of detoxifying enzyme CYP3A11 is less during daytime. Similarly, paracetamol caused more damage to liver when laboratory animals are exposed to the drug in dark period (night).

SAQ 3

a) State whether the following statements are true or false:

i) Vitamin deficiency and internal body temperature of animals may alter xenobiotic metabolism. [True/False]

ii) Polymorphism in xenobiotic metabolizing enzymes does not affect therapy using drugs. [True/False]

b) Fill in the blanks with appropriate words:

i) Components of food affecting metabolism of xenobiotics is an example of .... interaction.

ii) Increase in the concentration or amount of xenobiotic metabolism in cells is called................

5.5 SUMMARY

Let us summarize what we have learnt so far:

- In humans, systemic toxicants mainly get entry in the body by absorption through gastrointestinal tract, lungs and skin. After absorption from the site of exposure, some amounts of a toxicant may undergo-presystemic elimination (mainly due to metabolism). Thus, a fraction of the absorbed toxicant reaches systemic circulation. Once in the systemic circulation, toxicants are carried to various organs through a process known as 'distribution' and simultaneously, the toxicants are also slowly removed or excreted from the body.

- The rate and extent of absorption depends on the physicochemical properties of the toxicants such as lipid solubility and size, etc. In general, lipid soluble low molecular weight chemicals are absorbed well in the intestine by simple diffusion. Water-soluble toxicants use membrane transporters or pass through aqueous pores in the epithelial lining. Although, skin functions as a barrier to the entry of toxicants in the body, it can be easily traversed by lipid-soluble toxicants, whereas water-soluble toxicants get entry through dermal appendages. Absorption of toxic gases and solvent vapours via lungs depends on the blood-to-gas partition coefficient, whereas size and solubility of 'aerosols and particles' are the important factors determining theirabsorption and penetration in the respiratory tree. The extent of absorption is denoted by the parameter 'bioavailability', which is the fraction of absorbed toxicant that reaches systemic circulation.

- Distribution of toxicants to various organs depends on the rate of blood flow to an organ and lipid solubility of toxicants. A lipid-soluble toxicant is widely distributed in the body. Certain toxicants have the property to accumulate in tissues of various organs. These tissues or organs are known as 'storage depots'.The extent of distribution of toxicants in the body is measured by 'volume of distribution'.

- Metabolism of toxicants mainly takes place in liver, enterocytes and lungs. Several enzymes known as 'xenobiotic metabolizing enzymes' modify chemical structures of toxicants and are responsible for detoxification and activation of toxicants. Metabolism of toxicants is affected by genetic, physiological and environmental factors. Marked difference in the pattern of metabolism of toxicants is also observed between species.

- Toxicants are mainly removed or excreted from the body via urine, faeces, and lungs. The rate of removal of toxicants is denoted by the parameter ‘clearance’.

5.6 TERMINAL QUESTIONS

1. Mention various factors which affect absorption of xenobiotics via gastrointestinal tract.
2. Explain, why bioavailability of xenobiotics administered via routes other than intravenous route is low.
3. Genetic polymorphism in xenobiotic metabolizing enzymes causes variation in the toxic response in a population.
4. Discuss, how toxicants get entry into blood via skin.

5.7 ANSWERS

Self-Assessment Questions

1. a) i) False ii) False iii) True iv) True v) True vi) False

   b) i) Millilitres/minute or litres/hour

   ii) Facilitated transport, active transport and via aqueous pores

   iii) Total body burden

2. i) True ii) True

3. a) i) True ii) False

   b) i) Xenobiotic-food ii) Induction

Terminal Questions

1. Factors affecting absorption via gastrointestinal tract includes: physicochemical properties of toxicants (lipid solubility, size, etc.), attributes of the site where absorption takes place: i.e. surface area, properties of epithelium (presence of transporters, thickness of epithelium, etc.) and blood supply in the underlying tissue. Presence of food, various food components, motility of the intestine are other factors.
2. Xenobiotics administered via routes other that intravenous have low bioavailability due to incomplete absorption, and presystemic elimination i.e. metabolic degradation of xenobiotic and excretion from the body (such via bile).
3. Due to polymorphism, several alleles of one or more xenobiotics metabolizing enzymes exist in a population, which produce enzymes that catalyze reactions at different rates. Therefore, the ability to detoxify or activate a xenobiotic may vary amongst individuals of a population, resulting in variation in toxic response.

4. Fat-soluble xenobiotics can cross the stratum corneum layer by simple diffusion and reach dermis. Low molecular weight water-soluble xenobiotics enter dermis via hair follicles and opening of sweat glands at the surface of skin and reach dermis. Upon reaching dermis, toxicants get entry into blood via blood vessels in dermis.

5.8 FURTHER READINGS

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