

9. TRANSLOCATION OF TOXICANTS

The sufficient amount of toxicant must reach the target site in the biological system for a considerable length of time to induce adverse effect on a specific biological mechanism or the biological system as a whole. The biological system may be exposed to the high amount/concentration of a toxicant, but if due to certain reasons (may be due to defensive mechanism of the system), the concentration at the specific site remains low, it may result in little or no effect. Thus, in order to produce a measurable effect a chemical must be transported from the site of exposure to the specific site of action. *The process of transport of toxicant molecules from the site of their application to the specific site of action or to storage depots or to the sites of biotransformation including their elimination may be termed as translocation of toxicants.*

The initial step in the translocation of toxicant is its absorption across membranous barriers before it can be distributed either throughout the body or to a more specific site. For instance, a toxicant producing its effect at cellular level, at first must pass through a series of membranes. Entry of toxicants in the biological system occurs either by absorption through the skin or by uptake through the gastrointestinal tract during ingestion of food, or by inhalation through the lungs. After its passage across the membranes, the toxicants reach the blood stream from where they are distributed to various parts of the body or to a more specific site. Further at tissue level, the toxicant must pass through cellular, mitochondrial and nuclear membranes in order to reach the more specific site of action. If they are distributed as such, they induce toxic effects on account of their interaction with the receptors. Sometimes, during the course of passage, they are either directly stored in certain nontarget organs or first biologically converted into inactive substances and stored in certain parts. If they are bioconverted into

inactive forms and stored or converted into hydrophilic forms and excreted, they cause no harm to the organisms (Figure 9.1). However, if they get converted into more active forms, they cause more pronounced adverse effects.

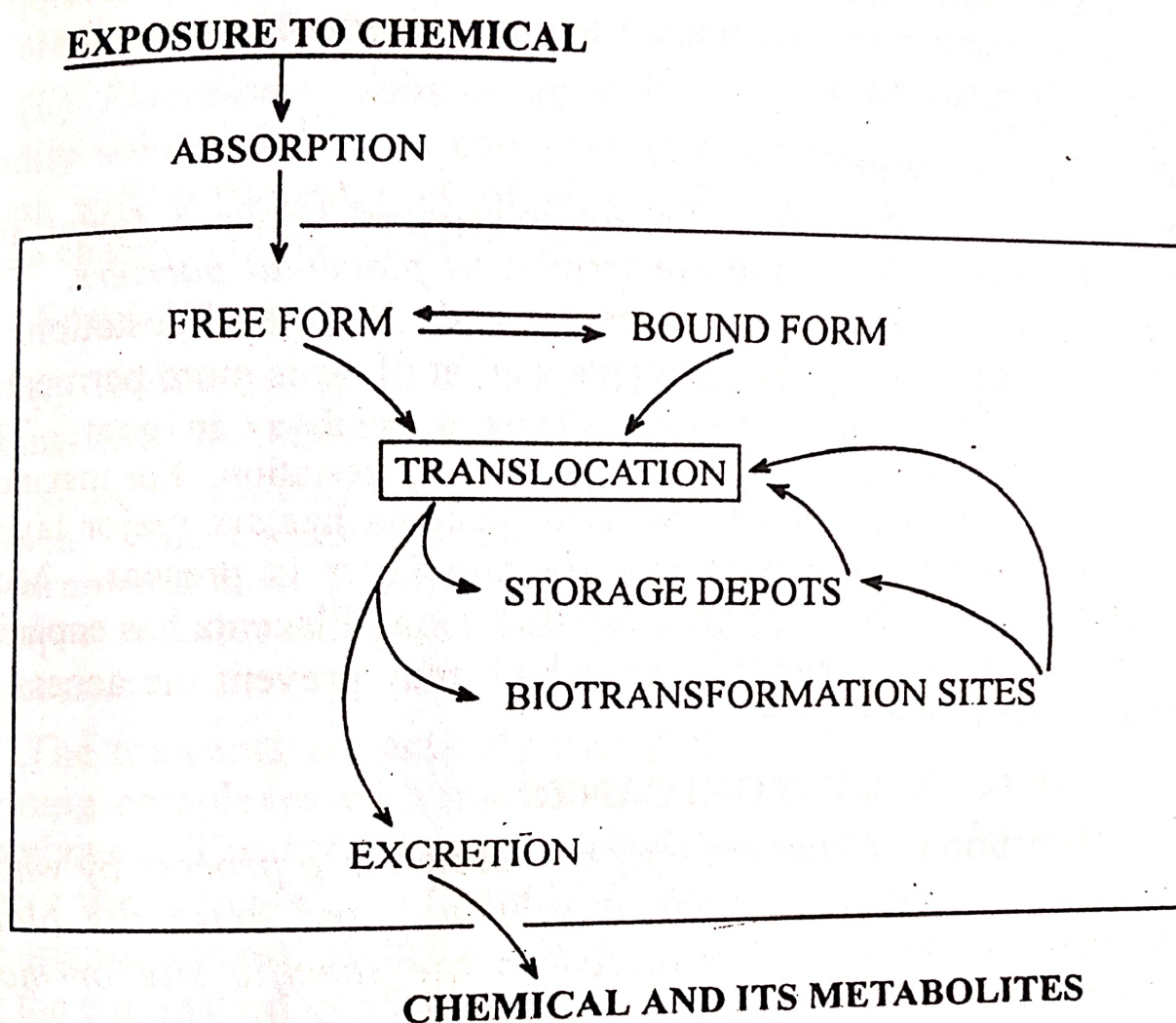


Figure 9.1 Schematic representation of the pathways through which a chemical may pass during its presence in the mammalian organism.

The biological conversion of toxicants is facilitated by certain nonspecific enzymes or enzyme systems located in certain organs of organisms, such as in the liver of mammals, in the fat bodies of insects, etc. Almost all the organs of body have the ability to biotransform the xenobiotic substances, but the major xenobiotic metabolizing enzyme systems are present mainly in the liver. Xenobiotic substance absorbed from gastrointestinal tract first passes through the liver, where extensive bioconversion takes place and the biotransformation products are then distributed to various parts of body. Because of this reason, xenobiotics substances administered orally often produce less effects than that of intravenous administration of similar doses of the chemical.

Membranous Barriers

The organisms are protected from their environment by specialized coverings which efficiently restrict the free passage of toxicants from the site of exposure to the internal organs and finally to the cells within those organs. Hence, a toxicant must pass through a number of membranous barriers (Figure 9.2) before achieving sufficient concentration in the organ where it produces its characteristic effect.

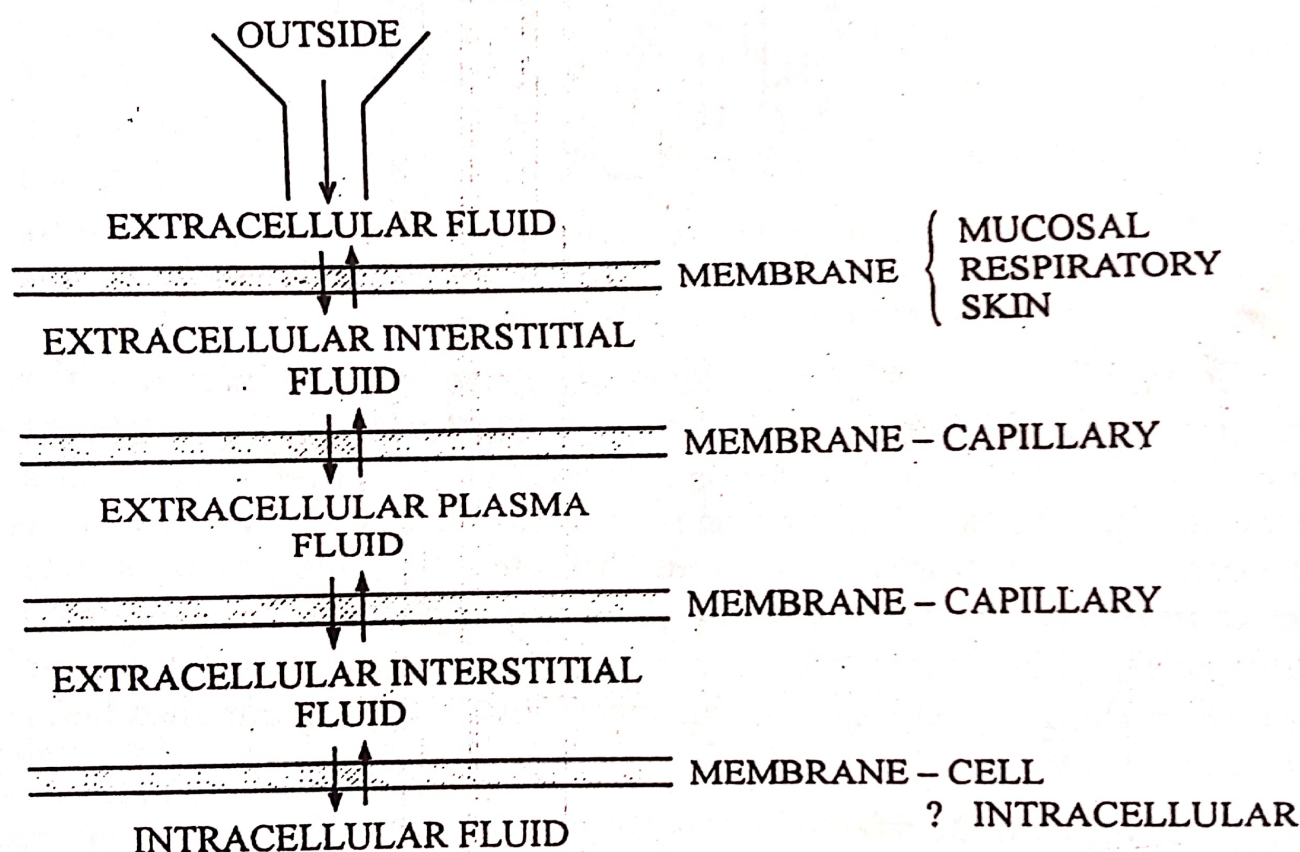


Figure 9.2. Schematic representation of membranous barriers involved in translocation of a foreign chemical from outside the mammalian organism to intracellular fluid in a tissue cell.

The toxicants gain access through various routes. Accordingly they may have to cross various membranous barriers. These include membranes of a number of cells, such as stratified epithelium of skin, thin layer in the lungs, gastrointestinal tract, the capillary endothelium and the cells of organ where the toxicant produces deleterious effects. The plasma membrane is approximately 70 Å and is composed of bimolecular layer of lipid molecules coated on each side with a protein layer (Figure 9.3).

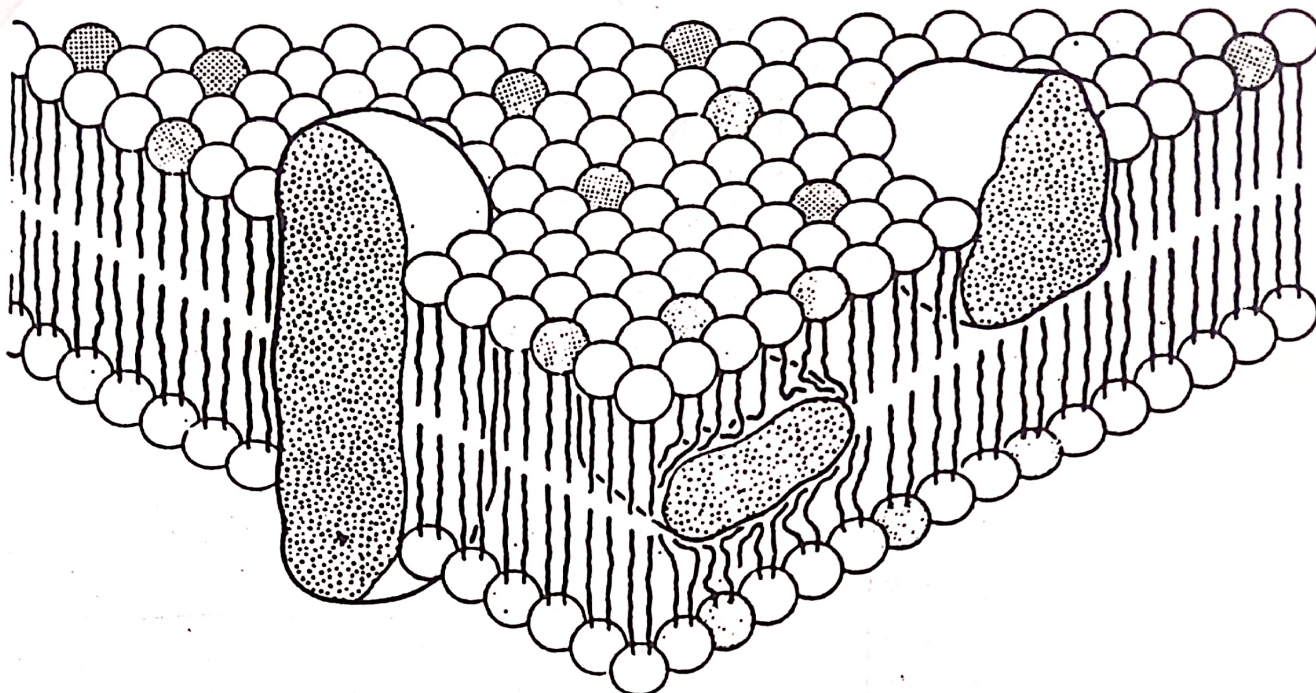


Figure 9.3. Schematic diagram of biological membrane. Head groups of lipids (as phosphatidyl-choline) represented by spheres, tail ends by zig-zag lines. Large bodies are associated proteins.

Certain branches of protein layer penetrate the lipid bilayer while the others extend through the membranes. The lipid layer of membrane consists of phosphatidyl choline, phosphatidyl ethanolamine and cholesterol. The fatty acids of the membrane are quasifluid in character at normal temperatures. The fluid character of membrane is largely determined by the structure and relative proportion of unsaturated fatty acids. The membranes containing more unsaturated fatty acids are more fluid like and active transport across such membranes takes place more rapidly.

(A) *Blood-brain barrier*

The blood-brain barrier prevents central nervous system (CNS) against the action of neurotoxicants. This site is less permeable than other areas of the body. The entry of toxicants to CNS is prevented because of the following three reasons :

- (i) capillary endothelial cells of CNS are tightly joined with a few or no pores between the cells;
- (ii) the capillaries of CNS are surrounded by glial cell processes, and
- (iii) protein concentration in interstitial fluid of CNS is much less than elsewhere in the body.

Thus, entry of toxicants in capillary endothelium and glial cell membranes, in order to gain access to interstitial fluid, is restricted. Low protein content in interstitial fluid further affects the distribution

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of toxicants to CNS. These adaptations protect the CNS from the action of toxicants. The effectiveness of blood-brain barrier varies from one area of the brain to another. The entry of toxicants into the brain follows the similar principle as in case of transfer across other cells in the body. The toxicants not bound to plasma proteins gain entry into the brain. The blood brain barrier is not completely developed at birth and because of this reason some toxicants are more toxic to new-borns than to adults.

(B) *Placental barrier*

A number of layers of cells are interposed between foetal and maternal circulations. These are termed as *placental barriers*. The number of layers varies with the species and the state of gestation. It is believed that relatively thin placenta as that of rat is more permeable to toxicants than those of thicker placenta, such as in goat. The number of layers in placenta also changes during gestation. For instance, in rabbit, at the beginning of gestation placenta has six major layers whereas at the end of gestation only one layer is present. Most toxicants pass through the placenta by diffusion. Placenta has capacity of biotransforming the xenobiotics, which may prevent the access of some toxicants to the foetus.

1. ABSORPTION OF TOXICANTS

The absorption of toxicant may be defined as *a process by which the toxicant crosses the body membranes and enter the blood stream from where it is distributed to the specific site or more diffused sites.*

The toxicants penetrate the body membranes by the processes affecting the physiological absorption of oxygen, food-stuffs, other nutrients and excretory substances. Thus, a toxicant may pass through a membrane either *by passive transport or by specialized transport*

(A) *Passive transport*

(i) *Simple diffusion* : It is simplest type of exchange mechanism effecting transport of substances in response to concentration gradient, i.e. from higher concentration to lower concentration. This type of diffusion does not involve expenditure of energy and during passive transport the membrane system practically offers no resistance.

Most toxicants cross membranes by simple diffusion. Small hydrophilic compounds diffuse across lipid membranes through aqueous channels, whereas larger organic molecules diffuse across lipid regions.

Lipophilic substances, such as ethanol, DDT, *etc.* readily pass through cell membranes by simple diffusion. The rate of transfer of toxicants across cell membranes is dependent on their lipid solubility. The ionized form of toxicants has low lipid solubility, hence is unable to penetrate the cell membrane, whereas unionized form is lipid soluble and diffuses across the membranes.

(ii) *Filtration* : Substances of low molecular weight, which are readily soluble in water, can pass through the pores of membranes along with sufficient flow of water. Passage of substances through these channels is known as filtration.

(B) Special transport

(i) *Active transport* : In active transport substances are moved against electrochemical gradients and maximum transport is exhibited at high substrate concentrations because of saturation of the transport system. The transport system is selective, certain basic structural requirements exist for the substances to be transported. The active transport requires energy, so the metabolic inhibitors block the transport process.

The toxicants are actively transported across cell membranes by forming complexes with macromolecular carriers on one side of the membrane. The complexes pass to the other side of the membranes, where the toxicants are released and thereafter the carriers return to the original surface. Active transport is especially important in toxicology for the elimination of xenobiotics from the body of organisms.

(ii) *Facilitated diffusion*: In facilitated diffusion, transport of toxicant is carrier mediated, but it does not take place against the concentration gradient and the process is not energy dependent.

(iii) *Additional transport* : Phagocytosis and pinocytosis are additional transport processes through which the cell membrane flows and engulfs the particles. This type of transport is important in toxicology for :

- (a) the removal of particulate matter from the alveoli by alveolar phagocytes, and
- (b) the removal of toxic substances from blood by the reticuloendothelial system of liver and spleen.

Sites of absorption

According to sites of exposure, there may be various sites of absorption. For instance, toxicants applied topically over the body get

absorbed through the skin or integument whereas toxicants ingested orally are absorbed in the gastrointestinal tract. Likewise, toxicants applied in vapour form are taken in through respiratory route and are thus absorbed in the lungs or gills. Substances ingested intravenously are directly distributed in the body.

(A) Absorption through skin

Fortunately, the skin is not very permeable and is, therefore, relatively a good lipid barrier. The skin represents an almost continuous barrier to xenobiotics. The outer nonvascular layer consists of cells packed with keratin, which limits the transport of toxicants. The underlying dermis is more permeable and vascularized, but in order to reach the systemic circulation through the skin, the toxic substances would have to pass several layers of cells in contrast to gastrointestinal tract where only two cells separate substances from the blood-stream.

Solvents, such as carbon tetrachloride, that are highly lipid soluble, absorbed percutaneously exert systemic toxicity following absorption by this route. Small hydrophilic substances, such as hydrazine, sufficiently absorbed through skin cause systemic toxic effects as well as local effects. Damage to outer horny layer of epidermis increases absorption and a toxic compound might facilitate its own absorption in this way.

(B) Absorption by gastrointestinal tract

Absorption from gastrointestinal tract is a major route of entry of many toxic substances into the body of organisms. Absorption may occur along whole length of the tract. The site of absorption for any particular toxicant depends on its physico-chemical properties and the conditions in that area of the tract. The lining of gastrointestinal tract presents a continuous lipid barrier.

Many environmental toxicants enter the food-chain. They are ingested by the organisms occupying higher trophic levels and are absorbed by their gastrointestinal tract. This site of absorption is of great interest to forensic toxicologists because suicide attempts involve an over dose of an orally ingested toxicant. Oral intake is also very common route by which children or enemies are poisoned.

The substances ingested by oral route do not produce systemic effects unless they are absorbed. The substances, which are weak acids or bases, get absorbed by diffusion in unionized form. The weak organic acids are absorbed in the stomach, whereas weak organic bases are absorbed in the intestine.

(C) Absorption by lungs

Absorption via lungs is an important route for toxic gases, such as volatile solvents, aerosols and in some cases air-borne particles. Large surface area of lungs is highly permeable to lipid soluble and even some lipid insoluble substances. The blood flows very rapidly and is separated by 10 μm from the air in the alveoli. Consequently, lipid soluble and volatile substances are rapidly absorbed by passive diffusion. The passive diffusion depends on their lipid solubility. Particulate matter, such as air-borne particles of uranium dioxide may be absorbed by pinocytosis.

The most frequent cause of death from poisoning by carbon monoxide and an occupational disease *silicosis* are the results and examples of absorption of air-borne poisons by the lungs. This site of absorption has been used in chemical warfare (e.g., chlorine gas, phosgene gas and mustard gas) and for executing criminals in gas chambers (chamber containing hydrogen cyanide). *Bhopal gas tragedy* is an example of industrial accident, which claimed thousands of lives and these killings may be cited as an extreme example of absorption of methyl isocyanate gas through the lungs.

(D) Absorption through special routes

Usually the toxicants enter the blood stream following absorption through skin, lungs and gastrointestinal tract. But, sometimes, the toxicants are administered in the body of laboratory animals through special routes, such as (i) intraperitoneal, (ii) subcutaneous (iii) intramuscular, and (iv) intravenous injections. The toxicants are directly administered into the blood stream through intravenous route. Administration of toxicants through intraperitoneal route results in rapid absorption of toxicants primarily through the portal circulation because of the large surface area and rich blood supply to the peritoneal cavity. Toxicants administered subcutaneously and intramuscularly are absorbed at slow rate. This can be altered by changing the blood flow to the area and the medium in which the toxicant is administered. Most of the toxicants administered intraperitoneally enter the liver via portal circulation before it reaches the general circulation of the animal. Therefore, sometimes the toxicant may be completely biotransformed and excreted into the bile and thus it never reaches the target sites.

2. DISTRIBUTION OF TOXICANTS

Some toxicants readily pass through membranous barriers and are easily distributed throughout the body, whereas others do not readily

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pass through these barriers and thus their distribution is restricted.

Following their absorption, the xenobiotics reach the blood stream. The part of vascular system into which the toxicant is absorbed largely depends on the site of absorption. Absorption through the skin leads to peripheral blood supply, whereas toxicants reach the pulmonary circulation following their absorption through the lungs. Absorption of toxicants through gastrointestinal tract results in their entry into the portal supply of the liver.

The distribution usually occurs rapidly. The rate of distribution of toxicants to the tissues is determined by the blood flow through the organ and ease with which toxicant crosses the capillary wall, and penetrates the cells of a particular tissue. The distribution into particular tissue involves crossing of biological membranes and the principle of absorption across the cell membranes has already been described earlier. Thus, the eventual distribution of toxicants is largely dependent on their ability to pass through cell membranes of various tissues and their affinity for these tissues.

The concentration of toxicant in plasma is very important as it reflects the distribution of toxicants into various tissues or the target tissue. Toxicants, which are highly soluble in lipids and readily distributed to tissues have low concentrations in the plasma. On the other hand, the substances that do not readily distribute into tissues may have high concentrations in the plasma. The volume of distribution can be quantified by the following formula :

$$VD (l) = \frac{\text{Dose (mg)}}{\text{Plasma concentration (mg/l)}}$$

The volume of distribution sometimes indicates that the xenobiotic compound is localized in a particular tissue or is mainly confined to the plasma. Thus, if a substance is distributed mainly in the adipose tissue, the plasma concentration will be low and in accordance with the above formula, volume of distribution will be more. During the course of distribution, the toxicant may get concentrated in a particular tissue or organ and such tissues or sites are termed as *storage depots*.

Another aspect of distribution having great toxicological implications is interaction of toxicants with plasma proteins. Several toxicants bind to the plasma proteins noncovalently. This binding affects the distribution of the substance because it is attached to a macromolecule, which restricts its passage across membranes, by diffusion. The distribution of toxicants to the site of their action is particularly an important

aspect in toxicology. For example, organochlorine, organophosphorus and carbamate pesticides, besides other neurotoxins, act on CNS. So, they must enter the CNS in order to produce toxic effects. But, the entry of such substances to the brain is restricted because of the *blood-brain barrier*. The highly lipid soluble substances enter the brain crossing this barrier.

Storage-depots (*Storage of toxicants in certain tissues*)

Quite often, the toxicants are concentrated in specific tissues. Some toxicants achieve their highest concentration at the sites of their action and such substances are highly toxic substances. Some others concentrate at sites other than their sites of action, hence cause less effects. For example, carbon monoxide has very high affinity for haemoglobin, hence accumulates in the blood occupying haemoglobin molecules. On the other hand, lead poisoning occurs due to the inhibition of α -aminolevulinic acid dehydratase (ALAD) activity present in soft tissues, whereas lead is accumulated in the bones. Another example is those of organochlorine pesticides, which are nerve poisons, but they accumulate in tissues having high lipid content. The compartment where toxicants are concentrated or accumulated are termed *storage depots*.

The stored toxicants hardly harm the organisms. The storage depots can, therefore, be regarded as protective mechanisms, which prevent the accumulation of toxicants at the site of their action, thereby reducing their toxicity. The toxicants in storage depots maintain an equilibrium with their free concentrations in plasma. The biotransformation and excretion of toxicants lead to their release from storage sites.

(A) Plasma protein as storage depot

The xenobiotics can bind to several plasma proteins. Their binding with plasma proteins is of special importance in toxicology. Because of their attachment with high molecular weight plasma proteins, they cannot cross capillary walls and other cell membranes. Hence, the bound form of toxicant is not available at the target site to produce injury. However, addition of another toxic agent can displace the first one from plasma proteins, thus making it available in free form. For instance, if a sulfonamide drug is given to a patient already taking a diabetic drug, the former replaces the latter and induces hypoglycemic coma.

The majority of toxicants bind to serum *albumin*. It is most

abundant protein in plasma, which serves as a protein depot and transport protein for numerous endogenous and exogenous substances. *Transferrin*, a β -globulin, is important for the transport of ions in the body. *Ceruloplasmin*, another plasma protein, carries most of the copper in the serum. α and β -lipoproteins are very important plasma proteins for the transport of lipid soluble substances.

(B) Fat as storage depot

Many xenobiotics, such as chlordane, DDT, poly-chlorinated biphenyls, polybrominated biphenyls, *etc.* are highly lipophilic substances, which are distributed and concentrated in body fat by simple physical dissolution in neutral fats. The toxicants with a high lipid/water partition coefficient may be stored in the body fat to a large extent. This storage lowers toxicant concentration in the target organ and serves as protective mechanism. The toxicity symptoms can be produced by short-term exposure of organisms previously exposed to persistent chlorinated poisons.

(C) Liver and Kidney as storage depots

Liver and kidney concentrate more toxicants than other organs of body. Both these organs have the capacity to excrete xenobiotic substances. In addition, the liver has great capacity to biotransform these substances. Intracellular binding proteins are important in accumulating and storage of toxicants within liver and kidney. *Ligandin*, a γ -protein, occurs in the cytoplasm of hepatic cells and has high affinity for organic acids. This protein plays an important role in the transfer of organic anions, azo dye and cortico-steroids from plasma to liver. *Metallothionin*, another binding protein, is present both in the liver and kidney, which binds to the cadmium. Liver has the ability to concentrate lead 50 times greater than plasma within 30 minutes after single administration.

(D) Bone as a storage depot

Bone also serves as a major storage site for certain toxicants, such as lead, fluoride and strontium. Of these toxicants, accumulation of lead in skeletal tissues is more pronounced, as it comprises of 90% of the total lead in the body. The deposition and storage of toxicants in bone may or may not be detrimental. For instance, lead is not toxic to the bones, but the accumulation of fluoride for long-time causes *skeletal fluorosis*. Likewise, long-term accumulation of strontium produces *osteosarcoma* and other neoplasms.

3. BIOTRANSFORMATION OF TOXICANTS

During the course of distribution, the toxicants reach certain organs/tissues having high enzymatic activity, hence greater biotransformation potential, such as liver. In the liver and certain other organs, these xenobiotic compounds are biocatalytically converted into hydrophilic forms to facilitate their excretion from the body. Almost all parts of the body possess some activity against these xenobiotic substances, but the major enzyme systems are present mainly in the liver. A detailed account of biotransformation of toxicants has been given in a separate chapter.

4. EXCRETION OF TOXICANTS

The excretion of toxicants from the body of organisms is an important step in the translocation of toxicants. As a result of excretion, the activity components of toxicants are eliminated outside the body of organisms and are thus not available to produce adverse effects. The rapid elimination reduces the duration of exposure of chemicals to the biological system. Hence, the rapid removal of toxicants reduces the extent of damage.

The plasma half-life or whole body half-life of toxicants reflects the rate of elimination of toxic substances from the biological system. The most important route of excretion of toxicants is their passage through the kidney into the urine. Other routes are biliary excretion, excretion of volatile and gaseous substances into the expired air from lungs and excretion through gastrointestinal tract, milk, sweat and other fluids.

(A) *Urinary excretion*

The kidneys receive around 25% of the cardiac output of blood. So, they are exposed to and filter out a significant proportion of toxicants. Large molecules, such as protein do not pass out through the intact glomerulus and lipid soluble molecules, such as bilirubin, are absorbed from the kidney tubules. Hence, the excretion of relatively small and hydrophilic molecules from the blood stream into the urine takes place and this occurs by any of the following three mechanisms:

- (i) filtration from the blood through the pores of glomerulus;
- (ii) diffusion from the blood stream into the tubules; and
- (iii) active transport into the tubular fluid.

The structure of kidney facilitates the elimination of xenobiotic substances from the blood stream (Figure 9.4). The functional unit of the kidney is nephron. The nephron permits the passage of small molecules from blood in the glomerulus into the ultrafiltrate and this

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 passage is facilitated by the large pores in the capillaries and the pressure of blood.

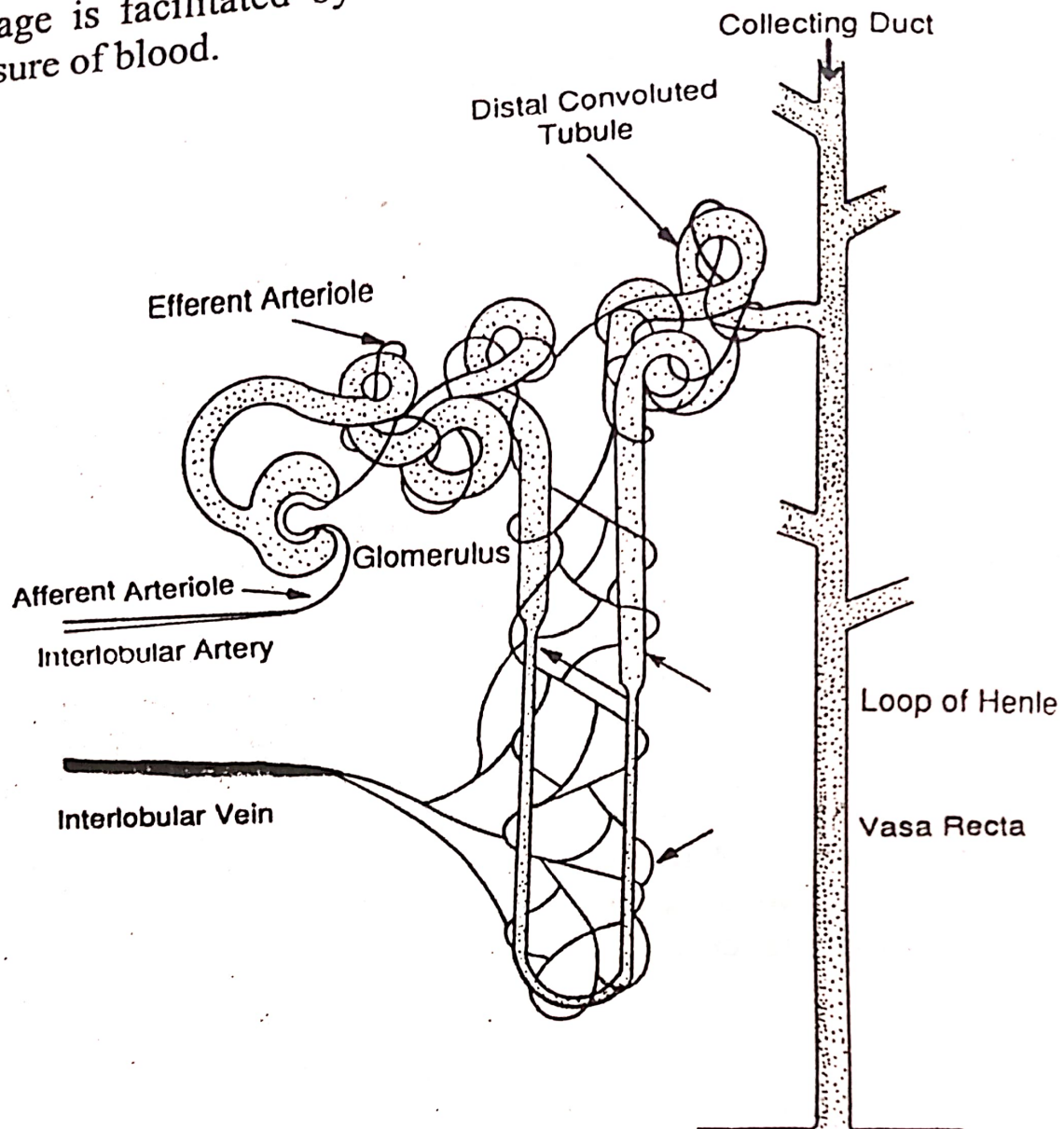


Figure 9.4. Structure of the mammalian kidney

Lipophilic substances passively diffuse out of the blood against concentration gradient. But, these substances are reabsorbed from the tubule back into the blood, by passive diffusion because of the concentration gradient from tubule to blood, if they are not ionized at physiological pH. However, the hydrophilic substances once passed out of the blood into the tubule cannot be reabsorbed by passive diffusion because they ionize at tubular pH. Hence, they pass out through the urine.

The passive diffusion of substances from the blood stream to the kidney tubule takes place against concentration gradient. Hence, greater the amount of substance in the blood, greater would be the rate of elimination. However, the passage of substances into the kidney tubules by means of active transport and facilitated diffusion occurs with the help of specific carriers. At high concentrations of toxicants in plasma, the carrier molecules are saturated. Consequently, the rate of elimination

remains constant. At high concentrations of toxic substances, if the excretion by means of passive diffusion against concentration gradient takes place, the rate of excretion would increase, whereas if excretion occurs via active transport, the rate of excretion would remain constant. This would lead to high levels of toxicants in the plasma and other body tissues, which may produce harmful effects. The binding of toxic substances with plasma proteins affects the excretion via passive diffusion, at the same time it has no effect on active transport.

(B) *Biliary excretion.*

The large polar substances having a molecular weight of 300 or so are eliminated through biliary excretion. This route is indeed a prominent route of elimination.

The bile is secreted in the liver by hepatocytes into the canaliculi from where it is transferred and stored in the gall bladder for some time. From gall bladder bile flows into the bile duct and eventually into the intestine (Figure 9.5). Thus, the toxic substances ingested orally reach the liver through the portal vein, where they are biotransformed and transported to the gall bladder with the bile, reach the intestine and finally eliminated in the faeces. Excretion of toxicants into the bile is an active process and there are three transport systems: one is meant for the transport of neutral compounds, the other for anionic substances and the third for cationic substances.

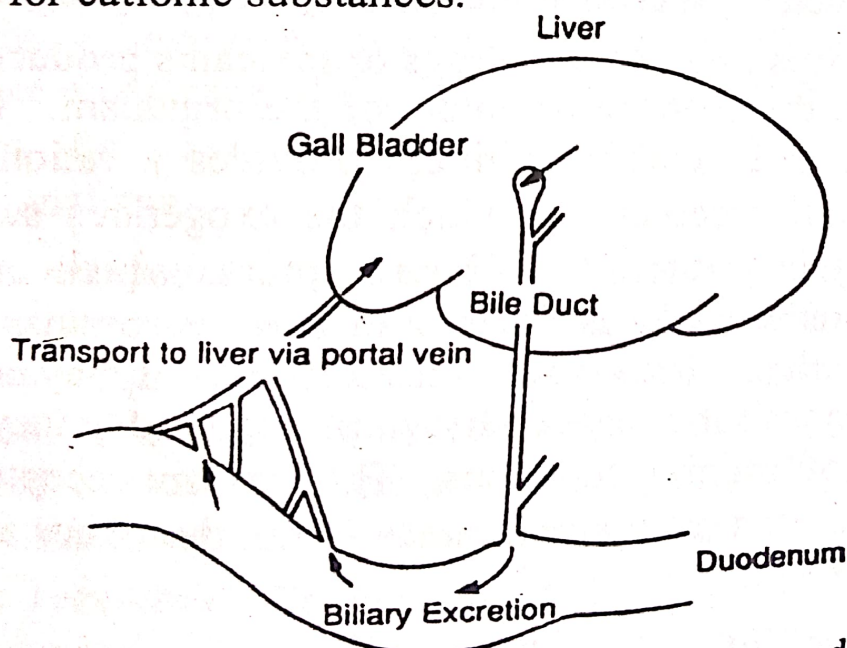


Figure 9.5. Biliary excretion route for foreign compounds.

The toxicants ingested orally and those routed through biliary excretion reach the gut, where they come in contact with the gut flora. Sometimes, the gut flora converts these toxicants into lipophilic forms, which are reabsorbed and transported back to the liver. This may lead to cycling (enterohepatic recirculation) of xenobiotic substances and may increase their toxicity. Sometimes, the gut microflora biotransform

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the chemical substances into more toxic products, which could be reabsorbed and may produce systemic toxicity.

For example, orally ingested normal glycoside, *cycasin*, is biocatalytically hydrolyzed into a potent carcinogen, methyl azoxy methanol. Thus, the biliary excretion may:

- (i) increase the half-life of xenobiotics;
- (ii) lead to production of toxic products;
- (iii) increase hepatic exposure; and
- (iv) lead to hepatic damage.

(C) *Excretion via lungs*

Those substances, which occur predominantly in gas phase at body temperature, are principally eliminated by the lungs. This route is very efficient route of excretion of lipophilic substances because of the fact that the capillary and the alveolar membranes are thin and in close proximity. Thus, the excretion of toxicants via lungs is related to their vapour pressure. The pulmonary excretion takes place by passive diffusion from the blood into the alveoli facilitated by the concentration gradient. There will be continuous concentration gradient between the blood and air in the alveolus due to rapid removal of gases from the lungs and rapid blood flow through the lungs.

(D) *Other routes of excretion*

(i) *Excretion through faeces*

Many toxic substances pass through faeces. The appearance of toxicants may be due to:

- (a) incomplete absorption of toxicants from gastrointestinal tract;
- (b) excretion through the bile, saliva, pancreatic secretion and gastric or intestinal secretion.

(ii) *Excretion through milk*

Lipophilic substances are secreted in the milk. The excretion of lipophilic toxicants through milk is important because:

- (a) they may pass from mothers to new-born individuals;
- (b) they may pass from cow's milk to human beings.
- (iii) Another possible route of excretion of a few toxicants is through cerebrospinal fluid. Such substances can leave the CNS with the flow of cerebrospinal fluid through the arachnoid villi. The lipophilic substances can cross the blood-brain barrier and eventually be excreted with the urine via kidney.
- (iv) Certain toxicants can also be eliminated through sweat, tears, semen, etc.