CHAPTER 2

# *Disposition of toxic compounds*

## *Chapter outline*

In this chapter the disposition of chemicals in biological systems will be discussed:

- The absorption of toxic compounds into biological systems
  - the cell membrane
  - transport of toxicants through cell membranes
  - sites of absorption of toxic substances and factors affecting absorption

    skin

    lungs

    gastrointestinal tract
- The distribution of toxic compounds in the body
  - volume of distribution
  - plasma level
  - half life
  - area under the curve
  - plasma protein binding
  - site of action
  - accumulation
- The excretion of toxic compounds and factors affecting excretion
  - urinary excretion
  - biliary excretion
  - excretion via lungs

The disposition of a toxic compound in a biological system may be divided into four phases: absorption, distribution, metabolism and excretion. These four phases are interrelated:

Absorption → Distribution → Metabolism
↘ ↙
Excretion

and we shall consider each of them in turn.

## *Absorption of toxic compounds*

Before a substance can exert a toxic effect it must come into contact with a biological system. Indeed the means, the rate and the site of absorption may all be important factors in the eventual toxicity of a compound. There are several sites for first contact between a toxic compound and a biological system but absorption necessarily involves the passage across cell membranes whichever site is involved. Consequently it is important to consider first the structure and characteristics of biological membranes in order to understand the passage of substances across them.

Membranes are composed mainly of **phospholipids** and **proteins** with the lipids arranged as a **bilayer** interspersed with proteins as shown in Figure 2.1. The particular proteins and phospholipids incorporated into the membrane vary depending on the cell type in which the membrane is located. The proteins may be structural or have a specific function, such as a carrier for membrane transport. The phospholipids may have one of several polar head groups (Figure 2.2) and the fatty acid chains may be saturated, unsaturated or a mixture of both. The degree of saturation will influence the fluidity of the membrane. Cholesterol esters and certain carbohydrates are also found in some membranes.

The structure of biological membranes determines their function and characteristics. The most important feature from a toxicological point of view is that they are selectively permeable. Only certain substances are able to pass through them, depending on particular physicochemical characteristics:

1 size
2 lipid solubility
3 similarity to endogenous molecules
4 polarity/charge

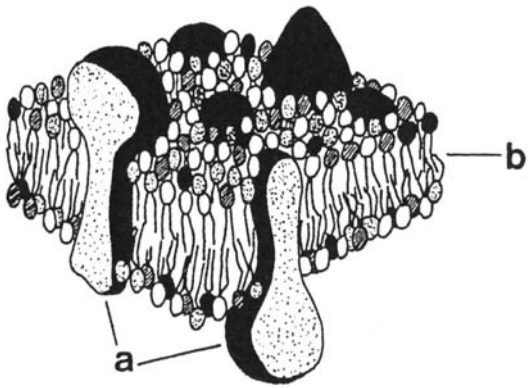


FIGURE 2.1 *The three-dimensional structure of the animal cell membrane. Proteins (a) are interspersed in the phospholipid bilayer (b).*

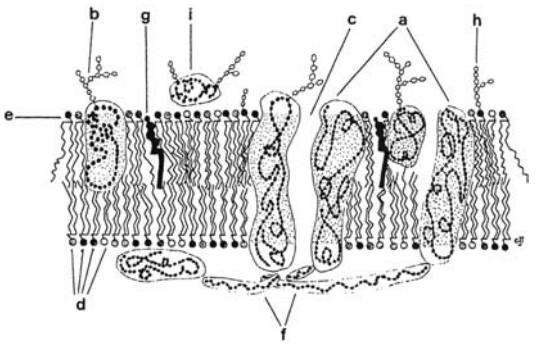


FIGURE 2.2 *The molecular arrangement of the cell membrane. a: integral proteins; b: glycoprotein; c: pore formed from integral protein; d: various phospholipids with saturated fatty acid chains; e: phospholipid with unsaturated fatty acid chains; f; network proteins; g: cholesterol; h: glycolipid; i: peripheral protein. There are four different phospholipids: phosphatidyl serine; phosphatidyl choline; phosphatidyl ethanolamine; sphingomyelin represented as ○; ◌; ▨; ●. The stippled area of the protein represents the hydrophobic portion.*

The lipid solubility of a chemical substance is usually represented by its **partition coefficient**: the oil/water partition coefficient is the comparative solubility of the chemical in aqueous versus organic solvents. It can be simply determined by shaking a solution of the chemical in water or buffer with an organic solvent such as chloroform or a biologically more relevant liquid such as olive oil. The concentration remaining in the aqueous phase and that in the organic phase are then measured and compared. The greater the lipid solubility of a compound, the greater is the value.

The ways in which foreign substances may pass through biological membranes are as follows:

1 **filtration through pores**

2 **passive diffusion through the membrane phospholipid**

3 **active transport**

4 **facilitated diffusion**

5 **phago/pinocytosis**

1 *Filtration*. Small molecules may pass through **pores** in the membrane formed by proteins. This movement will occur down a concentration gradient and may include substances such as ethanol and urea.

2 *Passive diffusion*. This is probably the most important mechanism of absorption for foreign and toxic compounds. For passive diffusion to occur certain conditions are required:

   a there must be a **concentration gradient** across the membrane
   b the foreign molecule must be **lipid soluble**
   c the compound must be **non-ionized**

These principles are embodied in the **pH-partition theory**: only non-ionized lipid soluble compounds will be absorbed by passive diffusion down a concentration gradient. Furthermore certain factors affect the rate at which foreign compounds passively diffuse. This rate of diffusion is described by **Ficks Law**:

$$\text{Rate of diffusion} = KA(C_2 - C_1)$$

where A is the surface area, $C_2$ is the concentration outside and $C_1$ the concentration inside the membrane, and K is a constant.

The above relationship applies to a system at constant temperature and for diffusion over unit distance. The concentration gradient is represented by $(C_2 - C_1)$. Passive diffusion is a **first order process**, that is the rate of diffusion is *proportional* to the concentration.

Normally biological systems are dynamic and the concentration on the inside of the membrane is continually reducing as the foreign compound is being removed by blood flow and possibly ionization (Figure 2.3). Consequently there is always a concentration gradient towards the inside of the membrane. As well as a concentration gradient, lipid solubility and ionization and, hence, the pH of the particular tissue fluid are also factors in passive diffusion. Lipid soluble compounds are able to pass across biological membranes by dissolution in the phospholipid and movement down the concentration gradient. Ionizable compounds will only do this if they are in the non-ionized form. The degree of ionization can be calculated from the **Henderson Hasselbach** equation:

$$pH = pK_a + \frac{\text{Log}[A^-]}{[HA]}$$

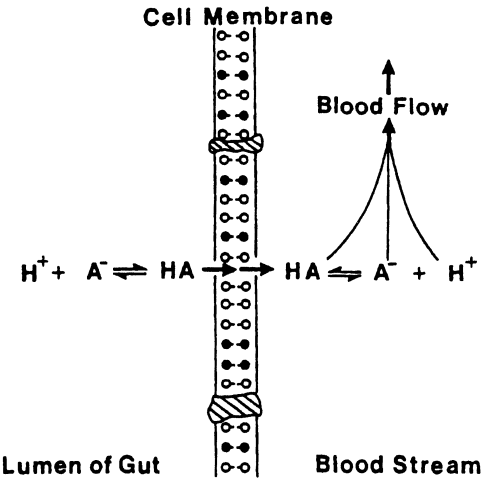


FIGURE 2.3 *Role of blood flow and ionization in the absorption of foreign compounds. Both blood flow and ionization create a gradient across the membrane.*

where $pK_a$ is the dissociation constant for the acid, HA. The ionization of an acid and base are shown in Figure 2.4. The role of ionization will be discussed more fully when the gastrointestinal tract is considered.

3 *Active transport.* Active transport of compounds across membranes has several important features:

a a specific membrane **carrier** is required
b metabolic **energy** is necessary to operate the system
c the process may be **inhibited** by metabolic poisons
d the process may be **saturated** at high substrate concentrations and hence is **zero order** rather than first order
e transport occurs against a **concentration gradient**
f similar substrates may **compete** for uptake

There are various kinds of active transport systems which involve carrier molecules operating in different ways. These are **uniports**, **symports** and **antiports**. The uniport transports one molecule in a single direction. Symports and antiports transport two molecules in the same or opposite directions respectively.

This type of membrane transport is normally specific for endogenous and nutrient substances but analogues and similar molecules or ions may be transported by the system. For example, the drug **fluorouracil**, an analogue of uracil and **lead** ions are absorbed from the gut by specific transport systems.

4 *Facilitated diffusion.* This has the following salient features:

a a specific membrane **carrier** is required
b a **concentration gradient** across the membrane is necessary
c the process may be **saturated** by high substrate concentrations

Unlike active transport, no energy expenditure is necessary. This type of transport system also normally applies to endogenous substances and normal nutrients but may apply to foreign compounds which are structurally similar to an endogenous compound. The transport of glucose from the cells of the intestine into the bloodstream involves this type of system.

5 *Phagocytosis and pinocytosis.* These involve the **invagination** of the membrane to enclose a particle or droplet respectively. This is the mechanism by which particles of insoluble substances such as **uranium dioxide and asbestos** are absorbed into the lungs.

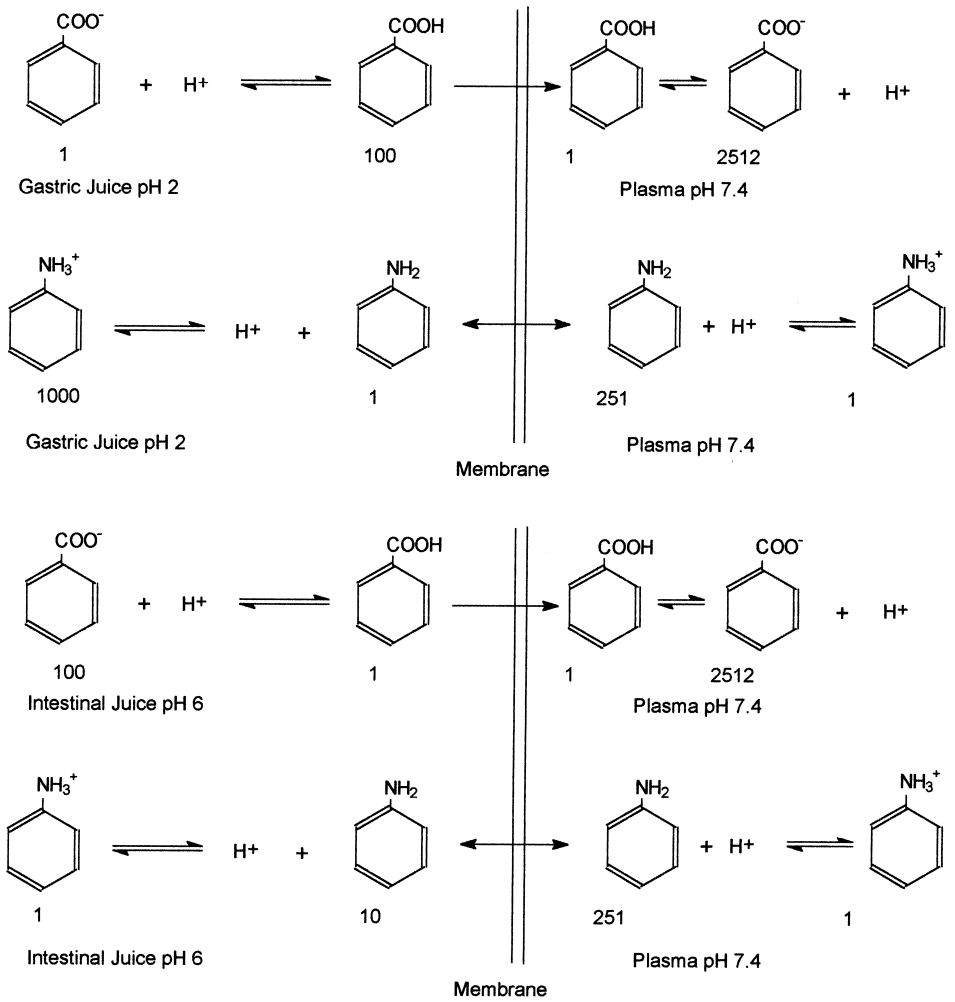


FIGURE 2.4 *Ionization of an acid and base in the stomach and intestine. From Timbrell, J. A.,* Principles of Biochemical Toxicology, *Taylor & Francis, London, 2000.*

## SITES OF ABSORPTION

There are three major sites for the absorption of foreign compounds: the skin, lungs and gastrointestinal tract. The gastrointestinal tract is the most important in toxicology as most foreign compounds are ingested orally. The lungs are clearly important for all airborne compounds whereas the skin is only rarely a significant site for absorption.

### Skin

The skin is constantly exposed to foreign compounds such as gases, solvents, and substances in solution, and so absorption through the skin is potentially an important route. However, although the skin has a large **surface area** for absorption, its structure is such as to present a barrier to absorption. This is because there is an outer layer of dead cells, a poor blood supply, and the outer cells of the epidermis are packed

with **keratin** (Figure 2.5). Although the dermis below is vascularized, it is several cells thick and this will also inhibit absorption.

Absorption through the skin is mainly limited to lipid soluble compounds such as solvents. Fatalities have occurred, however, following absorption of toxic compounds by this route, such as with the insecticide **parathion**.

## Lungs

Exposure to toxic compounds via the lungs is toxicologically more important than via the skin. The air we breathe may contain many foreign substances. These may be gases (**carbon monoxide**), vapours from solvents (**methylene chloride**), aerosols or particulate matter (**asbestos**) in an industrial or other workplace environment. Also, the air in an urban or home environment may contain noxious gases (**sulphur dioxide** and **nitrogen oxides**), particulates (**fibre glass** and **pollen**), and possibly solvent vapours and aerosols from home use. The lungs have a very large **surface area**, around 50–100 $m^2$ in man, they have an excellent blood supply, and the barrier between the air in the alveolus and the blood stream may be as little as two cell membranes thick (Figure 2.6). Consequently absorption from the lungs is rapid and efficient. Two factors which affect absorption via the lungs are blood flow and breathing rate. For compounds with low solubility in blood the absorption will be mainly dependent on the rate of blood flow. For compounds with high solubility in blood the absorption will be mainly dependent on the breathing rate. The rapid rate of blood flow means that foreign substances are continually removed from the absorption site and, therefore, there is always a concentration gradient.

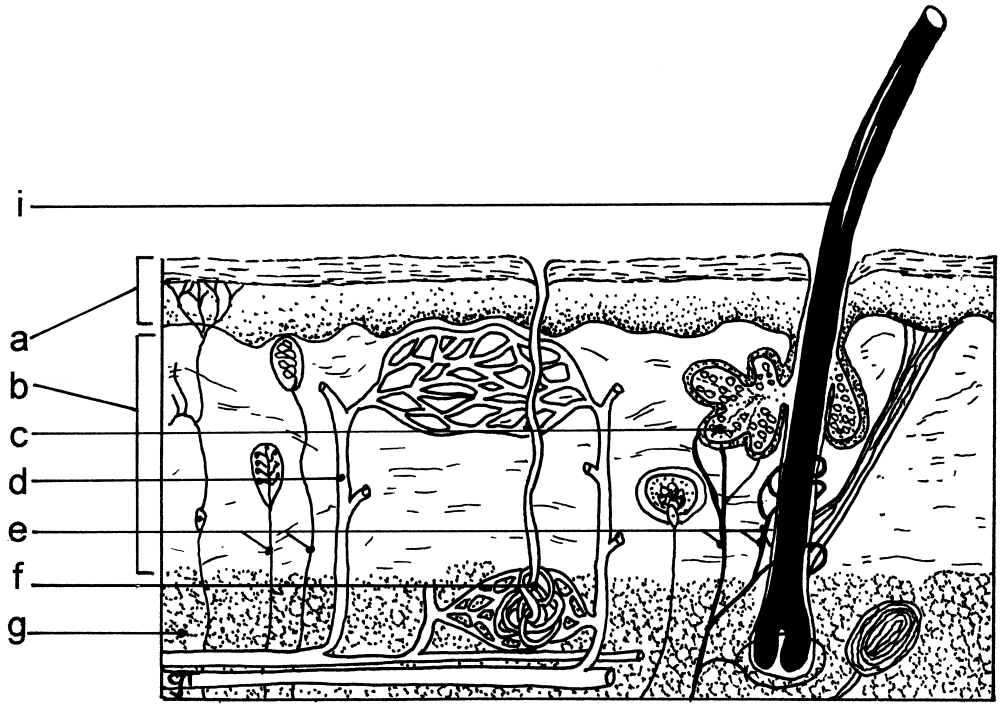


FIGURE 2.5 *The structure of mammalian skin. a: epidermis; b: dermis; c: sebaceous gland; d: capillary; e: nerve fibre; f: sweat gland; g: adipose tissue; i: hair. Drawing by C. J. Waterfield.*

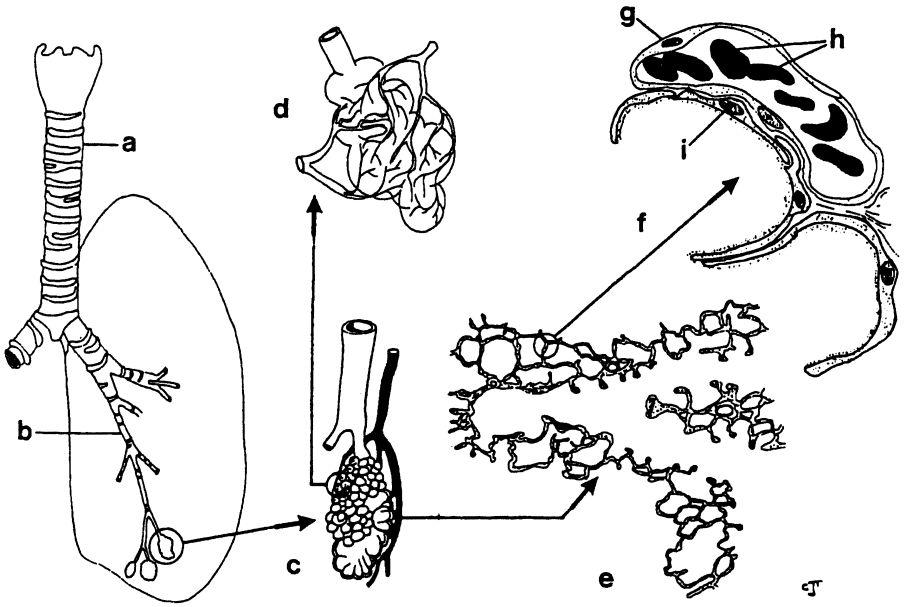


FIGURE 2.6 *The structure of the mammalian respiratory system. a: trachea; b: bronchiole; c: alveolar sac with blood supply; d: arrangement of blood vessels around alveoli; e: arrangement of cells and airspaces in alveoli showing the large surface area available for absorption; f: cellular structure of alveolus showing the close association between the endothelial cell of the capillary, g, with erythrocytes, h, and the epithelial cell of the alveolar sac, i. The luminal side of the epithelial cell is bathed in fluid which also facilitates absorption and gaseous exchange.*

Reaction with plasma proteins and for gases particularly dissolution in the plasma may also be factors.

Small, lipid soluble compounds, such as solvents, will be readily absorbed from the alveolus. For compounds which are absorbed via the lungs it is a very efficient and rapid route of entry to the body. Compounds in solution and particles may be absorbed by pinocytosis and phagocytosis respectively. For example, **uranium dioxide** particles, which are insoluble, are absorbed via the lungs and cause kidney damage. **Lead** is also absorbed in the particulate form from the air via the lungs. The size of particle is a major factor in determining where in the respiratory system it is deposited and whether it is absorbed. For example, lead particles of 0.25 $\mu$m diameter are absorbed but uranium dioxide particles of more than 3 $\mu$m diameter are not.

## Gastrointestinal tract

Numerous foreign substances are taken in via the diet, while many drugs are normally taken by mouth, and various poisonous substances taken either accidentally or intentionally are usually ingested **orally**. Consequently the gastrointestinal tract is a very important site of absorption for foreign compounds.

The internal environment of the gastrointestinal tract varies throughout its length, particularly with regard to the **pH**. Substances taken orally first come into contact with the lining of the mouth (buccal cavity), where the pH is normally around 7 in man, but more alkaline in some other species such as the rat. The next region of importance is the stomach where the pH is around 2 in man and certain other mammals. The substance may remain in the stomach for some time particularly if it is

taken in with food. In the small intestine where the pH is around 6, there is a good blood supply and a large surface area due to folding of the lining and the presence of villi (Figure 2.7).

Due to the change in pH in the gastrointestinal tract different substances may be absorbed in different areas depending on their **physicochemical characteristics**. Lipid soluble, non-ionized compounds will be absorbed along the whole length of the tract, but ionizable substances generally will only be absorbed by **passive diffusion** if they are **non-ionized** at the pH of the particular site and are also **lipid soluble**. The Henderson Hasselbach equation can be used to calculate the extent of ionization of aniline (a weak base) and benzoic acid (a weak acid) at the particular pH prevailing in the stomach and small intestine. It can be seen (Figure 2.4) that **weak acids** should be absorbed in the **stomach** and **weak bases** in the **small intestine**. However in practice weak acids are also absorbed in the small intestine due to the influence of blood flow and plasma pH. Although they exist mainly in the ionized form in the small intestine (Figure 2.4), the non-ionized form passing into the blood will immediately be removed by:

1 **blood flow**, and

2 **ionization** at pH 7.4.

These two factors ensure that weak acids are absorbed to a certain extent in the small intestine if they have not been fully absorbed in the stomach.

Another factor which may affect absorption from the gastrointestinal tract is the presence of **food**. This may facilitate absorption if the substance in question dissolves in any fat present in

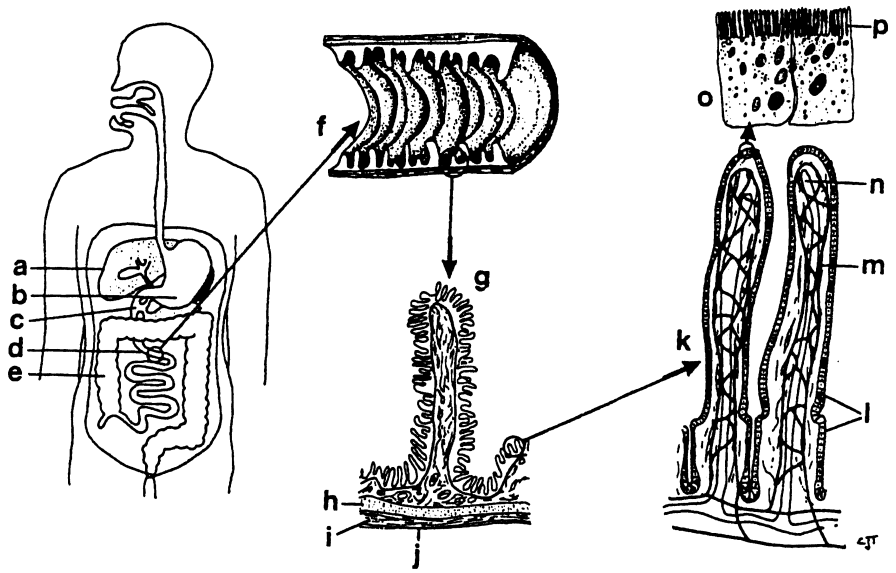


FIGURE 2.7 *The mammalian gastrointestinal tract showing important features of the small intestine, the major site of absorption for orally administered compounds. a: liver; b: stomach; c: duodenum; d: ileum; e: colon; f: longitudinal section of the ileum showing folding which increases surface area; g: detail of fold showing villi with circular and longitudinal muscles, h and i respectively, bounded by the serosal membrane, j; k: detail of villi showing network of capillaries, m, lacteals, n, and epithelial cells, l; o: detail of epithelial cells showing brush border or microvilli, p. The folding, vascularization and microvilli all facilitate absorption of substances from the lumen.*

the foodstuff but may delay absorption if the compound is only absorbed in the small intestine, as food **prolongs gastric emptying** time.

When drugs and other foreign compounds are administered the vehicle used to suspend or dissolve the compound may have a major effect on the eventual toxicity by affecting the rate of absorption and distribution.

The site of absorption itself may be important in the eventual toxicity because of the blood supply to that site as discussed in the next section.

The site of absorption and exposure to compounds may also be important in the fate of the compound. For example, the acidic conditions of the stomach may cause the substance to **hydrolyze**, or poisons such as **snake venom** may be **inactivated**. The **bacteria** in the gastrointestinal tract may metabolize foreign compounds as may enzymes in the gut wall. In the lungs phagocytosis sequesters some inert substances, such as particles of **asbestos** which can remain in the lung tissue for long periods of time with eventual toxic consequences.

## *Distribution of toxic compounds*

After a foreign compound has been absorbed it passes into the **bloodstream**. The part of the vascular system into which the compound is absorbed will depend on the site of absorption. Absorption through the skin leads to the **peripheral blood** supply, whereas the major **pulmonary circulation** will be involved if the compound is airborne and hence absorbed through the lungs. For the majority of compounds **oral** absorption will be followed by entry of the compound into the **portal vein** supplying the liver with blood from the gastrointestinal tract.

Once in the bloodstream the compound will then be distributed around the body and be diluted by the blood. Depending on the **physico-chemical properties** of the compound it may then be distributed into the tissues. As with the absorption of foreign compounds, distribution into particular tissues involves crossing biological **membranes** and the principles which have already been discussed earlier in the chapter again apply. Only the **non-ionized** form of compounds will pass out of the bloodstream into tissues by passive diffusion. Specific transport systems may operate for certain compounds, and phagocytosis and pinocytosis may transport large molecules, particles or solutions of large molecules. The **concentration** of the compound in the plasma and the plasma level profile (Figure 2.8) will reflect the distribution. For example, compounds which are distributed into all tissues, such as lipid soluble solvents like carbon tetrachloride, will tend to have low plasma concentrations, whereas substances which are ionized at the pH of the plasma and which do not readily distribute into tissues, may have much higher plasma concentrations. This can be quantified as the parameter known as apparent **volume of distribution**, $V_D$:

$$V_D(\text{L}) = \frac{\text{Dose}\ (\text{mg})}{\text{Plasma concentration}\ (\text{mg L}^{-1})}$$

(There are other means of determining $V_D$ which the interested reader may find in more advanced texts.)

This is the volume of body fluids into which the particular substance is apparently distributed. The determination is analogous to dissolving a known amount (dose) of a substance in an unknown volume of water (body fluids). A knowledge of the concentration of compound in the water (plasma level) allows us to determine the volume of water.

The volume of distribution may sometimes indicate that a foreign compound is localized in a particular tissue or is confined mainly to the plasma. Thus, if a substance distributes mainly into **adipose tissue**, the plasma concentration will be very low and from the above formula it can be seen that the volume of distribution will be large. The substance is not necessarily evenly distributed in body water however and may reach high concentrations in one particular tissue or organ.

The concentration of a chemical in the blood plasma and its change over time (Figure 2.8) is a reflection of the absorption, distribution, metabolism and excretion of the chemical. For example, after a drug is taken orally, the **plasma level** profile will be different from the profile of a drug given intravenously (Figure 2.9). The plasma level of a chemical and its change over time are vitally important pieces of information for a toxicologist. This is because:

a it reflects the concentration of the chemical in the tissues more readily than does the dose of the chemical which may be incompletely absorbed;

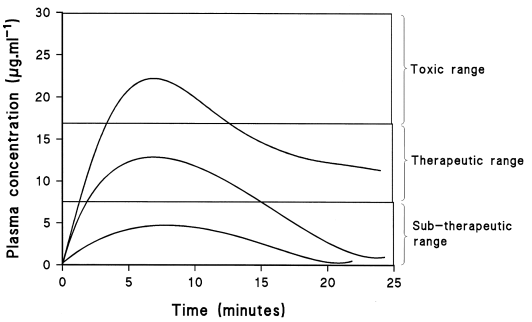


FIGURE 2.8 *The blood or plasma level profiles of three different doses of a drug after oral ingestion by an animal. The doses given are sub-therapeutic, therapeutic and toxic. The plasma level is plotted on a linear scale. The area under each curve (AUC) represents the overall exposure of the animal.*

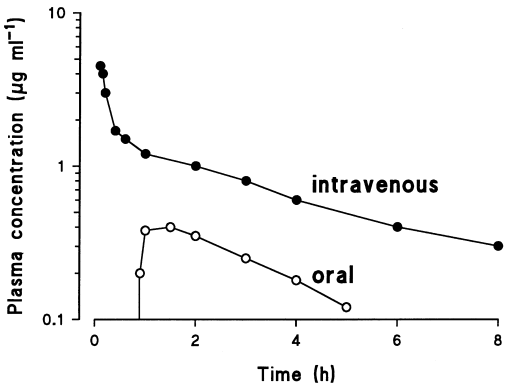


FIGURE 2.9 *The plasma level profile of a foreign compound after oral and intravenous administration. The marked difference in the areas under the curves (AUC) may indicate that first pass metabolism is occurring.*

b it may reflect the concentration of the chemical at the target site;

c it is necessary in order to calculate parameters such as **half-life**, $V_D$, **AUC** and **body burden**;

d it may indicate the type of distribution which the compound is undergoing (i.e. which compartments it is distributed to);

e the plasma level when plotted against time gives an indication of the duration of significant exposure (area under the curve or AUC; see Figure 2.8).

An indication of the overall exposure of the animal is given by the body burden, determined by $V_D \times$ plasma concentration.

For a drug, the plasma level indicates whether it has reached the therapeutic concentration and if so how quickly and for how long or whether the drug has reached a toxic concentration (see Figure 2.8).

The **half-life** ($t_{\frac{1}{2}}$) of the chemical in the blood can be calculated from the graph of the plasma

level plotted against time (if the plasma level is plotted on a log scale) (see Figure 2.9). The half-life is defined as the time taken for the plasma concentration to decrease by half and is determined by metabolism and excretion. Clearly, a substance with a long half-life will be in contact with the biological system for longer than a compound with a short half-life and is an indication of the likelihood of a substance accumulating with repeated or chronic dosing. It is normally a constant value but if not, one or both of the processes that determine the decline in plasma level, metabolism or excretion, is saturable.

The plasma level of a chemical is important information for the treatment of patients suffering overdoses of drugs. Thus it allows the clinical toxicologist to know the exact exposure rather than have to estimate an overdose and to estimate the elimination rate and time of dosing. It is important information for an experimental toxicologist who needs to know that absorption of the chemical has occurred and to what extent the organs and tissues of the animal have been exposed. It also allows the half-life to be calculated, which lets the experimental toxicologist design repeated dose studies.

It will be clear from Figure 2.9 that the **AUC** after oral dosing is much less than that after intravenous dosing. This may be because the drug or other compound is metabolized during the absorption process either in the gastrointestinal tract or in the liver. This is known as '**first-pass metabolism**' and means that less of the parent compound reaches the circulation after oral dosing.

Another indicator of the ability of the body to eliminate the compound is the total body clearance which is calculated as shown:

$$\frac{\text{dose}}{\text{AUC}}$$

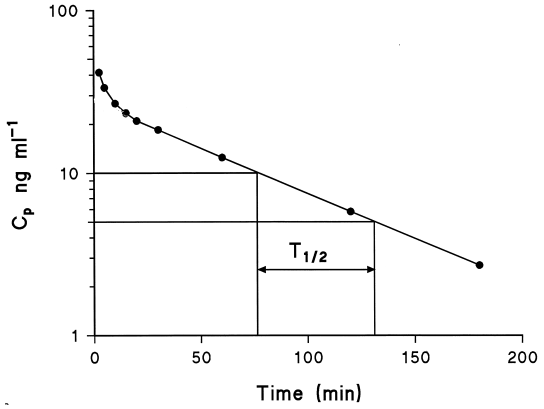


FIGURE 2.10 *Plasma level of a chemical after intravenous administration. The plasma level is plotted on a log scale. The half life ($T\frac{1}{2}$) can be determined as shown. The elimination rate constant ($k_{el}$) can be determined from the slope of the linear part of the line.*

(The units are ml min$^{-1}$ if the dose is in mg and the plasma concentration is plotted as mg ml$^{-1}$ against minutes.)

Another aspect of the distribution phase which may have important toxicological implications is the interaction of foreign compounds with **proteins** in plasma and various macromolecules in other tissues. Many foreign compounds bind to plasma proteins non-covalently and in doing so their distribution is altered. Distribution from the blood into the tissues is reduced by binding to such proteins as the foreign compound is now attached to a large molecule which limits its passage across membranes unless a specific transport system exists. Binding can also limit excretion as will be discussed later. Foreign compounds in plasma often exist in equilibrium between the bound and unbound form and the extent of binding and the tightness of that binding varies between different compounds. Binding may involve **ionic** forces, **hydrogen bonding**, **hydrophobic bonding** and **Van der Waals** forces. Foreign

compounds bind most commonly to **albumin** but some, such as **DDT**, which are lipophilic may associate extensively with plasma **lipoproteins**.

Distribution of foreign compounds to those tissues which may be the site of action is a particularly important aspect of their toxicology. For example, **barbiturates** act on the central nervous system and so must enter the brain in order to have a pharmacological, and if exaggerated, toxic, effect. The entry of substances into the brain is less readily attainable than passage into other tissues because of the so-called **blood-brain barrier**. This is due to the nature of the capillaries serving the brain. These are surrounded by cells which do not allow the ready passage of substances into the central nervous system. Lipid soluble compounds such as some of the barbiturates will enter the brain by passive diffusion. However, some barbiturates, such as phenobarbital, are weak acids and so ionize. In the treatment of barbiturate **poisoning** this ionization is utilized by increasing the plasma pH with infusions of **sodium bicarbonate**. This increases the ionization of the barbiturate in the plasma, changes the equilibrium and so causes more unionized drug to diffuse out of the tissues, including the brain, into the plasma. Another compound which is known to be toxic due to its effect on the central nervous system is **methyl mercury**, a lipophilic mercury derivative which is able to cross the blood-brain barrier.

Lipophilic foreign compounds localize particularly in body fat, sometimes to the extent that the plasma level is hardly detectable and the $V_D$ is very large. For example, **polybrominated biphenyls**, substances once used extensively in industry, are very persistent and highly fat soluble. This localization in body fat resulting in very long whole body half-lives may have important toxicological consequences. The drug **thiopental**, a barbiturate anaesthetic which is very lipid soluble, has an extremely rapid onset of action due to its ability to enter the brain very quickly.

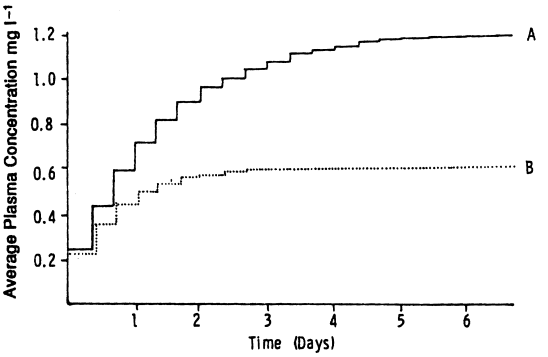


FIGURE 2.11 *Accumulation of two compounds after multiple dosing. Compound A has a half-life of 24 hours, compound B of 12 hours. Dosing interval is 8 hours.*
*From Timbrell, J. A.,* Principles of Biochemical Toxicology, *Taylor & Francis, London, 2000.*

Some toxic foreign compounds are chronically ingested or there is continuous exposure to them over shorter periods and this may alter their disposition. If the dosing interval is shorter than the **half-life** the compound will accumulate in the animal (Figure 2.11). The blood and tissue level may increase disproportionately and dramatically under certain circumstances, such as where excretion or metabolism is saturated. Otherwise the plateau level reached in the plasma is proportional to the plasma half-life, so that compounds with long half-lives could accumulate to significant levels on repeated dosing or exposure despite the low level of each dose or exposure (Figure 2.11).

## *Excretion of toxic compounds*

The elimination of toxic substances from the body is clearly an important determinant of their biological effect; rapid elimination will reduce the likelihood of toxicity occurring and

reduce the duration of the biological effect. In the case of a toxic effect, removal of the compound may help to reduce the extent of damage.

The elimination of foreign compounds is reflected in either the plasma half-life or the whole body half-life. However, the plasma half-life also reflects metabolism and distribution as well as excretion. The **whole body half-life** is the time required for half of the compound to be eliminated from the body and consequently reflects the excretion of the compound.

The most important route of excretion for most compounds is through the kidneys into the urine. Other routes are secretion into the bile, excretion into the expired air from the lungs for volatile and gaseous compounds and secretion into the gastrointestinal tract, milk, sweat and other fluids.

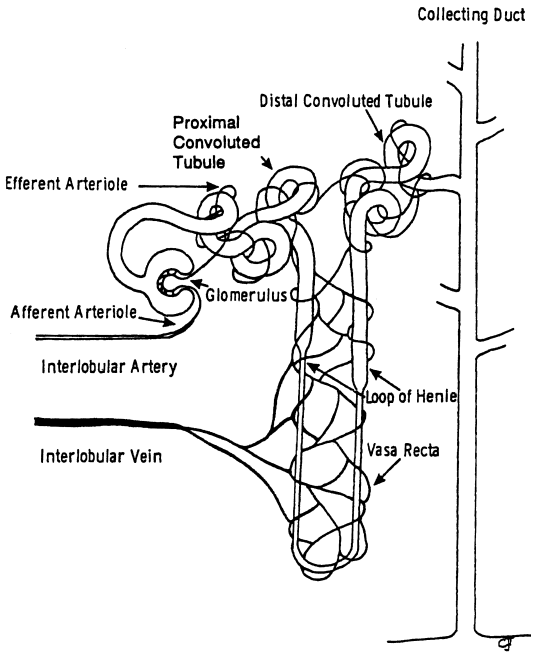


FIGURE 2.12 *Structure of the mammalian kidney. From Timbrell, J. A.,* Principles of Biochemical Toxicology, *Taylor & Francis, London, 2000.*

## URINARY EXCRETION

Excretion into the urine from the bloodstream applies to relatively small, water-soluble molecules; large molecules such as proteins do not pass out through the intact glomerulus and lipid soluble molecules such as bilirubin are reabsorbed from the kidney tubules.

The kidneys receive approximately 25 per cent of the cardiac output of blood and so they are exposed to and filter out a significant proportion of foreign compounds. Excretion into the urine involves one of three mechanisms: **filtration** from the blood through the pores in the glomerulus; **diffusion** from the bloodstream into the tubules; and **active transport** into the tubular fluid.

The structure of the kidney facilitates the elimination of compounds from the bloodstream (Figure 2.12). The basic unit of the kidney, the **nephron**, allows most small molecules to pass out of the blood in the glomerulus into the tubular ultrafiltrate aided by large pores in the capillaries and the pressure of the blood. Lipid-soluble molecules will passively diffuse out of the blood provided there is a concentration gradient. However, if such compounds are not ionized at the pH of the tubular fluid, they may be reabsorbed from the tubule by passive diffusion back into the blood as it flows through the vessels surrounding the tubule because there will be a concentration gradient in the direction tubule → blood. Water-soluble molecules which are ionized at the pH of the tubular fluid will not be reabsorbed by passive diffusion and will pass out into the urine.

Certain molecules, such as **ρ-aminohippuric acid**, a metabolite of ρ-aminobenzoic acid are actively transported from the bloodstream into the tubules by a specific anion transport system.

**Passive diffusion** of compounds into the tubules is *proportional* to the concentration in the bloodstream, so the greater the amount in the blood the greater will be the rate of elimination. However, when excretion is mediated via **active transport** or **facilitated diffusion**, which involves the use of specific carriers, the rate of elimination is *constant* and the carrier molecules may become **saturated** by large amounts of compound. This may have important **toxicological consequences**. As the dose of a compound is increased, the plasma level will increase. If excretion is via passive diffusion, the rate of excretion will increase as this is proportional to the plasma concentration. If excretion is via active transport, however, increasing the dose may lead to saturation of renal elimination and a toxic level of compound in the plasma and tissues may be reached. This is the case with **ethanol** where continuous intake leads to ever increasing plasma levels accompanied by the well-known effects on the central nervous system.

Another factor which may affect excretion is **binding to plasma proteins**. This may reduce excretion via passive diffusion especially if binding is tight and extensive as only the free portion will be able to passively diffuse into the tubule. Protein binding does not affect active transport however and a compound such as *ρ*-aminohippuric acid which is 90 per cent bound to plasma proteins is cleared in the first pass of blood through the kidney.

One of the factors which affects excretion is the **urinary pH**. If the metabolite excreted into the urine is ionizable it may become ionized when it enters the tubular fluid. For example, an acidic drug such as **phenobarbital** is ionized at alkaline urinary pH and a basic drug such as **amphetamine** is ionized at an acidic urinary pH. This factor is utilized in the treatment of poisoning by barbiturates **and aspirin** (**see below, page 77**). The pH of urine may be affected by diet; high protein diet for instance causes urine to become more acid. The rate of urine flow from the kidney into the bladder is also a factor in the excretion of foreign compounds; high fluid intake, and therefore production of copious urine, will tend to facilitate excretion.

## BILIARY EXCRETION

Excretion into the bile is an important route for certain foreign compounds, especially large **polar** substances. Indeed, it may indeed be the predominant route of elimination. Bile is secreted in the liver by the **hepatocytes** into the **canaliculi** and it flows into the bile duct and eventually into the intestine (Figure 2.13). Consequently compounds which are excreted into the bile are usually eliminated in the faeces. **Molecular weight** is an important factor in biliary excretion as can be seen from Table 2.1 and so for polar compounds with a molecular weight of **300** or so, such as **glutathione conjugates**, biliary excretion can be a major route of excretion. Excretion into the bile is an active process and there are **three specific transport systems**, one for neutral compounds, one for anions and one for cations.

As with renal excretion via active transport, biliary excretion may be saturated and this may lead to an increasing concentration of compound in the liver. For example, the drug **furosemide** was found to cause hepatic damage in mice due to saturation of the biliary excretion route which caused an increase in its concentration in the liver.

Another consequence of biliary excretion is that the compound comes into contact with the **gut microflora**. The bacteria may metabolize the compound and convert it into a more lipid-soluble substance which can be reabsorbed from the intestine into the portal venous blood supply and so return to the liver. This may lead to a cycling of the compound

TABLE 2.1 *Effect of molecular weight on the route of excretion of biphenyls by the rat*

| Compound | Molecular weight | % Total excretion | |
|---|---|---|---|
| | | Urine | Faeces |
| Biphenyl | 154 | 80 | 20 |
| 4-Monochlorobiphenyl | 188 | 50 | 50 |
| 4,4'-Dichlorobiphenyl | 223 | 34 | 66 |
| 2,4,5,2',5'-Pentachlorobiphenyl | 326 | 11 | 89 |
| 2,3,6,2',3',6'-Hexachlorobiphenyl | 361 | 1 | 99 |

Source: H. B. Matthews (1980), *Introduction to Biochemical Toxicology,* Hodgson and Guthrie (Eds) (New York: Elsevier-North Holland)

known as **enterohepatic recirculation** which may increase the toxicity (Figure 2.13). If the compound is taken orally, and therefore is transported directly to the liver and is extensively excreted into the bile, it may be that none of the parent compound ever reaches the systemic circulation. Alternatively, the gut microflora may metabolize the compound to a more toxic metabolite which could be reabsorbed and cause a systemic toxic effect. Compounds taken orally may also come directly into contact with the gut bacteria. For example, the naturally occurring glycoside cycasin is hydrolyzed to the potent carcinogen **methylazoxymethanol** by the gut bacteria when it is ingested orally.

Biliary excretion, therefore, may:

1 **increase the half-life** of the compound;

2 lead to the production of **toxic metabolites** in the gastrointestinal tract;

3 increase hepatic exposure via the **enterohepatic recirculation**;

4 be **saturated** and lead to hepatic damage.

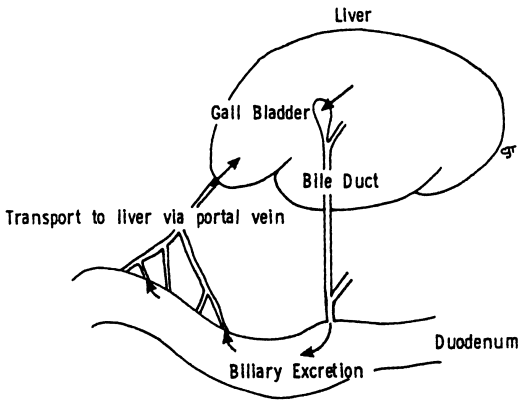


FIGURE 2.13 *Biliary excretion route for foreign compounds.*
*From Timbrell, J. A.,* Principles of Biochemical Toxicology, *Taylor & Francis, London, 2000.*

The importance of biliary excretion in the toxicity of compounds can be seen from Table 2.2 which shows that ligation of the bile duct increases the toxicity of certain chemicals many times.

## EXCRETION VIA THE LUNGS

The lungs are an important route of excretion for **volatile** compounds and **gaseous** metabolites of foreign compounds. For example, about 50–60 per cent of a dose of the aromatic hydrocarbon **benzene** is eliminated in the expired air. Excretion is by passive diffusion from the blood into the alveolus assisted by

TABLE 2.2 *Effect of bile duct ligation (BDL) on the toxicity of certain compounds*

| Compound | $LD_{50}$; mg/kg | | |
|---|---|---|---|
| | Sham operation | BDL | Sham:BDL ratio |
| Amitryptiline | 100 | 100 | 1 |
| Diethylstilboestrol | 100 | 0.75 | 130 |
| Digoxin | 11 | 2.6 | 4.2 |
| Indocyanine Green | 700 | 130 | 5.4 |
| Pentobarbital | 110 | 130 | 0.8 |

Source: C. D. Klaassen (1974), *Toxicology and Applied Pharmacology,* **24**, 37.

the concentration gradient. This is a very efficient route of excretion for lipid-soluble compounds as the capillary and **alveolar membranes** are **thin** and in very close proximity to allow for the normal gaseous exchange involved in breathing. There will be a continuous concentration gradient between the blood and air in the alveolus because of the rapid removal of the gas or vapour from the lungs and the rapid blood flow to the lungs. This may be a very important factor in the treatment of poisoning by such gases as the highly toxic **carbon monoxide**. Compounds may also be metabolized to volatile metabolites such as carbon dioxide for example.

### OTHER ROUTES OF EXCRETION

Excretion into **breast milk** can be a very important route for certain types of compounds especially lipid-soluble compounds. Clearly new born animals will be specifically at risk from toxic compounds excreted into milk. For example nursing mothers exposed to **DDT** secrete it into their milk and the infant may receive a greater dose, on a weight basis, than the mother. Foreign compounds may be secreted into other body fluids such as **sweat**, **tears** or **semen** and certain compounds may be secreted into the **stomach** or **saliva**.

## *Summary and learning objectives*

In this chapter you will have read about ***three*** of the ***four phases*** of disposition of a chemical in a biological system: the ***absorption*** through membranes into the system, the ***distribution*** throughout the system and the ***excretion*** and elimination from the system. In the next chapter you will learn about the metabolic fate of chemicals, which is the fourth phase.

All three phases of disposition require the chemical to cross ***biological membranes***. Membranes consist of phospholipid bilayers with proteins of various types interspersed. Depending on the chemical structure and its physico-chemical characteristics this will occur by one of a number of processes: ***filtration*** through pores (small molecules), ***passive diffusion*** (most foreign chemicals if lipid soluble), ***active transport*** (chemicals similar to endogenous substances such as amino acids), ***facilitated diffusion*** (similar chemicals to active transport), **phago/pinocytosis** (large molecules and particles).

Passive diffusion is the most important means of transport of foreign chemicals across biological membranes. It depends on three important factors that together form the ***pH partition theory***: the chemical must form a

***concentration gradient***, be ***lipid soluble*** (measured as partition coefficient) and be ***non-ionized***. The degree of ionization can be calculated using the ***Henderson Hasselbach*** equation.

Ficks Law defines the rate of diffusion through membranes and relates it to surface area and the concentration gradient.

Active transport and facilitated diffusion require ***carrier proteins*** and so can be ***saturated*** and may undergo ***competitive inhibition***. Active transport also requires ***energy***.

***Absorption*** occurs from three main sites: ***skin*** (large surface area, poorly vascularized, not readily permeable); ***gastrointestinal tract*** (major site, well vascularized, variable pH, large surface area, transport processes, food, gut bacteria), ***lungs*** (very large surface area, very well vascularized, readily permeable). Also chemicals (e.g. drugs) may be administered by direct injection (i.p., i.m., s.c., i.v.). The result of the absorptive phase is that the compound enters the blood. Absorption from the gastrointestinal tract may result in ***first pass metabolism*** occurring in the gut wall or liver.

***Distribution*** is the phase in which the compound is carried to the tissues by blood or lymph.

The ***plasma level*** reflects the concentration at the ***target site*** and is governed by distribution. It is a vitally important piece of information for the toxicologist. Distribution may be limited by ***binding*** to plasma proteins. This binding, which is usually non-covalent (ionic, hydrophobic, hydrogen, Van der Waals bonding), may be saturated or be subject to displacement by other compounds allowing threshold effects. The blood level of a chemical can be used to derive ***kinetic*** parameters such as ***half-life***, ***area under the curve*** (AUC) and ***volume of distribution***. Chemicals may be sequestered and ***accumulate*** in tissue compartments (e.g. adipose tissue) depending on physico-chemical characteristics such as lipid solubility.

***Excretion*** is the elimination of a chemical from the organism via the urine, bile or expired air. Excretion via the kidney into the ***urine*** is the major route involving filtration through the glomerulus and passive diffusion, filtration or active transport from the blood into the nephron. Extent of ***biliary excretion*** is influenced by molecular weight and may result in ***enterohepatic recirculation***. Exhalation from the ***lungs*** involves passive diffusion.

Chemicals may accumulate after repeated exposure if the frequency of dosing is shorter than the half-life or elimination (metabolism or excretion) is saturated.

## *Questions*

Q1. Choose one answer which you think is the most appropriate.
The oil/water partition coefficient of a chemical is an indication of:

a carcinogenicity
b long half-life
c potential to bioaccumulate
d low apparent volume of distribution
e chronic toxicity.

Q2. Choose one answer which you think is the most appropriate.
The absorption of which of the following is facilitated by the prevailing pH in the stomach:

a weak organic bases
b strong acids
c weak organic acids
d strong bases
e none of the above.

Q3. Choose one answer which you think is the most appropriate.
The parameter 'volume of distribution'

($V_D$) may be determined for a chemical *in vivo*. Is it:

a equal to the water solubility of the chemical
b sometimes larger than the total body volume
c equal to the volume of total body water
d smaller than the total body water if highly bound in tissues
e none of the above.

Q4. Choose one answer which you think is the most appropriate.
The half-life of a drug in the blood is determined by:

a the metabolism of the compound
b the volume of distribution
c plasma protein binding
d absorption of the drug
e urinary pH
f the total body clearance.

Q5. Choose one answer which you think is the most appropriate.
The term 'first-pass effect' means which of the following:

a the drug is excreted unchanged
b the drug is mostly metabolized by the gastrointestinal tract and/or liver before reaching the systemic circulation
c the drug is completely absorbed from the gastrointestinal tract
d the drug is excreted completely and very quickly by the kidneys
e none of the above.

Questions 6 and 7. Answer (a) if the statement is true and (b) if the statement is false.

Q6. The absorption of drugs into biological systems by passive diffusion is facilitated by ionization of the compound.

Q7. Binding of drugs to proteins in the blood involves the formation of covalent bonds.

Questions 8 and 9.

Select A if 1, 2 and 3 are correct
Select B if 1 and 3 are correct
Select C if 2 and 4 are correct
Select D if only 4 is correct
Select E if all four are correct

Q8. Which features of a chemical will favour accumulation in biological systems?

1 binding to plasma proteins
2 lipophilicity
3 limited volume of distribution
4 resistance to metabolism.

Q9. When considering the chronic toxicity (but not acute toxicity) of a chemical which of the following must be considered?

1 nature of the chemical
2 half-life in the body
3 dose of the chemical
4 frequency of dosing.

## SHORT ANSWER QUESTIONS

Q10. Write notes on three of the following:

a volume of distribution
b binding of drugs to plasma proteins
c first-phase effect
d Fick's law of diffusion.

Q11. Write notes on three of the following:

a the pH partition theory
b plasma half-life
c plasma clearance
d enterohepatic recirculation.

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