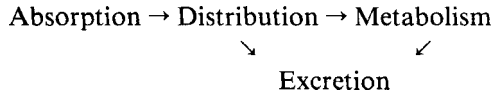
Chapter 2

Disposition of Toxic Compounds

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:



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

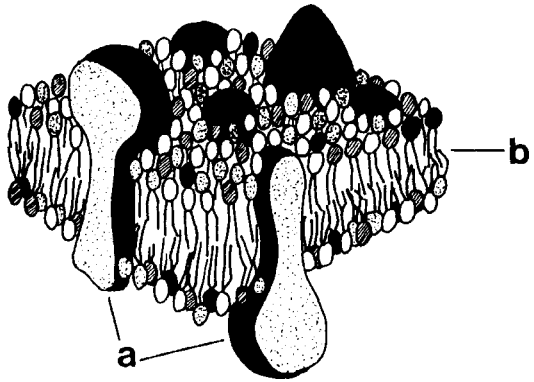


Figure 2.1. The three-dimensional structure of the animal cell membrane. Proteins (a) are interspersed in the phospholipid bilayer (b).

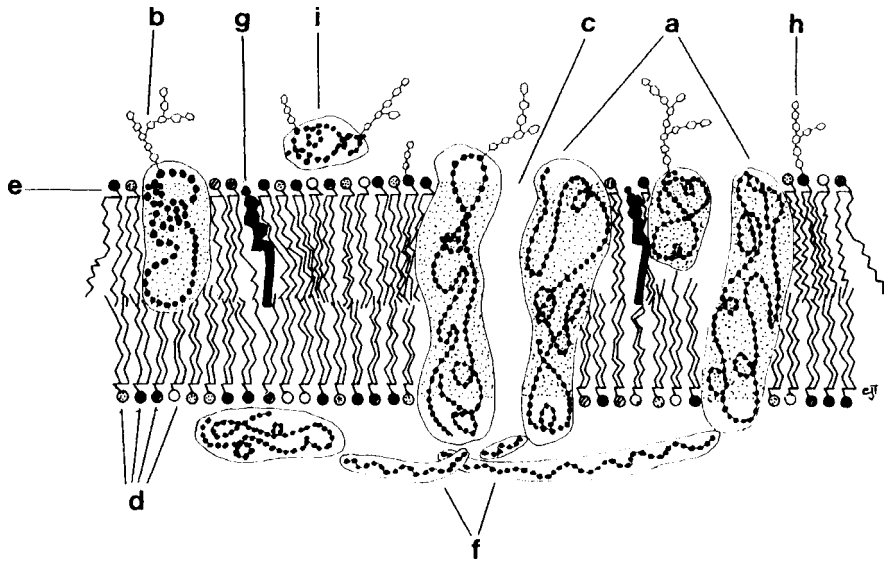


Figure 2.2. The molecular arrangement of the cell membrane, a: integral proteins; b: glycoprotein; c: pore formed from integral protein; d: various phospholipids with saturated fatty acid chains; e: phospholipid with unsaturated fatty acid chains; f: network proteins; g: cholesterol; h: glycolipid; i: 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.

is that they are selectively permeable. Only certain substances are able to pass through them, depending on particular physico-chemical characteristics:

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

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. lonizable compounds will only do this if they are in

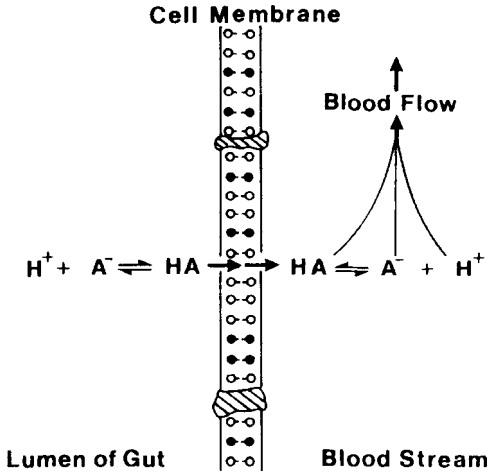


Figure 2.3. Role of blood flow and ionization in the absorption of foreign compounds. Both blood flow and ionization create a gradient across the membrane.

the non-ionized form. The degree of ionization can be calculated from the Henderson Hasselbach equation:

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

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 more fully discussed 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

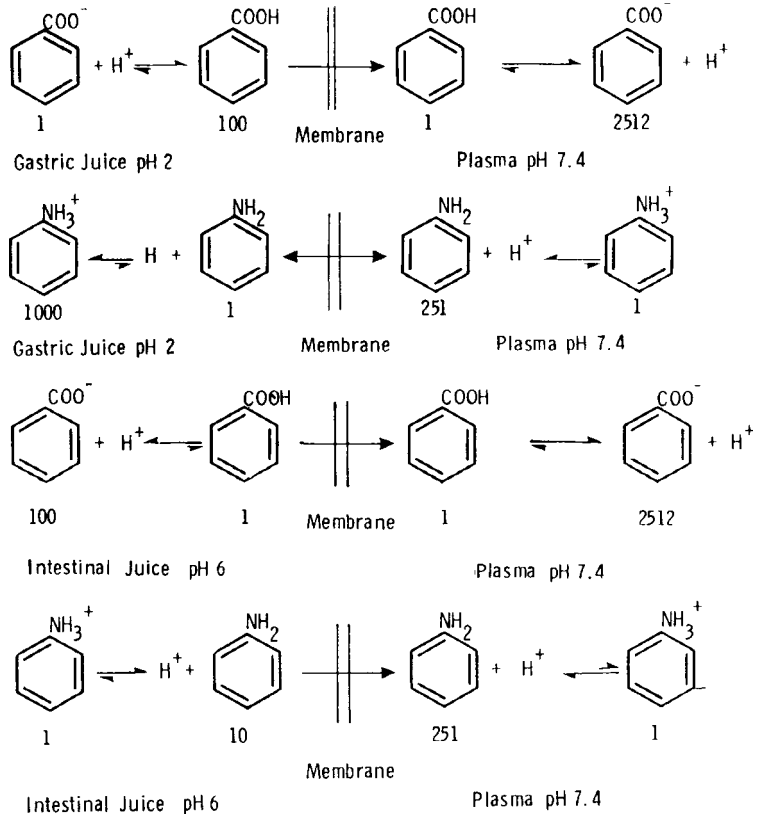


Figure 2.4. Ionization of an acid and base in the stomach and intestine.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

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.

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.

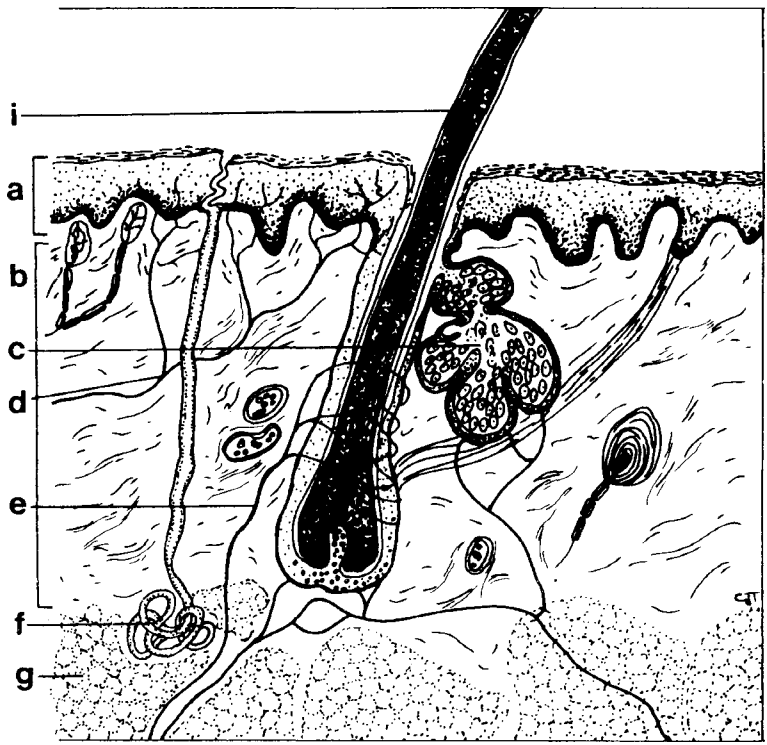


Figure 2.5. The structure of mammalian skin, a: epidermis; b: dermis; c: sebaceous gland; d: capillary; e: nerve fibre; f: sweat gland; g: adipose tissue; i: hair.

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

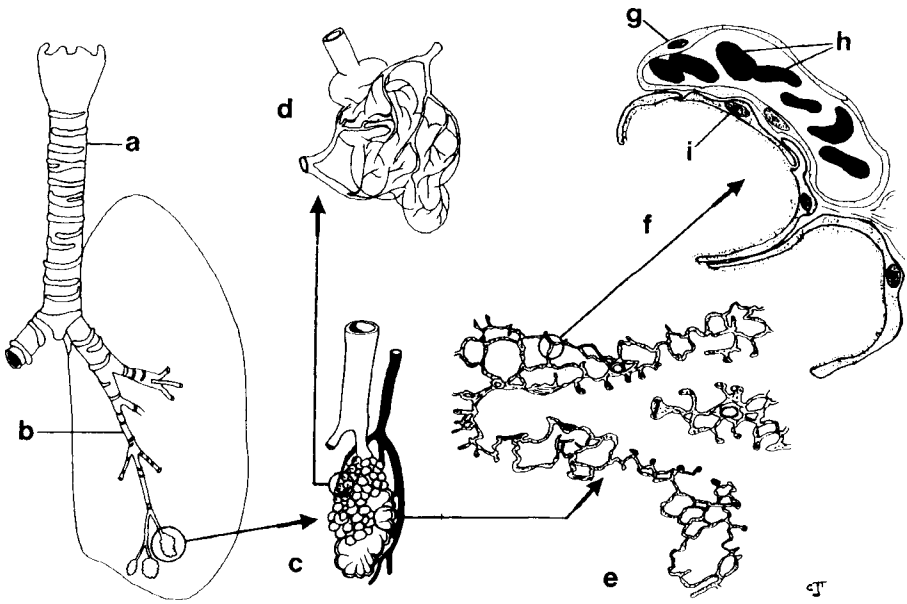


Figure 2.6. The structure of the mammalian respiratory system, a: trachea; b: bronchiole; c: alveolar sac with blood supply; d: arrangement of blood vessels around alveoli; e: arrangement of cells and airspaces in alveoli showing the large surface area available for absorption; f : cellular structure 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.

rate of blood flow means that foreign substances are continually removed from the absorption site and, therefore, there is always a concentration gradient. 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 μm diameter are absorbed but uranium dioxide particles of more than 3 μm 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 physico-chemical 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 Render son 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.

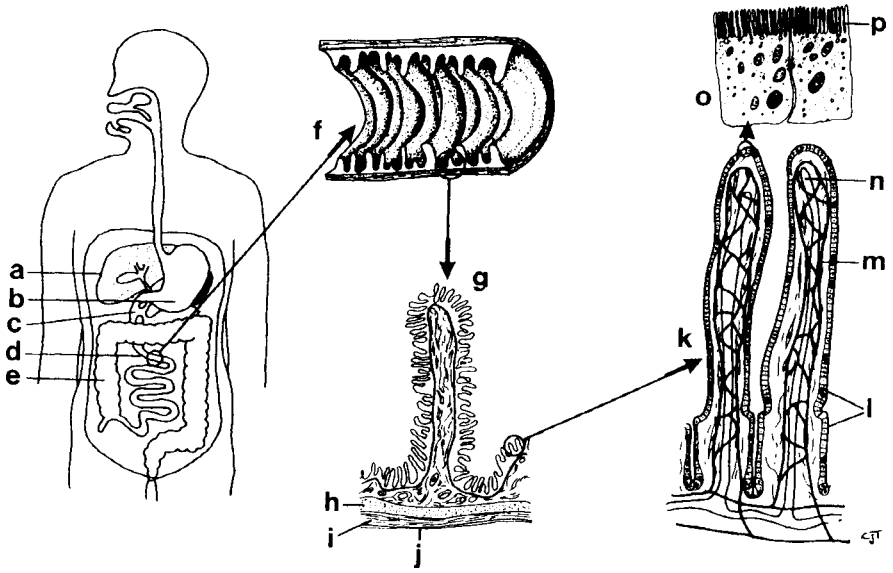


Figure 2.7. The mammalian gastrointestinal tract showing important features of the small intestine, the major site of absorption for orally administered compounds, a: liver; b: stomach; c: duodenum; d: ileum; e: colon; f: longitudinal section of the ileum showing folding which increases surface area; 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.

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 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:

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

(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 plasma concentration, or better the area under the plasma concentration versus time curve (AUC) (Figure 2.8) gives a much more meaningful indication

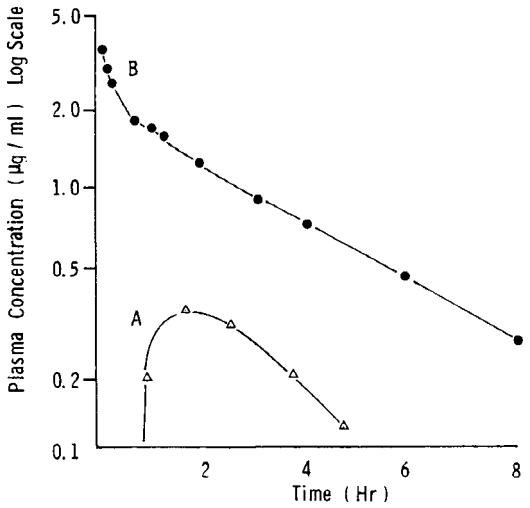


Figure 2.8. Plasma level profile of a foreign compound after oral (A) and intravenous (B) administration. The difference between the profile may indicate first-pass metabolism is occurring. From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

of the exposure of the animal than the dose as it indicates the concentration to which the tissue or receptor site may be exposed. This is usually the case but there are exceptions such as when a compound is sequestered or concentrated in a tissue. The plasma level also reflects the absorption, distribution, metabolism, and excretion of the compound. It is an essential piece of information for the treatment and management of drug overdoses and during chronic dosing as it may indicate when accumulation is occurring. The plasma level is therefore a very important parameter in toxicology. An indication of the overall exposure of the animal is given by the whole body burden, determined from $V_D \times$plasma concentration.

Another important value is the half-life of the compound $(t_{\frac{1}{2}})$. This may be the plasma half-life or the whole body half-life and indicates the length of time required for the concentration in the plasma or body to decrease by one half. This may be a very important factor in the toxicity of the compound as a substance with a long half-life can accumulate during chronic exposure. The longer a substance remains in the body of an animal, the more chance for toxic effects to occur. The half-life is determined by both the metabolism and elimination and as both of these processes may be saturable, dose dependent changes in the half-life will indicate saturation of either or both processes.

It will be clear from Figure 2.8 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:

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

(The units are ml min^{-1} if the dose is in mg and the plasma concentration is plotted as mg m^{-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 phenobarbitone, 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.

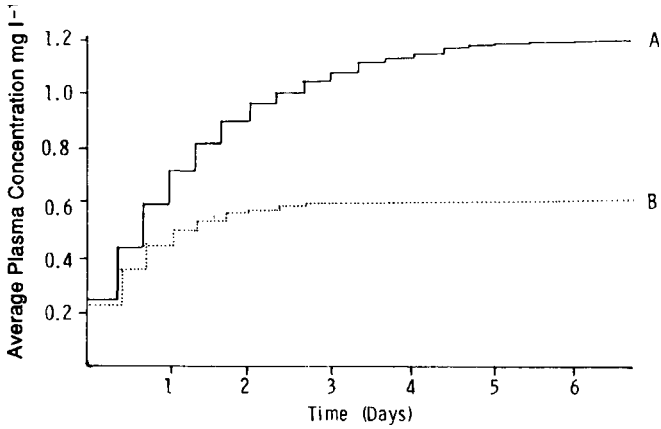


Figure 2.9. Accumulation of two compounds after multiple dosing. Compound A has a half-life of 24 hours, compound B of 12 hours. Dosing interval is 8 hours.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

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 2.9). 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 2.9).

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.

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 2.10). 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

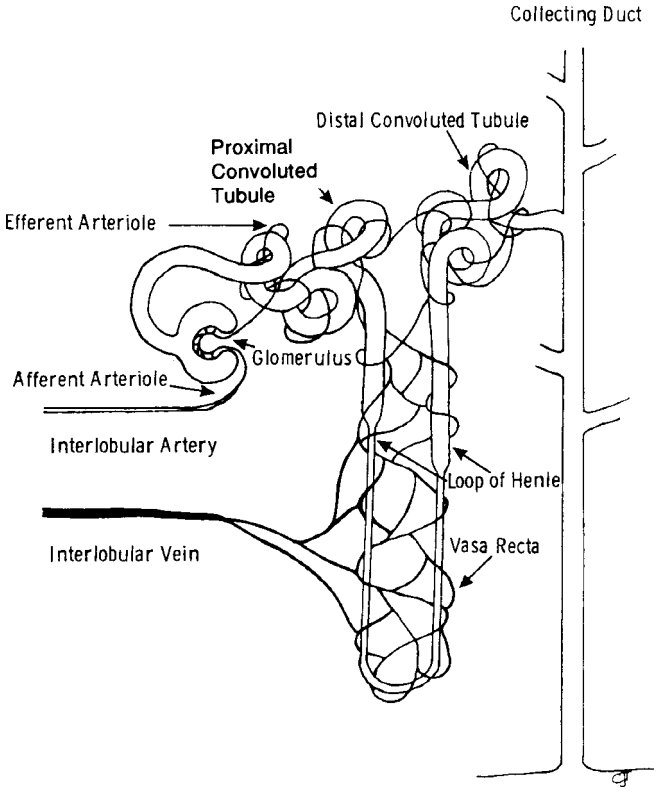


Figure 2.10. Structure of the mammalian kidney.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

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. 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 2.11). Consequently compounds which are excreted into the bile are usually eliminated in the faeces. Molecular weight is an important factor in

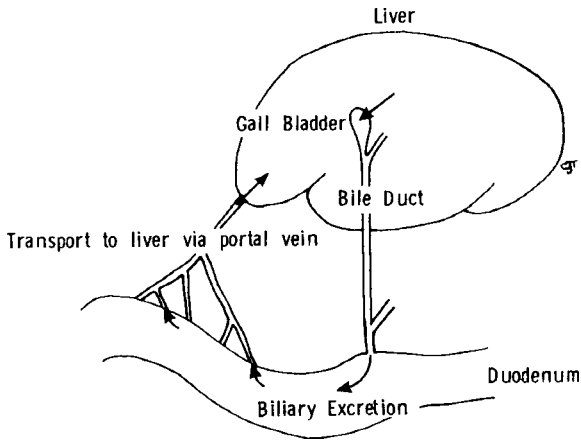


Figure 2.11. Biliary excretion route for foreign compounds.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

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 known as enterohepatic recirculation which may increase the toxicity (Figure 2.11). 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

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)

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.

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 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.

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.

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.

Metabolism of Foreign Compounds

As we have seen, foreign compounds absorbed into a biological system by passive diffusion are generally lipid soluble and consequently not ideally suited for excretion. For example, very lipophilic substances such as DDT (Figure 7.1) and the polychlorinated biphenyls are very poorly excreted and hence remain in the animal's body for many years.

After a foreign compound has been absorbed into a biological system it may undergo metabolism (also known as biotransformation). The metabolic fate of the compound can have an important bearing on its toxic potential, disposition in the body and its excretion. The products of metabolism are usually more water soluble than the original compound. Indeed, in animals biotransformation seems directed at increasing water solubility and hence excretion. Facilitating the excretion of a compound means that its biological half-life is reduced and hence its potential toxicity is kept to a minimum. Metabolism may also directly affect the biological activity of a foreign compound. For example, the drug succinylcholine causes muscle relaxation, but its action only lasts a few minutes because metabolism cleaves the molecule to yield inactive products (Figure 2.12). However, in some cases metabolism increases the toxicity of a compound as we shall discuss later in this book. There are numerous examples of this but a well-known one is ethylene glycol which is metabolized to oxalic acid, partly responsible for the toxicity (Figure 2.13).

Metabolism, therefore, is an extremely important phase of disposition as it may have a major effect on the biological activity of that compound, generally by increasing polarity and so water solubility and thereby increasing excretion. For example, the analgesic drug paracetamol (discussed in Chapter 4) has a renal clearance value of 12 ml min^{-1}, whereas one of its major metabolites, the sulphate conjugate, is cleared at the rate of 170 ml min^{-1}.

Therefore, in summary, metabolism leads to:

1. transformation of the molecule into a more polar metabolite;
2. possible increase in molecular weight and size;
3. facilitation of excretion and so elimination from the organism.

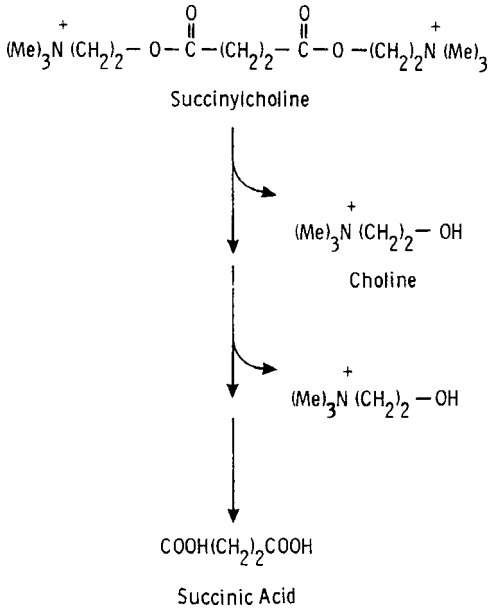


Figure 2.12. Hydrolysis of the drug succinylcholine.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

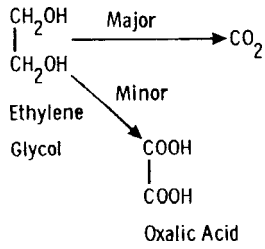


Figure 2.13. Metabolism of ethylene glycol.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

The consequences of these changes are:

a. the half-life of the compound is decreased;
b. the exposure time is shortened;
c. the possibility of accumulation is reduced;
d. a probable change in biological activity;
e. a change in the duration of the biological activity.

Sometimes metabolism may decrease water solubility and so reduce excretion. For example, acetylation decreases the solubility of sulphonamides in urine and so leads to crystallization in the kidney tubules causing necrosis of the tissue.

Metabolism can be simply divided into two phases: phase 1 and phase 2. Phase 1 is the alteration of the original foreign molecule so as to add on a

functional group which can then be conjugated in phase 2. This can best be understood by examining the example in Figure 2.14. The foreign molecule is benzene, a highly lipophilic molecule which is not readily excreted from the animal except in the expired air as it is volatile. Phase 1 metabolism converts benzene into a variety of metabolites, but the major one is phenol. The insertion of a hydroxyl group allows a phase 2 conjugation reaction to take place with the polar sulphate group being added. Phenyl sulphate, the final metabolite is very water soluble and is readily excreted in the urine.

Most biotransformations can be divided into phase 1 and phase 2 reactions, although some foreign molecules already possess functional groups suitable for phase 2 reactions, such as phenol for example. The products of phase 2 biotransformations may be further metabolized in what is sometimes termed phase 3 reactions.

Metabolism is usually catalyzed by enzymes and these are usually, but not always, found most abundantly in the liver in animals. The reason for this location is that most foreign compounds enter the body via the gastrointestinal tract and the portal blood supply goes directly to the liver (Figure 2.7). However, it is important to remember that (1) the enzymes involved with the metabolism of foreign compounds may be found in many other tissues as well as the liver; (2) the enzymes may be localized in one particular cell type in an organ; and (3) the enzymes are not always specific for foreign compounds and may have a major role in normal endogenous metabolism.

The enzymes involved in biotransformation also have a particular subcellular localization: many are found in the endoplasmic reticulum. Some are located in the cytosol and a few are found in other organelles such as the mitochondrion. The various types of metabolic reactions are shown in Table 2.3. For more information on the metabolism of foreign compounds the reader should consult the more detailed texts indicated in the bibliography.

Phase 1 Reactions

Oxidation reactions

The majority of these reactions are catalyzed by one enzyme system, the cytochrome P450 mono-oxygenase system which is located in the smooth endoplasmic reticulum of the cell, isolated as the so-called microsomal fraction obtained by cell fractionation. The liver has the highest concentration of this enzyme although it can be found in most, if not all tissues. The

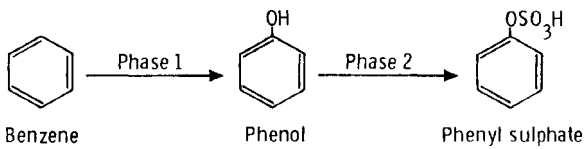


Figure 2.14. Metabolism of benzene.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

Table 2.3. The major biotransformation reactions.

Phase 1	Phase 2
Oxidation	Sulphation
Reduction	Glucuronidation
Hydrolysis	Glutatione conjugation
Hydration	Acetylation
Dehalogenation	Amino acid conjugation

reactions catalyzed also require NADPH, molecular oxygen and magnesium, and the overall reaction is shown below:

$$SH + O_2 + NADPH + H^+ \rightarrow SOH + H_2O + NADP^+$$

where S is the substrate.

The sequence of metabolic reactions is shown in Figure 2.15 and involves four distinct steps:

1. addition of substrate to the enzyme;
2. donation of an electron;

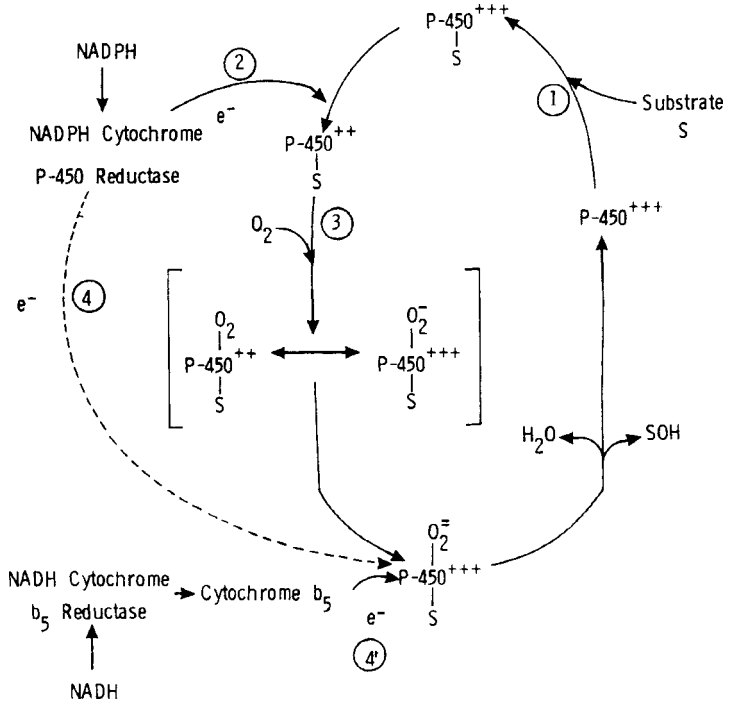


Figure 2.15. The cytochrome P450 mono-oxygenase system which catalyzes the phase 1 metabolism of many foreign compounds.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

3. addition of oxygen and rearrangement;
4. donation of a second electron and loss of water.

The cytochrome P450 system is actually a collection of isoenzymes all of which possess an iron atom in a porphyrin complex. These catalyze different types of oxidation reactions and under certain circumstances catalyze other types of reaction.

Let us look at the major types of oxidation reaction catalyzed by the cytochrome P450 system.

Aromatic hydroxylation, such as occurs with benzene (Figure 2.14) and aliphatic hydroxylation such as with vinyl chloride (Figure 2.16) involves adding oxygen across a double bond. Hydroxylation of the aliphatic moiety in propylbenzene may occur at one of three positions (Figure 2.17). Alicyclic and heterocyclic rings may also undergo hydroxylation.

Alkyl groups attached to N, O or S atoms may be removed by dealkylation reactions which involve oxidation of the alkyl group and then rearrangement and loss as the respective aldehyde (Figure 2.18). Nitrogen and sulphur atoms in xenobiotics may be oxidized by the microsomal enzymes (Figure 2.19) and sulphur and halogen atoms may be removed oxidatively (Figures 2.20 and 2.21).

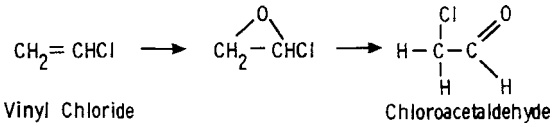


Figure 2.16. Epoxidation of vinyl chloride.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

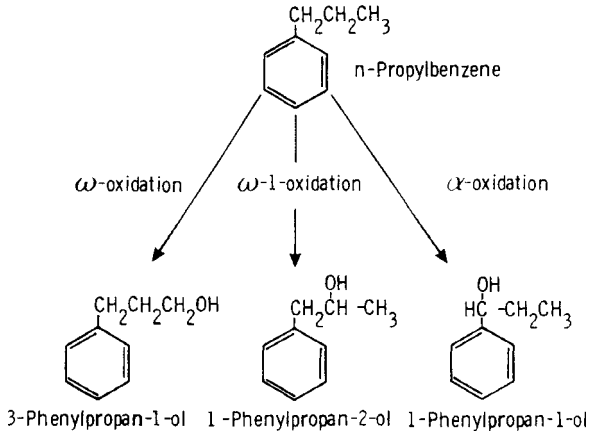


Figure 2.17. Oxidation of n-propylbenzene.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

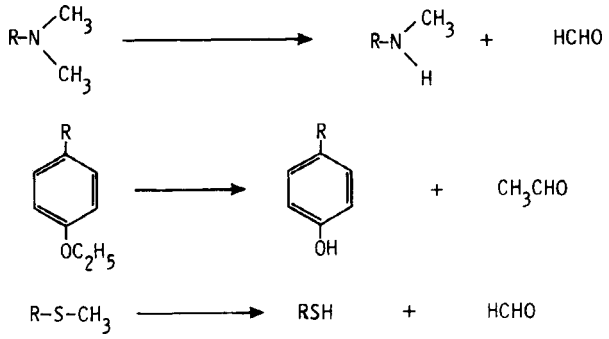


Figure 2.18. Dealkylation reactions.
From Timbrell, J.A., *Principles of Biochemical Toxicology*, Taylor & Francis, London, 1991.

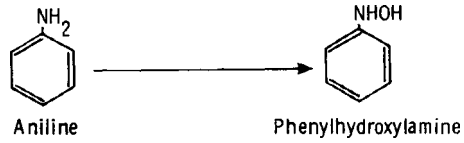


Figure 2.19. N-hydroxylation of an aromatic amino group.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

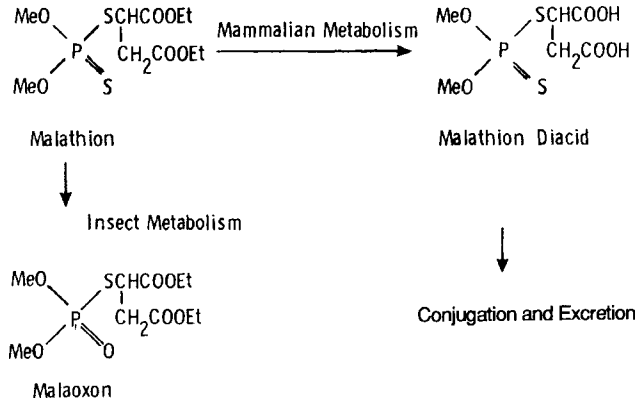


Figure 2.20. Metabolism of the insecticide malathion.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

Certain oxidation reactions are catalyzed by other enzymes such as alcohol dehydrogenase (Figure 2.22), xanthine oxidase, microsomal amine oxidase, monoamine and diamine oxidases.

Another important group of enzymes which catalyze oxidation reactions for foreign compounds are the peroxidases. For example, the toxic solvent benzene, which causes aplastic anaemia, is believed to be metabolized by peroxidases in the bone marrow. The drug hydralazine is also believed to be metabolized by this enzyme system (see Chapter 4).

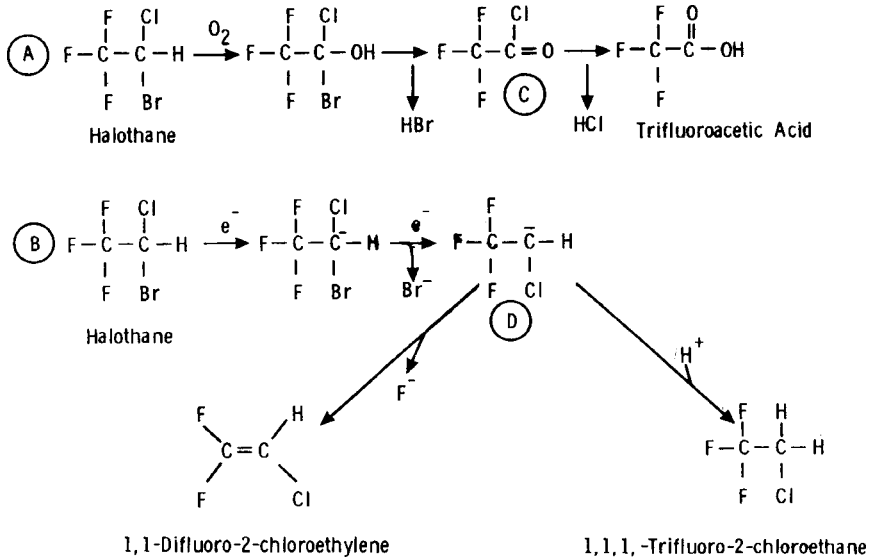


Figure 2.21. Metabolism of the anaesthetic halothane. A is the oxidative pathway, B the reductive pathway. C, trifluoroacetyl chloride is postulated as the intermediate which acylates membrane proteins. D is also a reactive intermediate and may also be involved in reactions with cellular macromolecules and lipid per oxidation.
Adapted from Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

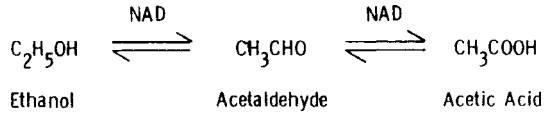


Figure 2.22. Oxidation of the primary alcohol ethanol.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

Reduction reactions

These reactions may be catalyzed by either microsomal or cytosolic reductases and by the gut bacteria, which also possess reductases. The most commonly encountered type of reductive reaction is the reduction of nitro and azo groups such as those present in the food colour tartrazine (Figure 2.23). Less common reduction reactions include reduction of aldehyde and keto groups, epoxides and double bonds.

Reductive dehalogenation, catalyzed by the microsomal enzyme system is an important route of metabolism for anaesthetics such as halothane (Figure 2.21) (see Chapter 4).

Reductive dechlorination is involved in the toxicity of carbon tetrachloride.

Hydrolysis

Esters and amides are hydrolyzed by esterases and amidases respectively, and

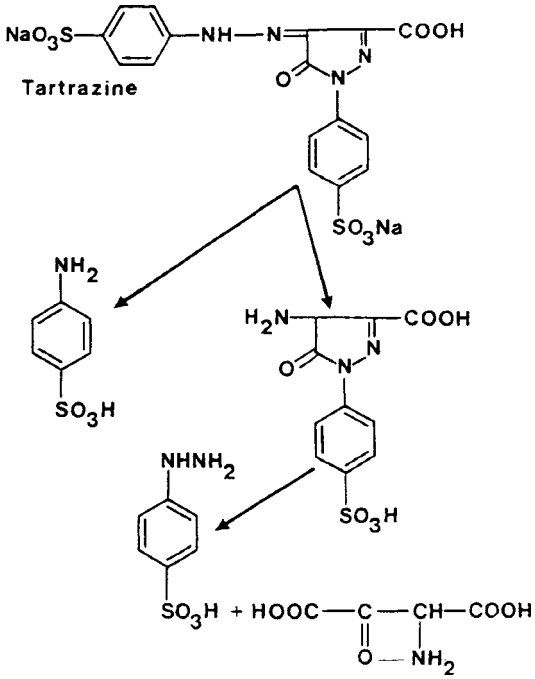


Figure 2.23. Metabolic reduction of the food-colouring agent tartrazine.

there are a number of these enzymes, which are usually found in the cytosol of cells in a variety of tissues. Some are also found in the plasma. Microsomal esterases have also been described. Typical esterase and amidase reactions are shown in Figure 2.24. An example of esterase action which is toxicologically important is that of the hydrolysis of the drug succinyl choline. The very short duration of action of this compound is due to it being very rapidly hydrolyzed in the plasma (see Chapter 4). Amidases have an important role in the toxicity of the drugs isoniazid and phenacetin, where hydrolysis is an important step in the metabolic activation.

Hydration

Epoxides, which can be stable metabolic intermediates, may undergo hydration catalyzed by the enzyme epoxide hydrolase located in the microsomal fraction. This is usually a detoxication reaction as the dihydrodiol products are normally much less chemically reactive than the epoxide.

Phase 2 Reactions

These reactions, also known as conjugation reactions, involve the addition of a polar group to the foreign molecule. This polar group is either conjugated to an existing group or to one added in a phase 1 reaction, such as a hydroxyl

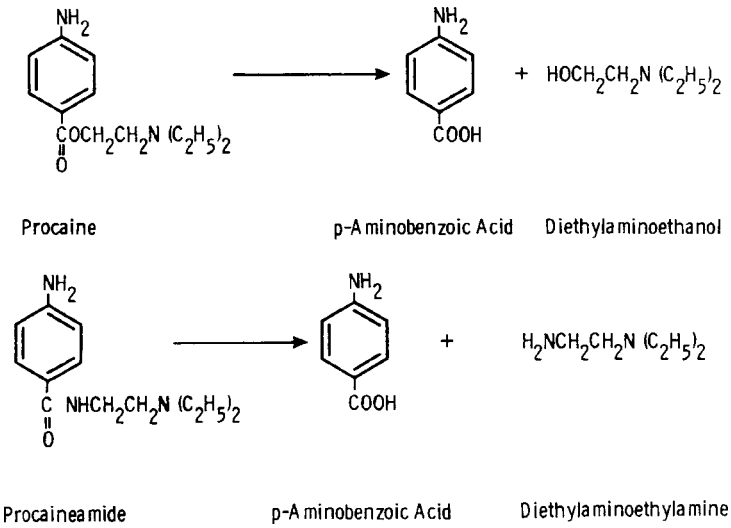


Figure 2.24. Hydrolysis of an ester (the drug procaine) and an amide (the drug procainamide). From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

group. The polar group renders the foreign molecule more water soluble and so more readily cleared from the body and less likely to exert a toxic effect. The groups donated in phase 2 reactions are commonly those involved in intermediary metabolism. Conjugation reactions are considered below:

Sulphation

The addition of the sulphate moiety to a hydroxyl group is a major route of conjugation for foreign compounds. It is catalyzed by a cytosolic sulphotransferase enzyme and utilizes the coenzyme phosphoadenosine phosphosulphate. The product is an ester which is very polar and water soluble. Both aromatic and aliphatic hydroxyl groups may be conjugated with sulphate as may N-hydroxy groups and amino groups (Figure 2.25).

Glucuronidation

Glucuronic acid is a polar and water soluble carbohydrate molecule which may be added to hydroxyl groups, carboxylic acid groups, amino groups and thiols (Figure 2.26). This process is a major route of phase 2 metabolism and utilizes glucuronosyl transferases, which are microsomal enzymes, with uridine diphosphate glucuronic acid as the cofactor. Other carbohydrates may also be involved in conjugation such as glucose, which is utilized by insects to form glucosides. Ribose and xylose may also be used in conjugation reactions.

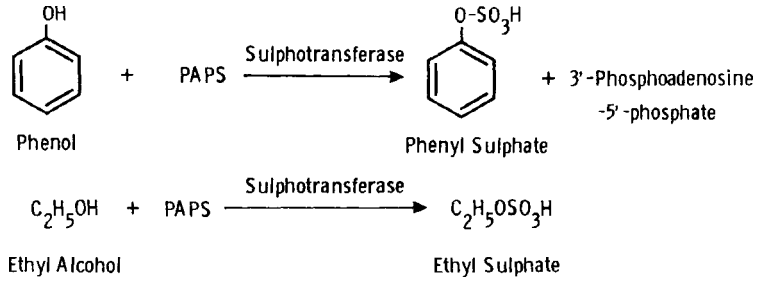


Figure 2.25. Conjugation of a phenol and an aliphatic alcohol with sulphate. PAPS is the sulphate donor, phosphoadenosinephosphosulphate.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

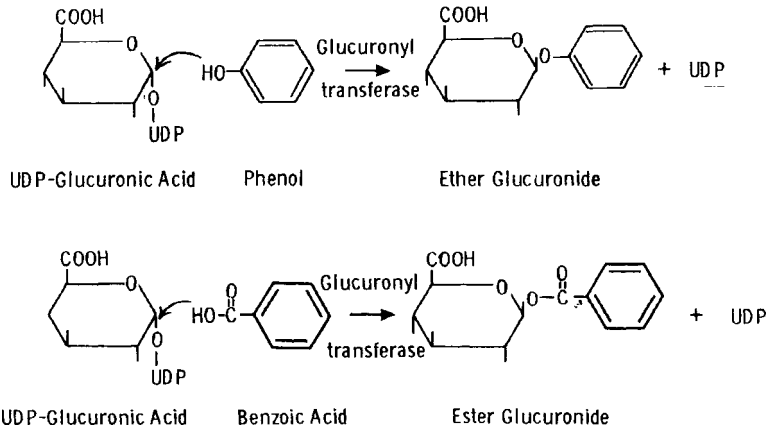


Figure 2.26. Conjugation of a phenol and a carboxylic acid with glucuronic acid.
From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

Glutathione conjugation

This is a particularly important route of phase 2 metabolism from the toxicological point of view as it is often involved in the removal of reactive intermediates. Glutathione is a tripeptide found in many mammalian tissues, but especially in the liver. It has a major protective role in the body as it is a scavenger for reactive compounds of various types, combining at the reactive centre in the molecule and so reducing or abolishing the toxicity. Normally, the sulphydryl group of glutathione acts as a nucleophile and either displaces another atom or attacks an electrophilic site (Figure 2.27). Consequently glutathione may react either chemically or in enzyme-catalyzed reactions with a variety of compounds which are either reactive or are electrophilic metabolites produced in phase I reactions. The reactions may be catalyzed by one of a group of glutathione transferases located in the soluble fraction of the cell. They have been detected also in the microsomal fraction. The substrates include aromatic, heterocyclic, alicyclic and aliphatic epoxides, aromatic halogen and nitro compounds and unsaturated aliphatic compounds. The

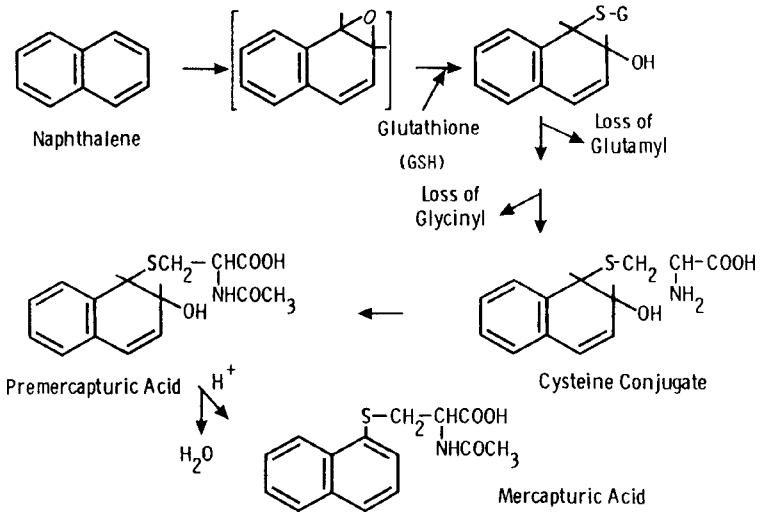


Figure 2.27. Metabolism of napthalene showing the conjugation of naphthalene epoxide with glutathione and the subsequent formation of a N-acetylcysteine conjugate (mercapturic acid). From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

conjugate which results may be either excreted into the bile unchanged or metabolized further, via so-called phase 3 reactions, to yield an N-acetylcysteine conjugate or mercapturic acid (Figure 2.27).

Acetylation

This metabolic reaction is unusual in that the product may be less water soluble than the parent compound. Substrates for acetylation are aromatic amino compounds, sulphonamides, hydrazines and hydrazides (Figure 2.28).

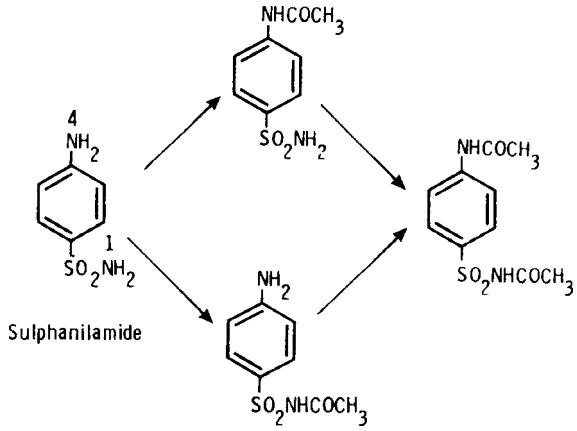


Figure 2.28. The acetylation of the amino and sulphonamido groups of the drug sulphanilamide. From Timbrell, J.A., *Principles of Biochemical Toxicology,* Taylor & Francis, London, 1991.

The enzymes involved are acetyltransferases and are found in the cytosol of cells in the liver, gastric mucosa and white blood cells. The enzymes utilize acetyl Coenzyme A as cofactor. There are two isoenzymes in the rabbit which differ markedly in activity and the same is probably true in humans. In both species the possession of a particular isoenzyme is genetically determined and gives rise to two distinct phenotypes known as 'rapid' and 'slow' acetylators. This has an important role in the toxicity of certain drugs such as hydralazine (see Chapter 4), isoniazid and procainamide, and these examples illustrate the importance of genetic factors in toxicology.

Amino acid conjugation

Foreign organic acids may undergo conjugation with amino acids (as well as with glucuronic acid, see above). The particular amino acid utilized depends on the species concerned and, indeed, species within a similar evolutionary group tend to utilize the same amino acid. Glycine is the most common amino acid used. The carboxylic acid group first reacts with Coenzyme A and then with the particular amino acid. The acylase enzyme catalyzing the reaction is found in the mitochondria.

Methylation

Hydroxyl, amino and thiol groups in molecules may be methylated by one of a series of methyltransferases. This occurs particularly with endogenous compounds but xenobiotics may also be substrates. As with acetylation this reaction tends to decrease rather than increase water solubility.

An important toxicological example is the methylation of heavy metals such as mercury. This may be carried out by micro-organisms in the environment (see Chapter 8). The importance is that this changes the physico-chemical characteristics of mercury from a water-soluble inorganic ion, to a lipidsoluble organic compound. There is also a corresponding change in the toxicity of mercury with mercuric ion being toxic to the kidney in contrast to organomercury which is toxic to the nervous system.

There are other reactions that a foreign molecule may undergo but the interested reader should consult one of the texts or reviews given in the bibliography. One important point to remember, however, is that although a molecule is foreign to a living organism, it may still be a substrate for an enzyme involved in normal metabolic pathways, provided its chemical structure is appropriate, and so this widens the scope of potential metabolic reactions. Foreign compounds can be metabolized by a number of different enzymes simultaneously in the same animal and so there may be many different metabolic routes and metabolites. The balance between these routes can often determine the toxicity of the compound.

Toxication Versus Detoxication

The metabolism of foreign compounds has been termed detoxication because

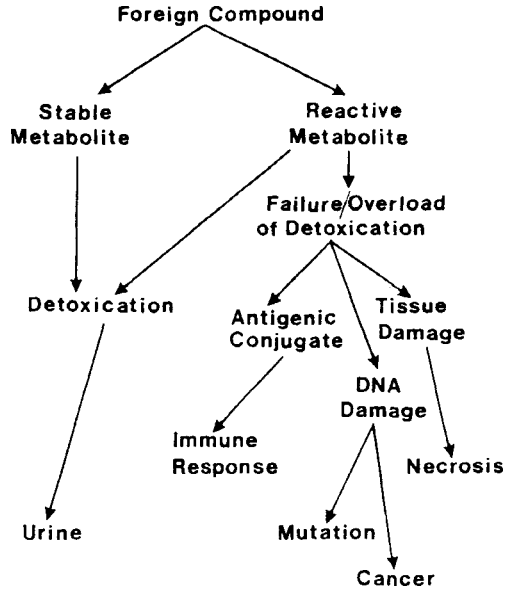


Figure 2.29. An illustration of the ways in which the metabolism of a compound may have a variety of consequences for the organism.

in general it converts these compounds into more water-soluble, readily excreted substances and decreases the toxicity. However, in some cases the reverse occurs and a metabolite is produced which is more toxic than the parent compound. A prime example of this is the drug paracetamol (acetaminophen) which is discussed in more detail in Chapter 4. However, in this case there are several pathways of metabolism that compete. Consequently, factors that alter the balance between these pathways will alter the eventual toxicity. This balance between toxication and detoxication pathways (Figure 2.29) is very important in toxicology and underlies some of the factors that affect toxicity. These will be discussed later in this chapter.

Factors Affecting Toxic Responses

As already indicated, metabolism is a major factor in determining the toxicity of a compound. Factors that affect the disposition will consequently affect toxicity. There are many such factors, which may be either chemical or biological. Chemical factors include the physico-chemical characteristics (pK_a, lipophilicity, size, shape) and chirality (various types of isomers). Biological factors are more numerous and include species, genetics, diet, age, sex, pathological state. Many of these factors affect metabolism and so may influence the toxicity of the compound. For example, different isomers may be metabolized differently and hence show different biological activity. In humans, genetic differences may affect metabolism and consequently toxicity.

Different species will have different metabolic capabilities and, therefore, may be more or less susceptible to the toxic effects of some compounds. Dietary constituents may influence metabolic pathways or rate of metabolism and, therefore, whether or not a compound is toxic. However, many of these factors will be discussed and highlighted in examples in later chapters and so will not be discussed in detail here.

Species

Species often vary widely in their responses to toxic compounds and this may be extremely important in relation to veterinary medicine, human medicine and environmental toxicology. For example, drugs are tested in animals for eventual use in man. If the response in the human animal is very different from that in rats or mice problems may arise when the drug undergoes clinical trials.

Similarly, veterinary products may be used on a variety of species and if there are big differences in toxicity this may lead to fatalities or pathological damage in farm animals or pets. In the environment very large numbers of widely different species may all be exposed to a pesticide and may react very differently. Indeed this difference in sensitivity is exploited in pesticides. Insecticides, such as organophosphorus compounds and DDT (see Chapter 7), are much more toxic to insects than to humans and other mammals; in some cases this is due to metabolic differences. For example, the insecticide malathion is metabolized by hydrolysis in mammals but is oxidized in the insect to malaoxon which then binds to and inhibits the enzyme cholinesterase (Figures 2.20 and 7.4; see also Chapter 7).

There are very many species differences in metabolism and it is beyond the scope of this book to discuss them in detail. The interested reader is recommended to consult the bibliography at the end of this chapter.

Strain of Animal

Just as different species may vary in their response to toxic compounds and in the way they metabolize them, different inbred strains of the same animal may also show variation. For example, different strains of mice vary widely in their ability to metabolize barbiturates and consequently the magnitude of the pharmacological effect varies between the strains (Table 2.4).

Sex

Males and females can also differ in their responses due to metabolic and hormonal differences. Males in some species metabolize compounds more rapidly than females, although this difference is not found in all species. As well as metabolic differences there are examples of sex differences in routes of excretion which underlie differences in susceptibility. For example, dinitrotoluene-induced hepatic tumours occur predominantly in males due to the differences in the route of excretion. Biliary excretion of a glucuronide conjugate is favoured in males while urinary excretion predominates in females.

Table 2.4. Strain differences in the duration of action of hexobarbital in mice.

Strain	Sleeping time
A/NL	48 ± 4
BALB/cAnN	41 ± 2
C57L/HeN	33 ± 3
C3HfB/HeN	22 ± 3
SWR/HeN	18 ± 4
Swiss (non-inbred)	43 ± 15

Source: G.E.Jay (1955), *Proceedings of the Society of Experimental Biology and Medicine,* **90,** 378

The glucuronide conjugate is broken down in the intestine by gut bacteria and the products are reabsorbed, causing the hepatic tumours. The difference in susceptibility to chloroform-induced kidney damage between male and female mice is an example of a sex difference which has a metabolic basis and hormonal basis. The male mice are more susceptible but this difference can be removed by castration and restored by androgens. It may be that testosterone is influencing the microsomal enzyme-mediated metabolism of chloroform to give greater metabolism in males.

Genetic Factors and Human Variability in Response

Genetic variation is particularly important in the human population which is genetically mixed. There are now many examples of toxic drug reactions which occur in individuals due to a genetic defect or genetic difference in metabolism. The best known example in man is that of the acetylator phenotype where the acetylation reaction (see page 46) shows genetic variations which are due to mutations giving rise to mutant alleles. This results in rapid and slow acetylators where the latter have less functional acetyltransferase enzyme. This is an important factor in a number of adverse drug reactions including the hydralazine-induced lupus syndrome discussed in Chapter 3, procainamide-induced lupus syndrome, isoniazid-induced liver damage and isoniazid-induced peripheral neuropathy.

Another important genetic factor in metabolism is that shown in the hydroxylation of debrisoquine, the details of which are discussed in Chapter 4. This variation in oxidation has now been shown for a number of other drugs such as phenytoin, sparteine and phenformin. In some cases, toxic reactions are associated with the 'poor metabolizer' status (see Chapter 4).

Toxic responses to foreign chemicals may show large variation between human subjects and some of this variation can be ascribed to the factors mentioned. As well as genetically determined metabolic differences, there may be genetic differences in a receptor or in an immunological parameter giving rise to variation in toxicological and pharmacological responses to drugs and other foreign compounds. Several examples will be discussed later in this book. In some cases, however, rare idiosyncratic reactions of unknown origin

may occur and in other cases a combination of factors may be necessary for a toxic reaction to occur (see Chapter 4; hydralazine). Unfortunately, much of the variability seen in humans is not encountered in inbred experimental animals and consequently rare but severe and life-threatening toxic reactions may not be encountered in toxicity studies in animals and may only become known after very large numbers of humans have been exposed to the particular chemical.

Environmental Factors

Another factor which affects the human population is the environment, in particular the other chemical substances to which people are exposed. Thus, chemicals in the diet, air or water may all influence the toxic response to another chemical. Unlike experimental animals, humans may be under medication with several drugs when exposure to an industrial chemical occurs for instance. These drugs can influence the way in which the body reacts to the chemical. The intake of one drug may affect the response to another. For example, overdoses of paracetamol are more likely to cause serious liver damage if the victim is also exposed to large amounts of alcohol or barbiturate, both of which induce drug metabolizing enzymes and thereby increase the *in vivo* activity.

Compounds which inhibit metabolic pathways by blocking particular enzymes may also be factors in toxic responses. For example, workers exposed to the solvent dimethylformamide seem more likely to suffer alcohol-induced flushes than those not exposed, possibly due to the inhibition of alcohol metabolism. The diet contains many substances which may influence the enzymes of drug metabolism such as the microsomal enzyme inducer ß-naphthoflavone found in certain vegetables. Cigarette smoking and alcohol intake also are known to affect drug metabolism and pharmacological and toxicological responses.

Pathological State

The influence of disease states on metabolism and toxicity has not been well explored. Diseases of the liver will clearly affect metabolism but different liver diseases can influence metabolism differently. Disease states such as influenza are also known to affect drug metabolizing enzymes, possibly via the production of interferon.

Questions

1. Describe the processes involved in the absorption of chemicals from various named sites of exposure in a mammalian system. Indicate what factors may affect these processes of absorption.

2. Write notes on three of the following:

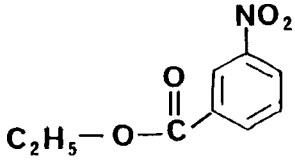
   (a) volume of distribution;
   (b) first pass effect;
   (c) binding of chemicals to plasma proteins;
   (d) Picks Law of diffusion.

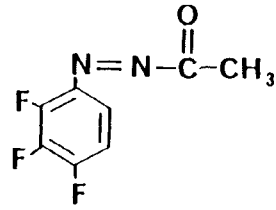
3. Discuss why a knowledge of the plasma level of a foreign compound is important for a toxicologist. What factors affect the plasma level and what information may be gained from it?

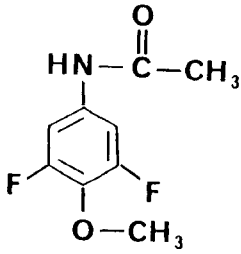
4. Using examples of substrates to illustrate your answers write short notes on three of the following:

   (a) cytochrome P450;
   (b) acetylation;
   (c) glutathione conjugation;
   (d) reduction;
   (e) alcohol dehydrogenase.

5. The compounds shown are hypothetical drugs. Indicate diagrammatically ways in which you think they might be metabolized by mammals. Name the enzyme(s) which catalyze the biotransformations you describe.







6. Describe the routes and mechanisms involved in the excretion of drugs from the mammalian body. What factors may affect excretion and how might they be toxicologically important?
7. Discuss the role of the physico-chemical properties of chemicals in their disposition.

Bibliography

Ballantyne, B., Marrs, T. and Turner, P. (Eds) (1993) various chapters in Part 1, vol. 1, *General and Applied Toxicology,* Basingstoke: Macmillan.

Bruin, A. DE (1976) *Biochemical Toxicology of Environmental Agents,* Amsterdam: Elsevier.

Caldwell, J. and Jakoby, W.B. (Eds) (1983) *Biological Basis of Detoxication,* New York: Academic Press.

Cassarett and Doull's Toxicology, Amdur, M.O., Doull, J. and Klaassen, C. (Eds) (1991) 4th edition, New York: Pergamon Press.

Clark, B. and Smith, D.A. (1986) *An Introduction to Pharmacokinetics,* 2nd edition, Oxford: Blackwell.

Gibson, G.G. and Skett, P. (1993) *Introduction to Drug Metabolism,* 2nd edition, London: Chapman and Hall.

Hathway, D.E. (1984) *Molecular Aspects of Toxicology,* London: The Royal Society of Chemistry.

Hawkins, D.R. (Ed.) (1988–1993) *Biotransformations,* vols. 1–5, London: The Royal Society of Chemistry.

Hodgson, E. and Levi, P.E. (1987) *A Testbook of Modern Toxicology,* New York: Elsevier.

Hodgson, E. and Levi, P.E. (Eds) (1994) *Introduction to Biochemical Toxicology,* Norwalk, Connecticut: Appleton & Lange.

Hodgson, E., Bend, J. and Philpot, R.M. (Eds) (1979–•) *Reviews in Biochemical Toxicology,* New York: Elsevier/North Holland.

Jakoby, W.R. (Ed.) (1980) *Enzymic Basis of Detoxification,* New York: Academic Press.

Jakoby, W.R., Bend, J.R. and Caldwell, J. (Eds) (1982) *Metabolic Basis of Detoxification,* New York: Academic Press.

Pratt, W.B. and Taylor, P. (Eds) (1990) *Principles of Drug Action: The Basis of Pharmacology,* 3rd edition, New York: Churchill Livingstone.

Timbrell, J.A. (1991) *Principles of Biochemical Toxicology,* 2nd edition, London: Taylor & Francis.

Williams, R.T. (1959) *Detoxication Mechanisms,* London: Chapman & Hall.

Zbinden, G. (1988) Biopharmaceutical studies, a key to better toxicology, *Xenobiotica,* **18,** suppl. 1, 9.