CHAPTER 4

Types of exposure and response

Chapter outline

This chapter will consider exposure of biological systems to chemicals and the pathological consequences of that exposure.

- Acute and chronic exposure. Routes of exposure
- Types of toxic response
  - Direct toxic action – the liver as a target organ; mechanisms of toxicity
  - biochemical lesions
  - pharmacological and physiological effects
  - immunotoxicity
  - teratogenicity
  - genetic toxicity
  - carcinogenicity
- Biomarkers

Types of exposure

There are two basic exposure conditions for toxic compounds: **acute** and **chronic** exposure. Acute exposure applies to a single episode where a particular amount of a substance such as with an overdose of a drug, enters the organism. Chronic exposure applies to repeated exposure to a substance which may then accumulate or cause a cumulative toxic effect.

Acute toxicity usually applies to a toxic event which occurs soon after acute or limited exposure; chronic toxicity may apply to an event which occurs many weeks, months or years after exposure to either repeated doses or pos-

sibly after an acute exposure to a particular toxic substance.

Route of exposure

Routes of exposure have already been discussed in Chapter 2 and so only brief mention will be made here. Exposure via the **gastrointestinal tract** is the most important route for most drugs, food additives and contaminants, natural products, and other potentially toxic substances. **Inhalation** is particularly important in an industrial environment both inside and outside the factory and pesticides may also be taken in this way during spraying. Absorption via the **skin** is also important in an industrial and agricultural setting.

The site and route of absorption is important from two points of view:

1 the **route** may influence the eventual systemic toxicity as already indicated in Chapter 2;

2 the **site** may be important if there is local toxicity at the point of absorption.

For example, substances which are irritant may cause inflammation at the site of absorption and this may depend on the conditions at this site. Particles such as **asbestos** will cause damage to the cells of the lung by being taken up into them, but will not particularly damage the skin. The skin will tend to be more resistant because of the outer layer of keratinized cells and its poor absorptive properties.

Drugs may also gain access to the body by routes other than those mentioned; in particular, intravenous and intramuscular **injection** are employed in human medicine while intraperitoneal and subcutaneous administration are commonly used in experimental animals. Intravenous and intraperitoneal injection lead to *rapid distribution* to most parts of the body whereas subcutaneous and intramuscular injection usually lead to *slow absorption*.

Types of toxic response

A biological system can respond in many different ways to a toxic compound and death of the cell or whole organism is only one response. Furthermore there may be many different causes of cell death. Although specific examples will be considered in more detail in later chapters, here we will consider the types of response in general. Many toxic responses will have a biochemical basis yet the expression of those responses can be very different. For example, the biochemical interaction between a toxic compound and a nucleic acid may lead to a tumour but in a developing embryo a birth defect could be the result. Alternatively, an interaction with a receptor could cause a major physiological effect such as loss of blood pressure or by inhibiting an enzyme might cause a sufficient biochemical perturbation to lead to tissue damage and necrosis. **Toxic responses** can be divided up into six categories on the basis of the end result:

i **direct toxic action: tissue lesions**

ii **biochemical lesions**

iii **pharmacological or physiological effects**

iv **immunotoxicity**

v **teratogenicity**

vi **genetic toxicity**

vii **carcinogenicity**.

There may be overlap between these types of toxic response and some chemicals may cause more than one type of effect.

For example in many cases biochemical effects will underlie the toxicity and can lead to tissue damage or some other pathological lesion (see paracetamol, Chapter 5 and snake venoms, Chapter 10). However some biochemical effects of drugs or chemicals do not lead to detectable pathological lesions but rather morbidity or death of the organism may occur through biochemical or physiological dysfunction (see salicylate, Chapter 5).

TARGET ORGAN TOXICITY

The **liver** is a common target organ for the effects of toxic compounds (especially direct toxic effects, see below) and serves to illustrate both the reasons that organs are targeted and the mechanisms underlying different types of toxic effect.

The **liver** is a **target** for toxic substances because of:

a its position in the body in relation to its blood supply (Figure 4.1)

b its structure

c its role in intermediary and xenobiotic metabolism

d its function.

Most toxic substances are ingested by mouth and following absorption from the gastrointestinal tract the blood supply is so arranged that the substance is transported straight to the liver via the portal vein (Figure 4.1). Hence the liver is the first organ exposed to the substance (after the gastrointestinal tract itself). The liver receives 25 per cent of the blood from the heart. Once in the liver the chemical will be taken up into the liver cells (hepatocytes) either actively or by passive diffusion depending on the chemical structure.

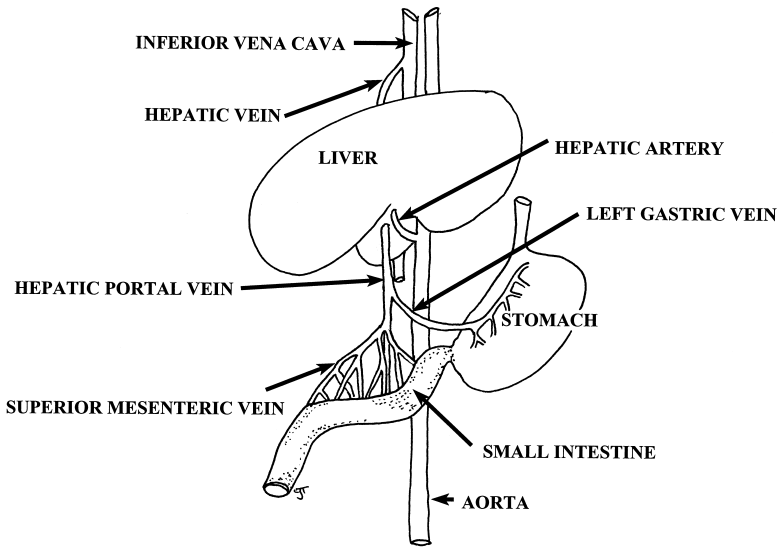

FIGURE 4.1 *The vasculature supplying and draining the liver and its relationship to the systemic circulation. From Timbrell, J. A., Biotransformation of Xenobiotics. From* General and Applied Toxicology, *2nd Edition, edited by Ballantyne, Marrs and Syversen, Stockton Press, USA. Drawing by C. J. Waterfield.*

The **hepatocytes**, which make up the majority of the liver structure, are metabolically very active. They carry out a variety of biochemical reactions essential to the functioning of the whole organism such as protein synthesis, removal of excess nitrogen (ammonia detoxication as urea) and lipid metabolism. Interference with such essential intermediary metabolic activity by exogenous chemicals can result in toxicity. Thus many chemicals that are toxic to the liver inhibit protein synthesis. Two chemicals that cause liver toxicity by interfering with intermediary metabolism are **galactosamine**, which interferes with uridine nucleoside synthesis, and **ethionine**, which blocks the recycling of adenosine in the methionine cycle. Many hepatotoxic chemicals reduce lipid transport out of the liver as a result of inhibition of protein synthesis. For example, the solvent **carbon tetrachloride** causes the accumulation of fat in the liver (**fatty liver** or **steatosis**) via this mechanism.

Hepatocytes are also very active in the metabolism of exogenous (xenobiotic) chemicals and this is another reason why the liver is a target. Many chemicals are metabolized and in the process reactive intermediates may be produced. As these are produced within the liver cell this is the first target. An example of a chemical that is toxic to the liver as a result of this type of mechanism is **carbon tetrachloride** which is metabolized to a reactive free radical that damages the endoplasmic reticulum, and hence disrupts protein synthesis (see above). Other examples are paracetamol (see Chapter 5) and vinyl chloride (see Chapter 6).

The final reason why the liver is a target organ for toxicity is because it also has an excretory function, producing **bile**, which incorporates and transports waste products. Therefore xenobiotics or their metabolites may be excreted by this route. As this is often an active process, the concentration reached in the bile itself may be quite high. This can lead to direct damage to the bile duct. Alternatively, high doses of a chemical normally excreted into the bile may saturate the excretory processes leading to accumulation and high concentrations in the hepatocyte. An example of a compound that causes toxicity by this route is the diuretic drug **furosemide** that causes dose-dependent liver necrosis in animals as a result of **accumulation** in the liver. Other common target organs for the toxic effects of chemicals are the **kidney** and the **lungs**. Like the liver, the kidney also has a relatively high metabolic activity and blood flow and is an excretory organ. Compounds that cause kidney damage are those which are concentrated there such as **cadmium** (see below, Chapter 6) and the drug **gentamycin**. The industrial chemical **hexachlorobutadiene** undergoes further metabolism in the kidney and the reactive metabolite produced damages the mitochondria in proximal tubular cells.

The lungs are targets for chemicals particularly as a result of direct exposure, being the organs of absorption for volatile and particulate substances such as asbestos (see below, Chapter 6). However, they also have a particularly high blood flow and significant metabolic activity. The lungs may be a target as a result of high oxygen concentrations or particular uptake mechanisms such as occurs with paraquat (see below, Chapter 8). In all three organs the cells comprising them are readily exposed to chemicals and able to take up the substances. This is not the case with some organs such as the brain which is organized to exclude many substances, having a '**blood–brain barrier**'.

The basic mechanisms underlying toxic responses are similar in all organs and can be divided into primary, secondary and tertiary. **Primary events** are those occurring at the molecular level such as **covalent binding** to crucial macromolecules or **lipid peroxidation**. These may cause enzyme inhibition or depletion of **thiols** (e.g. glutathione) for example.

Secondary events resulting from this are damage to macromolecules such as DNA or changes in the structure or function of organelles such as the mitochondrion or endoplasmic reticulum. These underlie the **tertiary events** such as blebbing, necrosis, apoptosis or steatosis.

Several of the types of toxic response that a tissue can undergo and the mechanisms underlying them can again be illustrated by the liver, with the obvious exception of teratogenesis because this specifically relates to the developing organism (embryo or foetus). Thus, the liver can undergo irreversible destruction of its cells (see Chapter 5, paracetamol), reversible biochemical disturbances such as fatty liver, immune-mediated damage (see Chapter 5, halothane) or develop cancer (see Chapter 6, vinyl chloride).

DIRECT TOXIC ACTION: TISSUE LESIONS

Direct toxicity to tissues, results in tissue damage often manifested as necrosis. This is a process in which the cells are destroyed, the surrounding tissue is often affected and an inflammatory response occurs which can be observed by microscopy. **Necrosis** is an irreversible process during which the cell degenerates, the nucleus may become fragmented and proteins denature. The cells swell, accumulating fluid, lyse and the contents leak out. The underlying mechanism may involve derangement of a biochemical pathway or the production of a reactive intermediate which interacts directly with cellular components such as enzymes or structural proteins or may have an immunological basis. Highly reactive compounds may also react with cell membranes and cause instant cell death by damaging the membrane sufficiently to allow rapid loss of contents and influx of external ions and other substances. Some toxic compounds interfere directly with vital cellular functions such as respiration, which usually leads to rapid cell death. Not all toxic compounds act in this way, however, and some cause cell death to occur more slowly (see lead, Chapter 9). However, the intermediate stages between the interaction of the toxicant or its metabolite with cellular constituents and destruction of the cell are often not clearly understood.

An alternative mode of cell death is **apoptosis**, also known as programmed cell death. This is part of normal tissue turnover and renewal but may also be stimulated by toxic chemicals. One apparent function of apoptosis is to remove damaged DNA which cannot be repaired and this may be one of the triggers that stimulates the process. Severe damage to the cell can compromise the apoptic process and then necrosis may follow. Apoptosis is a process which involves the production of specific proteins resulting from expression of particular genes (e.g. *Fos, myc, max* and *jun*.) The process involves condensation of chromatin and cytoplasmic components. DNA is broken up into small fragments and cell division is blocked. The end result is that the cell contracts away from its neighbours and is removed by phagocytosis. Consequently, there is no inflammatory response.

Skin disease is the most common injury associated with industrial chemicals (see Chapter 6) and most chemical-induced skin reactions are probably associated with direct toxicity leading to irritation. After a single insult to the epidermis the primary response is a local inflammatory reaction. Acute inflammation is the immediate response to irritant chemicals and it is characterized by dilation of blood vessels, increased blood flow, accumulation of fluid in the tissues and invasion of white blood cells. These changes give rise to redness, heat, pain and swelling.

Corrosive chemicals such as sodium hydroxide, cause destruction of tissues (see Chapter 11).

BIOCHEMICAL LESIONS

Biochemical lesions may lead to the development of pathological change such as cell degeneration but they may also simply cause death of the whole organism by interfering with some vital function such as respiration. For example, **cyanide** causes death of cells by interfering with the electron transport chain in the mitochondria such that oxygen cannot be utilized, leading to the death of cells in vital organs such as the heart and brain so that the whole organism dies. Some biochemical effects are reversible, such as the binding of **carbon monoxide** to haemoglobin which may not be at a sufficiently high level to cause death of the organism. Carbon monoxide does not normally cause pathological damage, except at high levels of exposure from which the victim rarely recovers (see carbon monoxide, Chapter 11).

A common toxic response in the liver resulting from a disturbance of normal intermediary metabolism of lipid is **fatty liver** (see above). Other tissues such as the heart and kidney may also show this response to chemical exposure. A more specific type of derangement is **phospholipidosis** in which phospholipids accumulate and which may occur in several tissues, but particularly the lungs and adrenal glands. The drugs **chlorphentermine** and amiodarone may cause this. As well as such pathological changes which can be demonstrable by microscopy, biochemical lesions can cause physiological effects or death by organ failure. For example, **aspirin** overdose leads to biochemical and physiological derangements (ATP depletion, acidosis, hyperthermia) which can lead to death (see below, Chapter 5). **Fluoroacetate**, the natural product which is used as a rodenticide, blocks Krebs' cycle (see below, Chapter 8). This fairly rapidly causes death to animals that have ingested it, probably due to heart failure.

PHARMACOLOGICAL AND PHYSIOLOGICAL EFFECTS

Pharmacological and physiological responses are those where a particular bodily function is affected. For example, some compounds cause a change in blood pressure by affecting **β-adrenoceptors** or by causing vascular dilatation or constriction. These clearly are toxic reactions if extreme and directly life threatening or when they occur in workers occupationally exposed to the drug for example. Alternatively, a drop in blood pressure may be sufficient to initiate another response such as ischaemic tissue damage due to insufficient blood flow. (See **debrisoquine** and **succinyl choline**, Chapter 5 and **tetrodotoxin** and **botulinum toxin**, Chapter 10.)

IMMUNOTOXICITY

Toxic reactions involving the immune system may be manifested in a number of ways: as **hypersensitivity** or **allergic reactions** and **autoimmunity**, which are categorized as indirect immunotoxicity and **immunosuppression** and **immunostimulation** which are categorized as direct immunotoxicity. Hypersensitivity or allergic reactions may occur when the immune system is stimulated after individuals are exposed to chemicals which may bind to or alter a macromolecule, commonly a protein. The protein must be large enough and have sufficient hapten groups attached to be regarded as foreign by the immune system and so act as an **antigen**. Most commonly the chemical (known as a **hapten**) reacts with and becomes

attached to an endogenous macromolecule such as a protein which is then regarded as foreign. Allergic reactions may develop at the site of exposure such as the lungs or skin or may cause a systemic reaction. For example, **toluene diisocyanate**, an industrial chemical, will cause allergic type reactions as a result of exposure of the lungs. Immune reactions take one of several forms, including stimulation of a physiological response such as bronchoconstriction, or cellular destruction by complement. The hepatotoxicity of **halothane** and the adverse effects of **hydralazine** are examples of autoimmune reactions where a body constituent is attacked by the immune system (see Chapter 5). An immune-mediated reaction may underly rare, idiosyncratic, responses (see halothane, Chapter 5) or more common adverse effects of drugs (see hydralazine, Chapter 5). **Penicillin** is the drug most commonly associated with allergic reactions which may take a number of different forms ranging from severe and possibly fatal anaphylactic shock to skin rashes.

There are four different types of hypersensitivity reactions as shown in Figure 4.2, but these will not be discussed in detail. **Type I reactions (anaphylaxis)** may be elicited by chemicals such as **penicillin** and **toluene diisocyanate**. Sensitization occurs after initial exposure and then subsequent exposures cause the anaphylactic reaction resulting in

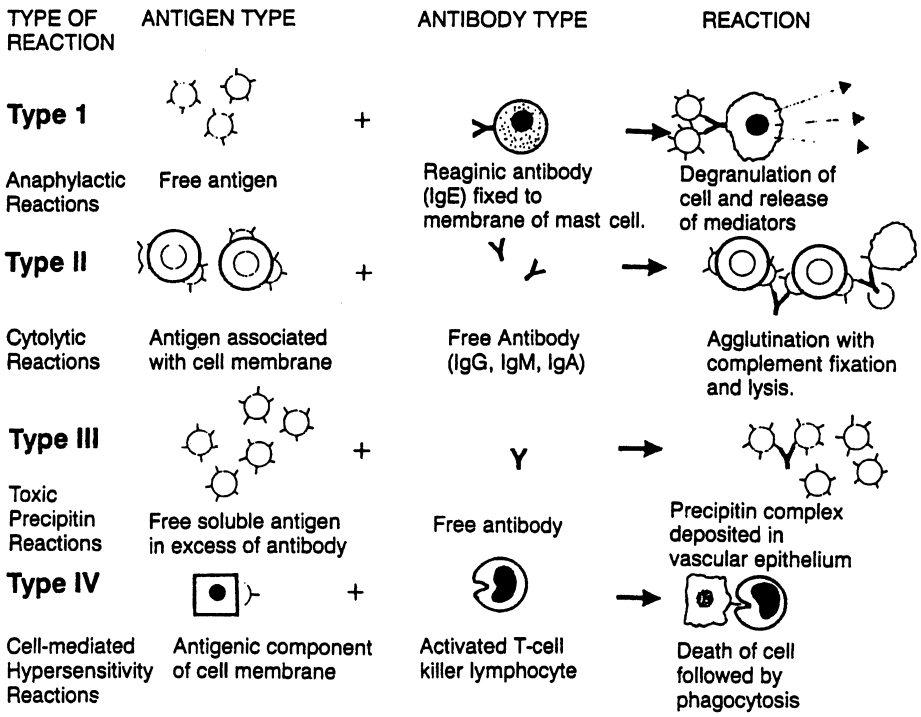

FIGURE 4.2 *Mechanisms for the stimulation of an immune response. The antigen is usually a foreign macromolecule such as a protein or an altered cell membrane as in Type IV reactions. Most foreign compounds are of low molecular weight and are not directly antigenic. They may act as haptens and so cause immune reactions by reacting with and thereby altering endogenous proteins or cell membrane components.*
Adapted from Bowman, W. C. and Rand, M. J., Textbook of Pharmacology, *2nd edition, Blackwells Scientific Publishers, Oxford.*

bronchoconstriction and asthma, for example. **Type II** reactions involve antigens bound to blood cells and so may result in loss of these cells by lysis or removal, such as may occur after administration of the drug **aminopyrine**. A **Type III** reaction may be responsible for the adverse effects of **hydralazine** (see below, Chapter 5). **Type IV** reactions may underlie contact dermatitis, which is a major industrial problem associated with exposure to **nickel** and **cadmium**.

Immunosuppression is the result of a direct effect on the immune system so that it does not function properly. This may be the result of damage to a component of the system such as the thymus, which produces B lymphocytes, or the bone marrow, which is responsible for the production of blood cells. The industrial and environmental contaminant **dioxin** (**TCDD**) is a powerful immunosuppressant causing damage to the thymus and hence reducing the production of lymphocytes.

Immunostimulation is where the immune system responds to an administered protein, which, although it may be similar to that found in a human, is recognized as an antigen. **Novel peptide drugs** such as those derived from recombinant DNA may elicit this type of immune response.

TERATOGENICITY

Teratogenicity is a very specific type of toxic response whereby the development of the embryo or foetus is affected. This may lead to a functional and/or structural abnormality of the foetus and the resulting animal. Although cytotoxic compounds may be teratogenic, in many cases the malformations are the result of a perturbation in the development of the organism rather than direct damage to the embryo or foetus, as this usually results in death and abortion.

Teratogens are often relatively non-toxic to the mother but interfere in some specific way with the development of a particular stage of the embryo. The timing of the exposure or dosing with a teratogen relative to the stages of pregnancy is therefore crucial (Figure 4.3; see thalidomide, Chapter 5).

Thus the embryo and foetus are especially sensitive to chemical exposure. The reason for this is that the sequence of events in embryogenesis and foetal development is easily disturbed. Consequently, the timing of the exposure is crucial. Therefore there are several characteristics of teratogenesis:

1 teratogens are generally **selective** for the developing organism rather than the maternal organism

2 the **susceptibility** of the **embryo** or **foetus** will vary depending on the stage in relation to exposure

3 the abnormalities observed will often be **specific** to the **stages** of exposure rather than the chemical

4 the **dose response** is often **steep** partly because of the mediation of the maternal organism.

In general terms the outcomes of exposure of a developing organism are limited to four:

1 **death** and **abortion**

2 **malformations**

3 **growth retardation**

4 **functional disorders**.

There are many possible mechanisms underlying teratogenesis and a small interference in cellular function may be all that is needed. Occasionally teratogenic effects may develop later in the life of the offspring rather than

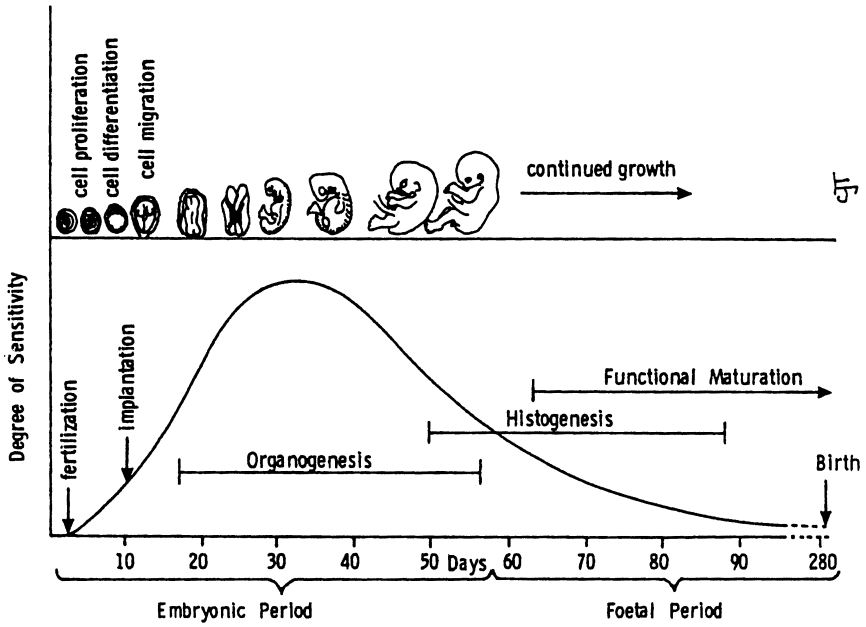

FIGURE 4.3 *The stages of mammalian embryogenesis indicating the periods of greatest susceptibility to teratogens. From Timbrell, J. A.,* Principles of Biochemical Toxicology, *Taylor & Francis, London, 2000.*

during the gestation period. A particularly distressing example of this is the case of **diethylstilboestrol**, a drug once given to pregnant women. The teratogenic effect eventually connected to use of this drug was the development of vaginal cancer in the female offspring of the women exposed to the drug. However, this did not occur until the girls were entering puberty, maybe 12 or more years after their birth and original exposure.

GENETIC TOXICITY

The mutation that may result from an interaction between a chemical and the genetic material is a heritable change in the cell genotype, and thus the error may be transferred to the daughter cell or next generation.

The interactions of chemicals with genetic material can be divided into three types: **aneuploidization**, **clastogenesis** and **mutagenesis**. Aneuploidy is the loss or acquisition of a complete chromosome; clastogenesis is loss, addition or rearrangement of parts of chromosomes; mutagenesis is the loss, addition or alteration of a small number of base pairs.

Mutations may be characterized as **base-pair transformations** and **base-pair additions** or **deletions**. These refer to small changes in one of the four bases that make up DNA. Thus, in base-pair transformations one base is replaced by another. This may involve replacement of a base by another of the same type (i.e. purine or pyrimidine), in which case it is a base-pair transition. Alternatively, a purine base may be replaced by a pyrimidine, which is a base-pair transversion. This may result in the erroneous coding for an amino acid.

Base-pair deletions or additions involving the loss or addition of a base pair are more serious because the whole base-pair sequence is then altered and the reading of genetic code is shifted. This may result in a **frame shift mutation**.

Large deletions and rearrangements may follow breakage and erroneous reconstitution of the DNA molecule. Similarly, whole segments of the chromosome may become inverted (clastogenesis). These types of changes inevitably lead to major adverse effects in the cell because of the number of genes potentially affected.

Chemicals such as the naturally occurring **vinca alkaloids** may interfere with the process of mitosis or meiosis and, for example, disturb the separation of chromosomes during mitosis (aneuploidization) leading to non-disjunction or unequal partition. This may be the result of interference with spindle formation or some other aspect of the process of cell division. The resulting cell may not be viable, however, leading to cell death and tissue damage.

There are many ways in which a compound may cause a mutation and consequently many different types of foreign compound have been found to be mutagenic. Thus, chemically reactive compounds, such as **alkylating agents**, may *react* directly with the DNA in the cell nucleus, or a compound, such as **bromouracil**, may be *incorporated* into the DNA during cell replication. This may then lead to mistakes occurring in the new DNA. In mammals mutations in the germ cells can lead to birth defects. Mutations in somatic cells are also believed to underlie the development of cancer in most instances (see below).

CARCINOGENICITY

It has been suggested that the majority of human cancers are caused by chemical carcinogens and although this is still a contentious issue, there are now many examples of chemicals which will reproducibly cause cancer in experimental animals.

Carcinogenesis is a specific toxic effect that leads to the uncontrolled proliferation of cells in a tissue or organ. It comes in many different forms, differing in malignancy and type of tissue affected.

Many, but by no means all, chemical-induced cancers are the result of a mutation in a somatic cell. Thus, toxic chemicals may be carcinogenic by interfering with the genetic control of cellular processes via mutation.

Cancer is now believed to be a **multi-stage process**. This in simple terms requires **initiation** followed by **promotion** and then finally **progression**. Exposure to a carcinogen, such as chemically reactive alkylating agents, **vinyl chloride** and **aflatoxins** (see Chapters 6 and 10, respectively) causes an initiating event. This is normally followed by several exposures to a substance which is a promoter. Experimental studies have indicated that the initiating event produces an irreversible change (such as damage to DNA) and must precede the promotion stage. For example, tumours of mouse skin can be caused by application of an initiator such as **benzo(a)pyrene**, a polycyclic hydrocarbon, followed by a **phorbol ester** (promoter). Several exposures to an initiator may result in tumours in the absence of a promoter. The initiation stage typically involves the interaction between DNA and a reactive chemical. The promotion stage involves an alteration in genetic expression and the growth of a clone from the original initiated cell.

During progression the **neoplastic cells** may change phenotype and become a **malignant tumour** involving increased growth and invasion of healthy tissue. However, not all carcinogens are mutagenic, for example **ethionine** and **asbestos** (see Chapter 6). Therefore mechanisms that do not involve a mutagenic event (**epigenetic mechanisms**) must be invoked to explain the cancer caused by such compounds. Furthermore, not all mutagens are carcinogens, although there is a sufficiently good correlation between mutagenicity and carcinogenicity for mutagenicity tests to be regarded as predictive of potential carcinogenicity (see Chapter 12).

Mutagenicity tests are also of use for prediction of germ cell defects and hence damage that is heritable.

One type of chemical carcinogen that is not mutagenic are **peroxisome proliferators**. This type of carcinogen has been extensively studied, especially as a number of drugs and industrial chemicals fall into this category. Compounds such as the drug **clofibrate** and plasticizers such as **phthalate esters** have been found to produce liver tumours in rodents after repeated exposures. Associated with this effect is the phenomenon of proliferation in the number of peroxisomes. The result is not only an increase in the number of peroxisomes, an intracellular organelle, but an increase in a number of the enzymes located in the peroxisome and an increase in liver size due to hyperplasia. However, clofibrate and other compounds are only carcinogenic in rodents. It appears that the phenomenon of peroxisome proliferation requires a cellular receptor and only those species that possess a functional receptor are responsive to these chemicals. Humans, it seems, do not possess a fully functional receptor.

The mechanism underlying the carcinogenicity of peroxisome proliferators involves a combination of increased oxidative stress due to increased production of **hydrogen peroxide** in the peroxisome and increased cell proliferation. As well as the requirement for a functional receptor, peroxisomal proliferators show a clear dose threshold for both the peroxisomal effects and the tumour induction therefore allowing a risk assessment to be made (see below, Chapter 12).

Biomarkers

Determination of the true exposure to a chemical substance, of the response of the organism to that chemical and its potential susceptibility to toxic effects are all crucial parameters in toxicology. Biomarkers are tools that facilitate measurement of these.

There are thus three types of biomarkers: biomarkers of **exposure** of the organism to the toxic substance, biomarkers of **response** of the organism to that exposure and biomarkers of **susceptibility** of the organism to the chemical. Thus, exposure may be crudely determined by measuring the dose but it cannot be assumed that all of the dose is absorbed. Therefore a more precise estimate of exposure is the blood level of the chemical. The level of a chemical in the blood approximates to the concentration in organs, which are perfused by that blood, one of which may be a target for toxicity. However, a metabolite may be responsible for the toxicity and therefore measuring the parent chemical may not be an appropriate biomarker. A more appropriate marker of exposure would be the **metabolite** itself, or if this was a reactive metabolite, a glutathione conjugate or one of its products which reflects the formation of a reactive metabolite could be measured in the urine. Unlike biomarkers of exposure, which are relatively few for a particular chemical, there are many biomarkers of response which may be measured. These include markers such as **enzymes** which appear in the blood when an organ is damaged, increased levels of an enzyme or stress protein (induction), urinary constituents, enzyme activity and pathological changes detected at the gross, microscopic and subcellular level. Indeed, a biomarker of response could be almost any indication of altered structure or function. The search for novel biomarkers now includes study of changes in genes (**genomics**), changes in the proteins produced from them (**proteomics**) and changes in the metabolites resulting from these proteins (**metabonomics**).

Finally, biomarkers of susceptibility can be determined for example in individual members of a population. This could be a **genetic defi-**

ciency in a particular enzyme involved in detoxication or xenobiotic metabolism such as CYP 2D6 or N-acetyltransferase for example. A less common type of susceptibility marker is that reflecting increased responsiveness of a receptor or resulting from a metabolic disorder, such as glucose 6-phosphate dehydrogenase deficiency, leading to increased susceptibility. These three types of biomarkers are all interrelated as indicated in Figure 4.4.

Summary and learning objectives

In this chapter ***types of exposure*** and ***types of response*** have been discussed. Exposure to chemicals may be either ***acute*** or ***chronic*** as will be the responses. Routes of exposure may affect the type and location of the response which can be local or systemic. Biological systems can respond in many different ways ranging from simple irritation to complex immunological reactions. The major types of toxic responses observed are: direct toxic action; tissue lesions; biochemical lesions; pharmacological or physiological effects; immunotoxicity; teratogenicity; genetic toxicity; carcinogenicity.

Certain organs may be ***targets*** for toxicity, such as the ***liver***. This is due to its position in the body, structure, role in metabolism and function. Hepatocytes are metabolically very active cells and the liver is crucial to the organism. Liver may suffer steatosis, necrosis, damage to the biliary system. Kidney is also often a tar-

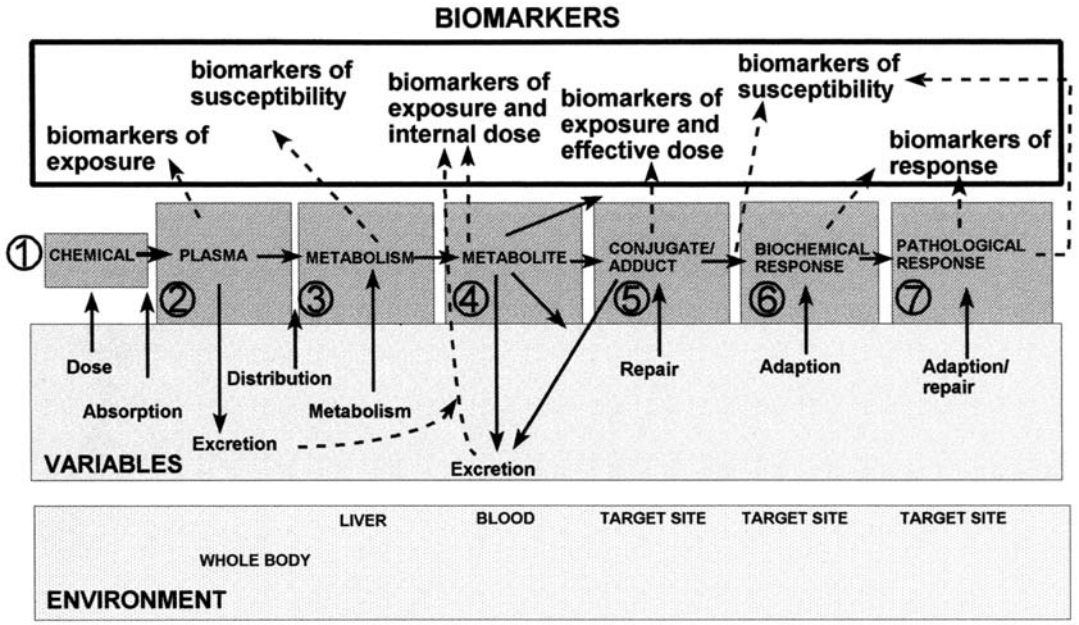

FIGURE 4.4 *The three types of biomarkers and their interrelationships. The numbers represent stages from initial exposure of the organism (1), through absorption into the plasma (2), metabolism of the chemical (3), distribution and interaction of a metabolite with body constituents (4), possible formation of conjugates and adducts with macromolecules (5), production of a biochemical response (6) leading possibly to a pathological response (7). Biomarkers may be measured at several different points in the sequence.*
From Waterfield, C. J. and Timbrell, J. A., Biomarkers – An overview, General and Applied Toxicology, *2nd Edition, edited by Ballantyne, Marrs and Syversen, Stockton Press, USA.*

get due to its high metabolic activity and role in excretion. Toxic effects in organs and tissues may involve primary (e.g. lipid peroxidation), secondary (e.g. damage to specific proteins or DNA) and tertiary events (e.g. necrosis).

Direct tissue damage and destruction can result from *local corrosion* or *systemic effects* leading to liver necrosis for example (see paracetamol, Chapter 5, snake venoms, Chapter 10). This is often due to direct interaction with macromolecules. Another form of cell death is apoptosis.

Biochemical lesions are due to interference with a specific enzyme or pathway which leads to cell dysfunction and possibly death of the organism (see Chapter 5, aspirin; Chapter 7, fluoroacetate; Chapter 10, cyanide). Some effects are reversible such as the interaction of carbon monoxide with haemoglobin with no apparent effects if not lethal. Pathological lesions such as steatosis and phospholipidosis are the result of biochemical lesions.

Pharmacological or physiological effects such as changes in blood pressure or vascular dilation are often due to overdoses of drugs (see debrisoquine Chapter 5) with specific biological actions. However, effects such as bronchoconstriction and production of excessive amounts of secretions may also occur with other chemicals such as organophosphate pesticides (see Chapter 8).

Immunotoxic effects may involve allergic reactions, autoimmune reactions, immunosuppression or hypersensitivity. Allergic reactions require an antigenic protein. Immune stimulation may be divided into four types, including anaphylaxis and cell-mediated reactions (e.g. hydralazine, halothane). Autoimmune reactions result in destruction of body constituents (see Chapter 5, halothane). Immunosuppression is a direct effect on a component of the immune system such as lymphocytes or bone marrow (e.g. dioxin).

Teratogenesis is the specific interference with the development of the embryo and foetus in the uterus resulting in a structural or functional abnormality. These can be manifested as abortion, malformations, growth retardation or functional disorders (see Chapter 5, thalidomide). Teratogens are selective, specific for the embryo/foetus, often showing steep dose–response relationships. The malformations that result will depend on the stage during exposure.

Genetic toxicity is the specific interference with the genetic material of the cell to cause a heritable change in the cell genotype. There are three types of interaction: aneuploidization (loss or acquisition of complete chromosome), clastogenesis (loss, addition or rearrangement of parts of chromosomes) and mutagenesis (loss, addition or alteration of a small number of base pairs). Aneuploidy and clastogenesis usually lead to major cellular effects. Base pair changes may lead to small changes or consequences such as frame shift mutations. Mutations can lead to tumours.

Carcinogenesis is the production of a malignant tumour, resulting from the uncontrolled proliferation of cells, as a response to chemical exposure (see vinyl chloride, Chapter 6; arsenic, Chapter 9; aflatoxin, Chapter 7) but carcinogens are not necessarily mutagens (epigenetic mechanisms may be involved, e.g. peroxisome proliferators). Carcinogenesis is a *multi-step process* involving initiation, promotion and progression. Initiation in which an irreversible change is produced, such as in DNA, must be followed by promotion in which there is an alteration of gene expression and growth of a clone of cells. During progression there may be a change in phenotype when the cells become a malignant tumour.

Determination of exposure, detection of responses and determination of susceptibility to chemicals involves the use of ***biomarkers***. Thus there are biomarkers of ***exposure,***

response and ***susceptibility***. Biomarkers of exposure include metabolites and adducts with proteins indicating internal exposure. Biomarkers of response are many and various and range from increases in serum enzymes to the up or down regulation of genes. Biomarkers of susceptibility are more limited and are individual indicators such as the presence or absence of an enzyme or isozyme.

Questions

Q1. Choose one answer which you think is the most appropriate.
Which of the following is the *most important* in determining the extent of toxicity of a chemical:

a chemical structure
b dose
c metabolism of the compound
d excretion of the compound
e metabolic detoxication of the compound.

Q2. Indicate which of the following are true.
The liver is a target organ for the toxic effects of chemicals because of:

a its highly complex structure
b its ability to metabolize chemicals
c its blood supply
d its excretory function
e its low levels of glutathione.

Q3. Choose one answer which you think is the most appropriate.
The most common toxic response the liver shows after exposure to chemicals is:

a cancer
b cholestasis
c blebbing
d necrosis of sinusoidal cells
e steatosis.

Q4. There are four general types of toxic effect involving the immune system. Indicate which of the following are included:

a anaphylaxis
b immunosuppression
c skin sensitization
d autoimmune reactions
e bronchoconstriction.

Q5. Choose one answer which you think is the most appropriate.
Chemicals which are active during the first week of pregnancy after fertilization of the egg are most likely to cause which effect in the embryo:

a death
b malformations
c functional abnormalities
d growth retardation
e sterility.

Q6. Match the following:

Aneuploidization	addition or alteration of the number of base pairs
Clastogenesis	loss or acquisition of a complete chromosome
Mutagenesis	loss, addition or rearrangement of parts of chromosomes

Q7. Indicate which of the following are true.
Biomarkers are used:

a to indicate that exposure has occurred
b to detect changes in genes
c to measure stress proteins
d to measure exposure, response or susceptibility.

SHORT ANSWER QUESTIONS

Q8. List the types of toxic response which a living system may undergo as a result of exposure to a chemical. Give an example of each type.

Q9. List the four different types of immune stimulation and give an example for one of them.

Q10. Carcinogenesis is a multi-stage process. Indicate what the stages are and explain each of them.

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