CHAPTER 7

General Aspects of Toxicology

A. Jernelöv, K. Beijer, and L. Söderlund

Swedish Water and Air Pollution Research Laboratory,
Box 21060, 10031 Stockholm, Sweden

7.1 INTRODUCTION 

As a result of new insights into biochemistry, cellular biology, cytogenetics, and pharmacology, increased attention has been paid to the ability of toxic agents, individually or collectively, to induce responses and pathological changes at the cellular level.

A large number of in vitro studies on the interaction of chemical agents with cellular organelles, DNA and RNA molecules and individual enzymes or enzyme systems etc., have been undertaken. However, due to the complexity of the living organism, results obtained in such studies do not necessarily explain effects observed in the organism which may be the result of the interference with several biochemical mechanisms. Thus it is important to establish the qualitative and quantitative relationships between effects demonstrated in an in vitro experiment and the effect in the organism, which they are supposed to explain. A thorough knowledge of the basic mechanisms by which toxic agents exert their injurious effects is fundamental for an understanding of biological responses of flora and fauna.
7.2. THE CELL

Toxic agents may disrupt the integrity of cellular structures thus impairing vital processes. Inside the cell they may interfere in some way with normal metabolic processes necessary to sustain life.

In order to enter the organism and reach some target organ, any material must penetrate one or more living membranes. The membranes form barriers with specific permeability properties which together with the mediated transport systems control the rate and extent of transfer of substances into and out of a cell and cell organelles. Toxic agents may in different ways modify the permeability of a membrane. A stabilization will occur when the pores are compressed by the penetration of a chemical agent with a decrease in membrane permeability as a result. Some insecticides affect the cell membrane in this way. When a chemical gives rise to an increased permeability a labilization is said to occur. DDT is believed to have this effect. The mediated transfer systems may be disturbed by chemicals interfering with carriers and ATP production. Thiol groups are often involved in the binding of substrates to specific carriers and the transport mechanism may be impaired by toxic agents such as for instance cadmium.

The outer membrane of mitochondria is freely permeable to most low molecular weight solutes including many toxic agents which may interfere with different processes within the organelle, whereas the inner membrane, where the enzymes of electron transport and energy conversion are located, is relatively impermeable and allows only certain substances to pass via a transport system. The permeability can, however, be altered by toxic agents, for example by carbon tetrachloride and some insecticides. Some chemicals produce a swelling of the mitochondria with a resultant unfolding of the membranes and a decrease in ATP production. Cadmium has been found to produce such a swelling in corn, probably through binding to sulphydryl groups on the membrane (Miller et al., 1973).

The lysosomal enzymes responsible for cell autolysis would cause a destruction of most cellular components if they were not separated from the rest of the cell by a membrane. Thus increased permeability or a rupture of the membrane by a toxic agent will injure or even kill the host cell. A well-known example of such an effect is what is called silicosis in humans. The digestive enzymes if released can attack the nucleic acids in the nucleus and cause chromosome damage. An increase of membrane stability so that the release of enzymes is retarded will impede the replacement of ‘old cells’. Toxic agents may change the properties of the lysosomal membranes so as to make it less suitable for fusion with other membranes, and thus seriously affect cellular nutrition.

The maintenance of normal cell volume and pressure depends on the sodium—potassium pump. If the pumping is inhibited, sodium will enter the cell along the concentration gradient and water will follow along the osmotic gradient. This will cause a swelling of the cell. The cation pump in cell membranes is a likely
point of attack by chemicals in their first interaction with the cell. The pumping
may also be inhibited by chemicals interfering with the production of ATP.

Mercury is one toxic agent which may affect living membranes and their
functions in several ways. For example, mercuric ions have been shown to cause a
decrease in the electrical potential across the membrane of new cells with a
resulting loss of cellular potassium and a significant change in nerve conduction.

Bacteria grown in the presence of mercuric chloride have been found to exhibit
extensive morphological abnormalities. Significant effects are the numerous
structural irregularities associated with cell wall and cytoplasmic membrane
synthesis and function (Vaituzis et al., 1975).

Methylmercury is soluble in phospholipids in the central nervous system which
leads to membrane lysis specific for those membranes that contain plasmalogens as
a major constituent of the phospholipid backbone in membrane structure. In vitro
experiments have shown that methylmercury can react both catalytically and
directly with a group of phospholipids that are important in membrane structure
for cells of the central nervous system. It has thus been suggested that this reaction
could have a significance in the neurotoxicity of methylmercury (Wood and Seagall,
1974).

The nervous system in particular serves as a target for many toxic agents. The
nerve impulses produced by the movement of sodium and potassium ions across the
cell membrane may of course be disturbed by chemical agents interfering with the
ion pump.

Chemicals may also inhibit or facilitate processes at the synapsis involving the
transmitter substance (usually acetylcholine, adrenaline, or noradrenaline) and
cause disturbances of the synaptic transmission by interfering with the synthesis or
storage of the transmitter substance, its release from the synaptic buttons by nerve
impulses, its permeability-changing action on the membrane of the next cell or its
removal and destruction.

One example of the last process is the action of organophosphorous compounds
(used as insecticides and also for instance for fireproofing of textiles) and
carbamates which inhibit acetylcholinesterase, the enzyme responsible for the
destruction of acetylcholine. Inhibition of the enzyme permits an abnormal
accumulation of acetylcholine. This will cause excessive activity of the para-
sympathetic system, give central nervous system effects and over-reactivity of the
voluntary muscles.

Lead may, besides affecting the activity of Na⁺−K⁺-ATPase, cause demy-
elination of the axon. Acrylamide is also believed to have this effect. Toxic levels of
triethyltin may cause enlarging of the oligodendrocytes, which develop large
fluid-filled vacuoles. Extracellular fluid will also accumulate between the myelin
rings with a resultant splitting of the sheaths from the axons. Triethyltin is thought
to exert its effect by inhibiting the ATPase normally present in astroglia and axonal
tubes (Toback, 1965). Hexachlorophene is believed to have similar effects.
Enzymes are of supreme importance in biology. They make up the largest and most highly specialized class of proteins. Life depends on a complex network of chemical reactions brought about by specific enzymes. The enzymes are the primary instruments for the expression of genetic action since they catalyse the thousands of chemical reactions that collectively constitute the intermediary metabolism of cells. Clearly any modification of an enzyme pattern may have far-reaching consequences for the living organism.

The consequences of enzyme inhibition for the metabolism will depend on the effects of the inhibition on the enzyme itself. The formation of enzymes is under different kinds of control. Repression and induction of enzyme synthesis by metabolites or substrate is one kind of control. Thus since inhibition of the enzyme will lead to a build-up of substrate and changed levels of metabolites, enzyme formation will be increased and the effects of the inhibition on the overall metabolic function will not be severe. The rate of enzyme formation may also be regulated by the actual level of enzyme present. If the enzyme–inhibitor complex or the inhibitor itself is mistaken for intact enzyme, no more enzyme will be synthesized and the impact of this suppression may be great.

Inhibition of enzymes in the usual sense, by a chemical agent, may be reversible or irreversible, competitive or non-competitive. A reversible inhibition is characterized by an equilibrium between enzyme and inhibitor and will give a definite degree of inhibition depending on inhibitor concentration. Reversible inhibitions are numerous, e.g. the action of cyanide on cytochrome oxidase and of malonate on succinate dehydrogenase.

An irreversible inhibition is characterized by a progressive increase with time, ultimately reaching complete inhibition even with very low concentrations of inhibitor, provided that the inhibitor is in excess of the amount of enzyme present. Examples of irreversible inhibition are the action of cyanide on xanthine oxidase and of the ‘nerve gases’ on cholinesterases.

Inhibition may involve a direct combination of the inhibitor with the enzyme, but inhibition will also be produced by substances that combine with the substrate, a coenzyme or metal activator.

There are some different mechanisms through which thiol groups of enzymes can be inhibited. Probably the most important is the formation of mercaptides. Heavy metals such as mercury, lead, cadmium, gold, and zinc are examples of metals that will attack thiol groups. Organic compounds of mercury and arsenic also form mercaptides as do trivalent arsenicals. Thus for example, inhibition of succinoxidase by lead has been demonstrated in dogs. The pyruvate oxidase system is inhibited by the action of arsenicals on the dithiol lipoate cofactor. Low concentrations of lead in blood cause a reduction of δ-aminolaevulinic acid dehydrogenase activity in erythrocytes (Prerovska and Teisinger, 1970).

Thiol groups are relatively easily oxidized by a wide variety of oxidizing agents.
For instance, selenium ions oxidize thiol groups rather than complex them and the same can be assumed for cupric ions:

\[ 4RS^- + 2Cu^{2+} \rightarrow 2RSCu + R-SS-R \]

Another possibility is the alkylation of the thiol group by halogenated carbon compounds like ethyl iodoacetate and chloroacetophenone. Certain ions are absolutely necessary for the activity of some enzymes while others are highly toxic to nearly all enzymes (e.g. Ag\(^+\), Hg\(^{2+}\) and Pb\(^{2+}\)). Some ions are poisons for some enzymes and activators for others, and some may even inhibit an enzyme at one concentration and activate it at another. Particularly with cations, the enzyme is inactive by itself and the requirement for a cation is usually fairly specific; in some cases only one particular cation is effective, in other cases two or three different cations can act.

For instance, riboflavin kinase in animal tissues is activated by Mg\(^{2+}\), Co\(^{2+}\), or Mn\(^{2+}\), and inhibited by Ca\(^{2+}\). In plants it is activated by Mg\(^{2+}\), Zn\(^{2+}\), or Mn\(^{2+}\), and inhibited by Hg\(^{2+}\), Fe\(^{2+}\), or Cu\(^{2+}\). Glycerol dehydrogenase in bacteria is activated by NH\(_4^+\), K\(^+\), or Rb\(^+\) and inhibited by Zn\(^+\).

There are several examples of displacement of essential metals by non-essential ones, either in the same or in contiguous periodic groups. The result may produce a conditioned deficiency as in chlorosis in plants which is caused by iron deficiency or by the displacement of iron by other metals, e.g. nickel or copper, in chlorophyll with an impairment of photosynthesis as a result.

This displacement is dependent on the strength of the individual ligand complexes, generally following the extended Irving-Williams series

\[ \text{Ca}^{2+} < \text{Mg}^{2+} < \text{Fe}^{2+} < \text{Co}^{2+} < \text{Zn}^{2+} < \text{Ni}^{2+} < \text{Cu}^{2+} \]

(which is independent of ligand type), and also on the concentrations of ligands and metal ions present in the system. It is also dependent on the relative strength of the complexes involved. The effect of this may be illustrated by the following example: According to the Irving-Williams series, cupric ions should replace ferrous ions in ligand complexes. However, this will not happen when the ferrous ion is complexed by an aromatic nitrogen-type ligand, where the ferrous ion is abnormally stabilized, resulting in a very small difference in stability constants for these types of complexes with cupric and ferrous ions, compared to those of almost any other complex possible in the system (Williams, 1953).

Zinc occurring in carbonic anhydrase will be replaced by administered cupric ions, with the consequent loss of activity for the enzyme (Williams, 1953). Cadmium inhibits peptidase and enhances esterase activity by displacing zinc from its nitrogen—sulphur ligands in carboxypeptidases (Perry et al., 1955). Large doses (0.25% in diet) of pure zinc salts retarded the growth of rats and caused hypochromic anaemia, probably resulting from displacement of copper and iron (Underwood, 1962). Nickel has the ability to displace beryllium from alkaline phosphatase and re activate the enzyme (Schroeder et al., 1962). The feeding of
tungstate ion in large quantities has caused displacement of molybdate in the body of experimental animals (De Renzo, 1962).

Metal ions in the active site of enzymes can be inhibited by substances forming stable complexes with the metal. Cyanide, hydrogen sulphide, azide, and carbon monoxide are examples of such compounds. Hydrogen sulphide and cyanide inhibit many of the enzymes which contain iron or copper as an essential part of their catalytic activity. They are, for instance, powerful inhibitors of the respiration of many tissues through interaction with the cytochrome oxidase system. Carbonic anhydrases both in blood and plants are inhibited by cyanide, azide, and hydrogen sulphide.

Cyanide may inhibit enzymes by several different mechanisms, such as combining with essential metals in the enzyme or removing a metal ion thus leaving the enzyme as an inactive complex, combining with carbonyl groups in the enzyme, a cofactor, a prosthetic group or the substrate, or by acting as a reducing agent breaking disulphide bonds. Carbon monoxide will only inhibit enzymes containing iron or copper and particularly those that react directly with oxygen, combining with the reduced form of the enzymes. Cyanide combines most stably with the oxidized forms.

In the formation of avian eggshell, carbonic anhydrase is believed to be necessary to supply the carbonate ions required for calcium carbonate deposition. It has been shown that the DDT metabolite DDE and also PCB may inhibit the formation of carbonic anhydrase and other transport enzymes in the shell gland and may thus be contributing to thinness.

7.4. METABOLISM AND REGULATORY PROCESSES

Metabolism, the sum of all enzymatic reactions occurring in the cell, can be disturbed by a great number of toxic agents, in many different ways. Chemicals may interfere with the enzymes essential for various processes. Hormones and other regulatory systems may be affected, resulting in an uncontrolled metabolic rate. Toxic agents may also cause genetic mutations which can result in a failure to synthesize an enzyme in its active form. Such defects, if they are not lethal, may result in an accumulation or excretion of the normal substrate of the defective enzyme.

(i) Carbohydrate Metabolism

One of the major biochemical lesions arising from the action of toxic compounds is the disturbance of carbohydrate metabolism. Since carbohydrate catabolism provides energy for other metabolic processes, deviations from the normal metabolic pattern might be expected to result in impairment of respiratory chain reactions. The kinds of deviation are numerous as are the possible mechanisms through which they are mediated. Only a few examples are given here.
In glycolysis the phosphorylation of glucose to yield glucose-6-phosphate is catalysed by two types of enzymes, hexokinase and glucokinase. Hexokinase may be inhibited by certain sulphhydryl reagents, especially arsenicals. Hexokinase has also been shown to be completely inhibited by copper in sheep (Agar and Smith, 1973). The conversion of 3-phosphoglycerate to 2-phosphoglycerate is catalysed by the enzyme phosphoglyceromutase. This enzyme may be inhibited by mercury ions in heart muscle (Diederich et al., 1970). The enzyme enolase catalyses the reaction in which 2-phosphoglycerate is transferred to phosphoenolpyruvate. Enolase may be inhibited by inorganic fluoride which forms a ternary complex with the essential activators Mg$^{2+}$ and inorganic phosphate. This complex competes with Mg$^{2+}$ for the active site (Smith, 1970). In the last step of glycolysis, pyruvate is reduced to lactate. This reaction is catalysed by lactate dehydrogenase, and this enzyme is inhibited by oxalate, a structural analogue of pyruvate.

Also, the tricarboxylic acid cycle may be disturbed in several ways. For example, the pyruvate dehydrogenase complex is characteristically inhibited by trivalent arsenicals or by arsenite, which can react with both thiol groups to yield an inactive cyclic derivative. A metabolite, namely fluorocitrate, of fluoroacetate, used as a rodenticide and also synthesized by certain plants growing on fluorine-rich soils, inhibits the metabolism of citric acid by aconitase. Thus the citric acid cycle is blocked and citrate is accumulated in the tissues.

(ii) Respiration

Biological oxidation proceeds through a sequence of intermediary carriers transferring electrons from substrates to molecular oxygen. The respiratory chain is of vital significance for supplying energy to living cells. The electron transport along this chain may be inhibited at specific sites by different toxic agents. For instance, cyanide, azide, and CO may inhibit the electron transport between cytochrome $a_3$ and $O_2$. Antimycin A and 2-alkyl-4-hydroxyquinoline-N-oxide may inhibit the electron transport between cytochrome $b$ and cytochrome $c_1$. Rotenone and barbiturates may inhibit the electron transport between flavoprotein and co-enzyme $Q$. Oxidative phosphorylation, through which energy released from the exergonic catabolic process is conserved for driving a multitude of endergonic biological processes, is tightly coupled to the respiratory chain. There are several toxic agents that do not inhibit respiration directly but prevent the associated phosphorylations. These agents are called uncoupling agents. Substituted phenols such as 2,4-dinitrophenol and pentachlorophenol belong to this group, as do triethyltin and triethyllead.

(iii) Lipid Metabolism

Disturbances of lipid metabolism resulting in impaired liver function are caused by many toxic agents, e.g. lindane, cobalt salts, and selenium. One example of this
Principles of Ecotoxicology

is that chronic exposure to both DDT and dieldrin has been shown to increase significantly lipogenesis in fish, and their effects are additive.

(iv) Microsomal Enzyme Systems

The multienzyme system located in hepatic microsomal fractions is responsible for the metabolism of, e.g. steroid hormones, fatty acids, alkylpurines, thyroxine, and bioactive amines, and also for the biotransformation of foreign compounds. This system is subject to modification by a vast number of chemical agents as well as by various physiological conditions such as hormonal derangement. The alteration in microsomal metabolic function is of two types - stimulation or inhibition.

The pattern of microsomal enzyme depression varies with the inhibitory agent and may be mediated through a variety of mechanisms. Indirect mechanisms like generalized hepatotoxicity with consequent damage to endoplasmic membrane structures may also be involved in chemically induced inhibition of microsomal metabolism.

Among the chemicals of environmental importance capable of impairing hepatic microsomal biotransformations are organophosphate insecticides, carbon tetrachloride, carbon disulphide, and carbon monoxide.

Microsome enzyme induction by organochlorines and by polyaromatic hydrocarbons is exhibited by a variety of species including mammals, fish, and birds. Also toxic elements, such as Ni, Cr, Br, Cd, Pb, in trace amounts affect the liver microsomal enzyme system.

For example, chlordane, DDT, dieldrin, and PCB’s have been noted to bring about an accelerated hydroxylation of androgens, estrogens, and glucocorticoids in several animal species thus causing a more rapid deactivation of these steroid hormones.

(v) Biosynthesis

Protein synthesis can be disturbed on many levels by a variety of mechanisms, either by affecting the nucleic acid metabolism or structure, or in the protein-forming system itself. Toxic agents acting directly on ribosomes, RNA, enzymes or coenzymes may also have a drastic influence on protein synthesis.

For example, all aminoacyl-tRNA synthetases have thiol groups in their active sites and are therefore, of course, especially sensitive to sulphur-attacking toxicants.

It is thought that carbon tetrachloride exerts its toxic effect on protein synthesis on single-unit ribosomes (Farber et al., 1971). The inhibition of amino acid incorporation in microsomes by ethionine is thought to be connected with a deficiency of available ATP in the cell. Trapping of cellular adenine and a diminution in the rate of ATP synthesis is brought about by the replacement of methionine by ethionine with the formation of s-adenosylethionine (Farber et al., 1971).
All biosynthetic processes can of course be disturbed in one way or another. The inhibition of carbon dioxide fixation — the synthesis of organic compounds in photosynthetic plants — by zinc and cadmium, which has been demonstrated for spinach, is an example from the plant world (Hampp et al., 1976).

(vi) Regulatory Processes and Growth

In general, the rate of catabolism is controlled by the second-to-second need for energy. The rate of biosynthesis of cell components is also adjusted to immediate needs.

The regulatory system may be disturbed by toxic agents in many ways. The structure or activity of regulatory enzymes may be altered and the synthesis, storage, release, or sequestration of hormones may be affected. For example, the release of peptide hormones such as insulin is strongly dependent on calcium ions. Magnesium ions can block the secretion of these systems.

Disturbance of the regulatory system may have serious effects on the organism. The rate of different metabolic processes may become uncontrolled. Such effects may be lethal for the organism. For example, when the herbicide 2,4-D penetrates the leaves of a plant, the result is a violent and uncontrolled growing to death. In this example growth is affected. All chemicals interfering with metabolic pathways may affect the growth.

The thyroid has many regulatory functions in all vertebrates. Its main function is control of metabolic rate. It also plays a major role in the control of growth rate and is essential for reproduction. It is believed that faulty thyroid activity and changes in vitamin A storage may be the cause of sublethal effects by organochlorine pesticides and PCB, which have structural similarities to thyroid hormones. This subject has been reviewed recently by Jefferies (1975). For example, in one experiment with pigeons an increase in metabolic rate was shown to occur with the administration of low doses of DDT and at higher doses a decrease was observed.

The pesticide dieldrin has been shown to decrease the growth of fawns that had parents whose diet incorporated dieldrin. Dieldrin was passed across the placenta to the fawns, and also was secreted with the mother’s milk.

D-Threose-2,4-diphosphate has been found to inhibit the activity of growth hormone and thereby the growth of certain microorganisms.

Also the growth of aquatic invertebrates has been found to be impeded by exposure to several pesticides.

Especially the impact of atmospheric pollutants such as ozone, sulphur dioxide, and nitrogen oxides adversely affects plant growth (Mudd and Kozlowski, 1975). For instance, using duckweed, carnations, corn, petunias, marigolds, chrysanthemums, and turf grasses, a reduction in growth rate, stem elongation, leaf area, general plant size, top and root weight, fruit and seed set, and floral productivity was shown, when the plants were grown in air with ozone levels ranging from 0.1 to 10 ppm (Feder, 1970).
Mutations occur spontaneously in nature in all living organisms, but they are also artificially induced. Ionizing radiation induces mutations and chromosome aberrations in all kinds of cells. Chemicals, however, may act as powerful mutagens in one kind of organism or type of cell and remain without effect in others.

Two main categories of molecular mechanisms lead to point mutations during DNA replication: base substitution and intercalation. Base substitutions may be brought about by alkylating agents, for instance diethyl sulphate and mustard gas ($\beta,\beta'$-dichlorodiethyl sulphide) alkylate guanine, and will result in the incorporation of an incorrect amino acid into the protein coded for, or possibly the synthesis of the protein may be stopped at a premature stage. This may be of little consequence if the correct amino acid(s) is unimportant for the activity of some enzyme, but on the other hand, it may cause severe damage to the enzyme activity if this is not the case. Alkylating agents such as mustard gas with two or more functional groups produce extensive crosslinking of guanine moieties in the opposite strands of the DNA molecule, so that they cannot separate for replication.

The intercalation of a chemical substance between base pairs results in a distortion of the sugar-phosphate backbone of the DNA molecule producing deletion or insertion of nucleotide bases. This will have a drastic effect on the activity of the gene in that the grouping together of the bases three by three will be shifted up or down along the DNA chain. The amino acid sequence of a protein synthesized will thus, from the point of mutation on, be completely changed, and this will lead to a completely non-functional protein unless the mutation affects only the very end of the protein.

A large number of chemicals cause breaks in the DNA chain and the chromosomes. When a chromosome is broken, the ends are usually fused together through special enzymes and the break will pass unnoticed. It may happen, though, that the chromosome is not repaired due to some fault in the enzyme system, leading to structural changes. Such changes may be due to translocations or inversion of fragments or the duplication of fragments. Dicentric bridges may be formed between different chromosomes in close proximity. Many chromosome aberrations will lead to cell death, or grave abnormalities may arise in the offspring if reproductive cells are thus affected.

The distribution of chromosomes at cell division may be disturbed, leading to different numbers of chromosomes in the daughter cells. Some metal compounds have been shown to have this effect, and organic mercury compounds are particularly powerful agents effective at very low concentrations.

Most chromosome mutations in reproductive cells will cause abnormalities or lethality in the offspring. The effects of mutations in somatic cells and disturbances of mitosis are not very well known or easily predictable, however. In the organism a faulty cell is most likely to be selected against, and replaced by a healthy one. Some data, however, indicate a connection between genetic defects in somatic cells and
the formation of tumours. Thus if a chemical is shown to cause mutations there is reason to suspect that it may cause cancer as well.

Many biocides have been tested for their mutagenicity and some have been shown to be mutagenic. For instance the insecticidal organophosphate ethyl esters can act as alkylating agents and are powerful mutagens. The fungicidal action of benzimidazole carbamic acid methyl ester (BCM) is thought to be due to some interference with spindle formation.

There are both ‘natural’ and artificial food additives that have been shown to be mutagenic. Aphlatoxin has been demonstrated to be a mutagen and it is also suspected of causing liver tumours. Cycasin is, after being metabolized in the intestine, a potent methylating agent.

Examples of artificial food additives with mutagenic and carcinogenic action are cyclamates, nitrite, and nitrofurazone.

In vitro experiments with DNA nucleosides and nucleotides have shown that Cu\(^{2+}\) binds to the N\(_{11}\) position of cytidine and to N\(_{7}\) in adenosine and thymine. Copper also binds phosphate, cleaving the phosphodiester bonds in polynucleotides (Eichhorn et al., 1966). The interaction of silver ion with guanosine has been investigated and it is found to form a bond at the N\(_{7}\) position (Tu and Reinosa, 1966). The manganous ion seems to have a marked preference for association with the guanine ring (Anderson et al., 1971); it also interacts with the phosphate groups. Such ligand complexes of metal ions with nucleotide bases will interfere with hydrogen bonding between bases in the DNA molecule.

In experiments with human diploid cells, namely leukocytes and fibroblasts, chromosome alterations were not observed with salts of cadmium, cobalt, nickel, iron, selenium, vanadium, and mercury. Chromosome breaks were shown to be induced by arsenic and tellurium salts, however. The chromatid breaks were frequent and often located close to the end of a chromosome chain, and cells with several damaged chromosomes were common.

Arsenic is known to block sulphydryl groups and it has been suggested that arsenic may inhibit DNA repair enzymes. The uptake of phosphate into DNA is reduced in the presence of arsenate ion which is thought to be incorporated into DNA in lieu of phosphate thereby forming weak bonds between the DNA strands.

Surveys on the mutagenic effects of environmental contaminants may be found in reviews and monographs (e.g. Fishbein et al., 1970; Voegel, 1970; Hollaender, 1971).

### 7.6. TUMOUR INDUCTION

Cancer can be induced by chemical carcinogens, oncogenic virus, or ionizing radiation. Chemicals are the most likely major cause of human cancer and environmental pollutants are thought to be responsible for the increasing tumour frequency observed in fish from contaminated waters.

It seems that carcinogenic agents are generally chemically reactive electrophilic...
compounds, such as the alkylating agents. These electrophilic reagents interact with a variety of informational macromolecules, for instance the proteins and nucleic acids of the cell. The reactive sites on DNA and RNA molecules have been shown to be the nucleotide bases and in proteins they seem to be tryptophan, tyrosine, methionine, and histidine.

It has been shown that even though procarcinogens are generally not mutagenic, their electrophilic metabolites are almost invariably potent mutagens. Alkylating agents are both highly mutagenic and potent carcinogens. Several agents — initiators, promoters, co-carcinogens etc., will often interact in the production of cancer.

There are non-carcinogenic chemicals that augment the effect of a primary carcinogen. This promotion involves the growth and development of dormant or latent tumour cells, resulting from the interaction of the primary carcinogen and specific receptors in susceptible cells — the initiation step. The promotion could be described as any situation yielding an increased growth rate of dormant cells, that is, hastening the mitotic rate.

The work that led to the important distinction between tumour initiation and tumour promotion described the increase in tumour yield and shortened latent period caused by, for example, croton oil applied subsequently to a dose of a carcinogenic polycyclic aromatic hydrocarbon (Sall et al., 1940; Berenblum, 1941).

Sulphur, sulphur dioxide, and some other compounds; dodecane, aldehydes, and phenols all stimulate the effects of polycyclic aromatic hydrocarbons and other carcinogens.

There are two types of chemical carcinogens. The first consists of those that are direct acting and do not require metabolic activation. Metabolic conversion of such compounds leads to detoxification and loss of effectiveness. These compounds usually act at the point of application. Examples are some active halogen derivatives (e.g. methyl iodide), strained lactones, unsaturated larger ring lactones, epoxides, imines, alkyl sulphate esters (e.g. dimethyl sulphate), and nitrogen mustard gas derivatives.

Most of the chemical carcinogens in the environment however, belong to the second type, which require some sort of activation — they must first be converted either directly or by chemical activation to an electrophilic compound with suitable properties of stability and reactivity. Many of these activation reactions consist of biochemical oxidation or hydroxylation. This type of chemical carcinogen can be divided into different groups, such as polycyclic aromatic hydrocarbons, aromatic amines and azo compounds, N-nitrosamines and amides, and metal compounds etc. They all give rise to tumours under certain conditions and at specific sites. The carcinogenic action of polycyclic aromatic hydrocarbons seems to be related to their binding to DNA and it has been postulated that an epoxide in the K-region formed through oxidation by microsomal enzymes may be the metabolically activated form responsible for the carcinogenic action (Boyland, 1950; Sims and Grover, 1974).
To induce cancer, aromatic amines need first to undergo N-hydroxylation. They are then changed into the highly reactive N-sulphate which reacts with proteins and guanine in nucleic acids. (Miller and Miller, 1974a). The chemical interaction of azo dyes and aromatic amines with tissue constituents has been extensively investigated (Miller and Miller, 1974b). For instance the carcinogenic nitrosamines are transformed either enzymatically or non-enzymatically into agents that donate methyl and ethyl radicals to RNA and DNA (Swann and Magee, 1968). Biochemical activation of nitrosamines appears to take place in all species so far studied. Many potent carcinogens are found among the organic nitroso compounds (Magee and Barnes, 1967). Nitrosamines can be formed \textit{in vivo} in the gastrointestinal tract by reaction of nitrite ion and secondary or tertiary amines and amides, causing tumours in other parts of the organism (Sander and Bürkle, 1969). It has been shown that nitrosamines may pass the placental barrier to the foetus and give rise to the development of tumours after birth (Ivankovic and Preussmann, 1970).

Aphlatoxin, a mycotoxin from \textit{Aspergillus flavus}, and cycasin, a plant toxin from cycad tree fern (methylazo methyl-\(\beta\)-D-glucoside) are examples of carcinogenic substances which occur naturally (Miller, 1974).

Metals and their compounds induce cancer after a long latent period during which the metals probably initiate irreversible cellular changes necessary for the subsequent development of tumours. It seems that they give rise to cancer at the point of application in most instances. Inhalation of arsenic compounds can result in tumour formation. Inhalation of nickel carbonyl has been shown to give rise to nasal sinus- and lung cancer. Chromium salts have been shown to be the cause of lung cancer in workers employed in the manufacture of dichromate from the ore.

\textbf{7.7. REPRODUCTION AND TERATOGENESIS}

Foreign compounds may interfere with the organs of reproduction so that the formation of their product is inhibited. Thus in mammals, chemicals may act to prevent implantation of the fertilized egg, causing its expulsion from the uterus. Or they may interfere with the normal development of the placenta, for instance by alteration of the enzymatic functions, or interfere with the transfer of nutrients. Several hormones are involved in ovulation and spermatogenesis and there are many direct and indirect effects of toxic agents that may disturb the hormonal balance. Compounds of very diverse structure may affect the reproductive system which is very sensitive to toxic agents.

Metals may cause disturbances of the reproductive functions. Cadmium, for example, can cause testicular damage in several species of animals, probably due to interference with the seminiferous epithelium and Leydig cells resulting in testicular degeneration.

Certain insecticides, notably the organochlorine compounds, have been shown to affect the male and female reproductive systems. Several workers have shown the connection between pesticide residues and viability of fish eggs. The organo-
chlorines are lipid soluble and may thus be **accumulated in fish eggs** and lead to the death of fry as the yolk-sac is absorbed at a critical stage of growth.

Pesticides may influence fish reproduction by inducing abortion, thus DDT, DDE, methoxychlor, aldrin, dieldrin, endrin, toxaphene, heptachlor, and lindane have all been shown to cause some degree of abortion in mosquito fish. The prostatic uptake of testosterone is decreased in male mice by continuous administration of dieldrin.

DDT has structural similarities with the synthetic oestrogen diethyl stilboestrol and oestrogenic activity has been shown to be affected by DDT in rats.

For many species of birds the organochlorines cause severe damage to the reproductive system with a decrease in egg production, thinning of eggshells, and reduced hatchability and fledgling success. In a second generation of pheasants whose parents received 4–6 mg of dieldrin per week for thirteen weeks, fertility and hatchability of eggs were found to be significantly lowered even though they only received the toxin through the egg.

The offspring may be born malformed mentally or physically as a result of chemical action on the reproductive system of either parent (a mutation) or on cell differentiation in the conceptus (a teratogenic change) or by toxic actions of chemicals on the developing organs of the foetus which can result in growth retardation or in degenerative toxic effects similar to those seen in the postnatal animal.

### 7.8. IMMUNE RESPONSE

The most powerful allergens are chromium, beryllium, nickel, and cobalt but many other industrial and environmental pollutants are also strongly allergenic. Some examples to illustrate other types of immune response will be given.

It is thought that bone marrow damage may be due to an allergic reaction involving antibodies to the precursor cells and a sensitizing chemical. This is suspected in some cases involving chloramphenicol.

Peripheral destruction of red cells may involve an allergic mechanism (autoimmune haemolytic anaemia) via sensitization by a chemical such as acetanilide.

Several environmental contaminants, e.g. lead, cadmium, mercury, DDT, and PCB, have been found to be synergistic to infectious agents through an immuno-suppressive action. Thus chronic exposure to lead has been shown to cause a significant decrease in antibody synthesis, particularly IgG globulin (Koller and Kovacic, 1974).

### 7.9. INTERACTIONS OF COMPOUNDS

The presence of large numbers of toxicants in the environment makes it necessary to consider interactions between such substances. Thus the concurrent presence of two or more foreign compounds in the organism often yields a toxic response which deviates from a simple additive one.
The interacting effect is said to be additive or substitutive, when the combined effects of two or more toxicants equal that expected when considering the toxicants individually. However, the additive effect does not imply a strict summation of the toxicity of the two substances. If the effect is greater than additive, it is said to be synergistic and means that one toxicant will potentiate the effect of the other. If the effect is less than additive, it is said to be antagonistic (see also Chapter 9).

As the toxicity of a compound depends largely on the efficiency of its biotransformation any interference with metabolism is likely to influence its toxic potential. Altered sensitivity of receptor sites, hormonal intervention, and competition for binding sites of biological receptors are other mechanisms which may contribute to potentiating or antagonistic effects.

A direct chemical interaction of exogenous compounds, which often results in a decreased response, is still another mechanism. One example of this is the precipitation of calcium fluoride which is not readily resorbed, upon the ingestion of sodium fluoride together with food rich in calcium. Often toxic agents concentrate at sites other than the site of toxic action. They may be stored in plasma proteins, in proteins in liver and kidney, in fat and in bone. Lead, for example, is stored in bone but exerts its toxic action in soft tissues. Displacement is another type of interaction. One chemical agent may displace another of similar structure from its binding site and thereby alter its toxicity. The binding capacity of the receptor may be influenced producing the same result. Thus lead stored in bone may be mobilized with a concomitant rise in the serum level of lead. Suppression or induction of microsomal enzyme systems can explain many of the metabolic interactions arising from the combined presence of different chemicals. The consequences of a suppression are usually a potentiation of the toxicity of foreign compounds. Formulations for this topic are to be found in Chapter 9.

Simultaneous intake of certain organophosphorus esters has been observed to result in potentiation of toxicity. Phosphothioates such as malathion are rendered much more toxic in combination with e.g. EPN (O-ethyl-O-p-nitrophenyl phenyl phosphoethionate). EPN competes with malathion for its hydrolysing enzyme thus impeding hydrolysis and resulting in a prolonged retention of malathion within the organism.

The increased toxicity of insecticides achieved by the addition of so called pesticidal synergists results from the inhibitory action of the synergists upon the microsomal mixed-function oxidase system. They have been developed to synergize insecticidal toxicity of pyrethrins but they have been shown to potentiate the toxicity of a number of other insecticides such as carbamates and DDT, and to be active in mammals as well as insects. Piperonyl butoxide, the most commonly used synergist also causes the formation of toxic metabolites of freons to occur. In insecticide spray bottles, 'Freons' occur together with the pyrethrins and the synergist.
7.10. DISCUSSION

Based on a knowledge of the biochemistry and physiology of the organisms and on previous experience of disturbances caused by toxic substances, various bioassay tests have been and can be worked out.

Obviously the total number of tests that would have to be performed in order to evaluate possible disturbances to any part of the metabolic system is enormous.

Sometimes arguments of resemblance and analogies can give some guidance as to what to test.

As an attempt to reduce the number of tests and yet to cover a variety of biochemical and physiological processes, bioassays are sometimes carried out to check net effects on functions composed of a large number of processes. Such functions may be reproduction or growth rate.

A further argument for such an approach is that even with the knowledge of how a substance will affect each process by itself, it may not be possible to predict the total effect on the function.

Regulating systems or feed-back or feed-forward type may have a major influence on the overall result.

It should also be kept in mind that the response of an individual organism provides only one part of the information required to predict effects on an ecological or population level.

Knowledge about the reaction of one species to a given chemical does not tell whether that species will increase or decrease in an exposed ecosystem (except when the result is death or total sterility).

The reaction of other species – competing ones, predators on prey – must also be known as the interactions between organisms often is a major regulating factor.

Obviously there is also a need for knowledge of the nature of these interactions.

Thus in addition to bioassays based on single biochemical or physiological reactions and those based on complex functions at the individual level, there is a need for testing procedures at higher levels of organization such as test ecosystems and computer-based mathematical models.

7.11. REFERENCES


