CHAPTER 1

CHEMICAL RADIATION PROTECTION

The work reported in this thesis deals mainly with the X-ray analysis of the crystal and molecular structure of some compounds that act as chemical radioprotectants. Radioprotectants are agents that alleviate the hazardous effects of ionizing radiation. An essential pre-requisite for understanding the molecular mechanism of action of the radioprotectants is a detailed knowledge of the molecular geometry of these compounds and the interactions they are likely to be involved in.

With a view to understand the mechanism of radiation protection on the basis of the structural characteristics of the molecules, a programme of X-ray investigations on some chemical radioprotectants was initiated in our laboratory and a part of the results has been included in this thesis. This chapter is meant to provide an introduction to the concept of chemical radio-protection. It is started with a very brief description of radiation and its effects on some typical systems. This is followed by a description of some important classes of chemical radioprotectants and the various existing theories of radioprotection. This chapter is by no means considered an exhaustive review on chemical radio-protection. More detailed information on the subject is available in the literature in the form of text books and review articles 1-6.

1.1 Radiation

Radiation could be broadly classified into two categories, viz.,

(i) electromagnetic and (ii) corpuscular. Electromagnetic radiation consists of
self-propagating electric and magnetic disturbances. The environment around us contains electromagnetic radiations in the form of radiowaves, infrared, visible, ultra-violet, X-rays and $\gamma$-rays. The effect of electromagnetic radiation, when it falls on a body, is a function of its energy. From the relation $E = \frac{hc}{\lambda}$, the energy is inversely proportional to $\lambda$, the wavelength of the radiation. The effect of radiation is also dependent on the condition and nature of the material being struck. The response of molecular structures to radiation could be compared to that of the action of a spring when a rhythmic force is imposed on it. Thus, the wavelength of the incident radiation determines the size of the structure that will resonate i.e., the radiation can excite structures whose dimensions are similar in magnitude to their wavelength. For example, radiowaves have long wavelength and they induce oscillations in structures that can be seen visibly and handled. X-rays and $\gamma$-rays with shorter wavelength possess higher energy and they can affect the electrons in the outer shells of atoms. They can produce excitation i.e., pulling of an electron to a higher energy orbit or, ionization i.e., removal of outer electrons, in the media through which they pass.

Corpuscular radiations consist of streams of various kinds of atomic or sub-atomic particles. They include electrically charged particles like electrons and positrons, the heavy electrically charged particles like the protons and deuterons, electrically neutral particles like the neutrons etc. These particles interact with matter by transferring their kinetic energy.
In dealing with the radiation effects on biological systems, we are concerned mainly with "ionizing radiations". As the name implies, these radiations ionize the matter through which they pass. Since the outer electron shell of atoms determines the properties of atoms and of molecules they constitute, the disturbance of the outermost electrons can result in changes in the properties of the media through which the ionizing radiations pass. Among the corpuscular radiations, electrons and $\alpha$-particles can cause ionization. Neutrons which are uncharged, produce ionization by transferring their energy to a hydrogen nucleus, in the form of kinetic energy. The result is a moving particle, viz., a proton, which is capable of ionizing the molecules of the medium. The ionization by protons is probably the most important mechanism by which biological effects are produced by neutrons.

The intensity of the radiation is measured in units of 'rads'. One rad is that amount of ionizing radiation, which causes the absorption of one hundred ergs of energy for every gram of irradiated matter. However, the practical unit for comparing different ionizing radiations and the biological effects they produce, is the 'linear energy transfer' or 'LET'. LET is the average energy transferred by the radiation per unit length of its track. It is usually expressed in units of Kev/$\mu$m, i.e., thousands of electron volts per micron of the path length. In Table 1.1, the LET values for some radiations have been compared. The quantity LET thus provides information about the relative efficiencies of radiations and is, therefore, useful in understanding the mechanism of action.
### TABLE 1.1

<table>
<thead>
<tr>
<th>Radiation</th>
<th>LET (keV/µm)</th>
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<tr>
<td>250 keV X-ray</td>
<td>3.0</td>
</tr>
<tr>
<td>3MeV X-ray</td>
<td>0.3</td>
</tr>
<tr>
<td>60 Co γ -radiation</td>
<td>0.3</td>
</tr>
<tr>
<td>Recoil proton from fission</td>
<td>45.0</td>
</tr>
<tr>
<td>5.3 MeV α -radiation from polonium</td>
<td>110.0</td>
</tr>
<tr>
<td>Fission fragment</td>
<td>4000-9000</td>
</tr>
</tbody>
</table>
of ionizing radiations. The sources of radiation on earth are varied in nature. Biological systems get exposed to radiation under different circumstances. With the increased use of atomic energy, with the use of radiation for medical diagnosis and therapy, the development of radioactive materials, radioactive fall-outs etc., the potential hazards to living systems by radiation have increased a great deal. With the rapid advances in space technology, radiation in space has become an important factor. The space vehicles on leaving the earth’s atmosphere are prone to lethal radiation, emanating from a variety of sources, the main source being the sun. The temperature of the sun varies from about 6000°C on the surface to \((2 \times 10^7)\)°C near the centre and therefore a continuous range of radiation from the short wavelength X-ray to the long wavelength radiowaves emanates from different depths in the sun. In addition, the sun-spots which are often found on the sun’s surface, are also known to emit short wavelength radiation and particles. Van-Allen belts, solar winds and solar flares are sources of electrically charged particles. They arise from the escape of electrons and protons from the sun’s corona. These electrically charged particles move very fast and reach the earth through the solar system. Powerful magnetic fields are also associated with these particles. In addition to the electrons and protons, cosmic rays are also released from solar flares.

1.2 **Effect of radiation**

(i) *The direct and indirect action theories*

The effect of radiation on chemical or biological systems could be of the direct or the indirect type. In the former category, a molecule is ionized by
receiving energy directly from the incident radiation. In the early days of radiobiology, only the direct effect of radiation was considered and it led to the formulation of the 'target theory' of radiation effect. According to this theory, radiation hits the vital site like a bullet and inactivates it i.e., radiation affects only specific target areas of the medium exposed to radiation. In the second category, viz., the indirect action, a molecule receives energy by transfer of energy from another molecule that has initially been affected by radiation. The indirect effect of radiation is relevant and important in systems involving aqueous media where the ionized water molecules act as intermediates transferring the acquired energy to another molecule. As most of the biological systems consist of large aqueous regions, the indirect action theory was found to be more suitable for studying the radiation effects. Thus the 'target theory' or the direct action theory was superseded by the indirect action theory of radiation effect. It is appropriate now to consider the effect of radiation on some specific systems.

(ii) Radiation effect on water and aqueous media

The radiation breakdown of water is known as radiolysis. The generally accepted reaction in the radiolysis of water is

\[ \text{H}_2\text{O} \rightarrow \text{H}^0 + \text{OH}^0 \]

(The symbol \( ^0 \) denotes a radical). The steps involved in the formation of these radicals are as follows:

\[ \text{H}_2 \rightarrow (\text{H}_2\text{O})^+ + e^- \]
i.e., an electron is ejected from the water molecule and is picked up by another water molecule, i.e.,
\[ \text{e}^- + \text{H}_2\text{O} \rightarrow (\text{H}_2\text{O})^- \]
The ions $(\text{H}_2\text{O})^+$ and $(\text{H}_2\text{O})^-$ can combine again to produce the radicals $\text{H}^0$ and $\text{OH}^0$, i.e.,
\[ (\text{H}_2\text{O})^+ \text{ with water} \rightarrow \text{H}^+ + \text{OH}^0 \]
\[ (\text{H}_2\text{O})^- \text{ with water} \rightarrow \text{OH}^- + \text{H}^0 \]
The ions $\text{H}^+$ and $\text{OH}^-$ do not contain large energy and they recombine to form water. However, the radicals $\text{H}^0$ and $\text{OH}^0$ are highly reactive. Two radicals of $\text{OH}^0$ can combine to produce $\text{H}_2\text{O}_2$, hydrogen peroxide. The $\text{H}^0$ and $\text{OH}^0$ radicals can further react with $\text{H}_2\text{O}_2$ to produce water, i.e.
\[ \text{H}^0 + \text{H}_2\text{O}_2 \rightarrow \text{OH}^0 + \text{H}_2\text{O} \text{ and} \]
\[ \text{OH}^0 + \text{H}_2\text{O}_2 \rightarrow \text{H}^0 + \text{H}_2\text{O} \]
Therefore, there is a higher production of the $\text{H}^0$ and $\text{OH}^0$ radicals compared to $\text{H}_2\text{O}_2$. When oxygen is present in the system, peroxy radicals $(\text{HO}_2)^0$ are also produced as follows:
\[ \text{H}^0 + \text{O}_2 \rightarrow (\text{HO}_2)^0 \]
Two of the $(\text{HO}_2)^0$ radicals can again react to form hydrogen peroxide as
\[ (\text{HO}_2)^0 + (\text{HO}_2)^0 \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]
The net effect of radiolysis of water is thus the production of the radicals $\text{H}^0$, $\text{OH}^0$ and hydrogen peroxide.
As mentioned in the beginning of this section, the effect of radiation on systems with large aqueous media is likely to be of the indirect nature i.e., through the components formed during the radiolysis of water. The \( \text{H}^0 \) radical is a powerful reducing agent i.e., it readily gives up its unpaired electron. The radical \( \text{OH}^0 \) is a powerful oxidizing agent i.e., it readily accepts an electron from another molecule to pair with its unpaired electron. The \( \text{H}_2\text{O}_2 \) molecule is also a powerful oxidizing agent. A typical reaction due to an oxidizing agent can be depicted as

\[
\text{RH} + \text{OH}^0 \rightarrow \text{R}^0 + \text{H}_2\text{O}
\]

where, \( \text{RH} \) is an organic molecule and is changed to the radical \( \text{R}^0 \). The \( \text{R}^0 \) type of radicals could be involved in a variety of reactions. For example, two types of radicals \( \text{R}^0 \) and \( \text{S}^0 \), produced from the same system by irradiation, can combine to produce a new type of molecular species i.e.,

\[
\text{R}^0 + \text{S}^0 \rightarrow \text{RS}
\]

Also \( \text{R}^0 \) can combine with oxygen to produce a radical of the type

\[
\text{R}^0 + \text{O}_2 \rightarrow \text{RO}_2^0
\]

which in turn can pick up a hydrogen atom as

\[
\text{RO}_2^0 + \text{R'}\text{H} \rightarrow \text{RO}_2\text{H} + \text{R'}^0
\]

to produce a resultant new molecule \( \text{RO}_2\text{H} \) and a radical \( \text{R'}^0 \). The net effect of radiation on an aqueous medium is therefore the production of chemically different molecules and radicals with properties different from that of the parent molecule.
(iii) **Specificity of some chemical groups to ionizing radiation**

Certain chemical groups show specific sensitivity to ionizing radiations. In particular, the -SH and the -SS groups have been found $^7,8,9$ to be more vulnerable to the effects of radiation. Barron and Flood $^7$ have shown that enzymes requiring -SH groups for their activity were readily inhibited by ionizing radiation. The oxidation of the -SH groups by the ionizing radiation led to subsequent inactivation of the enzymes. From a study of simple as well as complex organic molecules, it has been found $^{10,11,12,13}$ that the disulphide groups are specially sensitive to the attack of free radicals. Irradiation of the sulphur groups results in oxidation products like sulphinic and sulphonic acids. Shapiro and Eldjarn $^{14}$ have reported the formation of sulphonic and sulphinic acids by irradiation of solution of cystamine, which contains a disulphide group.

Chemical groups other than the -SH and the -SS groups are also sensitive to radiation, however, on a lower order of magnitude.

(iv) **Radiation effect on macromolecules**

Due to their large size, the effect of radiation on macromolecules could be quite varied and complex in nature. The radiation could produce chemical or structural changes which could in turn affect the functional role of the macromolecule in the system. In this section, some effects of ionizing radiation on proteins and nucleic acids are described.
(a) **Effect of radiation on Proteins**

Simple proteins are made up of chains of amino acids. The general formula for an amino acid could be represented as

\[
\begin{align*}
\text{R} & \\
\text{NH}_2 & - \text{CH} - \text{COOH}
\end{align*}
\]

where R represents the side chain which is different for different amino acids.

In a protein, the sequence of amino acids represents its primary structure. The adjacent amino acids are linked up by the formation of a peptide bond which is obtained by the interaction between the amino group of an amino acid and the carboxyl group of a neighbouring amino acid, with the loss of a water molecule as shown in Fig. 1.1. The proteins are characterised by a secondary and tertiary structure also and these are obtained by the coiling of the peptide chains and the bending or twisting of the coiled chains in the form of a three dimensional configuration. The three dimensional arrangement of the protein molecule is stabilized by secondary bonding like the disulphide linkages, hydrogen bonds and non-bonded molecular interactions between the side chains. The disulphide bonds are formed by interaction between the thiol groups of two cysteine units. Proteins being polypeptide chains, it might appear that the effect of radiation on proteins and on amino acids would be the same. However, the effect of radiation on an isolated amino acid is different from the effect on the same amino acid when it is part of a larger macro molecule. In an isolated amino acid, the amino group is known to be more sensitive to radiation. However, in a polypeptide, since the amino group and the carboxyl
Fig. 1.1 Formation of peptide chain from amino acids.
groups are involved in the formation of the peptide bond, they are no more available for the radiation. Also, peptide bonds, being strong in nature, are not easily affected by radiation. Therefore, the primary structure of a protein is seldom disrupted by irradiation. The side chains of the amino acids forming the polypeptide chain are, however, more prone to the radiation effects. Depending on the chemical characteristics of the side chain groups, specific effects like removal of a hydrogen atom, breaking of bonds etc., take place.

As the interactions between the side chains play a significant role in determining the secondary and tertiary structure of the molecule, whenever the chemical structure of the side chain is disturbed, the non bonded interactions between the side chains are affected. The hydrogen bonds, being weak in nature, are also easily disrupted by radiation. In addition, the -S-S-linkages are also most easily affected by radiation. Therefore, in effect, the three dimensional structure of the protein molecule is highly prone to be disturbed by the radiation induced effects. The changes in the three dimensional structure could be so large as to cause even unfolding of the polypeptide chain.

(b) Effect of radiation on nucleic acids

Nucleic acids are carriers of genetic information. They are composed of purine (adenine and guanine) and pyrimidine (cytosine, uracil and thymine) bases. The nucleic acids are aggregates of a purine/pyrimidine base, sugar and phosphoric acid i.e., as analogous to the amino acid in a peptide chain, the component unit in a nucleic acid is Base-Sugar-Phosphate, which is known as
a nucleotide. The linkage between adjacent nucleotides is in a step-wise pattern as shown below:

```
Base  ---  Sugar
      |     Phosphate
Base  ---  Sugar
      |     Phosphate
```

Depending on the characteristic of sugar and on the presence of uracil in the place of thymine, there are two general types of nucleic acids viz., ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The double helical structure of DNA as given by Crick and Watson, can be described as two intertwined polynucleotide chains, helically arranged about a common axis and held together by hydrogen bonds between paired purine and pyrimidine bases. A portion of the double helix showing the base pairing is shown in Fig. 1.2. Before considering the radiation effects on DNA, it is appropriate to mention the known radiation effects on the constituents of the nucleic acid, viz., the bases and some mononucleotide systems.

Both purine and pyrimidine bases are sensitive to radiation. It has been shown that under the influence of ultra-violet radiation, uracil gets converted to hydroxy uracil in an aqueous medium. When thymine is irradiated by ultra-violet radiation, formation of thymine dimer has been observed. Deamination of bases due to irradiation and the consequent increase in the level of
Fig. 1.2. A Representation of the Double Helix Structure of a DNA Molecule.

A, T, G, and C represent adenine, thymine, guanine, and cytosine, respectively.
ammonia has also been observed.\textsuperscript{19, 21, 22} Scholes and Weiss\textsuperscript{19} have made extensive investigations on the yield of ammonia from adenine, guanine and uracil, and their results are tabulated in Table 1.2. Slight yield of ammonia\textsuperscript{19} from irradiated thymine was observed whereas, from cytosine, the yield was more. Purine bases yielded small amount of oxalic acid\textsuperscript{19} which was isolated as a calcium salt. Guanine degraded with the formation of guanidine which was isolated as picrate. Table 1.2 lists the details on the formation of oxalic acid and guanidine. Irradiation is known to lead to the opening of the ring structure in bases.\textsuperscript{20} The deamination and the ring opening have profound bearing on the formation of hydrogen bonds by the bases.

Some investigations have been carried out on the effect of radiation on nucleotides. Raleigh, Greenstock, Kremers and Whitehouse\textsuperscript{23} have shown that in mononucleotides, irradiation produces cleavage of the 3' bond. The work of Raleigh et al\textsuperscript{17, 24} has shown that the 3' -nucleotide bonds are two to three times more labile i.e., they are prone to undergo displacement or change in nature, than the 5' bond i.e., the 3' and 5' phosphate bonds in mononucleotides are not equivalent with respect to radiation - induced breakage.

The effect of radiation on DNA has been the subject of a large number of studies. There is ample evidence to show that DNA is the site of primary radiation damage.\textsuperscript{25, 26} As DNA is the carrier of genetic code, any change in the sequence or chemical structure of DNA leads to very crucial damages to biological
<table>
<thead>
<tr>
<th>Bases</th>
<th>pH</th>
<th>Dosage x 10^-6</th>
<th>Ammonia formed x 10^4 /100ml</th>
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</thead>
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<tr>
<td>Adenine</td>
<td>2.0</td>
<td>4</td>
<td>7.74</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>4</td>
<td>5.75</td>
</tr>
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<td></td>
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<td>4</td>
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<td></td>
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<td>8.34</td>
</tr>
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<td></td>
<td>2.75</td>
<td>4</td>
<td>6.5</td>
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<td></td>
<td>4.8</td>
<td>3.2</td>
<td>3.07</td>
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<td>Guanine</td>
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<td>1.03</td>
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<td>4.74</td>
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<tr>
<td>Uracil</td>
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<table>
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<th>Bases</th>
<th>pH</th>
<th>Ammonia x 10^4 /100ml</th>
<th>Oxalic acid x 10^6 /100ml</th>
<th>Guanidine x 10^6 /100ml</th>
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<td>Adenine</td>
<td>1.75</td>
<td>8.34</td>
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<td>Guanine</td>
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systems. The mutations produced by the radiation induced changes in DNA are carried over and reproduced by the replication process. The nucleic acid is more sensitive to radiation damage than proteins. This is due to the fact that nucleic acid absorbs the energy of the electromagnetic radiation more easily than proteins. In particular, in the ultra-violet region of the electromagnetic spectrum, the absorption by the nucleic acid is sixty times more than that by proteins. Radiation effects on DNA could lead to the breakage of nucleotide linkage, breakage of hydrogen bonds, destruction of bases, cross linking of polynucleotide chains etc. Sedimentation data and electron micrographs have shown that the effect of radiation on DNA, is to break it into fragments of variable dimensions. A brief description of the major effects of radiation on DNA is given below.

1. Fragmentation or scission of DNA

Scissions could be either single strand scissions or double strand scissions. Single strand scissions in DNA could be due to the cleavage between a sugar and a phosphate group and is followed by deletions or alterations of bases. Single strand breaks are, however, considered to be less frequent, on account of the rigid nature of the structure of DNA. Also when single strand scissions occur, the broken ends of the single strand do not separate but they tend to rejoin. However, in the presence of oxygen, single strand breaks are known to lead to peroxidation, which would prevent the broken ends from rejoining. This is represented in Fig. 1.3(a).
Fig. 1.3 (a) Single strand break (b) Double strand break and (c) Cross linking in DNA.
Double strand scissions in DNA have been observed more commonly. Whenever two single chain scissions come into juxtaposition or when the two single chain breaks are not more than five nucleotides apart, double strand scissions tend to occur. When double chain scission occurs, the DNA molecule separates into fragments. This is schematically illustrated in Fig. 1.3(b).

(2) Cross-linking

Cross-linking is the formation of new bonds in the chains. When reactive groups are present at the points where the chain breaks, cross linking occurs. This is represented in Fig. 1.3(c). Cross-linking due to radiation could be of different types. It could be within the helix or between two DNA molecules. Within the helix, cross-linking could occur between the bases also. The net effect of cross-linking is to introduce structural changes in DNA.

(3) Breaking of hydrogen bonds

As mentioned in the beginning of this section, the double helical structure of DNA involves the formation of hydrogen bonds between pairs of bases. Hydrogen bonds, being weak in nature could be easily ruptured by radiation. Also, whenever deamination and ring opening of the bases occur due to irradiation, the chemical structure in that part of DNA is affected and the hydrogen bond scheme between base-pairs in that region is no longer possible. Therefore, with a single chemical event like the deamination of a base, several hydrogen bonds could be
broken and several changes could occur in the polynucleotide DNA chain. When sufficient number of hydrogen bonds are broken, the polynucleotide chains get freedom to move apart and the basic double helical structure of DNA is disturbed.

(4) **Breaking of ester linkages**

An increase in the inorganic phosphate, due to the breaking of ester linkages, has been observed in DNA and RNA and, the formation of inorganic phosphate was found to increase with the dosage in both RNA and DNA. Results of the experiments by Scholes and Weiss have been reproduced in Fig. 1.4(a) and 1.4(b) for RNA and DNA respectively. A similar increase in the yield of phosphate with radiation dosage has been observed by Kapp and Smith in DNA.

(5) **Deamination and ring opening of bases**

The yield of ammonia by irradiation of yeast RNA and DNA was studied by Scholes and Weiss. Results of their study up to a dosage of 30,000 r, under different conditions like in air, in the presence of oxygen, in the presence of hydrogen and in vacuum, have been reproduced in Fig. 1.5(a) and 1.5(b). The yield of ammonia was found to increase with the radiation. The yield was found to be highest in the presence of oxygen and lowest in the presence of hydrogen. Experiments on the sodium salt of thymo DNA and RNA, by Scholes, Stein and Weiss, Butler and Smith, Sparrow and Rosenfeld, Scholes and Weiss.
Formation of inorganic phosphate by irradiation of aqueous solutions of RNA (BY 3) with 200 kv X-rays. RNA soln., 0.05% w/v; pH ~ 5. Phosphate determined by method of Lowry & Lopez (1946). ○, in oxygen (1 atm.); ○, in air; ○, in vacuo; ○, in hydrogen (1 atm.).

Fig. 1.6 (b). Formation of inorganic phosphate by irradiation of aqueous solutions of DNA (Signer A) with 200 kv X-rays. DNA soln., 0.05% w/v; pH ~ 6.5. Phosphate determined by method of Lowry & Lopez (1946). ○, in air; ○, in vacuo; ○, in hydrogen (1 atm.).
Fig. 1.5(a). Formation of ammonia by irradiation of aqueous solutions of RNA (BY3) with 200 kv X-rays. RNA soln., 0·05%, w/v; pH~5. ○, in oxygen (1 atm.); ○, in air; ×, in vacuo; ●, in hydrogen (1 atm.).

Fig. 1.5(b). Formation of ammonia by irradiation of aqueous solutions of DNA (Signer A) with 200 kv X-rays. DNA soln., 0·05%, w/v; pH~6·5. ○, in oxygen (1 atm.); ○, in air; ×, in vacuo; ●, in hydrogen (1 atm.).
and Taylor, Greenstein and Hollander, gave ample evidence that the hydrogen bonds were broken by the deamination process and for the consequent ring opening of the bases.

(6) Formation of malonic aldehyde

Formation of malonic aldehyde due to irradiation of bacterial DNA has been observed by Kapp and Smith. The curve describing the relation between the formation of malonic aldehyde and radiation dosage is given in Fig. 1.6. The formation of malonic aldehyde is due to the breakage of C(3′) - C(4′) bond in deoxyribose. Scholes and Weiss, and Kapp and Smith have correlated the formation of malonic aldehyde with the denaturation of DNA, due to exposure to X-rays.

Formation of strand break at different stages of reproduction cycle has been investigated and it was found that the number of breaks produced were the same throughout the cell cycle.

The radiation-induced effects on DNA have been detected by physical and chemical methods. The fragmentation of DNA into small bits has often been detected by sedimentation method and by the use of electron micrographs. Sedimentation experiments are based on changes in molecular weight. Changes in the molecular weight of DNA have been observed by Studier. Scholes and Weiss have observed a decrease in the molecular weight of DNA due to
Fig. 1.6. Deposition of malonic aldehyde on irradiation of DNA.
Irradiation. They have correlated the change in molecular weight and the number of breaks, \((n)\), by the relation

\[
n = N \left( \frac{1}{M_T} - \frac{1}{M_0} \right)
\]

where \(N\) = Avogadro number

\(M_T\) = Molecular weight of DNA on irradiation

\(M_0\) = Molecular weight of DNA without irradiation

The number of chain breaks per strand of DNA was found to increase with dosage.

Results of the experiments made by Kapp and Smith\(^{32}\) are given in Fig. 1.7.

Similar results have been obtained by Lett, Caldwell, Dean and Alexander\(^{39}\), Brustad and Rup\(^{40}\), Corry and Cole\(^{11}\) and, Greenstein and Hollander\(^{12}\), using DNA's of different origins. Freifelder\(^{12}\) has studied the rate of production of single strand breaks. He has observed that 7.5 single strand breaks occur with every lethal hit of radiation. He has also shown that the major lethal event in DNA is due to double strand break. He further showed that the isolated DNA is fifteen times more sensitive to single strand break than DNA in situ.

When structural changes are produced in the polynucleotide chains, a change in the viscosity of the molecule could be expected. It has been observed\(^{18,34,35,36}\) that with irradiation, the viscosity of DNA decreased.

It was shown that changes produced were due to the chemical reactions effected by the free radicals. Degradation of the polynucleotide, due to the attack by free
radicals, was also confirmed by Scholes and Weiss, who measured the viscosity of DNA. The marked decrease in the structural viscosity was attributed to a loss of hydrogen bonding and liberation of amino groups from the bases, adenine, guanine and cytosine. Decrease of viscosity with doses of radiation has been studied in detail by Sparrow and Rosenfeld, using the sodium salt of nucleic acid i.e., sodium nucleate solution. The variation of viscosity with dosage, as observed by them, has been reproduced in Fig. 1.8. A sharp decrease in viscosity with radiation has been obtained. It has been found that 10,000 r of radiation dose would halve the viscosity in polynucleotide chains.

As is well-known, birefringence of DNA is due to the helical nature of the structure. When the helical structure is disturbed, changes in birefringence could be anticipated. Loss of streaming birefringence due to irradiation has been observed in DNA.

Great deal of work has been done on radiation induced effects on several enzymes, carbohydrates, lipids, unicellular systems like bacteria, yeasts, isolated cells of higher organisms etc. Details of such investigations are, however, not included here.
Fig. 1.8. Variation of relative viscosity of sodium nucleate with irradiation.
1.3 Chemical radioprotection

Since the 1940's a great deal of attention is being paid to the method of protection of mammals against radiation by chemical means. In 1942 it was first shown by Dale that, by administering some chemicals before exposure to radiation, the effect of radiation could be reduced. Chemicals used to produce this effect are known as 'radioprotectants'. The radioprotectants were found to be effective only when they were administered prior to the exposure i.e., they must be present in the system before the exposure to radiation. This feature indicates that the chemical radioprotectants influence the initial events in the radiation damage. The radioprotectants do not completely alleviate the effect of radiation but they decrease the effect of radiation and prolong the survival rate of the exposed system. In other words, the radioprotectants reduce the effective radiation dose received by the system. For example, when rats were treated with the maximum tolerated amount of the radioprotectant cysteine, it was found that cysteine reduced the radiation damage by 40 percent, i.e., when they have received, say, 1000 rads of radiation, they would show the response of the rats that have received only 600 rads, without cysteine. An important characteristic of the radioprotectants is that the effect of the chemical is not general; its radioprotective effect depends on the nature of the system on which it is administered. A chemical which is very effective with one system may only be partially effective or totally ineffective with another system in the
same animal. This is a consequence of the specific chemical properties of the compounds and the differences in the physiological properties of the various types of tissues in the animal. These differences lead to different types of distribution of the chemical within the same animal and therefore, the effectiveness of the radioprotectant also varies.

The effectiveness of a chemical is quantitatively described by the 'dose reduction factor', which is expressed as the ratio of the $\text{LD}_{50(30)}$ for the protected animal to that for the unprotected animal. The $\text{LD}_{50(30)}$ (lethal dose to 50 percent in 30 days) is the dose of radiation which will kill about 50 percent of the exposed organism within 30 days after exposure. The dose reduction factor is, therefore, the ratio between equieffective doses of radiation in the presence and absence of the protective agent.

A large number of compounds have been tested for radioprotective action and, a collective account of the results has been compiled by Tseunov, Vasilev and Paribok. In Table 1.3, a few representative examples of various types of radioprotectants have been listed and, a brief description of some salient features of each class of radioprotective compound is given in what follows in this chapter.

(i) **Thiols and thiol derivatives**

The thiols, especially the low molecular weight aminothiols and their
**TABLE 1.3**

*Some radioprotective compounds*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Protective effect*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine</td>
<td>3</td>
</tr>
<tr>
<td>Cysteamine</td>
<td>3</td>
</tr>
<tr>
<td>Cystamine</td>
<td>3</td>
</tr>
<tr>
<td>N-Dimethylcysteamine</td>
<td>2</td>
</tr>
<tr>
<td>N, N'- Tetra methyl cysteamine</td>
<td>2</td>
</tr>
<tr>
<td>N-Diethyl cysteamine</td>
<td>2</td>
</tr>
<tr>
<td>Cysteamine-N-acetic acid</td>
<td>2</td>
</tr>
<tr>
<td>N-Phenethyl cysteamine</td>
<td>3</td>
</tr>
<tr>
<td>3-Mercaptopropylamine</td>
<td>3</td>
</tr>
<tr>
<td>3-Mercaptopropyl guanidine</td>
<td>3</td>
</tr>
<tr>
<td>4-Mercaptobutylamine</td>
<td>2</td>
</tr>
<tr>
<td>N-Acetylcysteamine</td>
<td>1</td>
</tr>
<tr>
<td>S-Acetylcysteamine</td>
<td>3</td>
</tr>
<tr>
<td>S-Benzoylcysteamine</td>
<td>1</td>
</tr>
<tr>
<td>N-β-Allylcysteamine</td>
<td>1</td>
</tr>
<tr>
<td>Dithiouracil</td>
<td>1</td>
</tr>
<tr>
<td>2-Aminothiazoline</td>
<td>3</td>
</tr>
<tr>
<td>2-Mercaptothiazoline</td>
<td>1</td>
</tr>
<tr>
<td>2-Benzothiazolethiol</td>
<td>1</td>
</tr>
<tr>
<td>Compound</td>
<td>Protective effect*</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>2,3-Dimercaptopropane-1 sulfonic acid</td>
<td>2</td>
</tr>
<tr>
<td>2-Aminoethyl 2-aminoethane thiol sulphonate</td>
<td>3</td>
</tr>
<tr>
<td>2-Guanidino ethyl-2-guanidinoethane thiol sulphonate</td>
<td>2</td>
</tr>
<tr>
<td>2-(2-Aminoethylidithiobenzene sulphonic acid</td>
<td>3</td>
</tr>
<tr>
<td>S-2, Aminoethyl isothiouronium bromide hydrobromide</td>
<td>3</td>
</tr>
<tr>
<td>S,2-Aminoethyl-N-methyl isothiuronium chloride HCl</td>
<td>2</td>
</tr>
<tr>
<td>β-Mercaptoethyl guanidine</td>
<td>3</td>
</tr>
<tr>
<td>bis guanidinoethyl disulphide</td>
<td>3</td>
</tr>
<tr>
<td>S-3-Aminopropyl isothiuronium bromide</td>
<td>3</td>
</tr>
<tr>
<td>2-Guanidinoethyl trithio carbonate</td>
<td>3</td>
</tr>
<tr>
<td>S, 2-guanidino ethyl phosphorothioate</td>
<td>3</td>
</tr>
<tr>
<td>2-Amino-1-Pentanethiol</td>
<td>3</td>
</tr>
<tr>
<td>1-Amino-1-Propanethiol</td>
<td>3</td>
</tr>
<tr>
<td>Homocysteine</td>
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</tr>
<tr>
<td>Homocysteine thiolactone</td>
<td>2</td>
</tr>
<tr>
<td>2-Mercaptoethylamino propane sulphonic acid</td>
<td>3</td>
</tr>
<tr>
<td>Glutathione</td>
<td>3</td>
</tr>
<tr>
<td>N, N'-Bis (mercaptoacetyl) hydrazine</td>
<td>3</td>
</tr>
<tr>
<td>Ammonium dithiocarbamate</td>
<td>3</td>
</tr>
<tr>
<td>Ethylene-bis (N, N'-dimethyl dithiocarbamate)</td>
<td>2</td>
</tr>
<tr>
<td>Compound</td>
<td>Protective effect*</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Diethyl dithiocarbamate</td>
<td>3</td>
</tr>
<tr>
<td>2-piperazino ethyl dithiocarbamic acid</td>
<td>2</td>
</tr>
<tr>
<td>Thiourea</td>
<td>2</td>
</tr>
<tr>
<td>Histamine</td>
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</tr>
<tr>
<td>Tryptamine</td>
<td>3</td>
</tr>
<tr>
<td>Serotonin</td>
<td>3</td>
</tr>
<tr>
<td>Serotonin creatinine sulphate</td>
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</tr>
<tr>
<td>Tyramine</td>
<td>3</td>
</tr>
<tr>
<td>Epinephrine</td>
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</tr>
<tr>
<td>Reserpine</td>
<td>3</td>
</tr>
<tr>
<td>Sodium cyanide</td>
<td>2</td>
</tr>
<tr>
<td>Malononitrile</td>
<td>3</td>
</tr>
<tr>
<td>Hydroxy acetonitrile</td>
<td>1</td>
</tr>
<tr>
<td>2-Cyano-3, 3-acrylonitrile dithiol, zinc salt</td>
<td>1</td>
</tr>
<tr>
<td>β-aminopropiophenone (PAPP)</td>
<td>3</td>
</tr>
<tr>
<td>Selenocysteine</td>
<td>2</td>
</tr>
<tr>
<td>Seleniumurea</td>
<td>2</td>
</tr>
<tr>
<td>Glycerol</td>
<td>2</td>
</tr>
<tr>
<td>Fructose</td>
<td>2</td>
</tr>
<tr>
<td>Glucose</td>
<td>1</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>3</td>
</tr>
<tr>
<td>Formic acid</td>
<td>2</td>
</tr>
<tr>
<td>Pyruvic acid</td>
<td>1</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>2</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>1</td>
</tr>
</tbody>
</table>

*Protective effect: Grading of the protective effect is according to an arbitrary scale

1 = Slight protective effect

2 = Moderate protective effect

3 = Strong protective effect
derivatives, have been the most extensively studied group of compounds. (The
term 'thiol' refers to the -SH group. The term 'mercapto' or 'sulphhydryl' also
refers to the same group). As the name implies, this aminothiol group of
compounds have an -SH and -NH$_2$ groups, which are either free or unstably
bound to the molecule. Specific examples of this group of compounds are
cysteine$^{47}$, $\beta$-mercaptoethylamine (MEA)$^{49}$, S-aminoethylisothiuronium
bromide hydrobromide (AET Br.HBr)$^{50}$ etc. The general formula for this class
of compounds can be represented as in Fig.1.9.

In this formula, $x \leq 3$, $R_1$ and $R_2$ are either hydrogen atoms or
alkyl residues. It has been found that the aminothiols possess high degree of
structural specificity with regard to their radioprotective property. For example,
the compounds cysteine and isocysteine have the same chemical formula but
different structural arrangement of atoms. This is shown in Fig.1.10. Cysteine
is a very effective radioprotectant whereas, isocysteine does not offer any
protection against radiation. From vast researches, some important conclusions
relating the chemical structure of this class of compounds have emerged and they
are as follows:

(a) As mentioned earlier, the sulphur atom in the radioprotectant should be
included as a free -SH group. The radioprotective property of some compounds
$^{49,51,52,53}$ where there is no -SH group has been shown to be due to the conversion
of the compound, after administration to a form with a free -SH group. The
Fig. 1.9 The general formula for the thiol group of compounds.
HS - CH$_2$ - CH - COOH
   \( \text{NH}_2 \)

Cysteine

H$_2$N - CH$_2$ - CH - COOH
   \( \text{SH} \)

Isocysteine

Fig. 1.10 Structural formulae
conversion could be by hydrolysis, reduction, rearrangement or by even some metabolic action. For example, cystamine is reduced to cysteamine in vivo. Both cystamine and cysteamine (MEA) are radioprotective compounds. Chemical formulae for some radioprotectants have been represented in Fig. 1.11. From the figure, it is obvious that there is no -SH group in the molecule of AET, a well-known aminothiol radioprotectant. It has been shown by Shapira et al. that, when administered, AET rearranges itself into S, 2-mercaptopethyl guanidine (MEG) (Fig. 1.11) which is its active form and which has a terminal reactive -SH group in the structure. Similar observations have been made on the compounds 2-homo-cysteine thiolactone and S-acetyl cysteamine, where a -SH group is not available.

Alkylation of the sulphur atom has been found to remove the activity of the aminothiol group of compounds. On the other hand, acylation has been found to provide some active compounds.

In addition to the thiols, some of their oxidized derivatives have also been found to be active. For example, cystamine which is obtained from cysteamine and bisguanidinoethyl disulphide (GED), which is obtained from \( \beta \)-mercaptoethyl guanidine (MEG), (Fig. 1.11) are known to provide the same order of protection as the parent thiols themselves. Thus the presence of an unblocked thiol group or a thiol derivative that can be converted to a free thiol by hydro-
Fig. 1.11 Some of the radioprotective chemicals and their structural formulas
lysis, reduction, rearrangement or metabolic action appears to be an essential criterion for radiation protection.

(b) The presence of an amino group (in vivo) is another characteristic feature of the aminothiol group of compounds. The presence of an amino group enables the formation of hydrogen bonds with the biomolecules of the system to which the radioprotective compound is administered. The replacement of the amino group by another basic group, like guanidine, has been found to increase the radioprotective activity of the compound. For example, when the amino group in MEA is replaced by a guanidine group, MEG (Fig. 1.11) is obtained. MEG is known to be more active than MEA. Similarly, 3-mercaptopropyl guanidine (Fig. 1.11), is known to be more active than the corresponding amine. Replacement of the amino group by an amidino group is also known to provide radioprotective compounds. Acylation or arylation of the amino nitrogen atom has been found to abolish the activity of the compound.

(c) The number of carbon atoms or the length of the carbon chain between the amino and the thiol group, has been found to have profound influence on the protective property of the chemical. It has been found that the amino and the thiol groups should not be separated by more than two or three carbon atoms. An increase in the length or any branching of the carbon chain reduces the activity. Results of the experiments, on MEA and its propyl, butyl, pentyl and hexyl homologues by Gredan and Copeland, have proved that the length of carbon atoms
should not be more than three carbon atoms. A graph relating the survival
rate of mice and the days of irradiation has been obtained by Grenan and Copeland and is given in Fig. 1.12. It can be seen that the survival points of C$_3$ to C$_6$
compounds scatter about the control survival curve. Increasing the carbon length
of MEA (x=2) (Fig. 1.9) to three carbons, gave a compound β-mercaptopropy-
amine (MPA) which was twice as active as MEA. However, further increase in the
carbon chain length to x=4, gave a compound mercaptobutylamine,(MEA), whose
activity was found to be 1/5 that of MEA. It can be concluded from these experi-
ments that x has to be two or three to obtain the maximum protection.

Similar results have been obtained by increasing the carbon chain
length in AET Br.HBr (Fig. 1.13). When the number of -CH$_2$ groups is increased
to three, i.e., in S, 3-aminopropyl isothiuronium bromide hydrobromide (APT Br.
HBr) (Fig. 1.13), the compound was found to be as effective as AET Br.HBr.

However, in d-and 1 forms of s-2-aminobutylisothiuronium bromide hydrobromide,
(d-and 1 ABT Br.HBr), where there are four -CH$_2$ groups, no radioprotective effect
was observed. $S_3$-aminopropyl-$N'$-methylisothiuronium bromide hydrobromide,
(APMT Br.HBr), which was obtained by the addition of a methyl group to APT Br.
HBr was found to be radioprotective, though not as effective as AET. However,
the relative absence of the pharmacological side effects and the low toxicity
render APMT Br.HBr more suitable for future use in humans.
Effect of C₁–C₆ aminothiols on mouse survival after 875 rads gamma-irradiation. Mice were injected with 0.7 mM/kg of the aminothiols 15 minutes prior to $^{60}$Co gamma-irradiation. Daily mortality checks were made. Control (saline) (--); C₂ (MEA) ---; C₃ (○); C₄ (▲); C₅ (■); C₆ (●); and C₇, ignoring acute combined toxicity (◇).
Fig. 1.13  AET and related radioprotective compounds
(d) The presence of carboxylic group in addition to the amino group in the molecule has been found to reduce the protective capacity of the molecule. For example, cysteine in which a carboxylic group is present is less active compared to cysteamine where no carboxylic group exists.

(e) The effect of inclusion of methyl groups has been investigated by Loman, Voogd and Block. The radioprotectant MEG and its methyl derivatives viz., N-methyl MEG, N'-methyl MEG and N', N'-di-methyl MEG were studied and it was found that they offered the same amount of radioprotection as the parent compound MEG. However, derivatives of MEG having three methyl groups at N, N' and N'', were found to be toxic and no more protective.

(ii) Miscellaneous sulphur containing compounds

Apart from the aminothiols and their derivatives described in group (i) a large number of compounds containing sulphur atom have been found to offer radioprotection. The effect of thiourea and thiosulphates on mammals is known since the early days of the discovery of chemical radiation-protection. However, the extent of protection is very small and therefore, these compounds are less important than the cysteine, cysteamine derivatives.

A large number of dithiocarbamates have been found to provide radiation protection to mammals. The thiocarbamates are again less effective than the cysteamine derivatives. Dithio acids are also known to be radioprotective.
Several thiazoles, thiazolines and thiazolidines have been studied. Among these, the compound which offers significant radioprotection is 2-aminothiazoline (2-AT). As in the case of the aminothiol AET, 2-AT under physiological conditions is known to be converted into an open-chain compound with a free -SH group. Similar opening of the ring has been reported in the radioprotective thiazolidines also. The thiazolidines then rearrange to form aminothiols.

Among the other sulphur containing compounds, mention may be made of sodium sulphide, diammonium amido phosphorothioate, sodium tetra thioate, dimethyl sulphoxide, thio amido diphosphate, thio glycolic acid and glutathione. A large number of thioates have been found to be radioprotective.

(iii) Substances with pronounced pharmacological activity and toxic compounds which inhibit metabolism

A large number of compounds with pronounced pharmacological action have been found to be effective radioprotectants, on account of their ability to lower the oxygen pressure in the tissues, i.e., by introducing tissue hypoxia. Serotonin and reserpine are very effective drugs of this kind. Reserpine is considered to be as effective as cysteamine. Drugs like amphetamine, tryptamine, oxytryptamine, methamphetamine, acetyl choline, epinephrine and tyramine are also radioprotective. 4-aminopropiophenone offers radioprotection by producing a state of anoxia (no access to oxygen). Sodium nitrite.
Aniline and carbon monoxide affect the transport of oxygen by haemoglobin and thus reduce the oxygen pressure. Some estrogens have also been found to protect from radiation.

Some toxic compounds like potassium cyanide, malononitrile, sodium fluoroacetate are known to be radioprotective by virtue of their action on cellular respiration. However, the inherent toxicity of these compounds limits their practical use.

(iv) Metal binding agents

This group of radioprotectants has been formed, based on their typical mode of action involving a metal ion. The dithiocarbamates and aminothiols mentioned in groups (i) and (ii) belong to this group of radioprotectants. Some iron complexes of polyamines and the zinc complexes of MEA and MEG are also known for their radioprotective property.

(v) Amines and amino acids

Unlike the aminothiols, very few amines and aminoacids have been found to have radioprotective properties. Among the amines, methylamine, dimethylamine, trimethylamine, propyldiamine, L-guanidino-5-aminopentane form some examples. Among the amino acids, cysteine and \(\alpha\)-alanine are known to be radioprotective. In general, the amines are known to be more active than the corresponding amino acids.
(vi) **Hydroxylated substances**

Substances like glycerol, propylene glycol, phenols, catecholamine, pyruvic acid, formic acid, esters of gallic acid are some examples of compounds belonging to the group of hydroxylated substances. Glycerol is protective in a number of systems including mammals.

(vii) **Other compounds**

Some polysaccharides, mucopolysaccharides have been found to be radioprotective. Pyridoxal phosphate, which is a coenzyme of the amino acid metabolism, is known to offer moderate protection. It has been found that selenium compounds protect biological systems against ionizing radiation with a greater efficiency than the analogous sulphur containing radioprotectants.

Biologically active substances like selenoamino acids are also found to be radioprotective. Organic substances with aromatic groups strengthen the action of compounds containing selenium. Several nitroaromatic compounds were found to have protective property.

(viii) **Combinations of radioprotectants**

Use of combinations of radioprotective agents has been found to increase the radioprotective effect. Treatment with a mixture of two or more dissimilar radioprotective compounds has been extensively studied by Wang, Kereiakes and Gentz. A combination of 5-hydroxytryptamine, AET
and MEA was found to offer higher protection than any one of the individual compounds. Maisin and co-workers have worked on the various combinations of glutathione, serotonin, cysteine, MEA, AET, histamine, PAPP, dimethyl sulfoxide and cystamine to study their additive ability towards radiation protection. The most effective combination was found to be glutathione, cysteine, AET, MEA and serotonin.

1.4 Mechanisms of radioprotection

In as much as the nature of the primary biological effects of radiation on complex systems like the living body or even a single cell is not completely understood, the exact mechanism of action of chemical radiation-protection on these systems is less completely understood. A number of theories have been proposed to explain the phenomenon of chemical radiation-protection. However, no theory is applicable to all the systems. While attempting to correlate the experimental results with the theory, inadequacies in the various hypotheses are noticed. In this section, some of the well known theories of radiation-protection are described.

(i) Theory of radical scavenging

The 'radical scavenging' hypothesis is based on the indirect action theory of radiation. In section 1.2, it was mentioned that oxidizing and reducing radicals are produced from the radiolysis of water and these radicals cause damage to the system exposed to radiation. According to the 'radical scavenging' theory, the
radioprotectant competes for the radiation-induced free radicals from water, before the radicals enter into any further reaction. As an example, the radical scavenging by the aminothiol radioprotectant, cysteamine, is considered here. The cysteamine molecule, in the zwitterion form (Fig. 1.14) is oxidised by free radicals such as OH obtained by the radiolysis of water. The free radicals are thus prevented from entering any further interaction with the molecules of the system exposed to radiation. By oxidation of cysteamine, resonance stabilized free radicals of cysteamine that are incapable of reacting with cell components are produced. This is depicted in Fig. 1.14. The radicals of cysteamine could further react with another oxidizing free radical, to form an acid or they can regenerate into the original molecule. They could also form a disulphide by reacting with a similar radical.

The spatial relationship between the -NH\textsubscript{2} and the -SH groups in the cysteamine molecule is favourable for the formation of such resonance stabilized structure. At this juncture, it is worth referring to section 1.3(c), where it was mentioned that the number of carbon atoms between the amino and the thiol groups, \(x\) in Fig. 1.9), has a profound influence on the protective property of the compound. In cysteamine, the formation of the resonance stabilized structures is favoured by the fact that the value of \(x\) is 2. With \(x = 2\) or 3, five or six-membered ring structures (resonance stabilized) can be obtained and, it is well known that five or six membered rings are energetically most stable.
Fig. 1.14 Formation of free radical from cysteamine by interaction with $\ddot{\text{OH}}^\circ$
According to the theory proposed by Doherty et al. for the mechanism of action of the aminothiol radioprotectors, an intramolecular S......N interaction is necessary for the radioprotective action. The non-bonded S......N interaction facilitates the formation of resonance stabilized structures of the type mentioned above.

In general, the protective effect due to the free radical scavenging is dependent on the concentration of the radioprotectant in the system and its reactivity towards the products of radiolysis. The aminothiols and sulphur containing protective agents are generally known to react with free radicals. 7, 10, 13

Large number of electron paramagnetic resonance studies have unambiguously established the radical scavenging power of -SH containing radioprotectants. 104-103

The analogous seleno compounds are also known to act as free radical scavengers.

The radical scavenging theory finds support from the results of Greenstock and Chapman 109 who worked on mononucleotide components and -SH containing radioprotectants.

(ii) Theory of mixed disulphide formation

The hypothesis of mixed disulphide formation was first suggested by Phl and Eldjarn. 110-112 According to this theory, radioprotectants with free SH groups form transient mixed disulphides with the thiol groups of proteins or enzymes. In Fig.1.15, an example of mixed disulphide formation by the
Fig. 1.15 Formation of mixed disulphides by cysteamine
radioprotectant cysteamine, is illustrated in a schematic way. In (ii) of figure 1.15, one of the sulphur atoms is from the radioprotectant and the other is from the protein; hence the name 'mixed disulphide'. According to Pihl and Eldjarn, when the protein with the mixed disulphide bond is attacked by a free radical, one of the sulphur atoms is oxidized and the other is reduced. If the sulphur atom from the protective agent is oxidized as in (iii) (b) of Fig. 1.15 and the sulphur atom from the protein is reduced, the protein is left undamaged by the radiation. However, the sulphur atom from the protein has also an equal probability of getting oxidized. In any case, the probability of the sulphur atom from the protein getting damaged is only 50 percent. Therefore, according to this theory, at least 50 percent of the protein could be expected to be left undamaged.

Experiments to verify the theory of mixed disulphide formation have been carried out by Eldjarn and Pihl. They administered cystamine dihydrochloride and MEA to mice and have shown that only a minute fraction of the radioprotectant was left in the free form, thus proving that the radioprotectants were involved in cellular interactions. They further suggest that the thiols form mixed disulphides with the thiol groups of tissue proteins and that the resulting disulphide may protect the protein from free radicals or from direct radiation energy. Eldjarn and Pihl have also shown that the mixed disulphides were formed within a few minutes after the administration of the radioprotective compounds. A relation between the increase of cellular non protein
-SH content and radioresistance was obtained by Laszlo and Bergstrand. Their results suggested that the mechanism of radiation-protection by MEA depends on the concentration of the non-protein -SH level in the cells. Measurements of cellular -SH levels by Laszlo and Bergstrand support the formation of mixed disulphides. The hypothesis of protection by the formation of mixed disulphides is supported by electron paramagnetic resonance studies of Gordy, Ard and Shield.

It may be mentioned that the binding of a protective agent to a protein does not necessarily imply radioprotection. It has been found that when the radioprotective mercaptoethyl guanidine (MEG) bound to urease, it failed to protect urease against radiation. Free GED, on the other hand, was protective. Also when cysteamine bound to aldolase it failed to protect aldolase against radiation inactivation.

(iii) Production of tissue hypoxia

The general observation has been that when the oxygen level is low in the system, the radiation damage is small. Hypoxia, or the state of inadequate availability of oxygen to the tissue, has an important role in decreasing the radiation damage. The simple theory is that reduction in oxygen pressure decreases the possibility of the formation of oxidizing radicals. Thus by introducing certain chemicals, the oxygen pressure could be reduced and the
radiation damage could be correspondingly reduced. The thiol group of radioprotectants have a tendency to get oxidized readily. Therefore when the thiols are present in the system, they get oxidized fast and reduce the oxygen level in the system.

Protective compounds like serotonin, reserpine etc., mentioned in section 1.3, are known to be radioprotective by virtue of their ability to produce tissue hypoxia. Metabolic inhibitors like cyanide, effectively inactivate cytochrome C oxidase which controls the oxygen consumption in mammals. Compounds like 4-aminopropiophenone (PAPP) are radioprotective because of their ability to affect the transport of oxygen by haemoglobin.

(iv) Donation of hydrogen atoms

When a molecule loses a hydrogen atom by either the direct or indirect action of radiation, it could undergo a variety of reactions as shown below:

A macromolecule RH on irradiation forms a free radical R^0.

\[ RH \rightarrow R^0 + H \]

A variety of reactions such as cross linking occurs.

\[ R^0 + R^0 \rightarrow R - R \]

In the presence of oxygen, peroxy radical is formed.

\[ R^0 + O_2 \rightarrow RO_2^0 \]
If a hydrogen atom from a radioprotective compound PH is donated to the radical R\(^{\circ}\) to restore it to the original molecular state RH, the radiation-induced effects are reduced

\[
\text{i.e. } R^{\circ} + PH \rightarrow RH + P^{\circ}
\]

\(P^{\circ}\) is the radical of the radioprotectant. The presence and the amount of radicals are usually detected by electron spin resonance. Comerod and Alexander have shown by using cysteamine in irradiated DNA from sperm nuclei that in the presence of cysteamine there was an increase in the cysteamine radicals, thus confirming that the radioprotectants donated hydrogen atoms to the DNA molecules.

Transfer of hydrogen atom from -SH compounds to simple organic free radicals has been observed directly by pulse radiolysis. Repair action by the radioprotectant cystamine, by hydrogen atom donation was reported by Adams, Naughton and Michael. Johansen, Howard and Flander have studied the radiation-protection of bacteria and mammalian cells by -SH containing compounds and they suggest that the transfer of hydrogen atom from the -SH group is responsible for radiation-protection. Evidence was obtained by Sanner and Pihl that in frozen bacteria, hydrogen transfer played a significant role in radiation-protection. Loman et al found that in the case of cysteine, protection was offered by hydrogen atom transfer from the -SH group to the organic radical.
(v) **Transfer of energy**

The hypothesis of transfer of energy is based on the formation of a disulphide bond. The disulphide bond, as mentioned in section 1.2, is very sensitive to oxidation. Therefore, energy is likely to be absorbed at that site and the absorbed energy could either be transferred along the adjacent carbon atoms in the chain or the energy could even split off the -SS- link. When the disulphide bond is of the mixed type, with the rupture of the -SS- bond, the radioprotectant is split from the protein. The radioprotective chemical subsequently gets oxidized and the radiation energy is eliminated.

(vi) **Binding or chelating to the metal ions**

Another way of interaction of the radioprotectants is by binding or chelating to the metal ions. It has been observed that inhibition of some enzymes is achieved by radioprotective chemicals. In particular, with the enzymes lactate dehydrogenase and catalan, the radioprotectant is believed to react with the metal ions of the enzymes. By forming complexes with the metal ions, the metal ions are prevented from getting either oxidized or reduced by irradiation. The radioprotectants belonging to the thiol family are good chelating agents. It has been suggested that the thiol radioprotectants chelate to copper in copper enzymes and prevent its oxidation. It is also proposed that radioprotectants scavenge the ions of copper or iron by binding to them and thus interrupt
the cellular oxidation\textsuperscript{126} initiated by radiation. By complexing with the metal ions in a transient fashion, the radioprotective chemicals also protect the enzymes from the attack of free radicals.

(vii) **Introduction of energy traps**

When ionizing radiation is incident on the body, secondary radiations arise from the scattering of energy of the primary quanta or particles. The nucleic acids are known to absorb the energy of these secondary radiations more than other biological compounds. Thus, as mentioned in section 1.2, the nucleic acids are more prone to radiation damage. In particular, the pyrimidine bases which are the structural elements of nucleic acids are sensitive to the harmful photochemical reactions, which are initiated by the secondary radiations mentioned above.

The sensitivity of the nucleic acid components to radiation has been successfully utilised in affording radiation protection by providing chemical energy traps i.e., substances with similar sensitivity are introduced in the system as traps for the radiation. Amides of aromatic mercapto acids and compounds of pyrimidine series have often been used as energy traps\textsuperscript{2}. Pyrimidine bases, in nucleic acids have high photochemical sensitivity. They have maximum absorption in the ultraviolet region of the spectrum.
Energy traps are also formed by protective thiols which enter into reversible reactions with certain \( \geq C = 0 \) groups, which are commonly present in most biological systems. The new atomic groups or heterocyclic ring systems formed by these reactions may themselves take up a part of the energy from the incident radiation or the secondary radiation and the biomolecules get protected from the radiation effects. As an example, the uracil residue is considered (Fig. 1.16).

The aminothiol radioprotector enters into reaction with the \( 0 = C \) groups, in two ways viz., (i) with the formation of semithioacetals, as shown in Fig. 1.16 (i); or (ii) with the formation of a new heterocyclic nucleus (say, thiazolidine) as in Fig. 1.16 (ii). In either case, the uracil residue is temporarily prevented from enolising to form the chromophore \( - C = C - C = N - \) responsible for the absorption of energy. As a consequence, changes produced in the nucleic acids due to irradiation are likely to be less pronounced.

When the aminothiol radioprotectants are used to provide such indirect energy traps, the structural requirements are very stringent. For example, with more than three carbon atoms between the amino and the thiol group, seven membered or higher order rings are to be formed. However, as such rings are energetically unstable, they are not formed and, it is therefore imperative that the carbon chain in the aminothiol radioprotectants should be only two or three atoms long.
Fig. 1.16 Formation of energy traps
(viii) **Mechanism of action of aminothiols on DNA**

The aminothiols have been shown\textsuperscript{127, 128} to bind and stabilise that part of DNA not covered by histones. It has been found\textsuperscript{129} that the disulphide form of aminothiol radioprotectors bind strongly to DNA. This enables the loose ends, resulting from single strand rupture of DNA, to be held in place. Consequently, further damage arising from shortening or chemical alteration is prevented. The aminothiols also decrease the replication rate so that repair processes can set before radiation-induced alterations are replicated.

From the above description of the various theories of radiation-protection, it appears that the structure and the conformation of the radioprotectants are very pertinent to their protective properties. In particular, the relationship between the \(-\text{SH}\) and the \(-\text{NH}_2\) groups and the length of the carbon chain are important factors affecting the radioprotective property of the compound. In the ensuing chapters of this thesis, results of structure analysis of four radioprotective compounds, viz., sodium fluoracetate, cystamine dihydrochloride, 2-aminothiazoline hydrochloride and bisguanidino ethyl disulphide bromide hydrobromide are described. In Table 1.4, details such as the dose of radiation, quantity of radioprotectant administered before irradiation and the percentage of protection offered are given for these four compounds and a few other closely related radioprotectants. Three of the four radioprotective compounds investigated contain sulphur atom in their structure. The objective of this investigation
<table>
<thead>
<tr>
<th>Compound</th>
<th>Radiation dose (r)</th>
<th>Details of the intraperitoneal administration of the compounds</th>
<th>Percentage protection offered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium fluoroacetate</td>
<td>750-775</td>
<td>4-5 mg/kg 2-5 hours before</td>
<td>60</td>
</tr>
<tr>
<td>Cystamine</td>
<td>800</td>
<td>7.1 mg/mouse 10 minutes before</td>
<td>100</td>
</tr>
<tr>
<td>AET</td>
<td>800</td>
<td>250 mg/kg 15 minutes before</td>
<td>88</td>
</tr>
<tr>
<td>MEG</td>
<td>150-300</td>
<td>9 mg/mouse 15 minutes before</td>
<td>70</td>
</tr>
<tr>
<td>2-AT</td>
<td>800</td>
<td>2.2 mg/mouse 10 minutes before</td>
<td>70</td>
</tr>
<tr>
<td>GED</td>
<td>1000</td>
<td>200 mg/kg 10 minutes before</td>
<td>70</td>
</tr>
</tbody>
</table>
is not only to obtain the crystal structures but also to elucidate the more valuable information on the molecular conformation and to compare the results with those of similar radioprotectants.
REFERENCES


