INTRODUCTION

The history of modification of the biological effects of radiation by chemical substances has been closely inter-linked with the discovery of oxygen effect. The presence of oxygen during irradiation enhances the deleterious effects of radiation. This observation raised a question as to whether chemical compounds which remove or add oxygen would be useful in the modification of radiation damage. Consequently, a variety of naturally occurring as well as synthetic chemicals have been recognised. These compounds either increase (radiosensitisers) or decrease (radioprotectors) the injury in animals resulting from exposure to radiation. It is now known that many chemicals which modify radiation damage do not necessarily act via oxygen pathway.

Investigation on the effects of radioprotectors or radiosensitisers in living organisms by different methods have in general three aims. The first is to gain more information and understanding of basic radiation effects. The second is to search for compounds that may be of practical and clinical value in the protection from and treatment of radiation sickness. The third is to get more insight into the mechanism of the action of chemical modifiers.
A large number of sensitive methods have been developed to assess the radioprotective and radiosensitising potentials of chemical substances both under in vitro and in vivo conditions. In 1942, Dale found that colloidal sulphur and thiourea protect some enzymes in vitro against inactivation by X-rays. Laterjet and Ephrati (1948) provided evidence that thiglycollic acid, GSH, cysteine and cystine protect bacteriophages against radiation damage. In 1949, Patt, Tyree, Straube and Smith observed that cysteine given to rats prior to irradiation offered protection using survival as a criterion. Mole, Philpot, and Hodges (1950) showed that thiourea which protects enzymes in solution reduces mortality of mice if injected before irradiation. Subsequent demonstrations have shown that GSH, cysteamine, AET, provide protection against death of whole body X-irradiated mice (Chapman and Cronkite, 1950; Bacq, Harve, Lecomte, Fisher, Blavier, Dechamps, LeBihan and Rayet, 1951; Doherty and Burnett Jr., 1955). Patt, Smith and Jackson (1950) found a protective action of cysteine on the peripheral blood of the irradiated rat. Cysteine also confers protection on thymocytes irradiated in vitro over wide range of X-ray doses (Patt, Blackford and Straube, 1952). They used eosin stainability
as a criterion for the degree of protection. Smith (1957) studied the protective effect of MEG.Cl on 101 x C3.BPF1 male mouse bone marrow cells X-irradiated in vitro. He has shown that the depression in the number of nucleated bone marrow cells of a femour bone is less in X-irradiated mice protected with MEG than in unprotected controls. Cytologically, there is a wide spectrum of cell types in bone marrow a situation not well suited for studies involving quantitative relationship among dose of radiation, number of bone marrow cells and the degree of protection. Thus search for other methods had begun.

Chemical protection of the alimentary tract of A-1 mice against a dose of 1500 R by prior treatment with AET has been demonstrated (Maisin, Novelli, Doherty and Congdon, 1960). They provided a co-ordinated account of several aspects of protection against radiation, for example, changes in weight, histology of the cells in relation to nucleic acid and protein syntheses. Maisin and Doherty (1963) demonstrated the dependence of increased survival time of mice on increased protection of bone marrow by combined treatment of AET and serotonin. A mixture of AET, GSH and serotonin (assuming that GSH or serotonin added to AET decreases the toxicity of AET) also offers protection against death to C+ male mice
subjected to whole body X-irradiation at 2000 R (Maisin, 1964). A better radioprotection of the short and long term survival of BALB/c male mice treated with mixtures of chemical protectors (AET + GSH + Serotonin or AET + GSH + Serotonin + Cysteine) before exposure to X-irradiation has also been reported (Maisin and Mattelin, 1967). Maisin (1968) observed that radioprotection in mice following the administration of mixture of thiol compounds is due to a 'biochemical shock' (as defined by Bacq and Alexander, 1964). Such 'biochemical shock' is then further expressed in decrease in DNA synthesis and a prolongation of the cell cycle in the stem cells of the small intestine. He has also demonstrated that the administration of isogenic bone marrow to mice treated with former mixture increases the LD$_{99/30}$ (DRF 3.6). Baughet-Mahieu, Goutier and Semal (1967) reported an increase in activity of thymine phosphorylating kinase during liver regeneration of total body X-irradiated mice pre-treated with AET.

Mouse L-cells has been utilized to study the effect of caffeine on irradiated cells (Rauth, 1967). The effect is concentration-dependent in the range of 0.3 to 0.5 mM and it is a post-irradiation phenomenon being dependent on the time after irradiation that
caffeine is added to the cells. An immediate post-treatment of UV-irradiated cells in earlier S-phase with 1 mM caffeine for 22 hr caused a remarkable inhibition in the frequency of sister chromatid exchanges in a pseudoploid Chinese hamster cell line, "D-6, a clonal derivative of the Don line (Kato, 1973).

This finding appears to indicate a certain correlation between the cause of UV-induced sister chromatid exchanges and a process pertinent to the post-replication repair. The influence of a total body irradiation with 500 rads together with caffeine sodium benzoate pre-treatment has been investigated to understand the mode of action of caffeine in the reduplication process of DNA in irradiated mouse liver (Mitznegg, Heim, Hach, and Sabel, 1971). It has been suggested that ionizing radiation and caffeine have similar mode of action in inhibition of DNA reduplication.

The protective effect of ascorbate on the modification of radiation sensitisation of E. coli B/r by KI, stable free radical TAN and NEM has been evaluated (Joshi, Singh, Gopal-Ayengar and Ehrenberg, 1973). E. coli B/r grown to stationary phase suspended in 0.1 M buffer at pH 7.0 has been irradiated with 60Co gamma rays at 0°-4°C.
either in air or nitrogen in the presence of ascorbate with or without sensitizers. They used survival as the criterion to calculate DMP. From the results it appears that ascorbate does not exhibit any net protective effect.

Yuhas and Storer (1969) presented a report of a comparison of seven radioprotective drugs (PAPP, AET, MEA serotonin, WR-638, WR-2721 and WR-44923) on three important modes of radiation-induced death namely, haematopoietic, gastrointestinal and CNS death in female C 57 BL/6J mice. LD$_{50/30}$ as an index of haematopoietic resistance, LD$_{50/7}$ as an index of gastrointestinal resistance after whole body exposure to a dose of 2000 R and death immediately following the exposure to repeated 10000 R as an index of CNS death have been used as parameters. The results presented show that the protective effect of a given agent is a function of the syndrome under consideration.

MFG was reported to be radioprotective for the first time by Koyama, Ikeda, Nomura and Miyazaki (1965). Mice received an X-ray dose of 100 R and 800 R everyday. Injection of MFG at the dose level of 0.01, 0.1 or 1.0 mg per mouse one hour before or after every irradiation was given. Pretreatment with MFG recorded a slight
increase in survival at 100 R. The increase was seen to be higher with higher dose of the compound.

Sugahara, Tanaka, Nagata and Karo (1970) and Nagata, Sugahara, Tanaka (1972) carried out a series of experiments in mice to show the radioprotective effect of MPG. In one series, MPG was injected to mice intraperitoneally at a dose of 0.5 mg/animal (which is equivalent to roughly 20 mg/kg body weight) 15 minutes before exposure to radiation. At this dose the LD$_{50/30}$ of treated animals is 880 R as compared to 620 R for the control. DRF is 1.4. It gets reduced to 1.22 when the dose of MPG is raised to 1.0 mg/mouse i.e. approximately 40 mg/kg body weight. It is thus, evident that MPG is a better protector at a lower dose (20 mg/kg body weight).

Harris and Phillips (1971) have investigated the radioprotective efficacy and the cellular metabolism of several phosphorylated derivatives of cysteamine with particular reference to comparison between normal and malignant cells. Two compounds WR-2721 and WR-638 protect euoxic marrow-colony forming units (CFUs) in vivo better than cysteamine when the cells are treated with both drug and X-irradiation prior to transplantation.
Under these conditions, DMF for WR-2721 is 3.0 and for WR-638, 2.3. Hypoxic CFUs are protected only slightly showing lower OER of 1.1 with WR-2721 and 1.3 with WR-638. Both compounds also protect eugenic tumour cells (Ehrlich Carcinoma and P-388 leukemia growing as ascites tumours) with a DMF of approximately 2. Anoxic tumours are not protected.

Injection of the radioprotective drug WR-2721 (500 mg/kg) 15 minutes before X-ray exposures increases the dose required to induce LD$_{50/30}$ by 160-170%, increases the dose required to induce skin ulceration in 50% of the mice by 140%, but increases the dose required to inhibit tumor transplantability by only 15% (Yuhas and Storer, 1969). WR-2721 doubles the radiation resistance of the normal tissues exposed to 137-cesium X-rays (68 R/min). But, it does not protect against urethan (1000 mg/kg body weight) induced lung adenomas (Yuhas, 1972). The number and size of the tumours has been determined with a clearing staining technique.

Vos, Budke and Grant (1976) used a heteroploid human kidney cell line (T-cells) to study the radioprotective-ability of compounds with covered -SH group (WR-638 and WR-2721). The compounds show no protection when dissolved in tissue culture medium but developed a
protective activity when dissolved in rat blood. Thiol measurements demonstrates that in rat blood the compounds are partly hydrolysed to thiols. This suggest that dephosphorylation of these compounds to uncover the -SH group is a necessity to show protection. Sharma, Kesavan and Srivastava (1982) have shown that dephosphorylation of WR-2721 is however, not essential at least in barley seeds for eliciting protection against γ-ray induced oxic damage.

Revesz, Modig and Monstantinova (1972) have reported that the treatment of Chinese hamster cells with MPG, 30 minutes before irradiation, does not affect the slope of radiation survival curves either under oxic or anoxic conditions. But the MPG treatment raises significantly the extrapolation number of anoxic survival curve. The same treatment for the cells irradiated under oxic conditions did not bring any change.

The effect of the cysteamine and MPG on induction and repair of single strand breaks in the DNA of Chinese hamster cells line, V-79, by irradiation in vitro in the presence or absence of oxygen has been investigated by Modig, Edgren and Révész (1977). The compounds are effective in protecting DNA against the formation of
single strand breaks by irradiation with doses of 39 and 91 Gy (1 Gy = 100 rads) given underoxic, or anoxic condition, Cysteamine inhibits the rejoining of breaks when added to the cells after irradiation.

It has been established that the efficiency of agents which modify radiation damage, namely, cysteamine, thioglycollic acid which are classed as weak electrolytes, is dependent on pH (Vexler, Korystove, Kublik and Eidus, 1980). The effect of caffeine is found to independent of pH. Survival of Chinese hamster fibroblast has been scored. The post-irradiation treatment of Chinese hamster cells with MEA, caffeine-benzoate (CB) and caffeine sharply inhibits the repair of SSB after exposure to a dose of 200 Gy (Vexler and Eidus, 1980). The efficiency of MEA and CB, which are weak electrolytes is also found to be pH dependent.

Thus, a great number of experimental systems have been used for testing the radioprotective action of several compounds. These test systems differ greatly in their complexity. The range is from single, isolated enzymes or other biomolecules, isolated cell organelles, bacteria, plants, cells in tissue culture to whole animals.
Another aspect of investigation that has, of late, received considerable attention is whether the chemical agents which modify radiation damage exert an influence on the synthesis of macromolecules and if so, whether these represent the cause or an effect. Pirie and Lajtha (1950) observed a 60 to 80 percent depression of DNA synthesis by cysteine (1 mM) in human bone marrow cells cultured in vitro.

AET and MEA inhibits DNA synthesis in murine bone marrow cells (La Salle and Billen, 1964). The rate of DNA synthesis has been shown to be inhibited in mice by sulphur containing organic radioprotective compounds (AET, DTD, Cysteamine and Histamine) in bone marrow but not in gut (Simons and Davis, 1965). Ginsberg (1966) found an immediate inhibition of protein synthesis but not DNA synthesis in MEA treated logarithmic phase cultures of E. coli strain 15 TAU-bar.

DNA synthesis proceeded, with an arrest of RNA synthesis in cysteine-treated exponentially growing cultures of E. coli 15 T meaning thereby RNA and/or protein synthesising systems are sensitive to cysteine (Nagy, Hernadi, Kovacs and Valye-Nagy, 1968). Depression
of DNA synthesis in rat thymus and spleen by MEA and AET and in regenerating liver by AET has been reported (Goutier and Baugnet-Mahieu, 1969). Similar observation has also been made by Mitznegg and Sabel (1973) in liver of MEA-treated mice. MEA suppresses RNA synthesis in exponentially growing E. coli cells and mouse bone marrow cells in vivo and mouse spleen lymphocytes in vitro (Ehrenberg, Fedorcsak, Harms-Ringdahl and Näslund, 1974; Forsberg, Harms-Ringdahl and Ehrenberg, 1978).

Thus, there is reason to study the sequence of synthesis and metabolism of DNA, RNA and protein in a cell of an animal body during the process of modification of radiation damage by chemicals. However, it is necessary that alterations in the mode of magnitude of changes in the synthesis of macromolecules are correlated with well-defined biological parameters. At present, the involvement of DNA, RNA and protein syntheses in the development and manifestation of modification by various chemicals of the radiation-induced lesions is not precisely known and is largely controversial.

The basic mechanism whereby chemicals modify radiation damage are mostly conjectural which still need experimental proofs. Hence, Caffeine, Ascorbic acid, MPG, and WR-2721, have been chosen from the plethora of chemicals.
These chemicals may alter the synthetic pattern of DNA, RNA and protein in irradiated and unirradiated tissues. This alteration is expected to have a role in chemical modification of radiation damage.

There is hardly any drug that affects the genetic material in so many different ways as caffeine, which is a unique water soluble substance. It is purine derivative and is thus related to important bases present in informational biopolymers. Adenine and guanine are the key components of DNA, the carrier of genetic information, and of various RNAs which are involved in the translation of this information into protein structure. Adenine is also a component of many coenzymes and ATP, which plays a part of primary importance in biological exchange of energy. Thus, there must be something special about the purines since they have such predominant roles in fundamental biological process. Caffeine, has therefore, become an important tool in molecular genetic research.

Caffeine, not only produces mutation and chromosomal aberrations but also strongly potentiates the lethal mutagenic and chromosome damaging effects of the other
physical and chemical agents. The study of its biological action has undergone a renaissance of interest and is now the subject of a huge literature crowned with contradictory and complex reports. It is relevant to point out that the target sites of action of caffeine on various biologically important material has been dealt with in several papers (Kihlman, 1977).

Caffeine inhibits the normal semi-conservative DNA synthesis in *E. coli* (Lieb, 1961). Bendigkeit and Hanawalt (1968) found caffeine, at a concentration of 500 mg/litre \((2 \times 10^{-3} \text{M})\), has little effect on normal DNA replication. However, Grigg (1968) and Cleaver (1969) reported a marked inhibition of semi-conservative DNA synthesis by caffeine concentration between \(10^{-3}\) and \(10^{-2} \text{M}\) in *E. coli* and human cells, respectively. Similar caffeine effect has been observed in mouse P-388 cells and in first instar larvae of *Drosophila melanogaster* and in Opossum lymphocytes (Boyd and Presley, 1974). Meneghini (1974) observed a 50% inhibition of \((^{3}\text{H})\)-thymidine incorporation into DNA by semi conservative DNA synthesis in the presence of caffeine at a concentration above \(10^{-3} \text{M}\). Roberts and Ward (1973) also found a dose-dependent depression in the rate of DNA synthesis in asynchronous cultures of Chinese hamster
and HeLa cells. Lehmann and Kirk-Bell (1974) conclusively demonstrated inhibition of thymidine incorporation into DNA of L 5178 mouse lymphoma, LS 929 mouse fibroblast and V 79-4 Chinese hamster cells.

Caffeine elicits a three fold decrease in repair replication in UV-irradiated E. coli at a concentration of $2.3 \times 10^{-3}M$ (Bendigkeit and Hanawalt, 1968). A strong inhibition of DNA repair synthesis has been found to occur at a concentration of caffeine which has no effect on semi-conservative DNA synthesis in acellular slime mold, Physarum polycephalum and bacterium Bacillus subtilis respectively (McCornic, Marks and Rusch, 1972; Harris, 1973). Also, caffeine has no effect on repair replication induced by UV, X-ray or alkylating agents in first instar larvae of Drosophila melanogaster (Boyd and Presley, 1974). The unscheduled DNA synthesis induced by UV in HeLa cells has been reported by Cleaver (1969) to be unaffected by $10^{-2}M$ caffeine and Maneghini (1974) observed no significant effect of $10^{-3}M$ Caffeine on UV-induced repair replication in Opossum lymphocytes. Roberts and Ward (1973) found no effect of $1-5 \times 10^{-3}M$ caffeine concentration on the non-conservative repair synthesis in Chinese hamster and HeLa cells. In contrast to these observations, the
unscheduled DNA synthesis induced by UV and X-rays in HeLa cells is found to be inhibited by caffeine at a concentration of $10^{-2} \text{M}$ (Semenova, Volkova and Zhestyanikova, 1974; Zhestyanikova, Semenova and Volkova, 1974). Caffeine has also been found to inhibit the unscheduled DNA synthesis induced by nitrogen mustard in human leukemic leucocytes (Maurice and Lederrey, 1974).

Since caffeine has been found to modify semi-conservative DNA synthesis, excision repair in bacteria and post-replication repair in mammalian cells, it may exert an effect on enzymes involved in these processes. W. Cragg, Carr and Ross (1967) found a 50% inhibition of the activity of semi-purified DNA polymerase extracts from human embryo lung cells by caffeine ($10^{-2} \text{M}$). But, Grigg (1968) and Mouton and Fromageot (1971) observed no effect of caffeine on the activity of DNA polymerase purified from E. coli. Lang and Nuske (1973) found no effect of $8 \times 10^{-7} \text{M}$ to $8 \times 10^{-3} \text{M}$ caffeine on the activity of an in vitro polymerase system purified from the bacterium Proteus mirabilis. Roulland Dussoix (1967) reported that in the presence of $5.15 \times 10^{-2} \text{M}$ caffeine, the degradation of $^{32}\text{P}$ labelled DNA of E. coli by exonucleases I, II and III was strongly inhibited whereas
Lindahl (1971) found no effect of $2 \times 10^{-3}$ M caffeine in the activity of two UV-specific exonucleases, DNAse III and DNAse IV, isolated from rabbit liver nuclei. There are several DNA polymerases with different properties and functions in both bacterial and mammalian cells. It may be possible that caffeine affects the genetic material in eukaryotic cells by its action on some type of polymerase activity.

Caffeine has also been reported to inhibit RNA and protein syntheses in prokaryotic and eukaryotic cells. In the presence of $(5 \times 10^{-3}$ M) caffeine the amounts of RNA and protein synthesised by E. coli B/r during a 70 minutes incubation is found to be significantly reduced. Lieb (1961), Kuhlman, Fromme, Heege and Ostertag (1968) found that caffeine inhibits the incorporation of $^{14}$C-orotic acid into RNA in HeLa cells. An inhibition by caffeine of the incorporation of $^{3}$H-uridine and $^{3}$H-leucine into RNA and protein respectively in Saccharomyces cerevisiae and E. coli has been reported (Putrament, Baranowska, Bilinski and Prazmo, 1972). Waldren (1972) and Zuk and Swietlinska (1973) have demonstrated similar effect of caffeine in Chinese hamster cells and bean root tips respectively.

Very often caffeine is quoted as a potent radio-sensitizer. Ahnstrom and Natarajan (1971) found a
potentiation of the radiation-induced damage in barley seeds when caffeine is added within 4 to 5 hours of neutron irradiation. Similar observation has been reported by Balschandran and Kesavan (1974) in $\gamma$-irradiated barley seeds.

Caffeine-treatment of the virulent phage $V_r$ ($Proteus mirabilis$) or of indicator bacteria inhibits host cell inactivation of this phage after UV-irradiation (Witte and Bohme, 1972). An inhibitory effect on the survival of UV-irradiated diploid and tetraploid Chinese hamster cells have also been reported (Rommelaere and Errea, 1972).

Following UV-irradiation caffeine post-treatment enhances reproductive death in mouse $L$ cells (Rauth, 1967; Domon and Rauth, 1969). Caffeine potentiates 60 $^{60}$Co $\gamma$-ray induced lethality in human $T$-1 and Chinese hamster $V_79$ cells when present at 2.0 to 2.5 mM for 21 to 48 hr after irradiation (Schroy and Todd, 1975). It sensitises Chinese hamster cells to alkylating agents and X-rays by inhibiting repair replication (Gaudin, Gregg and Yielding, 1972).

Caffeine ameliorates a number of the responses of cells to ionising radiation. The survival after
irradiation reduces only slightly in caffeine treated Chinese hamster cells.

Post-irradiation treatment of HeLa S3 cells with 1 mM caffeine results in a synergistic lethality of survival as scored by colony formation (Busse, Bose, Jones and Tolmach, 1977). The sensitivities of other two cell lines, CHO and EMT 6, also have been examined; both are substantially less sensitive to caffeine. A 16-20 hr post-irradiation incubation with caffeine enhances X-ray killing of rodent and human cells (Waldcren and Rasko, 1978).

In a series of papers, Kesavan and co-workers (Kesavan, Trasi and Ahmad, 1973; Kesavan, 1973; Kesavan and Afzal, 1975; Kesavan and Dodd, 1976) have reported that caffeine affords significant radioprotection against γ-ray induced oxic damage in barley seeds but it dramatically potentiates anoxic damage. Post-treatment of caffeine does not inhibit split dose recovery in human or hamster cells (Schroy and Todd, 1975).

This wealth of data when viewed collectively indicate that many of the effects of caffeine are connected with macromolecular synthetic pattern in general and DNA synthesis in particular. But a great deal of controversy
exists regarding the molecular mechanism of the modification of radiation damage by caffeine, whether it is primarily due to the inhibition of repair replication or a repair process confined to the replicative synthesis of RNA and protein but not DNA synthesis (Zuk and Sweitlinska, 1973) or vice versa (Mitznegg, Heim, Hach and Sabel, 1971) remains to be settled.

L(+) ascorbic acid, the form in which it occurs in nature is an important biologically active reductant. So it can act as a radioprotective agent in several tissues by preventing radiation-induced oxidations. Ascorbic acid and dehydro-ascorbic acid are found to be potent protectors of erythrocytes (Shapiro and Kollman, 1969). In fact, the protective effect can be observed to persist even when erythrocyte cells treated with ascorbate are washed before irradiation. On the other hand, ascorbic acid is found to be devoid of protective ability against radiation lethality of bacterial cells (Joshi, Singh, Gopal-Ayengar, Ehrenberg, 1973) and animals (Bacq, 1965). If however, the bacterial cells are irradiated in air in the presence of certain sensitizers such as tetracycline or chloral hydrate, ascorbic acid not only reduces the sensitization but also protects
the cells against radiation-induced death. This protective effect is specific to some sensitizers but not to others such as sparsomycin and 2-fluoro-adenosine (Pittillo and Lucas, 1967). In vitro model chemical systems such as bilirubin (Barac, Beamerage, Cuvelier and Notay, 1961), DNA and enzymes in aqueous solutions (Shapiro, Kollman and Friedman, 1967) are shown to be protected by ascorbate. In this case, the protection by ascorbate might be due to its ability to scavenge free radicals.

The best protective sulphydryl compounds are characterised by a thiol and basic amino or guanidine group that are separated by not more than three carbon atoms (Doherty, Burnet Jr. and Shapira, 1957). Protective activity is inversely proportional to chain length (Eldjarn and Pihl, 1958; Vos, Grant and Budke, 1970). The proximity of the amino or guanidine group to the -SH group will lower the dissociation constant of the protector. This will help the -SH groups to be largely in ionised form at physiological pH. The ionised sulphydryl group will have the characteristics of:

(i) a good electron donor, (ii) be active in the formation of disulphides, (iii) have a low redox potential. Thus,
it can act as a good reducing agent. The substance must be present in the cell at the time of irradiation. Added after irradiation, it is without protective action (Vos, Budke and Vergroesen, 1962).

Not long ago, some sulphydryl compounds have been synthesised taking into account of all the above mentioned characteristics. The two sulphydryl compounds, namely MPG and WR-2721, used in this work, belongs to this group of chemicals. MPG which was introduced in Japan as a chelating drug in the treatment of liver diseases and mercury and lead poisoning, has been shown to protect whole body irradiated mice to the same extent as cysteamine, but with less side effects (Sugahara, Tanaka, Nagata, Kano, 1970). Urano and Tsukiyama (1976) have recently used MPG in mammary carcinoma in C3H/He mice to study its protective effect. The tumour has been locally irradiated with 4000 R of X-rays with or without MPG (20 mg/kg) pre-treatment under normal (air) and hypoxic (by local application of brass lamp above the tumour) conditions. The DMF in air is found to be 1.19. In normal mice, a dose reduction factor of 1.4 is achieved after using 20 mg/kg body weight of MPG. Under hypoxia or fractionated irradiation no significant protection has been observed.
WR-2721 is reputed to protect selectively normal tissues against ionising radiation onslaught but offers little or no protection for solid tumours (Harris and Philips, 1971; Yuhas, 1972; Yuhas and Storer, 1969). This differential function and low toxicity of the compound in human (Czerwinski, Czerwiniski, Clark, and Whitsett, 1972), suggests that WR-2721 might reduce damage of normal tissues in patients undergoing radiotherapy. Yuhas (1972) found that WR-2721 was highly effective (DMF = 3.46) in protecting splenic plaque forming cells (a complex T-cell dependent B-cell end point) in mice. Recently, Harris and Meneses (1978) measured the efficacy of WR-2721 in protecting splenic T lymphocytes in irradiated mice. Injection of WR-2721 (400 mg/kg) 20 min before irradiation protected both viable cell recovery (an end point that reflects both cell survival and proliferation) and CTL activity in mixed leukocyte culture. It has been demonstrated that pre-treatment of malignant tumor cells by RO-07-0582, is compatible with and does not reduce the protection of normal rat parotid gland afforded by pre-irradiation treatment of WR-2721 (Sodicoff, Conger, Pratt, Sinesi and Trepper, 1979). The ability of WR-2721 to protect against acute and chronic radiation injury in blood vessels, skin and muscle of normal rats has been
demonstrated (Utley, Quinn, White, Seaver and Bloor, 1981). This indicates that an increased therapeutic gain can be expected when this drug is used in clinical radiation therapy. If the radioprotective effect of WR-2721 on normal tissues can be reliably predicted and it does not protect tumours, then this compound would be of great value in clinical therapy. For this reason, Phase I and Phase II clinical trials with WR-2721 are already underway in the United States and Japan (Kligerman, Shaw, Slavik and Yuhas, 1980; Tanaka and Sugahara, 1980).

Many hypotheses have been put forward to interpret radioprotective function of -SH compounds. Predominance of the physicochemical explanation e.g. the free radical scavenging hypothesis, the hydrogen donation hypothesis and the molecular complex hypothesis (Pihl and Eldjarn, 1958), has been there for long time. It was often argued whether the above-mentioned physicochemical pathways alone would be sufficient to account for the observed magnitude of radioprotection or else certain biochemical pathways too should be implicated.

Protective thiols and disulphides induce pronounced biochemical and physiological effects in mammals (Bacq, 1965;
Bacq and Goutier, 1967; Eldjarn and Jellum, 1969). Bacq and Alexander (1964) termed these effects as "biochemical shock" and proposed that the biochemical changes may be the cause of protection. They pointed out that the existence of a correlation between the ability of compounds to protect and their capacity to bring about characteristic biochemical changes by mixed disulphide formation between protective agent and tissue sulphydryl group.

From the foregoing survey of literature, it is obvious that there are varied observations on the biochemical effects of the chemical modifiers of radiation damage. Apart from the more basic question viz., whether chemical radioprotection is physico-chemical or biochemical in nature, there is the even more complex argument on the molecular pathways of the biochemical explanation. Whether aminothiol brings about radioprotection via autooxidation, formation of $\text{H}_2\text{O}_2$ and then inhibition of RNA synthesis (Naslund, Fedoresak and Ehrenberg, 1976; Naslund, Ehrenberg and Djalali-Behzad, 1976; Naslund and Ehrenberg, 1978), and whether this pathway is counteracted by another radioprotector such as sodium ascorbate (Forsberg, Harms-Ringdahl and Ehrenberg, 1978; Naslund and Ehrenberg, 1978) is yet to be settled. Related to
this is the other question whether non-SH radio-protectors (non-aminothiols) also follow the same biochemical route or other pathway(s) to afford radioprotection. Following the pioneering demonstration of Kesavan and co-workers that one and the same chemical (e.g., Caffeine, NEM) afford radioprotection against oxic component, but potentiate the anoxic pathway of radiation damage in barley seeds (Sharma and Kesavan, 1975; Kesavan and Srivastava, 1979) and that cysteine protects only against oxic damage but has no effect on the anoxic radiation damage, there arises a question on the nature of synthetic profiles of DNA, RNA and proteins under the oxic and anoxic irradiation conditions. Kesavan and co-workers (Kesavan, 1973; Kesavan, Trasi and Ahmad, 1973; Kesavan and Ahmad, 1974a, b; Kesavan and Afzal, 1975; Kesavan and Ahmad, 1976; Kesavan and Dodd, 1976; Kesavan and Nadkarni, 1977; Afzal and Kesavan, 1977; Kesavan, Sharma and Afzal, 1978; Afzal and Kesavan, 1979a, b) have shown that chemical radioprotection in plant seeds involves mutually annihilatory reaction of the protector molecules with the radiation-induced, oxygen-sensitive sites (An sites). There is indeed overwhelming data to suggest that in animal cell systems too, chemical radioprotection is manifested only under
oxygenated conditions (Littbrand and Revesz, 1969; Modig, Edgren and Revesz, 1974; Roots and Smith, 1975; Yuhaç and Li, 1978). No demonstration of radioprotection of an unambiguous nature against anoxic damage has been shown so far. Then there are the clear-cut demonstrations that in experiments involving equimolar concentration of caffeine and cysteine, the former determines the mode and magnitude of modification of radiation damage (Kessavan and Ahmad, 1974). Based on biochemical interpretations, one should then expect that caffeine reverses the cysteine effect, if any.

It is for these variety of reasons, studies have been undertaken herein to assess the effect of known radioprotectors (e.g. WR-2721, MPG, ascorbic acid) and a known radiosensitiser (e.g. Caffeine) and their combinations on the synthesis of DNA, RNA and protein at specific intervals of time after treatment. The data have been discussed in terms of their consistency or inconsistency under various experimental conditions and their overall relevances to protection or potentiation of radiation damage.