DISCUSSION

1. **DNA SYNTHESIS**

Exposure of total body to radiation depresses the incorporation of $^3$H-thymidine into liver-DNA of two-week old mice. It appears that the DNA synthetic activity is comparatively radioresistant in the liver of four-week old mice. $^3$H-thymidine incorporation decreases below the normal level after 24 hours with 600 R whereas it increases continuously up to 72 hours with 300 R. Previous observations show that DNA synthesis is inhibited by 70% immediately after irradiation and returns to normal level after 40 hours (Sabel and Mitznegg, 1971). Autoradiographic studies indicate that the radiation-induced decrease in DNA synthesis in the liver is directly related to time (Unger and Gidali, 1971). Bhatavdeter et al. (1973) also noticed the decrease in DNA metabolism. Fractionated gamma-irradiation decreases the intake of $^{14}$C-orotic acid into liver DNA at small doses and increases after cumulative dose of 1000 R, exceeding the level by 6-13 times (Kiselgov and Muskacheva, 1977).

Present study shows the inhibition of $^3$H-thymidine incorporation into kidney DNA of two-week
and four-week old animals exposed to radiation. This is in agreement with results obtained by Lohman et al. (1966). However, 300 R does not affect the DNA synthesis appreciably in the kidney of four-week old mice.

The DNA content and synthesis in brain decrease 4-10 days after exposure to 700 R and one day after 2 KR (Caster et al. 1958; Altman et al. 1970). Our results also reveal that DNA synthesis is inhibited in two-week old animals after irradiation. However, in four-week old animals it increases continuously. Merits and Cain (1969) noticed rapid disappearance of the newly formed radioactive DNA. The findings of the present study do not agree with this observation. Here instead of continuous fall there is increase in labelled DNA content after a transient fall.

The synthesis of DNA and cell division involve a sequence of biochemical reactions occurring throughout the cell cycle. The rate and direction of reactions depend on the concentration of the molecular substrates and specific enzymes. The radiation may interfere with these reactions in a variety of ways and inhibit DNA synthesis in
the liver, brain and kidney of mice. The alteration of the size of the precursor pools may play an important role in changing the rate of DNA synthesis. The radiation induces drastic variation in the free thymidine pool of the various tissues of rat and mice thereby decreases the rate of DNA synthesis (Moskaleva, 1975). Similar results are also observed in cultured cells (Smets, 1966a, 1966b; Smets and Brohee, 1970; Little, 1970; Weiss, 1971; Gurley and Walter, 1971; Walters et al. 1974). Numerous investigators (Watanabe et al. 1974; Walter and Hildebrand, 1975; Painter and Young, 1975; Maniko and Okada, 1975; Painter and Young, 1976; Dahale et al. 1979) have demonstrated that radiation reduces the rate of DNA synthesis in cultured mammalian and other cells by transiently depressing the rate of replicon initiation. Moreover, the products of DNA breakdown dilute radioactivity in the precursor pool and this results in a lower specific activity of newly synthesized DNA (Myers and Skov, 1966; Ord and Stocken, 1968; Ivannick et al. 1976). It is possible that further increase in inhibition of DNA synthesis in the liver may be because of this phenomenon.
Since the synthetic processes in the nucleus are dependent on energy generating reactions in the nucleus and cytoplasm it is likely that the impairment of this energy production in the cells may be responsible, in part, for the observed effects on DNA synthesis, following irradiation. Studies on the mitochondria of spleen and thymus of rat and mouse demonstrated a depression of phosphorylation after total body X-irradiation (Van Bekkum, 1956). Depressed levels of deoxyribonucleoside triphosphates could result from depressed adenosine triphosphate (ATP) synthesis and this would retard DNA synthesis (Painter, 1962; Klouwen, 1964; Looney et al. 1965).

Nuclear proteins represent another factor influencing DNA synthesis. Longan (1959); Longan, et al. (1959) and Lehner and Okada (1966) have found striking changes in the incorporation of amino acids in irradiated nuclei. The transient loss of histones and globulins from nuclei is observed 10-24 hours after an exposure to 1000 R (Hagen, 1960). It is not known whether the bond between histones and DNA is rendered more labile by irradiation.
Looney et al. (1965) have suggested that the reduction in DNA synthesis after irradiation could, in part, be enzyme-dependent. The change in the concentrations of labelled thymidine monophosphate (TMP), thymidine diphosphate (TDP) and thymidine triphosphate (TTP) may result in the depression or inactivation of the enzymes thymidine kinase, thymydilate kinase or DNA-polymerase, which may also contribute to the eventual depression of DNA synthesis after irradiation. Harrington (1964) found that the activity of DNA as primer for RNA and DNA polymerase activities was severely depressed by X-radiation. It indicates that the final step of DNA synthesis may be an important site for the action of radiation.

Present investigation also shows that irradiation induces increase in DNA content in certain tissues at certain intervals. This increase could be due to new DNA synthesis by activation of enzymes involved in the synthesis. This unscheduled DNA synthesis seems to be associated with repair mechanisms (Lohman and Bootma, 1974; Zakrzhyskaya, et al. 1976; Hagen, 1977), which occurs at all stages of cell cycle after irradiation.
(Bhatavdekar et al. 1977). The decrease in DNA synthesis in the liver of four-week old and in the brain of two-week old animals after 24 hours irradiation could be probably due to failure of repair system.

Treatment of MPG diminished or checked the radiation-induced changes in $^3$H-thymidine incorporation pattern in all three tissues. $^3$H-thymidine incorporation is near normal level as compared to that in irradiated animals. However, mechanism of protective action of MPG is not fully known. Intraperitoneal injection of MPG alone inhibits the DNA synthesizing activity. It suggests the correlation between radioprotective action of MPG and its inhibitory effect on DNA synthesis. It seems possible that MPG activates, as do many endogeneous substances, specific repressor molecules which interrupt the DNA template activity required for DNA replication (Mitzneff and Sabel, 1973).

It is known that a metabolically-active DNA molecule ready for new DNA synthesis is in the S-phase of mitotic cycle and is more sensitive to ionizing radiation than DNA molecule in the post-mitotic $G_1$-phase (Langendorff and Langendorff,
The action of MPG is related to cell-cycle and it affects S-phase (Kawasaki et al., 1975; Kawasaki, 1977) thereby transforming DNA into radioreistant state.

Transformation of DNA from a metabolically active into a resting state plays a major role in radiation protection (Bacq, 1965). Radiation injury induced in this resting DNA could be repaired more easily in protector-treated than in only irradiated animals. The normalization is also much earlier in the former case. MPG-induced DNA inhibition and its radioprotective action may be mediated through cyclic 3',5'-AMP. Mitznegg (1973) has shown that cysteamine is able to increase the intracellular concentration of cyclic 3',5'-AMP which is sufficient to initiate radioprotective action.

The main effect of cyclic 3',5'-AMP administration is a suspension of DNA-synthesis without affecting either transcription process or translation process (Ryan and Heidrick, 1968; Mitznegg, 1973). On the other hand, cyclic 3',5'-AMP initiates cell division and thus counteracts mitotic delay (Scaife, 1971). This indicates that cyclic
3',5'-AMP promote DNA synthesis when cell division is delayed by ionizing radiation.

The radioprotectors after injection into animals form adsorption, thioether, amide and disulphide bonds with proteins. The mixed disulphide phenomenon also plays the decisive role in the inhibition of the DNA synthetic process (Romantsev et al. 1977). The administration of radioprotector induces the increase of the intracellular non-protein bound glutathione (Modig, 1969; Dontsova, 1972) by mixed disulphide reaction (Graevsky et al. 1969; Modig et al. 1972). This released glutathione seems to be another messenger, besides cyclic 3',5'-AMP, which also inhibits DNA synthesis. Exposure of the cells to MPG increases the intracellular amount of glutathione in a direct proportion to the concentration of the drug in medium (Revesz, et al. 1972). Glutathione itself is a well known radioprotector. Its main effect is also an inhibition of DNA synthesis.

The influence of the glutathione and cyclic 3',5'-AMP on each other is not known. Therefore, it is difficult to infer whether they act independently or in combination in radioprotective action.
The oxidation and reduction of the thiol and disulphide groups of histones alter the ability of these basic proteins to suppress the priming (template) activity of DNA (Ord and Stocken, 1967). It is likely that the addition of radioprotective thiol or disulphide may disturb biochemical regulatory mechanism in the nuclei. The thiol and disulphide groups of nucleohistone form mixed disulphide with radioprotectors. This binding may offer extra protection against radiation damage to the DNA-moiety of the complex.

The thiol-radioprotector binds reversibly with DNA chain (Jellum, 1965; Brown, 1967). This DNA binding with thiol maintains the DNA in repressed state, i.e., temporarily prevents the transcription of messenger RNA and inhibits enzyme synthesis (Vandergoten and Goutier, 1966; Baugnet-Mahieu et al. 1967).

also arise from the delay in the synthesis of the relevant enzymes.

It is clear that radioprotective action and mitotic delay by thiol protectors constitute a complex phenomenon. However, increase of cellular glutathione and release of cyclic 3',5'-AMP seems to be important.

The DNA synthetic rate is more in the liver, brain and kidney of two-week old animals than in those of four-week old animals. Burdman (1972) Itze et al. (1973) and Mares and Lodin (1974) also reported similar results. It may be because of percursor pool and rapid phosphorylation of nucleotides to triphosphate form (Grav and Smellie, 1963; Chang and Looney, 1965; Mori et al. 1970). This may partly be because of the fact that younger animals show greater degree of growth.
2. **RNA SYNTHESIS**

Both the radiation doses induce decrease in RNA synthesis in the liver of four-week old animals up to 48 hours and increase above the normal level at 72 hours. In eight-week old animals, 600 R elicits continuous increase in RNA up to 72 hours, whereas 300 R inhibits the same during 24 and 48 hours intervals. These findings are not consistent with the earlier reports in rats (Hedvegi et al. 1968; Subba Rao, et al. 1971; Chetty et al. 1971). This may be because they used higher doses of radiation. Sabel and Mitznegg (1971) observed no modifying effect of radiation on RNA synthesis. Cytochemical and biochemical studies show the decrease of RNA content in rat and mouse liver following the irradiation (Kiseldof and Mushkacheva, 1977; Bhatavdeker et al. 1977).

The kidney is an organ of highly specialized function; doses up to 1000 R do not affect the histological appearance and even enzymatic activities of adult kidney (Altman et al. 1970). Bhatavdeker et al. (1977), however, noticed reduction in RNA content up to 72 hours post-irradiation. The effects of ionising radiation on RNA have not been as vastly studied.
as has been on DNA. The probable reason for this may be the radioresistant nature of kidney and heterogeneity of RNA. However, we have observed radiation-altered RNA synthesis in four-week and eight-week old animals. Both 300 R and 600 R induced high incorporation of \(^3\)H-uracil at 12 hours and decreased below the normal level later on up to 48 hours. It again increased 75-88% above the normal level at 72 hours. In eight-week old animals also radiation caused alteration in RNA synthesis.

Exposure of total body to radiation increases incorporation of \(^3\)H-uracil into brain RNA of four-week and eight-week old mice. However, 600 R causes decrease of RNA below the normal level at 48 and 72 hours in four-week old animals. Decrease was also observed at 12 hours in eight-week old animals. Autoradiographic studies show \(^3\)H-cystidine incorporation into RNA at normal rate after irradiation at 500 R or 5 KR (Yamamoto et al. 1964). On the other hand, incorporation of \(^32\)P into RNA reduces after one day and again 3-5 days after exposure to 2 KR (Sopin et al. 1965).

The RNA synthesis is intimately linked with the protein synthesis. The change in protein
synthesis stems mainly from primary radiation effect at transcriptional level (Hidvegi et al. 1970; Chetty, et al. 1971; Subba Rao et al. 1974; Patil et al. 1975). Therefore, it seems suppression in RNA synthesis elicit decrease in protein synthesis due to restricted supply of mRNA and ribosomes (Chetty et al. 1976), and general stimulation in RNA synthesis elevates the protein synthesis (Hidvegi et al. 1968). Decrease in RNA levels after the irradiation in the present study could, at least partly, be due to damage to machinery associated with transcription. The radiation induced alteration in chromatin functions are associated, in some way, with non-DNA component of chromatin and its is likely that proteins may have some role in radiation-induced modification in chromatin function (Subba Rao et al. 1971; Subba Rao et al. 1974).

RNA is synthesized by DNA-directed RNA polymerase from ribonucleosides 5' triphosphates. Slight change in enzyme system activity by radiation will affect RNA synthesis. Subba Rao et al. (1971) and Chetty et al. (1976) have observed that any change in RNA-polymerase titre would alter the RNA synthetic level.
Chetty et al. (1978) have noticed that besides changes at the transcriptional level, whole body exposure of rat to radiation stimulates the synthesis of ribonucleotides in the liver. This shows probable link between RNA synthesis and precursor pool in cells.

It is suggested that RNA synthesis is adrenal mediated particularly from 4-18 hours after irradiation (Pradhan et al., 1974; Abdel-Halim and Yatvin, 1976). The administration of hydrocortisone to rat increases template activity of liver chromatin (Dahmus and Bonner, 1965; Murthy et al., 1971). However, our observations show that RNA synthesis is decreased during the period of 4-18 hours. It is likely that endocrine mechanism may not be involved at all or the endocrine gland itself may be affected by radiation. Suppression of RNA synthesis is not noticed by removal of adrenal prior to radiation exposure (Chammarano et al., 1969; Chetty et al., 1971).

The administration of MPG prior to irradiation suppresses the radiation effects on RNA synthesis. It accelerates the recovery and brings the \(^3\)H-uracil incorporation to near normal level within
72 hours. Injection of MPG alone causes an alteration in $^3$H-uracil incorporation. The mechanism involved in this protective action is not known. The nature of the correlation of the radiosensitivity with the rate of RNA synthesis is also not clear. It is possible that the suppression of the RNA synthesis by thiols, reflects on the arrest of metabolic activity transforming the cell into resting condition (Romantsev et al. 1977), which display decreased radiosensitivity (Goutier and Baugnet-Mahieu, 1970). Therefore, it seems that the observed decrease in RNA synthesis is related to depressive effect of MPG on DNA synthesis. According to Nuslund et al. (1976), radioprotectors generate hydrogen peroxides which inhibit RNA synthesis and thereby give protection to the system. The radioprotective action of hydrogen peroxides is possibly related to the protective action, in mammals, of catalase inhibitors. Though peroxides are generated naturally during various metabolic reactions, suggested radiobiological role of peroxides as mediator of radiation protection is a matter of controversy. There is report that RNA synthesis is inhibited earlier than DNA synthesis (Nagy et al. 1968). It is likely that MPG may be protecting RNA synthesis by some other mechanism(s) also.
Our observations show that MPG influences RNA synthesis and protein synthesis almost in similar manner. These findings suggest that MPG probably acts at the transcription level. Goutier and Baugnet-Mahieu (1970) also observed the effect of 2-amino-ethylisothiourea (AET) at the transcription level, rather than at the translation level. The injection of AET to rat does not modify the amino acid incorporating capacity of isolated liver polysomes or distribution pattern of liver polysomes centrifuged on sucrose gradients. Addition of AET to suspension of liver polysomes also has no effect on their amino acid incorporation capacity.

It is uncertain whether the altered RNA synthesis is due to interference of MPG with RNA polymerase or chromatation or both.

The RNA synthesis in the tissue of four-week old animals is more sensitive to radiation as compared to that in eight-week old animals. Probably it may be because of difference in mitotic and metabolic activities which are generally more in the younger animals. RNA synthesis is age-dependent and it decreases with increasing age (Devi, et al. 1966).
3. **PROTEIN SYNTHESIS**

Data obtained in the present study show quite interesting changes in protein synthesis pattern in the liver of two-week, four-week and eight-week old animals on exposure to radiation and the influence of MPG on it. Exposure of total body to radiation inhibits $^3$H-leucine incorporation in the liver protein. These findings support earlier reports on the subject (Nakazawa et al. 1965; Natario et al. 1965; Antov et al. 1969; Mitznegg and Sabel, 1973; Bhatavdeker et al. 1977). However, 300 R induces in $^3$H-leucine incorporation into liver proteins of eight-week old mice. It also increases in two-week old animals at 12 and 24 hours.

Both the radiation doses enhance the incorporation of $^3$H-leucine into kidney protein of two-week and eight-week old animals. In four-week old animals, surprisingly the protein synthesis is inhibited considerably. Incorporation is more at 72 hours with 300 R only. Eight-week old kidney also shows lowered incorporation first and then it increases. Rappoport (1960) and Edward et al. (1964) also noticed initial inhibition.
Incorporation of $^3$H-leucine increases continuously up to 72 hours into brain proteins of two-week, four-week and eight-week old animals. These results are not in agreement with previous report (Nakazawa et al. 1965). Unlike our irradiation schedule, they irradiated animals at 150 R each day for four days. However, 600 R inhibits $^3$H-leucine incorporation up to 48 hours and increases at 72 hours in four-week old animals.

The change in the protein synthesis stems mainly from the change in mRNA and ribosomes (Chetty et al. 1976). The radiation induced change in protein biosynthesis also associates with transport of amino acids through the plasma membrane into the intracellular precursors pool (Mukerjee and Goldfeder, 1974). In all experiments parallelism is observed to some extent between RNA and protein syntheses. Therefore, it seems, at least in part, changes observed in protein synthesis are due to radiation-altered RNA synthesis.

Our data also presents evidence to the fact that the effects of radiation on $^3$H-leucine incorporation into proteins are dependent upon organ and age of the animal. The protein synthesizing
machinery does not respond to radiation in cells killed most easily by radiation, while in the most resistant cells this machinery shows large response (Chammarano, 1962, 1963). E. coli which can rapidly initiate new protein synthesis after irradiation are most radioresistant (Stapleton and Fisher, 1967).

The brain is usually considered to be extremely radioresistant. This may be due to high efficiency in initiating protein synthesis required for repair and this kind of thing may not prevail in kidney and liver (which hence show lower uptake of $^3$H-leucine).

The age of the animals at irradiation is an important factor in determining their radiosensitivity. Lethal dose (LD) of 700 R is recorded for two-week old animals. Thereafter, radioresistance increases to some 950 R by 50 weeks of age and decline later on (Crossfill et al. 1959; Lindop and Rotblatt, 1962). The sensitivity is related to mitotic rate which decreases with increasing age from 2 to 50 weeks. Therefore, simple linear relationship is expected between the age and radiation induced protein synthesis in various tissue to support the view of Cammarano (1962, 1963) and Stapleton and
Fisher (1967). In the case of inhibition, it will be inversely proportional to the age of the animal. With few exceptions, our findings are almost consistent with the observations of above-cited authors.

Administration of MPG prior to irradiation diminishes the radiation effects. Incorporation level of $^{3}$H-leucine is near normal. The deviation, if any, becomes almost normalized around 72 hours.

The precise reaction of MPG with protein also is not known. Dickens and Shapiro (1961), Shapiro and Kollmann (1969), Horvath et al. (1972), Horvath and Csagoly (1974) and Romantsev et al. (1977) have shown the binding between proteins and thiol radioprotectors. Revesz and Modig (1965), Modig (1969), Graevsky et al. (1969) and Modig et al. (1972) have suggested that the formation of mixed disulphides releases glutathione from the binding of cellular proteins and provides protection against radiation. Released glutathione may be acting as secondary messenger in the inhibition of mitotic process. Though glutathione itself is a well-known radioprotector, its biochemical role is not yet clear.
Glutathione acts as a co-enzyme in glyoxalase enzyme system which transforms glyoxal derivatives into lactic acid. The glyoxals (which are dicarbonyl in nature) and glutathione play an important role in the regulation of cell division (Szent-Gyorgyi, 1973a, 1973b, 1974, 1977). Glyoxal and its derivatives inhibit cell division in a very low (0.001 M) concentration. In tissue, the greater part of carbonyls bind to protein structure. Glutathione catalyses the transfer of electrons from proteins to carbonyls but in excess it inhibits strongly. Only a small part of carboxyls is free in equilibrium with bound one (Szent-Gyorgyi, 1973b). The slight change in glutathione quantity may lead to change in the concentration of carbonyls which ultimately interfere with mitotic activity. It appears that increased intracellular glutathione by radioprotectors has a very important role to play in the regulation of cell division. MPG increases the cellular amount of glutathione in a direct proportion to the concentration of the drug (Modig et al. 1972). Glyoxal derivatives on the other hand, inhibit protein synthesis at the ribosomal level (Egyud and Szent-Gyorgyi, 1966; Otsuka and Egyud, 1968).