REVIEW OF LITERATURE
Increasing concern is being expressed regarding the depletion of stratospheric ozone layer and the ensuing climatic changes and enhanced dermal risks due to higher ultraviolet (UV) incidence (Koehler and Hajost, 1990; Yoytek, 1990). The human health hazards due to photochemical activation of dermally exposed occupational and environmental xenobiotics is a problem in tropical countries, necessitating further studies in this direction. Formation of active oxygen species especially singlet oxygen and superoxide anion have been implicated as a factor in photochemotherapy and phototoxicology (Pathak and Joshi, 1983; Joshi and Pathak, 1984). In the photosensitizing effect of psoralens also, oxygen dependent type II photodynamic reactions involving $^{1}\text{O}_2$ and $\text{O}_2^-$ were found (Pathak and Joshi, 1984) to cause membrane damaging reactions including oedema or inflammation. Data from the toxic effects of chemicals have indicated that the membrane damage by active oxygen radical formation could lead to *inter alia*, peroxidative degradation of polyunsaturated fatty acids (Fraga et al., 1987; Kakkar and Viswanathan, 1990).

**Biological Implications of Active Oxygen Species**

A major portion of the biological consumption of molecular oxygen occurs during reduction to water via oxidative phosphorylation. However, it has been found
that a small portion of the total oxygen consumed is reduced in a univalent pathway yielding superoxide, \(\cdot \text{OH} \) free radical and \(\text{H}_2\text{O}_2\), all of which are potentially damaging to biological systems (Fridovich, 1978). The protective enzymes, superoxide dismutase, catalase and glutathione peroxidase usually prevent excessive oxidative damage unless the flux of univalent oxygen reduction becomes so large as to over-ride the capacity of the enzymes (Fridovich, 1978; Floyd, 1983). Superoxide is the primary radical produced by stimulated polymorphonuclear neutrophils in the respiratory burst (Lynch, 1983). This species along with several other unstable radicals (some of them potent oxidants) are believed to be responsible for phagocytosis (Marenyi et al., 1985).

Excess amounts of \(\text{O}_2^-\) or \(\text{H}_2\text{O}_2\) may cause oxidative damage in biological systems. The iron-catalyzed Haber Weiss reaction may be responsible for oxidative damage.

\[
(\text{Fe})
\]

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

This \(\cdot \text{OH}\) formed is a very strong oxidant. This mechanism of the Haber-Weiss reaction is as follows:

\[
\text{O}_2^- + \text{Fe (III)} \rightarrow \text{Fe(II)} + \text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{Fe (II)} \rightarrow \text{Fe (III)} + \cdot \text{OH} + \text{OH}^-
\]

clearly, iron plays an essential role in catalyzing the reaction (Floyd, 1983). Lipid peroxidation begins when a radical species attacks and removes an allylic hydrogen from an unsaturated fatty acid, causing a radical chain reaction:

\[
\text{LH} + \cdot \text{R} \rightarrow \text{L} + \text{RH}
\]

\[
\cdot \text{L} + \text{O}_2 \rightarrow \text{LOO}; \text{LOO}^- + \text{LH} \rightarrow \text{LOOH} + \cdot \text{L}
\]

\[
\text{LOOH} + \text{Fe}^{**} \rightarrow \text{LO}^- + \text{OH}^- + \text{Fe}^{***}
\]

\[
\text{LOOH} + \text{Fe}^{***} \rightarrow \text{LOO}^- + \text{Fe}^{**}; \text{LOO}^- + \text{LH} \rightarrow \cdot \text{L} + \text{LOOH}
\]

\[
\text{LO}^- + \text{LH} \rightarrow \text{LOH} + \text{L} \quad (\text{Cervato, 1990})
\]
Fatty acids with single double bond have been found to be effective inhibitors of peroxidation. It is seen that fatty acid inhibition is partially reversed by increasing the iron concentration in the system. Peroxidation induced by methods independent of Fe are not inhibited by fatty acids. Therefore, it has been suggested that certain fatty acids inhibit peroxidation by chelating available free iron. They may also be involved in competing with the esterified fatty acids in membrane lipids which are substrates for peroxidation (Balasubramanian et al., 1989).

Tocopherols (Vit. E) are indispensable components of biological membranes located in such a way that their hydrophobic hydrocarbon chain is buried into the phospholipid bilayer, whereas the chromanol ring bearing the -OH group which is the essence of its antioxidant function is exposed near the membrane surface and can interact with substances in the aqueous phase (Stoyanovsky et al., 1989). According to the Mehlhorn et al. (1989), the biological activity of vitamin E is believed to be due to its antioxidant action to inhibit lipid peroxidation in biological membranes by scavenging the chain propagating peroxyl radicals (ROO').

\[
ROO' + TOC-OH \rightarrow ROOH + TOC-O' 
\]

The antioxidant function of vitamin E per se is localized in the chromanol nucleus, where phenolic hydroxy group donates an H- atom to quench lipid radicals.

\[
TOC-O' + RH \rightarrow TOC-OH + R' \\
TOC-O' + AH_2 \rightarrow TOC-OH + AH (Serbinova et al., 1991). 
\]

According to Fukuzawa et al. (1985) egg yolk phosphatidyl choline liposomes are shown to be rapidly oxidized in presence of chelated iron and superoxide generating system. Alpha-tocopherol incorporated in the bilayer is oxidized at the same time. The antioxidant does not inhibit lipid peroxidation until its concentration reaches a critical level, which depends on effectiveness of oxidative stress. Beyond this level, peroxidation is inhibited completely and simultaneously, rate of oxidation of tocopherol is lowered. Thus, it is suggested that antioxidant efficiency of alpha-tocopherol depends on its ability to react with chain initiating or propagating lipid radical. Ascorbate is seen to inhibit the consumption of alpha-tocopherol possibly by generating its
reduced form. Recent evidence has shown (Stoyanovsky et al., 1989) that alpha-tocopherol acts in two ways. It either specifically binds iron or may disorder the phospholipid interface which allows greater iron association with the bilayer. Thus, antioxidant effect of tocopherol may be enhanced due to sequestering of free iron, which catalyzes the initiation and propagation of lipid peroxidation into membranes, and thus stopping the initiation of lipid peroxidation.

Dousset et al. (1990) have shown that short ultraviolet radiations, like iron treatment, have been shown to induce a strong lipid peroxidation of low density lipoprotein (LDL), characterized by formation of peroxidation products like thiobarbituric acid reactive substances (TBARS), conjugated dienes and fluorescent lipid-soluble pigments as well as by a dramatic decrease in the contents of polyunsaturated fatty acids (Juliano et al., 1989). Natural antioxidants, Vitamin E and carotenes are almost completely consumed after ultraviolet radiation. This decrease is less marked in iron treated low density lipoproteins. However, ultraviolet radiations induce only minor or no changes in the apolipoprotein moiety (Negre-Salvayre et al., 1990). While iron catalyzed peroxidation results in formation of apolipoprotein-B alterations (Jurgens et al., 1987; Yakode et al., 1988).

**Tocopherols as Antioxidants**

The protective effect of vitamin E against oxidative damage is well documented in literature (Chow, 1991). This activity has been shown to be in part due to efficient radical scavenging. Sugiyama et al. (1992) have observed that the reduction of survival of chinese hamster V-79 cells by UV-B light is markedly inhibited by pre treatment with vitamin E. In other studies, Black and Chan (1975) have reported that dietary supplements of an antioxidant mixture, containing vitamin E, butyrate hydroxy toluene, ascorbic acid and glutathione produce a marked reduction in the numbers and severity of ultraviolet induced squamous cell carcinomas. Other animal studies by Mathews-Roth (1983) and Mathews-Roth and Kinsky (1985) have shown that antioxidant carotenoids are antitumor agents in the case of ultraviolet-B light. Sugiyama et al. have demonstrated that vitamin E accentuates the cytotoxicity of ultraviolet B light, indicating that Vitamin E has a protective effect against the cytotoxicity caused by this
wavelength region in sunlight. Their results have also showed that vitamin E has no effect on DNA breaks, chromosomal aberrations or mutation in V-79 cells exposed to ultraviolet-B light. Therefore, they have opined that the extent of ultraviolet B induced genotoxicity may not be associated directly with cytotoxic effect of ultraviolet B light.

Thiols and Oxidative Stress

Glutathione is an important endogenous antioxidant and fulfils various protective functions in the skin. The experiments of Tyrrell and Pidoux (1986) have shown that glutathione, which quenches intermediate oxygen radicals, protects human skin fibroblasts against cytotoxic action of ultraviolet-A (334 and 365 nm) and ultraviolet-B (313 nm), but not ultraviolet-C (254 nm) radiations. Tyrrell and Pidoux (1988) have studied the correlations between endogenous glutathione contents and sensitivity of cultured human skin cells to radiation at defined wavelengths in the solar ultraviolet range. They have suggested that endogenous glutathione is involved in protecting human skin cells against a wide range of solar radiation damage and have suggested that while free radical scavenging is involved at the shortest wavelength (303 nm) tested, a more specific role of glutathione is involved in protection against radiation at longer wavelengths.

Conner and Wheeler (1987) have studied the effect of ultraviolet irradiation on the levels of glutathione in the skin. They have reported that irradiation of hairless mice with ultraviolet B or ultraviolet A wavelength or with ultraviolet A combined with photosensitizing psoralen can deplete skin glutathione levels. Ultraviolet-B irradiation can cause rapid transient fluctuations in the epidermal glutathione level and the relative amount present as the oxidized form. Ultraviolet-A irradiation has been found to deplete epidermal and dermal glutathione for several hours but requires much higher doses than ultraviolet B. Treatment with photosensitizing psoralen may lead to extensive and prolonged depletions of epidermal and dermal glutathione, the severity of which is dependent on psoralen dose and may last several days. These changes in glutathione levels are seen to be compatible with a role for glutathione as an endogenous photoprotective agent in the skin.
The results obtained from all these experiments conducted with vitamin E and glutathione show that ultraviolet induced cytotoxicity is mediated by the formation of free radical species.

The studies of Kligman et al. (1982, 1985, 1986) Plastow et al. (1988) and Orkari nen and Kallioinen (1989) have demonstrated that ultraviolet irradiation plays a significant role in the cutaneous photoaging related changes which have been described in terms of connective tissue damage. Collagen and elastin are the major fibrillar components of lasting proteins of skin and are subject to ultraviolet exposure. Active oxygen radicals in the skin increase with ultraviolet irradiation and result in harmful effects on surrounding tissues (Danno et al., 1984). Miyachi et al. (1987) have evaluated the defences against oxidative damage from ultraviolet-B radiation, like superoxide dismutase, glutathione redox enzymes and other antioxidants.

**Active Oxygen Species and UV Mediated Effects**

Maeda et al. (1991) have studied the effects of chronic exposure of ultraviolet-A including 2% ultraviolet-B on free radical reduction system in hairless mice. They have observed an increase in insolubility of collagen and the amounts of elastin after six weeks of irradiation. Both these events are associated with photoaging. An increase in the activities of glutathione peroxidase and superoxide dismutase has been reported. Continued ultraviolet irradiation is found to result in the steady decline in superoxide dismutase and lipid soluble antioxidants while glutathione peroxidase remains elevated. Thus, Maeda et al. (1991) have suggested that superoxide dismutase and lipid soluble antioxidants in skin may be involved in protecting it from ultraviolet damage, and they deteriorate with chronic irradiation.

Among skin surface constituents, squalene has been suggested as significant source of malonaldehyde following ultraviolet exposure. Ultraviolet A irradiation produces low levels of malonaldehyde from squalene. Dennis and Shibamoto (1989) have reported that malonaldehyde production varies with the energy of irradiation, on irradiation with ultraviolet-B and ultraviolet-A.
Reactive oxygen species also play an important role in cutaneous pathology. Enzymic and nonenzymic antioxidants can prevent oxidative damage, but may be overcome by a strong pro-oxidative stimuli. Fuchs et al. (1989) have examined the acute effect of a single exposure to ultraviolet A (> 320 nm) on various skin antioxidants in hairless mice immediately after irradiation. They have observed the impairment of cutaneous catalase and glutathione reductase activity. Superoxide dismutase and glutathione peroxidase have not been found to be significantly influenced. Catalase has been shown to be the most susceptible component in skin to single exposure of ultraviolet A and visible light.

Isocaloric feeding of diets varying in lipid content to albino hairless mice has shown that their susceptibility to skin tumorigenesis induced by simulated solar ultraviolet light is not affected by the level of polyunsaturated fat 5% or 20% (Reeve et al., 1988). However, the experiments of Reeve et al. (1988) have demonstrated a qualitative effect of dietary lipid. Mice fed 20% of saturated fat have been found to be almost completely protected from ultraviolet tumorigenesis when compared with mice fed 20% polyunsaturated fat. Multiple latent tumors were detected in the saturated fat fed mice by subsequent dietary replenishment, suggesting that a requirement for dietary unsaturated fat exists for the promotion stage of ultraviolet induced skin carcinogenesis.

In addition to the DNA damaging effects of ultraviolet which result in the carcinogenic effect (Granstein and Sober, 1982), a second hypothesis implies the involvement of endogenously formed free radicals in tumor aetiology. Unsaturated bonds of fatty acids are a prime source of ultraviolet induced free radicals. Antioxidants have been shown to minimise lipid photooxidation and the cascade reaction producing free radicals, to reduce ultraviolet erythema and also to suppress ultraviolet tumorigenesis in mice (de Rios et al., 1978).

Cholesterol is often found in high concentration in a variety of membrane systems and can reduce membrane permeability both in model membranes (Shmidt and Raftery, 1973) and biomembranes (Legrimellec and Leblanc, 1978). Therefore, cholesterol tends to rigidify the membrane phospholipids above their phase transition temperature and is likely to alter the relationship between lipid peroxidation and
membrane permeability after irradiation with ultraviolet radiation (McIntosh, 1978).

Cepharanthin, a bisboccaurin alkaloid is a clinical drug with antihemolytic activity. Nagatsuka and Nakazawa (1982) have reported that the presence of cholesterol in the liposomal membrane decreases the degree of radiation induced changes in permeability in spite of increased lipid peroxidation. Cepharanthin is seen to suppress both radiation induced lipid peroxidation and changes in membrane permeability in liposomes.

Kocevar (1990) has studied the effect of ultraviolet radiations on erythrocyte membranes and has reported protein crosslinking on poly acrylamide gel electrophoresis along with lipid peroxidation. She has reported that the major effects of ultraviolet radiation on cell membranes are alterations of proteins and has stated that tryptophan is the major chromophore for these alterations.

The studies of Salmon et al. (1990) on the effect of solar ultraviolet radiations (mostly ultraviolet-B) on lipoproteins have demonstrated that exposure of lipoproteins to ultraviolet-B leads to destruction of the tryptophan residue of apolipoproteins, and lipid peroxidation, whereas ultraviolet-A is ineffective. In the early stages of irradiation, antioxidant bleaching also takes place. They have suggested that tryptophan residue excitation plays an important role in the initiation of lipid peroxidation in lipoproteins. Salmon et al. (1990) have therefore stated that ultraviolet-B induced peroxidation of lipoprotein lipids can be amplified by metal ions such as Cu++. Ultraviolet-B (280-320 nm) is readily absorbed by cornea and epidermis and is known to cause skin cancer in mammals (Tyrrell and Pidoux, 1987). Although ultraviolet-A (320-400 nm) is not as potent as ultraviolet-B, it is capable of producing erythema and pigmentation of skin and damage to the eyeball (Zigman, 1986). Light sources emitting UVA are used in industry, dentistry and medicine for photopolymerization, phototherapy of diseases and cosmetic tanning of skin (Urbach and Gange, 1986). Ultraviolet-A irradiation has been shown to damage DNA (Peak and Peak, 1990; Churchill et al., 1991; Larcom et al., 1991) and to impair cutaneous antioxidant defence system (Fuchs et al., 1989).

Vincent and Muller-Eberhard (1987) and Prinsze et al. (1990) have shown that ultraviolet-A is capable of causing photosensitized protein damage. Enzymes that
contain heme moiety and flavin adenine diphosphate are self photosensitizing (Fuchs et al., 1989; Kramer and Ames, 1987). Ultraviolet-A irradiation is seen to activate catalase in Salmonella typhimurium and in vitro (Kramer and Ames, 1987).

The above authors have attributed the inactivation to photosensitization by the heme moiety of catalase, since a non ultraviolet absorbing enzyme, alkaline phosphatase, is not inactivated by ultraviolet-A. In addition to catalase. Fuchs et al. (1989) have observed that ultraviolet-A irradiation of hairless mice also impairs glutathione reductase. As glutathione reductase contains ultraviolet-A absorbing flavin, it is likely that ultraviolet-A may only inactivate enzymes that contain ultraviolet-A absorbing molecules.

Hu and Tappe! (1992) have studied protein oxidation by ultraviolet-A irradiation in the absence of photosensitizers on 2SH enzymes not containing ultraviolet-A absorbing Heme or FAD. They have reported that while the enzymes are not appreciably inactivated by ultraviolet-A irradiation, synergistic inactivation occurs when hydrogen peroxide and Fe ions are present during ultraviolet-A irradiation. Thus, ultraviolet-A may be instrumental in enhancing the generation of hydroxyl ion by hydrogen peroxide and Fe ions.

Many studies have been conducted on the effect of ultraviolet radiations on lens protein in mammals and the subsequent cataract formation. Murakami et al. (1989) have conducted on ESR study on the ultraviolet photolysis of human and canine lens nuclei at room temperature. They have reported the generation of at least two kinds of free radicals and have detected two spin adducts upon irradiation of canine lens in presence of a spin trapping reagent. DMPO (5,5-dimethyl-1-pyridine N-oxide).

Andley and Clark (1989) have reported the effect of near ultraviolet radiation, which is one of the possible risk factors in cataract formation, on beta-crystallines, which comprise nearly half the protein of the human lens. They have reported protein intersubunit crosslinking, change in the charge of protein subunits to more acidic species, and changes in protein tertiary structure by 300 nm irradiation. Their results have provided evidence for the generation of hydrogen peroxide in the irradiated human beta-crystallin solution by the Typel photosensitizing action of the chromo-
phores absorbing at 300 nm. This hydrogen peroxide is generated via the intermediate production of superoxide anion. The latter spontaneously dismutates to hydrogen peroxide, presumably via superoxide ion-protein interactions.

Haemoglobin plays an essential role in the transport of oxygen to the tissues and is thus vital for maintaining life. The studies of Kollias et al. (1992) have shown that both haemoglobin and oxyhaemoglobin undergo photochemical changes with ultraviolet-B radiations in vitro as well as in erythrocytes. Similar changes to those found in oxyhaemoglobin occur in human skin in vivo with ultraviolet-B but not with ultraviolet-A. The authors have also reported that ultraviolet photons penetrate to the capillary plexus in the dermis and interact directly with erythrocytes. The experiments conducted by both Abd-El Baset (1986) and Kollias et al. (1992) have shown that the changes produced in the oxyhaemoglobin by ultraviolet A and B radiation could be consistent with the formation of methaemoglobin.

Various drugs are seen to have a protective effect against ultraviolet radiations. Peak et al. (1987) have reported that chlorpromazine (2-chloro-N,N-dimethyl 10-phenothiazine-10 propanamine) which is commonly used as a sedative, tranquilizer and antiemetic and is also photoactive, is seen to inhibit ultraviolet induced skin carcinogenesis in hairless mice.

The experiments of Dumont et al. (1992) with EGb 761 an antioxidant drug composed of a mixture of a dosed and standardized extract of Ginkgo biloba and mannitol, have shown that EGb761 protects the different polyunsaturated fatty acids of a biological membrane system from peroxidative damage. The extract is a complex mixture composed of terpenes and flavonoid substances, the latter being reponsible for the antiradicalar properties of the extract.

Exposure to ultraviolet-C irradiation induces preferential degradation of polyunsaturated fatty acids. Linolenic acid shows the strongest resistance to ultraviolet-C irradiation induced peroxidation. Arachidonic acid and dioicosahexaenoic acid are more susceptible. Ultraviolet irradiation produces both superoxide radicals and peroxyl radicals (Melo and Mahmoud, 1988). The total protection of microsomal polyunsaturated fatty acids afforded by the highest concentrations of EGb 761 shows
that antiradicalar compounds of the extract are capable of scavenging the superoxide radicals (Roback and Gryglewski, 1988) and providing an efficient protection of polyunsaturated fatty acids within the membrane structure.

Ultraviolet radiations are also known to have an adverse affect on the immune responses of the body. Many experiments have been conducted to establish the fact. Noonan and Fabo (1990) have reported that irradiation with ultraviolet-B radiation \textit{in vivo} suppresses cell mediated immune responses. This form of suppression is seen to decrease immune response to contact sensitizers, certain viruses and parasites and appears to be critical in the outgrowth of ultraviolet induced tumors (Fabo \textit{et al.}, 1990).

Denkins \textit{et al.} (1989) have exposed groups of mice to a single dose of ultraviolet radiation before or after immunization with Candida albicans. They have found that the delayed type hypersensitivity response is markedly depressed in ultraviolet irradiated mice. The experiments of Noonen \textit{et al.} (1981) have established that the promotion phase of ultraviolet carcinogenesis is regulated immunologically. Irradiation by ultraviolet is seen to induce a defect in la antigen presentation in the epidermis, which is accompanied by the generation of specific suppressor T lymphocytes rather than effector T-cells. By the mechanism the epidermal tumor cell evades recognition (Fischer and Kripke, 1982).

Ultraviolet radiations may also have a hand in the spread of the dreaded disease, AIDS. Valerie \textit{et al.} (1988) have reported that 254 nm ultraviolet radiation can induce the HIV promoter. The same authors have observed that ultraviolet radiations can stimulate the growth of the complete virus in human cells. Stein \textit{et al.} (1989) have shown that DNA damage is a pre-requisite for ultraviolet-C induced activation of the promoters of HIV and other genes. Valerie and Rosenberg (1990) have suggested that a change in the chromatin structure is involved in the promoter induction by ultraviolet radiations in the early post exposure stages. The suppression of immune response by ultraviolet radiations may also play a major role in the induction of HIV. The demonstration that HIV can be activated by ultraviolet radiation attracts special attention for several reasons. Firstly, as of today, HIV infection leads invariably to a fatal disease. Secondly, all humans are exposed to solar ultraviolet radiations which are becoming increasingly hazardous due to ozone depletion. Thirdly, ultraviolet radiations are
being increasingly used in phototherapy for cosmetic purposes (Zmudzka and Beer, 1990).

**Active Oxygen Species and Genotoxicity**

Free radicals that are formed in the body as a consequence of aerobic metabolism can produce oxidative damage to macromolecules in somatic cells. Oxidative damage to DNA is caused by reactive by products of normal metabolism, as well as by radiation. This type of damage may be an important factor (Tolmasoff et al., 1980) in aging and age dependent diseases such as cancer, arthritis and heart disease (McCord, 1974; Floyd, 1981). Circumstantial evidence implicating free radicals in aging includes the impressive inverse correlation between the specific metabolic rate and life span of the species. It is seen that smaller animals with higher metabolic rates consume greater quantities of oxygen and therefore produce harmful free radical by products at a higher rate, leading to a higher rate of damage to critical cellular targets.

Many uncertainties remain about the role of free radicals in aging. DNA seems particularly plausible as a critical target in aging. Adelman et al. (1988) have measured the oxidized DNA bases excised by repair enzymes and excreted in the urine of four different species (mice, rats, monkeys and humans). They have reported that mice excrete eighteen times more thymine glycol and thymidine glycol and monkeys secrete four times more than humans. Thus, it is seen that specific metabolic rate correlates highly with oxidative DNA damage.

A wide spectrum of products are formed in various stages of peroxidation of polyunsaturated fatty acids. Many of these products interact with proteins, or RNA and DNA to be mutagenic or to be metabolized to epoxides, compounds known to be DNA-alkylating agents with mutagenic activity (Vaca and Harms-Ringdahl, 1989). \( \text{H}_2\text{O}_2 \) is known to be an ubiquitous oxidative species produced by metabolic and pathologic cellular processes (Viullame, 1987; Adelman et al., 1988; Joenje, 1989) and also by near UV radiation (Miguel and Tyrrell, 1983; Peak and Peak, 1989). \( \text{H}_2\text{O}_2 \) is implicated in mutagenic processes and cell killing, DNA being one of the main cellular
targets (Mello-Filho et al., 1984). DNA strand breaks were shown to be induced by 
$H_2O_2$ within cellular DNA (Schraufstatter et al., 1988).

The pathway likely to be involved in the $H_2O_2$ mediated decomposition of 
isolated DNA implies a radical decomposition of $H_2O_2$ via a fenton type reaction 
leading to the production of $OH$ or related reactive species. It has been proposed that 
neither $O_2^-$ nor $H_2O_2$ under physiological conditions cause any damage to DNA. Their 
toxicity arises from their metal ion-dependent conversion to highly reactive $OH$ and 
have shown that base damage is predominant over single strand break induction 
within DNA exposed to $H_2O_2$. A large amount of base modifications occur (Mouret et 
al., 1991).

According to Dizdaroglu (1991), 'OH produces a large number of sugar derived 
and base derived products in DNA as well as DNA-protein cross links in mammalian 
chromatin. Reactions of 'OH, e'aq and $H^+$ with DNA bases are characterized by addition 
to double bonds of these molecules to give adduct radicals of bases. Abstraction 
of $H^+$ by 'OH from CH$_3$ groups of thymine also occurs when oxygen is present, pyrimidine radicals add oxygen to give corresponding peroxyl radicals. This phenomenon, 
however, has not been seen with purine adduct radicals. Subsequent reactions form a 
variety of products from each of the DNA bases (Blakely et al., 1990).

The degradative effect of $H_2O_2$ peroxide and 'OH radical on DNA is well 
documented. Transforming DNA is inactivated and decreases in UV absorption and 
mean melting temperatures have been observed. Decreases in sedimentation velocity 
and viscosity have also been seen to occur as a result of base liberation and subsequent 
sugar phosphate backbone breakage and transition metal ions have been shown to 
accelerate the rate of some of these reactions (Massie et al., 1972). On examining the 
changes in primary and secondary structure of DNA in presence of $H_2O_2$, Massie et al. 
(1972) have found that base destruction is the most important event. They have found 
the apparent rate of base destruction to be 20% bases/hour of the original molecule at 
37 °C in presence of 0.088 M $H_2O_2$. The relative magnitude of other reaction rates were, 
base destruction > single stand breaks > double strand breaks > crosslinking. Denat-
tured DNA is observed to be more sensitive to base destruction than native DNA. RNA base destruction rates exceeded those of DNA by a factor of three or more.

Vaca and Harms-Ringdahl (1989) have investigated the interaction of LPO products with nuclear macromolecules, when lipid peroxidation reactions are driven both non enzymatically (with ascorbate Fe++) and enzymatically (with NADPH-ADP-Fe"+++). They have observed that nuclear membrane lipid peroxidation products formed during incubation interact with DNA and total nuclear proteins (Shires, 1982). Non-enzymatic LPO processes induce a 40% larger association of oxidation products to DNA compared to processes driven enzymatically, whereas the corresponding interaction with the nuclear proteins have been found to be similar in both peroxidation systems. Inhibition of LPO by iron chelators prevent association of oxidation products to nuclear macromolecules.

In the eukaryotic cell nucleus, nuclear envelope is a reported site for LPO. LPO in vitro requires iron in hepatic microsomal fractions stimulated by NADPH. Fe seems to have a role in all stages of complex lipoperoxidative sequence (initiation, propagation and degradation of peroxide products), but initial activity appears to require maintenance of Fe as Fe(II) by membranes NADPH, cytochrome c reductase (Shires, 1982). Iron in biological systems is very precisely regulated such that there is very little "free" iron present. Floyd and Lewis (1983) have shown that ADP and ATP, but not AMP will ligate Fe(II) in such a way that •OH is produced from H₂O₂ in large quantities. Floyd (1983) has demonstrated that in general di and triphosphate linkednucleosides ligate Fe(II) in such a way that it catalyzes H₂O₂ breakdown to form a large amount of •OH radicals. Mg++, on the other hand even in higher than normal levels, does not prevent Fe(II), nucleotide catalyzed H₂O₂ breakdown to yield •OH radical.

Ionizing radiation causes heterogenous types of DNA damage. Some of this damage is due to the generation of oxygen radicals in the vicinity of cellular DNA (Cerutti, 1976; Ward and Kuo, 1976; Scholes, 1983). Transitional metal ions induce formation of lipid hydroperoxides and propagate free radical species generated in this process (Barak et al., 1983). The metal ions Fe and Cu are seen to be bound to the phosphates of DNA backbone and to certain aminoacids of various proteins including
Ionizing radiation induced formation of thymidine hydroperoxides and some of their decomposition products.
those in chromatin (Floyd, 1981; Floyd, 1983; Tien et al., 1982; Rowley and Halliwell, 1983; de Mello Filho and Meneghini, 1985). These metal ions result in the formation of \( \cdot \text{OH} \) from \( \text{H}_2\text{O}_2 \) (Aust et al., 1985; Samuni et al., 1981). These \( \cdot \text{OH} \) radicals result in DNA strand breaks and modification of DNA bases (Kasai et al., 1986; Frenkel et al., 1986; Frenkel and Chizan, 1987). The thymine moiety is the most susceptible among bases to the effects of ionizing radiation (Scholes, 1983; Cadet and Teoule, 1978) forming thymine hydroperoxides. The decomposition of these thymine hydroperoxides generates other stable products and active oxygen species which oxidize other DNA bases. Tofigh and Frenkel (1989) have analyzed the effects of free and chelated metal ions on the degradation of 5-hydroperoxymethyl -2'-deoxyuridine (HPMdU). They discovered that two products were formed 5-hydroxymethyl -2'-deoxyuridine (HMDU) and 5-formyl-2'- deoxyuridine (FdU). Fe(II) was seen to cause instant HPMdU degradation and formation of equal amounts of both HmdU and FdU. Fe(III) is found to be inactive. In case of Fe(II), formate inhibits HmdU formation, but enhances FdU formation. Tofigh and Frenkel (1989) discovered that EDTA enhances Fe(III) activity with time dependent formation of FdU. EDTA and diethylenetriamine pentaacetic acid (DTPA) are seen to cause instant Fe(II) mediated decomposition of HPMdU to FdU. Only desferal is found to partially inhibit Fe(II) activity whereas activity of Fe(III) is blocked by desferal and DTPA.

**Photochemical Damage to DNA**

The photodamage produced when DNA is irradiated with 254 nm ultraviolet light has been documented. The two major photoproducts formed are covalent linkages between adjacent pyrimidines. These are cyclobutane pyrimidine dimers and (6-4) photoproducts like 6-(1,2-dihydro-2-oxo-4 pyrimidyl) pyrimidine class of ultraviolet light induced dimer photoproducts. Mitchell (1988) has estimated the relative frequency of these products in DNA and found the ratio of cyclobutane dimers to (6-4) photoproducts to be about 3:1. Husain et al. (1988) have reported that both these photoproducts distort the DNA helix and are substrates for repair enzymes found in different organisms.
Ultraviolet light induced pyrimidine base hydration forms products at isolated cytosine and thymine positions in DNA (Wood, 1989). Most hydration products are unstable and revert to unmodified pyrimidines (Kittler and Lober, 1977). Other products are repaired by enzymes with pyrimidine hydrate DNA glycosylase activity. Duker and Gallagher (1988) have reported that ultraviolet light also forms photoproducts involving purine bases, but in double stranded DNA these occur at a very low frequency and account for less than 1% of the total photodamage. Ultraviolet induced pyrimidine dimers are perhaps the most frequent damage inflicted on DNA by the environment. The structure of DNA is altered by the formation of these dimers. Husain et al. (1988) have observed that the thymine photodimer introduces a bend of 30° in DNA. This change causes anomalous slow migration of DNA fragments in polyacrylamide gels and facilitates the formation of covalent circles. This anomalous migration is arrested by repair of thymine dimers by DNA photolyase.

Wood (1989) has described the process of repair of DNA damaged by ultraviolet radiation, by mammalian cells. He has reported that human cells remove pyrimidine dimer photoproducts from DNA by a nucleotide excision repair process. (6-4) photoproducts are removed faster, thus showing that the greater part of the repair synthesis is likely to be caused by (6-4) pyrimidine dimer photoproducts. This class of lesions is rapidly repaired by mammalian cells. Their removal is considered to be necessary for the survival of cells after UV irradiation. It has been found that cells from patients with inherited Xeroderma pigmentosum disease (a form of skin cancer) have defects in the repair of cyclobutane pyrimidine dimers and (6-4) photoproducts. Due to this defect, thymine dimers cannot be repaired and any exposure to sunlight can prove to be dangerous for the patient.

Lyamichev et al. (1990) have analysed the formation of cyclobutane and (6-4) pyrimidine dimers which are major products of ultraviolet irradiated DNA. They have analysed the photofootprints of fragments produced by the cleavage of the DNA chain near (6-4) pyrimidine dimers and found that a homopurine-homopyrimidine insert is a good target for ultraviolet-induced pyrimidine dimer formation. However, they have seen that dimerization is suppressed when pyrimidine oligonucleotides are added to DNA containing inserts. This effect is dependent on the type of oligonucleotide used and is site specific. The protection occurs under acidic conditions that favour the
formation of intermolecular triplexes between homopurine homopyrimidine inserts and homologous oligopyrimidines. Thus, Lyamichev et al. have concluded that triplex formation effectively protects the DNA duplex from ultraviolet induced damage.

The question remains as to why triplex formation has such a dramatic effect on the UV induced dimerization of duplex DNA. DNA photodamage is structure dependent. Becker and Wang (1989) have reported a 5-10 fold decrease in the photodamage caused to the duplex after its transition from B to A form. In triplexes the duplex is in the A form and this acts as a protection against photodamage. The occupation of the major groove of DNA by an oligonucleotide and the formation of Hoogsteen pairs with the purine strand further hinder the internal motion in DNA so as to prevent adjacent pyrimidines from acquiring positions that favour their dimerization under UV irradiation (Becker and Wang, 1984).

**Environmental Implications of Photochemical Damage to DNA**

The ozone depletion and the subsequent increase in the ultraviolet-B radiations reaching the earth is posing a hazard to both terrestrial and marine organisms. Stratospheric ozone depletion may result in the increased solar ultraviolet-B radiation to the oceans upper layers and may cause deleterious effects on marine organisms. Recent experiments on solar radiation in Antarctica during the 1987, Ozone hole by Karentz and Tutze (1990) have used an Escherichia coli mutant lacking DNA repair activity. Their experiments have measured killing but the fact that the organism they have used is incapable of DNA repair has made it highly likely that the mortality observed is due to DNA damage. Regan et al. (1992) have quantified the ultraviolet-B induced photoproducts (cyclobutane pyrimidine dimers) in DNA molecules exposed to solar ultraviolet at the surface and at various depths in clear, tropical marine waters. They have found that the pyrimidine dimer induction is linear with time but accumulation of dimers in DNA varies greatly with depth. Accentuation of dimer formation with depth of water is exponential. Eg. DNA at 3 metres depth has only 17% of the pyrimidine dimers found at the surface.
Gallagher and Duker (1989) have investigated the formation of DNA base damages by broad spectrum ultraviolet irradiation (250-400 nm) using a defined sequence of human DNA. Analysis of reaction products by sequencing gels have showed enzymic incision of purine containing photoproducts as well as pyrimidine cyclobutane photodimers. Purine photoproducts are mainly produced at biologically significant wavelengths between 260 to 300 nm. These results have suggested that purine containing photoproducts could be of consequence in ultraviolet carcinogenesis.

Hanawalt et al. (1979) have stated that far ultraviolet irradiation produces mostly pyrimidine dimers, which are of great biological importance. To a far lower extent, far ultraviolet induces photohydration of pyrimidines by a reaction mechanism which is not clearly established. In the mid ultraviolet region (290-320 nm), formation of pyrimidine dimers is still very substantial (Niggli and Cerutti, 1983), although indirect mechanisms gain in importance (Cerutti, 1985; Moore et al., 1987), which also result in the formation of a comparable density of monomeric ring saturation products as well as single strand breaks. In the ultraviolet-A region, pyrimidine dimers are formed in very low abundance (Freeman et al., 1987). The formation of pyrimidine (6-4) pyrimidine photoproducts by far and mid ultraviolet light may play an important role in the cellular response to ultraviolet irradiation in certain biological systems (Niggli and Rothlisberger, 1988).

The experiments of Brash (1988) on ultraviolet induced DNA photoproducts have shown that the major ultraviolet mutagenic photoproducts are the (6-4) photoproduct and the cyclobutane dimer both in E. coli and in human cells. The (6-4) photoproduct may be more important in E. coli and the cyclobutane dimer more important in mammalian cells. In human cells, mutations occur at the cytosine site of the Thymine-cytosine, cytosine-thymine or cytosine-cytosine cyclobutane dimer, but not at thymine-thymine cyclobutane dimers. They appear to occur less frequently at the cytosine site of the thymine-cytosine and cytosine-cytosine (6-4) photoproducts. The local structure of DNA is more important in determining the frequency of mutation at a site, than is the photoproduct frequency at that site. The effect of DNA structure, therefore, appears to be due to site specific lethality.
Strong evidence is available that ultraviolet light in the UVA range (320 nm or longer) induces damage to DNA largely through indirect photosensitization reactions involving molecular oxygen and ultraviolet-A absorbing sensitizer molecules (Cadet et al., 1986; Peak and Peak, 1986, 1989). DNA may be damaged by reactive oxygen species generated by Type I reactions in which excreted sensitizers are reduced by interaction with substrate and in the semireduced state react with oxygen to produce superoxide anion and other active oxygen species (Peak et al., 1988). Alternatively, in a type II process, absorption of photons by the sensitizer converts it to the triplet $T_1$ state, which then transfers the energy to oxygen to produce mainly singlet oxygen ($^{1}\text{O}_2$) and superoxide anion. The importance of molecular oxygen in photosensitization is evident from the large oxygen enhancement ratios observed for photosensitized DNA breakage in vitro (Peak et al., 1984, 1986). Studies with isolated DNA (Peak et al., 1986, 1988) have not excluded a role for superoxide anion, hydrogen peroxide, singlet oxygen or hydroxyl radical in ultraviolet-A photosensitized DNA breakage. Several naturally occurring organic molecules like 2-thiouracil, NAD+, NADH and riboflavin have been shown to act as sensitizers that enhance ultraviolet-A mediated DNA damage (Peak et al., 1984) and to produce superoxide anion and other active oxygen species when irradiated with ultraviolet-A irradiation (Cunningham et al., 1985; Czochralska et al., 1984; Peak and Peak, 1989).

Czochralska et al. (1984) have shown that direct excitation of NADH or NADPH in aqueous medium at 254 nm or at a wavelength longer than 320 nm leads to generation of NAD$^+$ or NADP$^+$. Under aerobic conditions the reaction is accompanied by the formation of $\text{H}_2\text{O}_2$. Thus Czochralska et al. have reported that the overall findings for the photooxidation of NADH in the presence of oxygen are consistent with the following mechanism.

\[
\begin{align*}
\text{Hv} \\
\text{NADH} &\longrightarrow \text{NADH}^+; \text{NADH}^+ + \text{O}_2 \longrightarrow [\text{NADH}^+ + \text{O}_2^-] \longrightarrow \text{NAD}^+ + \text{HO}_2^- \\
\text{NAD}^+ + \text{O}_2 &\longrightarrow \text{NAD}^+ + \text{O}_2^- \\
\text{HO}_2^- + \text{O}_2^- + \text{H}^+ &\longrightarrow \text{H}_2\text{O}_2 + \text{O}_2
\end{align*}
\]
p-aminobenzoic acid and related compounds have found widespread use in commercial sunscreens because of their proven ability to reduce erythema subsequent to exposure to sunlight (Willis and Kligman, 1970). Several recent studies have, however, shown that DNA is damaged after ultraviolet irradiation in presence of the sunscreen agent, p-aminobenzoic acid, both in vivo and in vitro. One type of damage has been shown to be the result of increased yields of pyrimidine cyclobutane dimer formation (Sutherland and Griffin, 1984). However, it has been suggested that the other types of lesions are produced as well. Shaw et al. (1992) have studied the photochemistry of thymine-PABA and thymidine-PABA systems and have reported the presence of thymine-PABA and thymidine-PABA photoadducts. This study has assumed importance after reports that more and more ultraviolet rays are reaching the earth with the depletion of the ozone layer.

Psoralens have been found to interact with many biological targets in presence of ultraviolet light. However, it is their interactions with nucleic acids, particularly DNA, which have dominated photobiochemical studies. The most predictable reaction is a (2+2) cycloaddition between a pyrimidine base and one double bond of the psoralen, either in the furan or pyrone moiety. When a monoadduct is first formed on the furan side, further irradiation of the remaining coumarin chromophore may also produce a cycloadduct on the pyrone side, generating a cross-linked DNA (Gasparro, 1988; Potapenko, 1991; Dall'Acqua and Martelli, 1991). One mode of photoreactivity in psoralens which does not lead to the formation of photoadducts with DNA is the sensitized generation of reactive oxygen species, which may produce lesions in DNA causing DNA cleavage (Kochevar and Dunn, 1990). Kagen et al. (1992) have reported that in presence of ultraviolet radiation, psoralens are capable of photosensitizing direct DNA strand breaking in vitro in competition with adduct formation. Strand breaking is more prominent in absence than in presence of oxygen.

The damage caused to DNA by ultraviolet radiation is an important cause for the rise in cases of skin cancer. The ras family of genes in Eukaryotes encodes regulatory GTP binding proteins. At least some ras proteins appear to play a role in the control of cell proliferation (Hall, 1990). Ultraviolet radiation is a potent DNA damaging agent that causes skin cancer in experimental animals and man (Emmett, 1973). Activated ras oncogenes have been identified in some ultraviolet radiation induced skin tumors by
The deleterious effects of ultraviolet radiations on RNA have been investigated by Dobrov et al. (1989), who have determined the efficiency of RNA-protein cross link and RNA chain break formation under nanosecond or picosecond ultraviolet laser pulse irradiation of tobacco mosaic virus. They have found that on high intensity ultraviolet laser irradiation, the quantum yields of both reactions increase considerably as compared to low intensity ultraviolet-irradiation.

With the rapid increase in the ultraviolet B radiation reaching the earth, the impact of the radiations on plants has been investigated only recently. Reduction in leaf area, fresh and dry weight lipid content and photosynthetic activity in a number of ultraviolet-B sensitive higher plant species have been reported (Briggs et al., 1981; Teramura, 1983). Ultraviolet-B has also been reported to affect many metabolic processes, pigmentation and community composition of several biological systems (Renger et al., 1989; Hader et al., 1986, 1988; Döhler et al., 1986; Döhler, 1985; Tyagi et al., 1992; Newton et al., 1979; Stewart, 1980).

Concluding Remarks

The above implications suggest the importance of UV irradiation mediated formation of active oxygen species and their interaction with biomolecules as a major environmental risk. Therefore, some aspects of this problem were taken up for study in this dissertation.