DISCUSSION
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(I) **Dry barley seed in radiobiology**

The choice of dry barley seed as the test system is due to the following attributes as detailed by Kesavan (1987):

(a) The most elegant feature of the barley seed system is that it could be rendered metabolically inert so that all sorts of experimental abuses (freezing, thawing and heating) could be levelled against it and then normal metabolism and biochemistry could be restored so that the overall effects of induction, fixation, bypass/repair and modification of radiobiological damage can be assessed in terms of $M_1$ seedling injury on the eighth-day of germination, $M_1$ chromosomal aberrations in the early mitoses of shoot-tip cells and $M_2$ chlorophyll mutations. The genes for chlorophyll mutations on various linkage groups are already known.

(b) In case of barley seeds, the radiation doses required are much lower and hence more realistic
towards the therapeutic doses as compared to those required for inducing the same degree of damage in spores.

(c) The post-irradiation oxygen-dependent (oxic) damage can be easily developed by immersing the "dry" seeds in oxygenated water (Caldecott et al. 1957). It is now well known that as the seed moisture content is increased, the oxygen-dependent damage [and consequently the oxygen enhancement ratio (OER)] decreases, and it abruptly disappears at seed moisture content between 10.7 - 11.0 per cent (Conger et al. 1966, 1968, Kesavan 1973). At about 11.0 per cent seed moisture content there is no "post-irradiation oxygen enhancement ratio" (post-irradiation OER) (Kesavan 1973). In the barley seeds of low water content, the free radicals are known to persist for long periods of time (Zimmer et al. 1957, Ehrenberg and Ehrenberg 1958) permitting ample time to study their behaviour relative to their ability to react with oxygen. The present observations (tables 1, 2 and 3) that oxygen-dependent damage decreases with increase in
seed moisture content and that oxygen-independent damage is not influenced by seed moisture content are in accordance with the abovesaid reports.

(d) The thermal annealment of oxygen effect has been extensively studied in barley seeds (Konzak et al. 1960, Kamra and Kesavan 1969, Kesavan and Kamra 1970, Kesavan and Nadkarni 1977). Both wet heat-shock (at 60°C for 90 sec.) and microwave (2450 MHz, 12.25cm, 9.5 W) greatly eliminate the post-irradiation oxic damage (Kesavan and Kamra 1970, Kesavan et al. 1973). The present data (table 26) show that wet heat-shock (at 60°C for 90 sec.) gives substantial radioprotection against the oxygen-dependent damage in seeds of about 3.6 per cent moisture content, but potentiates the oxygen-independent damage (tables 26 and 27).

(e) The water and oxygen content can be accurately controlled in the barley seed system. The importance of controlling the oxygen content needs no over emphasis in view of the fact that the magnitude and mode of modification of the radiation damage by chemical scavengers

(f) The data on the nature of DNA lesions induced by ionizing radiations, their enzymatic repair or their inhibition by certain chemical agents are also available in the radiobiological literature of barley seed (Yamamoto and Yamaguchi 1969, Yamaguchi and Tatara 1977).

(II) Oxic and anoxic pathways of radiation damage:

In dried spores of Bacillus megaterium, an oxygen-independent (class I) and two oxygen-dependent (class II and class III) components of radiation damage have been described by Powers et al. (1960). The class II (the "immediate") and class III (the "post-irradiation") oxygen effects are known to decrease and finally disappear with increasing moisture and temperature (Powers 1965, Powers and Tallentire 1968).

In the eukaryotic spores of Osmunda regalis and barley seeds, the class I (oxygen-independent
component) is clearly evident, but the two oxygen-dependent components (class II and class III) could not be resolved as distinctly different classes (Dodd and Ebert 1970, 1972, 1973, Kesavan and Dodd 1976, Kesavan 1979). What is, however, of interest is that the post-irradiation oxic damage, although could not be resolved into an "immediate" and "post" entities in the seeds, still responds to moisture and thermal energy in exactly the same way as the two oxic components do in spores. The present data show that seed moisture content greatly influences the oxygen-dependent damage (tables 1, 2 and 3) and the post-irradiation oxygen enhancement factor (table 4). It could be surmised that the wet heat-shock (at 60°C for 90 sec.) exerts radioprotection in the present study (table 26) due possibly to thermal annealment of the post-irradiation oxic damage, as deduced from numerous publications (Conger et al. 1966, 1968, Kamra and Kesavan 1969, Kesavan and Kamra 1970, Donaldson et al. 1979a, 1979b) in the literature. That thermal annealment involves the radiation-induced, O₂-sensitive (Aₙ) sites is evident from the fact that there is no protection by thermal energy in seeds of about 11.5% moisture
in which the $A_n$ decay far too rapidly (Table 27).

Development or abolition of post-irradiation oxygen-effect in barley seeds came to be understood better with the data derived from the kinetic experiments (Nadkarni and Kesavan 1975, Afzal and Kesavan 1979a). The results from several of these studies clearly showed that initial moisture content of the seeds and the post-hydration temperature greatly influence the rates of reaction of the radiation-induced oxygen-sensitive sites with oxygen or their rates of decay in the absence of oxygen. Apart from this, the time after irradiation when decay of $A_n$ sites is initiated has also been shown to depend on initial seed moisture content and post-hydration temperature (Afzal and Kesavan 1979a).

The data presented in figure 4 represent the rate of development of oxic damage when oxygen is available, and its rate of elimination progressively when seeds are first post-hydrated in oxygen-free (means $N_2$-or $N_2O$-saturated) water prior to their transfer to oxygenated water. The computation of the rates of reactivity of gamma-ray-induced oxygen-sensitive ($A_n$) sites with oxygen and their decay in the absence of oxygen (based on the $t_{1/2}$ values) in seeds of about 3.2 per cent moisture reveals
that the A\textsubscript{n} sites react with oxygen about 8-9 times faster at the post-hydration temperature of 4±1°C (figures 5, 6, 7 and 8 and table 5). Thus the present study largely reconfirms the earlier findings in this regard and in addition, these results also suggest that the t\textsubscript{1/2} for the decay of the oxygen-sensitive sites in N\textsubscript{2}- and N\textsubscript{2}O-saturated water does not widely differ. The t\textsubscript{1/2} for the decay of oxygen-sensitive sites in N\textsubscript{2}- and N\textsubscript{2}O-saturated water is 88 min. and 92 min. respectively (figures 7 and 8). Further, the reactivity of the oxygen-sensitive sites (OSS) with O\textsubscript{2} in any given circumstance is faster than the rate of their decay in the absence of oxygen. However, this trend is reversed in seeds of higher (∼10%) moisture content.

The other aspect of these studies concerns the possibility that the oxygen-sensitive sites are a heterogeneous population in the Osmunda spores (Dodd and Ebert, 1972, 1973). In barley seeds also, the heterogeneity of the radiation-induced O\textsubscript{2}-sensitive sites (OSS) has been experimentally deduced (Ahnström and Sanner 1971, Kesavan and Ahmad 1974a, 1976).
The 50 kVp X-ray response of aqueous suspension of \textit{Bacillus megaterium} spores to varying concentrations of oxygen published by Ewing and Powers (1976) has been a subject of considerable discussion. One reason for that is that these authors observed an "OH"-component" within the oxic damage at low (10^{-4}\text{M} - 10^{-5}\text{M}) oxygen-concentration. This was subsequently reconfirmed by Kesavan and Powers (1987) who employed caffeine, in addition to \textit{t-BuOH}, as the hydroxyl radicals (OH') scavenger. The importance of these findings is ascribed also to the plateau at certain intermediate oxygen-concentrations. In other words, there is no absolute linearity between oxygen-concentration and oxic damage from "zero" concentration of oxygen upto 100 per cent oxygen. Studies carried out by Donaldson \textit{et al.} (1979a, 1979b) in barley seeds reveal that for observing any plateau in the relationship between oxygen-concentration and post-irradiation oxic damage, the former in the post-hydration medium ought to be \leq 10 per cent or \geq 80 per cent. In the present studies, there have been no oxygen-concentrations between 0\% and 10\%, and therefore, no plateau is observed. However a plateau is observed between
80% and 100% of oxygen-concentration. Hence, the data in the present study (table 6 and figure 9) are considered to be largely in conformity with those of Donaldson et al. (1979a, 1979b) and Kesavan et al. (1990). In the current study, the oxygen-concentrations in the gas phase of the soaking medium (OCHG) of 0%, 10%, 30%, 50%, 80%, and 100% approximately correspond to $1.0 \times 10^{-6}$ M, $1.0 \times 10^{-4}$ M, $5.0 \times 10^{-4}$ M, $9.0 \times 10^{-4}$ M, $1.4 \times 10^{-3}$ M, and $1.8 \times 10^{-3}$ M respectively.

(III) Effects of chemicals on the post-irradiation damage in $O_2$, $N_2$, and $N_2O$:

Kesavan et al. (1973) and Kesavan and Ahmad (1974a, 1974b) were the first to demonstrate that caffeine and cysteine bring about differential modification of the post-irradiation oxygen-dependent and-independent components of damage. While both these chemical agents exert varying degrees of protection against the oxic component, caffeine potentiates the anoxic component, whereas cysteine exerts no effect whatsoever. Soon they assessed the effects of widely unrelated chemicals on the oxic and anoxic components of radiation
damage (Kesavan 1973, Kesavan et al. 1973, Kesavan and Ahmad 1974a, 1974b, Sharma and Kesavan 1975, Kesavan and Dodd 1976, Afzal and Kesavan 1977, 1979b, Sharma et al. 1982, Sharma and Kesavan 1988). These experiments, in particular the ones dealing with caffeine, clearly showed that event(s) other than the much-reported inhibition of DNA repair (Ts'O and Lu 1964, Bendigkeit and Hanawalt 1968, Yamamoto and Yamaguchi 1969, Harm 1967, Cleaver and Thomas 1969, Domon and Raugh 1969, Mitznegg et al. 1971, Fabre 1972, Gaudin et al. 1972, Rommelaere and Errera 1972, Witte and Bohme 1972, Žuk and Swietlinńska 1973, Ahnström 1974, Kihlman 1977) are possibly involved in the differential modification of the radiation-induced oxic and anoxic components of damage. This rationale advocated by Kesavan et al. (1978) received experimental verification through pulse radiolysis (Kesavan and Powers 1985). The most significant finding was that caffeine competes with oxygen for electrons \( k = 1.5 \times 10^{10} \text{ M}^{-1}\text{s}^{-1} \) and that it also appreciably reacts with hydroxyl radicals \( k = 6.9 \times 10^9 \text{M}^{-1}\text{s}^{-1} \). These studies have brought out the importance of the reactivity of chemical additives towards the radiation-induced free radical
species in the modification of the oxic and anoxic damage. The physicochemical reactions of this sort precedes the biochemical effects, if any, of the chemicals on the cellular metabolism. Dry seed experiments discussed here largely signify such physicochemical reactions.

Since the physicochemical reactions occurring in the irradiated systems are greatly influenced by the nature of the gas present during- and post-irradiation, a discussion of these here is appropriate.

(a) **Radiation chemistry of water, when present, in \( \text{O}_2, \text{N}_2 \) and \( \text{N}_2\text{O} \).**

When water is irradiated following radicals will be formed:

\[
\text{H}_2\text{O} \xrightarrow{\text{A}} \text{H}^+, \text{OH}^+, \text{e}^-_{\text{aq}} \tag{1}
\]

In the absence of oxygen, the following recombinations possibly occur:

\[
\text{H}^+ + \text{H}^+ \rightarrow \text{H}_2 \tag{2}
\]

\[
\text{H}^+ + \text{OH}^+ \rightarrow \text{H}_2\text{O} \tag{3}
\]

\[
\text{OH}^+ + \text{OH}^+ \rightarrow \text{H}_2\text{O}_2 \tag{4}
\]

\( (k=5.5\times10^9 \text{M}^{-1}\text{s}^{-1}) \) (Dorfman and Adams 1973)

of these, reactions (2) and (3) are entirely harmless. When oxygen is present, many of
the reactions leading to the formation of hydrogen peroxide ($\text{H}_2\text{O}_2$) and superoxide anion ($\text{O}_2^-$) take place as follows:

$$e_{aq}^- + \text{O}_2 \rightarrow \text{O}_2^-$$  \hspace{1cm} (5)

$$\text{H}^+ + \text{O}_2 \rightarrow \text{HO}_2^-$$  \hspace{1cm} (6)

The HO$_2^-$ formed in reaction (6) can lead to production of $\text{H}_2\text{O}_2$ as follows:

$$\text{HO}_2^- + \text{HO}_2^- \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$$  \hspace{1cm} (7)

The superoxide anion radicals ($\text{O}_2^-$) could also be formed by deprotonation of HO$_2^-$ depending upon pH.

$$\text{HO}_2^- \xrightarrow{\text{H}^+} \text{O}_2^-$$  \hspace{1cm} (8)

(McCord and Fridovich 1969)

HO$_2^-$ and O$_2^-$ could react as follows to result in $\text{H}_2\text{O}_2$:

$$\text{HO}_2^- + \text{O}_2^- \rightarrow \text{O}_2 + \text{HO}_2^- \rightarrow \text{O}_2 + \text{H}_2\text{O}_2 + \text{OH}^-$$  \hspace{1cm} (9)

($k = 1.0 \times 10^8 \text{M}^{-1}\text{s}^{-1}$)  (Bielski 1978)
When the degassed water is saturated with \( \text{N}_2\text{O} \), then the hydrated electrons (\( e^-_{\text{aq}} \)) are converted into hydroxyl radicals (OH\(^+\)) as follows:

\[
\text{N}_2\text{O} + e^-_{\text{aq}} \rightarrow \text{N}_2 + \text{OH}^- + \text{OH}^+ \quad (10)
\]

\[ (k = 5.6 \times 10^9 \text{M}^{-1}\text{s}^{-1}) \text{ (Keene 1964)} \]

The generation of OH\(^+\) from the above-said reaction has been held responsible for the increased radiosensitivity observed in bacterial and mammalian cells in \( \text{N}_2\text{O} \) over the level seen in \( \text{N}_2 \) (Powers and Cross 1970, Ewing et al. 1974, Brustad and Wold 1976, Samuni and Czapski 1978, Watanabe et al. 1981, Roots et al. 1982, Kesavan and Powers, 1987). Watanabe et al. (1982) have shown that cell concentration, dose-rate and irradiation temperature affect the radiosensitization of \textit{Pseudomonas radiora} 0-1 by \( \text{N}_2\text{O} \). It has, of course, been already pointed out that OH\(^+\) alone without the sequential action of hydrogen peroxide does not probably cause radiosensitization (Powers et al. 1972, Powers 1972).

These foregoing considerations leave one question for enquiry. That is whether \( \text{N}_2\text{O} \) can possibly enhance the radiosensitivity in biological
systems with greatly diminished water content. Data presented in this thesis (tables 1-3, 8-12, 14, 15, 17-22 and 25 and figures 2, 3, 11, 12, 14 and 15) clearly suggest an opposite effect—namely, significantly reduced damage (radioprotection) than that seen in even N₂. This unexpected result has been discussed in terms of radiation chemistry by Singh and Kesavan (1990a) which is briefly as follows:

The direct action of gamma-photons on vital target molecule (RH₂) results in the following:

\[ \text{RH}_2 \xrightarrow{\gamma} \text{RH}^-, \text{H}^-, \text{e}^-\text{(trapped)} \]  

(Kesavan et al. 1990)

Anbar et al. (1975) have reported that N₂O possibly reacts with H⁻, although at a very low rate constant as follows:

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

(k= 1.3x10⁴ M⁻¹ s⁻¹) (Anbar et al. 1975)

Thus, with N₂O being present, there could be some OH⁻ involvement as against possibly nothing at all in presence of N₂. This radiation chemical consideration could be relevant only if some
OH' (as against negligible) could account for the radioprotective action of \( N_2O \) in the dry seed. There is no direct experimental evidence for this as yet, but it is plausible that the reaction of \( N_2O \) with \( H^+ \) in the irradiated dry seeds restores at least to some small extent an equilibrium between electrons and hydroxyl radicals. When aqueous cell suspensions are irradiated in the presence of \( N_2O \), the conversion of \( e^-_{aq} \) into \( OH^- \) upsets the equilibrium among the water-derived radiolytic species. Powers (1972) has emphasized that an equilibrium between electrons and hydroxyl radicals could promote protective reaction as follows:

\[
e^{-}_{aq} + OH^+ \rightarrow OH^- \quad (12)
\]

"Electron sequestration" (Powers 1972) results in enhanced damage by the hydroxyl radicals. Several ways that oxidizing and reducing species might mutually annihilate each other have been suggested by Ewing (1980). Thus, the lack or inadequate formation of \( OH^- \) due to direct action of radiation in dry seeds could result in more reducing than oxidizing species. If so, anoxic radiation damage in dry seeds could
possibly be due to excessive reducing events, and the $N_2O$, which can generate a small amount of OH', could effectively balance these out (Singh and Kesavan 1990a). In support of this contention, it has been observed by Madhavi and Kesavan (unpublished) that $N_2O$ has no effect whatsoever on metabolizing seeds. It has already been pointed out that $N_2O$ exerts no influence at all in certain biological systems under certain circumstances (Mullenger and Ormerod 1969, Antoku 1977, Simone and Quintiliani 1977, Tilby et al. 1982, Ewing et al. 1986).

(b) **Modification of the post-irradiation damage** in $O_2$, $N_2$ and $N_2O$ by caffeine, catalase, glutathione, cysteine, potassium permanganate, hydrogen peroxide, t-butanol, ethanol and ascorbic acid.

Irrespective of the varied biochemical effects which these chemicals possibly exert on the cellular metabolism, all these chemicals at certain concentrations exert radioprotective effect against the post-irradiation oxygen-dependent damage (tables 8-26 and figures 11,12,
14, 15 and 16). The reaction rate constants of these chemicals with electrons and hydroxyl radicals are given in the Table 30.

### Table 30. Reaction rate constant (k) of chemicals with electrons and hydroxyl radicals.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Reaction rate constant (k) (M⁻¹ s⁻¹) with</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>electrons</td>
<td>hydroxyl radicals</td>
</tr>
<tr>
<td>Caffeine</td>
<td>1.5x10⁹</td>
<td>6.9x10⁹</td>
</tr>
<tr>
<td>Catalase</td>
<td>3.0x10¹⁰</td>
<td>1.4x10¹¹</td>
</tr>
<tr>
<td>Glutathione</td>
<td>3.4±0.3x10⁹ (at pH 6.8)</td>
<td>Not known</td>
</tr>
<tr>
<td></td>
<td>2.1±0.2x10⁹ (at pH 12.6)</td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>1.1x10⁹</td>
<td>1.3x10⁹</td>
</tr>
<tr>
<td></td>
<td>(at pH 5.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.0x10⁹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(at pH 7.0)</td>
<td></td>
</tr>
<tr>
<td>Potassium permanganate</td>
<td>2.2x10¹⁰</td>
<td>--</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>1.2x10¹⁰</td>
<td>4.5x10⁷</td>
</tr>
<tr>
<td>t-butanol</td>
<td>~10⁸</td>
<td>5.2x10⁸</td>
</tr>
<tr>
<td>Ethanol</td>
<td>&lt;10⁵</td>
<td>1.8x10⁹</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>-</td>
<td>1.2x10¹⁰</td>
</tr>
</tbody>
</table>
Among the chemicals which afford radioprotection against post-irradiation oxic damage, potassium permanganate and ascorbic acid represent the two extremes. For instance, potassium permanganate reacts only with electrons, whereas ascorbic acid reacts only with hydroxyl radicals.

Radioprotection by potassium permanganate against post-irradiation oxic damage observed in the present study (tables 11, 12, and 19) confirm the earlier studies of Kesavan and Co-workers in barley seeds (Kesavan and Afzal 1975, Kesavan and Dodd 1976, Kesavan et al. 1978) and bacterial spores (Raghu and Kesavan unpublished). In a more recent paper (Kesavan et al. 1990), we have briefly discussed the radioprotection by potassium permanganate at high oxygen concentrations. In metabolizing seeds, potassium permanganate possibly removes \( \text{e}^-_{\text{aq}} \) in competition with oxygen, this, of course, implies that superoxide anion (\( \text{O}_2^- \)) formed by the reaction of oxygen with \( \text{e}^-_{\text{aq}} \) is toxic. The other possibility, more relevant for the dry seeds, is that \( \text{MnO}_4^- \) reacts with \( \text{RH}_2^- \) formed by the reaction of \( \text{RH}^- \) with \( \text{O}_2 \) and \( \text{HO}_2^- \) formed by the reaction of \( \text{H}^- \) with \( \text{O}_2 \). In this regard, attention is drawn to the data (tables 28 and 29) that potassium permanganate does not protect against oxic damage in slowly-metabolizing seeds. It does
afford radioprotection against oxic damage in the normally-metabolizing seeds (Kesavan and Dodd 1976, Kesavan et al. 1978). The hydration factor here possibly operates in ways yet to be precisely elucidated. More experiments need to be undertaken.

Ascorbic acid, an OH\(^{-}\)-scavenger, also affords radioprotection against oxic damage (table 10), as already reported by Afzal and Kesavan (1977) and, Jha and Kesavan (1986). This observation in the simplest sense suggests that there is an "OH\(^{-}\)-component" within the oxic damage developing in \([O_2]\) of \(\sim 10^{-3} M\) and the removal of OH\(^{-}\) is, therefore, the basis of radioprotective action by ascorbic acid. This view is, however, not supported by the numerous reports (Ewing and Powers, 1976, Kesavan and Powers 1987) dealing with the 50 kVp X-irradiated *Bacillus megaterium* spores. In spores, OH\(^{-}\)-scavenging results in radioprotection only when the \([O_2]\) is in the range of \(10^{-5} M - 10^{-4} M\), and the irradiation source is 50 kVp X-rays. Whether the discrepancy observed between the present data and those of Ewing and Powers (1976) has something to do with the test-systems and/or the Linear Energy Transfer (LET) of the 50 kVp X-rays and \(^{60}\)Co-gamma-photons, cannot at present be resolved.
All the rest of the chemicals tested here fall between these two additives discussed above in view of their reactivity with both electrons and hydroxyl radicals.

All the six concentrations of both ethanol and t-butanol afford substantial radioprotection against post-irradiation oxygen-dependent damage (tables 8 and 9 and figures 11 and 12). However, t-BuOH present during irradiation of aqueous suspension of Bacillus megaterium spores with 50 kVp X-rays does not protect the fully oxygenated spores. It does, however, protect them only under low concentrations ($10^{-5}$ M - $10^{-4}$ M) of oxygen (Ewing and Powers 1976, Kesavan and Powers, 1987). As pointed out in the foregoing paragraph, the discrepancy might be related to the LET of the radiation, since t-BuOH affords discernible radioprotection to spores even under fully oxygenated conditions if $^{60}$Co-gamma-rays, instead of 50 kVp X-rays are used (Lajos and Tallentire 1982, Raghu and Kesavan unpublished). The protective action of t-BuOH, under these circumstances, possibly reveals the presence of an "OH· component" within the oxic damage.

Caffeine affords significant radioprotection against the post-irradiation oxygen-dependent damage
(tables 1-3, 18-21, 23 and 26). These results are in agreement with the earlier reports of protection against oxygen-dependent damage in barley seeds (Kesavan et al. 1973, Kesavan and Ahmad 1974a, 1974b, Nadkarni and Kesavan 1975, Kesavan and Afzal 1975, Kesavan and Dodd 1976, Kesavan and Ahmad 1976, Kesavan et al. 1978), Bacillus megaterium spores (Kesavan and Powers 1985, Raghu and Kesavan 1986) and CHO cells (Kesavan and Natarajan 1985). In the earlier papers, Kesavan and co-workers have interpreted the protection of oxygen-dependent damage, on the basis of mutually annihilatory reaction of caffeine molecules and radiation-induced, oxygen-sensitive sites (OSS). Further, the protective action of caffeine depends upon the availability of oxygen-sensitive sites for mutually annihilatory reaction with caffeine as shown by Nadkarni and Kesavan (1975), Kesavan and Afzal (1975) and Kesavan and Ahmad (1976). Kesavan and Powers (1985) working with Bacillus megaterium spores have shown that caffeine reacts quite appreciably with electrons (k = 1.5x10^{10} M^{-1} s^{-1}) and its radioprotective action could possibly be due to its competition with oxygen for electrons. Consequently the harmful products of reaction of oxygen with electrons (e.g. RH0\textsuperscript{2}, O\textsuperscript{2} and HO\textsuperscript{2}) would be greatly reduced.
The radiation chemical reactions involving caffeine (Kesavan et al. 1990) are as follows:

\[
\text{Caffeine} + \mathrm{HO}_2^+ \rightarrow \text{Caffeine}^+ + \mathrm{H}_2 + \mathrm{O}_2 \quad (13)
\]

(Restoration)

\[
\text{Caffeine} + \mathrm{HO}_2^- \rightarrow \text{Caffeine}^- + \mathrm{H}_2 + \mathrm{O}_2 \quad (14)
\]

\[
\text{Caffeine}^- + \mathrm{O}_2 \rightarrow \text{Caffeine} + \mathrm{O}_2^- \quad (15)
\]

(mutual annihilation of caffeine and oxygen)

Data presented in tables 14& 15 and figures 14&15 show that catalase affords significant radioprotection against oxygen-dependent damage. These results are entirely in agreement with those of earlier reports (Sah and Kesavan 1987, Singh and Kesavan 1990b). While interpreting the results largely on the basis of decomposition of radiolytically formed \( \mathrm{H}_2\mathrm{O}_2 \) by catalase, Sah and Kesavan (1987) briefly referred also to the reactivity of the catalase towards the electrons (\( \mathrm{H}^- \) and \( \mathrm{e}_{\text{aq}}^- \)) and hydroxyl radicals (\( \mathrm{OH}^- \)).

The reactivity of catalase towards electrons and hydroxyl radicals, in addition to its enzymatic decomposition of \( \mathrm{H}_2\mathrm{O}_2 \), has to be taken into consideration. Its reaction rate constants for electrons
and OH' radicals are $3.0 \times 10^{10} \text{ M}^{-1} \text{s}^{-1}$ and $1.4 \times 10^{11} \text{ M}^{-1} \text{s}^{-1}$ respectively (Dorfman and Adams 1973, Behar et al. 1975). In the present study involving 100-600 units/mg of catalase, the calculations suggest an overall scavenging efficiency ($kC$) in the range of $1.1 \times 10^3 \text{s}^{-1}$ to $6.7 \times 10^3 \text{s}^{-1}$ and $5.3 \times 10^3 \text{s}^{-1}$ to $3.1 \times 10^4 \text{s}^{-1}$ for $e_\text{aq}$ and $\text{OH}'$ respectively. Obviously, the dissolved oxygen could have better affinity for electrons ($kC = 1.6 \times 10^7 \text{s}^{-1}$) than catalase in these experiments, and therefore, the observed radioprotection possibly results from partial removal of electrons, hydroxyl radicals and enzymatic decomposition of $\text{H}_2\text{O}_2$. 

The data presented in table 13 show that $\text{H}_2\text{O}_2$ in the concentration range of $1 \times 10^{-6} \text{M}$ to $1 \times 10^{-4} \text{M}$ affords significant protection against the post-irradiation, oxygen-dependent damage and a combination treatment (catalase 300 units/ml + $\text{H}_2\text{O}_2$ $1 \times 10^{-5} \text{M}$) exerts protection that is significantly greater than either of these additives individually (table 16 and figure 16). In $\text{E.coli}$, Näslund et al. (1976b) have shown that radiolytically produced small amount of $\text{H}_2\text{O}_2$ could afford radioprotection. This radioprotection by $\text{H}_2\text{O}_2$ in $\text{E.coli}$ has been correlated with a metabolic block demonstrated as inhibition of RNA synthesis (Näslund et al. 1976, Näslund and Ehrenberg 1978).
The chemistry pertaining to the radiolytic production and annihilation of $\text{H}_2\text{O}_2$ in well oxygenated aqueous cell suspensions is now much better understood. Various modes of their formation in the irradiated cells have been presented by Raghu and Kesavan (1986) and Singh and Kesavan (1990b). From the point of view of radiolytic breakdown of $\text{H}_2\text{O}_2$, the following reactions are important:

$$\text{H}_2\text{O}_2 + e^-_{\text{aq}} \rightarrow \text{OH}^- + \text{OH}^- \quad (16)$$

$$k = 1.2 \times 10^{10} \text{M}^{-1}\text{s}^{-1} \quad \text{(Anbar et al. 1973)}$$

$$\text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{OH}^- + \text{H}_2\text{O} \quad (17)$$

$$k = 6.0 \times 10^{7} \text{M}^{-1}\text{s}^{-1} \quad \text{(Behar et al. 1975)}$$

$$\text{H}_2\text{O}_2 + \text{OH}^- \rightarrow \text{H}_2\text{O} + \text{HO}_2^- \quad (18)$$

$$k = 4.5 \times 10^{7} \text{M}^{-1}\text{s}^{-1} \quad \text{(Dorfman and Adams 1973)}$$

$$\text{H}_2\text{O}_2 + \text{O}_2^2+ \rightarrow \text{O}_2 + \text{OH}^- + \text{OH}^- \quad (19)$$

$$k = 0.5 \text{M}^{-1}\text{s}^{-1} \quad \text{(Weinstein and Bielski 1979)}$$

In the dry seeds irradiated in vacuo, the yield of $e^-_{\text{aq}}$ and OH$^-$ ought to be negligible (please see reaction 10). With the presence of oxygen during the post-hydration, HO$_2^-$ and RHO$_2^-$ will be formed. The HO$_2^-$ can lead to the production of $\text{H}_2\text{O}_2$ as shown in reaction (7).
When $\text{H}_2\text{O}_2$ in the range of $1 \times 10^{-6} \text{M}$ to $1 \times 10^{-4} \text{M}$ is added to the oxygenated post-hydration medium, a competition between $\text{H}_2\text{O}_2$ and oxygen for the electrons possibly occurs (Singh and Kesavan 1990b). The overall scavenging efficiency ($kC$) of oxygen for $\text{H}^+$ works out to be $1.6 \times 10^7 \text{s}^{-1}$ (table 32). Whether radioprotection against oxic damage by $\text{H}_2\text{O}_2$ ($1 \times 10^{-6} \text{M} - 1 \times 10^{-4} \text{M}$) results from the removal of a fraction of the $\text{H}^+$ and also $\text{OH}^-$ is not clear. However, Singh and Kesavan (1990b) have pointed out in its support that there is no concentration-dependent protection by $\text{H}_2\text{O}_2$. This argument is countered by the observation that increasing $\text{H}_2\text{O}_2$ concentration results in enhanced seedling injury involving oxygen-independent pathway. Data (table 13) show that $\text{H}_2\text{O}_2$ concentrations of $1 \times 10^{-3} \text{M}$ and $1 \times 10^{-2} \text{M}$ significantly potentiate both the $\text{O}_2$-dependent as well as the $\text{O}_2$-independent components of radiation-induced seedling injury in a concentration-dependent manner. So, this is possibly an evidence for a gradual increase in the oxygen-independent pathway of seedling injury with a concomitant reduction in $\text{O}_2$-dependent pathway within a certain range of $\text{H}_2\text{O}_2$ concentrations. Since, $1 \times 10^{-2} \text{M}$ of $\text{H}_2\text{O}_2$ has no adverse effect, whatsoever, on the growth of unirra-
diated seeds, a general toxicity unrelated to radiation-induced events can not be evoked. At present the exact nature of the reaction of $H_2O_2$ with the radiation-induced precursors of $O_2$-independent pathway of damage is not known.

Data presented in tables 22 and 23 show a concentration-dependent protective action of cysteine on oxygen-dependent damage. These results are in accordance with the earlier reports (Kesavan and Ahmad 1974b, Kesavan and Afzal 1975). This radioprotection against $O_2$-dependent damage could possibly be due to the elimination of radiation-induced, oxygen-sensitive sites. Cysteine is well known to react with electrons and hydroxyl radicals at a rate constant of $8 \times 10^9 M^{-1}s^{-1}$ and $1.3 \times 10^{10} M^{-1}s^{-1}$ (Anbar and Neta 1967). Thus, the competition of cysteine with oxygen to react with electrons, could be the reason for the protection of oxygen-dependent damage.

Radioprotection by glutathione against oxygen-dependent damage (table 24) is also possibly due to its competition with oxygen for the electrons.

While the radioprotection to varying degrees against the oxic damage by the structurally-unrelated
chemical additives is more universal as discussed above, their influence on the anoxic damage is not so. In other words, some of these chemical additives potentiate the anoxic damage depending on the initial seed moisture content, whereas some other additives do not exert any effect whatsoever. Table 31 gives a classification of these chemicals as also the seed moisture content in the given experiment.

Table 31: Influence of chemicals on post-irradiation oxygen-independent damage in barley seeds.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Seed moisture content</th>
<th>Chemical and post-hydration temperature</th>
<th>Concentrations of chemical</th>
<th>Effect</th>
<th>Table No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>4.0%</td>
<td>Caffeine - 4°C</td>
<td>3.8x10^{-4} M and 3.8x10^{-3} M</td>
<td>Potentiation</td>
<td>18</td>
</tr>
<tr>
<td>02</td>
<td>3.2%</td>
<td>Catalase -25°C</td>
<td>100,200,300,400 units/ml and 500 units/ml</td>
<td>No effect</td>
<td>14</td>
</tr>
<tr>
<td>03</td>
<td>4.0%</td>
<td>Catalase - 4°C</td>
<td>100,300,500 and 600 units/ml</td>
<td>Potentiation</td>
<td>15</td>
</tr>
<tr>
<td>04</td>
<td>3.3%</td>
<td>Glutathione - 4°C</td>
<td>1x10^{-4} M, 1x10^{-3} M and 1x10^{-2} M</td>
<td>Potentiation</td>
<td>24</td>
</tr>
<tr>
<td>05</td>
<td>4.0%</td>
<td>Cysteine - 4°C</td>
<td>3.8x10^{-4} M and 3.8x10^{-3} M</td>
<td>No effect</td>
<td>22</td>
</tr>
<tr>
<td>06</td>
<td>2.6%</td>
<td>Cysteine - 4°C</td>
<td>3.8x10^{-4} M and 3.8x10^{-3} M</td>
<td>Potentiation</td>
<td>23</td>
</tr>
<tr>
<td>No.</td>
<td>Solution</td>
<td>Temperature</td>
<td>Concentration</td>
<td>Potentiation</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>------------------</td>
<td>-------------</td>
<td>------------------------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>07</td>
<td>3.6% Potassium permanganate</td>
<td>-4°C</td>
<td>$1 \times 10^{-4}$ M, $1 \times 10^{-3}$ M</td>
<td>Potentiation 11 and $1 \times 10^{-2}$ M</td>
<td></td>
</tr>
<tr>
<td>08</td>
<td>4.0% Hydrogen peroxide</td>
<td>-4°C</td>
<td>$1 \times 10^{-6}$ M, $1 \times 10^{-5}$ M</td>
<td>No effect 13 and $1 \times 10^{-4}$ M</td>
<td></td>
</tr>
<tr>
<td>09</td>
<td>4.0% Hydrogen peroxide</td>
<td>-25°C</td>
<td>$1 \times 10^{-3}$ M and $1 \times 10^{-2}$ M</td>
<td>Potentiation 13</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4.0% t-BuOH</td>
<td>-4°C</td>
<td>$1 \times 10^{-4}$ M, $5 \times 10^{-4}$ M, No effect 8 $1 \times 10^{-3}$ M, $5 \times 10^{-3}$ M, $1 \times 10^{-2}$ M and $5 \times 10^{-2}$ M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>3.3% Ethanol</td>
<td>-4°C</td>
<td>$1 \times 10^{-4}$ M, $5 \times 10^{-4}$ M No effect 9 $1 \times 10^{-3}$ M and $5 \times 10^{-3}$ M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>3.3% Ethanol</td>
<td>-4°C</td>
<td>$1 \times 10^{-2}$ M and $5 \times 10^{-2}$ M</td>
<td>Potentiation 9</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>3.5% Ascorbic acid</td>
<td>-4°C</td>
<td>$1 \times 10^{-4}$ M and No effect 10 $1 \times 10^{-3}$ M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>3.5% Ascorbic acid</td>
<td>-4°C</td>
<td>$1 \times 10^{-2}$ M</td>
<td>Potentiation 10</td>
<td></td>
</tr>
</tbody>
</table>

Right from the first report (Kesavan et al. 1973), caffeine potentiation of the anoxic damage was attributed to both physicochemical and biochemical pathways. Kinetic experiments of Nadkarni and Kesavan
(1975) showed that in barley seeds the caffeine-sensitive species either react (e.g. enzymes) or decay (e.g. free radicals) with a half life-time (t½) of about 100 min. So far as the free radical mechanisms are concerned, Raghu and Kesavan (1986) and Kesavan et al. (1990) have considered several possibilities.

In the bacterial spores, Kesavan and Powers (1985) and Raghu and Kesavan (1986) observed that caffeine enhances the yield of H₂O₂ under anoxic irradiation. Therefore, they have suggested that anoxic sensitization occurs via the removal of electrons and the consequent increase in the damage to target molecules by hydroxyl radicals. This proposal is analogous to the "electron sequestration" model of Powers (1972), but one drawback is that t-BuOH does not desensitize the caffeine-enhanced radiosensitivity of anoxic component (Raghu and Kesavan 1986). Hence, Kesavan et al. (1990) have proposed an alternate model in which the well-known binding of caffeine with denatured regions of the DNA (Ts'O and Lu 1964, Witte and Böhme 1972) is taken into account. Hence, the RH⁺ (resulting from the direct
action of gamma-photons on the target molecule (RH₂) complexes with caffeine to form "caffeine - RH⁺ complex" which is a potentially lethal lesion. This complex might interfere with restoration by "charge transfer reaction" (Adams 1967) and/or enzymatic repair of the DNA (when RH₂ refers to DNA).

So far as catalase is concerned, it potentiates the oxygen-independent component of damage in seeds post-hydrated at low (4°C) but not at metabolizing (25°C) temperature (tables 14 and 15 and figures 14 and 15). Considering the fact that it reacts quite appreciably with both electrons and hydroxyl radicals, free radical reactions at low temperature might be important for radiosensitization. At high temperature, both the temperature and higher hydration rates possibly accelerate the decay of electrons and hydroxyl radicals. In dry seeds when OH⁺ yields are negligible, catalase at low temperature can simulate the action of caffeine and cause 'electron sequestration' of Powers (1972). This explains its radiosensitization of the O₂⁻-independent damage.

Cystein has no effect on oxygen-independent pathway of damage (table 22) in the seeds of about 4.0 per cent moisture content but it potentiates the same in the seeds of about 2.6 per cent moisture content. This shows that seed moisture content
plays an important role in mediating the oxygen-independent pathway of damage. Kesavan and Ahmad (1974b) also did not find radiosensitization of the anoxic damage by cysteine in the seeds of about 7.0 per cent moisture content. It may be that the radiation chemistry involving cysteine and free radicals varies with seed moisture content, but its exact nature at present is not clear.

Potassium permanganate has been shown to enhance the oxygen-independent damage in the present study (tables 11 and 12) and this confirms the earlier reports (Kesavan and Afzal 1975, Kesavan and Dodd 1976, Kesavan et al. 1978). Tallentire and Jones (1973) have also observed the radiosensitization of oxygen-independent damage by potassium permanganate in Bacillus megaterium spores. They have proposed a mechanism, not involving OH·, for the same. Kesavan et al. (1990) have adopted this scheme with minor changes as follows:

\[
\begin{align*}
RH^+ + \text{MnO}_4^- & \rightarrow RH^+ + \text{MnO}_2^2- \quad (20) \\
RH^+ + H_2O & \rightarrow \text{Stable lethal products} \quad (21) \\
H^+ + \text{MnO}_4^- & \rightarrow \text{MnO}_4^{2-} + H^+ \quad (22)
\end{align*}
\]
The above scheme suits the situation obtained in the dry seeds irradiated in vacuo, since RH' are the potentially lethal lesions formed by the direct action of radiation on RH₂.

Hydrogen peroxide potentiates the anoxic radiation damage only at about and higher than millimolar concentrations (table 13). Similar results were obtained by Sah and Kesavan (unpublished). The precise radiation chemistry operating in anoxic radiosensitization is not clear. However, that H₂O₂ can react with H' in the dry irradiated seeds and generate OH' as shown in reaction (17) is important.

The combination of OH' and H₂O₂ can promote sequential double oxidation proposed by Powers (1972) as follows:

\[
3\text{MnO}_4^{2-} + \text{H}_2\text{O} \rightarrow 2\text{MnO}_4^{-} + \text{MnO}_2 + 2\text{OH}^{-} \quad (23)
\]

\[
2\text{OH}^{-} + 2\text{H}^{+} \rightarrow 2\text{H}_2\text{O} \quad (24)
\]

\[
\text{RH} + \text{H}_2\text{O}_2 \rightarrow \text{R} + \text{OH}^{'} + \text{H}^{+} + \text{OH}^{-} \quad (26)
\]

(lethally altered molecule)
The formation of lethally altered molecule $R$ could possibly be the reason for the potentiation of oxygen-independent pathway of damage.

Ascorbic acid exerts no effect on $N_2$- and $N_2O$-mediated damage (tables 6 and 10) at lower concentrations ($\leq 1 \times 10^{-3}$ M), which is well in agreement with the earlier reports (Afzal and Kesavan 1977, Jha and Kesavan 1986, Kesavan et al. 1990). The absence of any effect at lower concentrations possibly suggests its lack of reactivity towards the precursors of oxygen-independent component of damage. However, at higher concentrations it potentiates the $N_2$- and $N_2O$-mediated damage. Whether radiosensitization of anoxic damage at higher concentration is mediated via biochemical pathways, not involving the free radicals is not known.

t-BuOH does not produce any discernible adverse effect on the post-irradiation $N_2$- and $N_2O$-mediated damage (table 8 and figure 11). The present results are at variance with the earlier report of Afzal and Kesavan (1979b) in which a potentiation of anoxic damage has been reported. This discrepancy between the present and the previously reported results
could possibly be due to differences in seed moisture content, having been \(~ 4.0\) per cent and \(~ 3.0\) per cent in the present and previous studies respectively.

Ethanol, unlike \textit{t}-BuOH, potentiates the \(N_2^-\) and \(N_2O\)-mediated damage at higher concentrations (\(\geq 1 \times 10^{-2} \text{M}\)) but not at lower concentrations (\(\leq 5 \times 10^{-3} \text{M}\)) (table 9 and figure 12). The difference in the influence of ethanol and \textit{t}-BuOH on \(N_2^-\) and \(N_2O\)-mediated radiation damage could be due to the fact that the moisture contents of the seeds used in the \textit{t}-BuOH and EtOH experiments were \(~ 4.0\) per cent and \(~ 3.3\) per cent respectively. These studies, however, reveal that the more efficient \(\text{OH}^-\)-scavengers (ascorbic acid and ethanol) potentiate the anoxic damage in seeds of low water content.

In the present study glutathione is observed to potentiate the \(O_2^-\)-independent damage. Glutathione is known to react with electrons (Anbar and Neta 1967, Hoffman and Hayon 1972) but its reaction rate constant with hydroxyl radicals is not available. What is of significance is that it simulates the action of cysteine in respect of potentiating the anoxic radiation damage in seeds of very low moisture content.
Table 32: Scavenging efficiency of additive(s) for electrons and hydroxyl radicals and protection against post-irradiation O₂-dependent damage.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Additive(s)</th>
<th>*Scavenging efficiency (kC) for</th>
<th>Percentage seedling injury</th>
<th>Total peroxides (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Electrons (H⁺)</td>
<td>hydroxyl radicals (OH⁻)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None (only O₂)</td>
<td>1.6x10⁷ s⁻¹</td>
<td>-</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>Catalase (300 units/ml)</td>
<td>3.4x10³ s⁻¹</td>
<td>1.6x10⁴ s⁻¹</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>H₂O₂ (1.0x10⁻⁵ M)</td>
<td>1.2x10⁵ s⁻¹</td>
<td>4.5x10² s⁻¹</td>
<td>41</td>
</tr>
<tr>
<td>4</td>
<td>Catalase (300 units/ml) + H₂O₂ (1.0x10⁻⁵ M)</td>
<td>4.0x10⁸ s⁻¹</td>
<td>7.2x10⁶ s⁻¹</td>
<td>30</td>
</tr>
</tbody>
</table>

Seed moisture content ~ 3.9%
Dose = 350 Gy
Post-hydration at 25°C for 8 hrs.
Unirradiated seeds have no seedling injury, and their total peroxide content is 2.25x10⁻⁵ M.

*Scavenging efficiency (kC) is defined as the product of scavenger's molar concentration and the bimolecular rate constant for the reaction between the scavenger and the radicals.
Data presented in table 16 and figure 16 show that a combination treatment of catalase (300 units/ml) and \( \text{H}_2\text{O}_2 (1\times10^{-5} \text{M}) \) affords much better radioprotection than either of these two additives individually. This has been explained in terms of radiation chemistry (Singh and Kesavan 1990b). Both \( \text{H}_2\text{O}_2 \) and catalase individually is less effective in competing with oxygen for electrons (table 32). Catalase (300 units/ml) and \( \text{H}_2\text{O}_2 (1\times10^{-5} \text{M}) \) would be expected to remove 2 and 75 electrons respectively for every 10,000 electrons reacting with \( \text{O}_2 \). The reaction involving \( \text{H}_2\text{O}_2 \) with \( \text{H}^+ \), the consequent production of \( \text{OH}^- \) and their rate constants are given in reactions (17) and (18). Protection which is still seen is possibly due to partial removal of \( \text{H}^+ \) and \( \text{OH}^- \). The combination treatment on the other hand, would be expected to remove 25 electrons per electron removed by oxygen.

Data presented in table 17 show that both catalase and SOD exert radioprotection against oxygen-dependent damage. The radioprotection by catalase has already been discussed. SOD has been shown to protect the *Acholeplasma laidlawii* B from ionizing radiation (Petkau and Chelak 1974) and bacteriophage, bacteria, and enzymes from UV-radiation (Lavelle et al. 1973).
However, endogenous induction of an increased level of SOD does not necessarily lead to radioprotection in *E. coli* (Goscin and Fridovich 1973). The radioprotection by SOD wherever observed, has been explained on the basis of its removal of superoxide radical anion (O$_2^-$) as follows:

$$2O_2^- + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

$$\text{(k= 2.37x10}^9\text{M}^{-1}\text{s}^{-1}) (\text{Fielden et al. 1974})$$

In the abovesaid experiments reported in the literature, the gaseous conditions during irradiation were not controlled. In the present study where the gaseous conditions are controlled, it is noted that SOD potentiates the N$_2^-$ and N$_2$O-mediated damage (Table 17) in terms of seedling injury, but not in terms of peroxidase activity and total peroxides. This observation is at variance with the report of Petkau and Chelak (1974) in which SOD has no effect on the anaerobic radiation damage in *Acholeplasma laidlawii* B. There is, of course, no formation of O$_2^-$ under anoxic irradiation conditions. The SOD at the concentration tested exerts no effect on the unirradiated seeds. Therefore, there is a clear role of the radiation-induced electrons
and hydroxyl radicals; more work is necessary to elucidate the possible radiation chemistry.

Buthionine sulfoximine (BSO) and glutathione (G-SH) individually and in combination with each other protect against the \( O_2 \)-mediated damage, but potentiate the \( N_2 \)- and \( N_2O \)-mediated damage (table 25). There are several reports in the literature on the protective effects of glutathione (Meister and Anderson 1983, Rotstein and Slaga 1988) and the nullification of these effects by depletion of glutathione by BSO (Bump et al. 1982, Dethlefsen et al. 1988). In rapidly metabolizing mammalian cells, these effects are indeed biochemical; the present studies reveal that the BSO exerts a different effect at least in the dry seeds irradiated in vacuo.

(c) Radiation damage as a function of varying \( O_2 \)-concentration and the influence of ascorbic acid and caffeine.

The influence of \( O_2 \)-concentrations on the magnitude of post-irradiation oxic damage has been discussed in previous section (II). From the data (table 6 and figure 9), it is seen that the mode and magnitude of chemical modification of post-irradiation damage
is clearly dependent upon the oxygen concentrations in the post-hydration medium.

Livessey and Reed (1987) have shown that ascorbic acid can donate electron to the damaged molecule and cause restoration as follows:

\[ \text{RH}^- + \text{ascorbic acid} \rightarrow \text{RH}_2 + \text{ascorbic acid}^- \] (28)

Ascorbic acid exerts no effect at \( \leq 30\% \) \( \text{O}_2 \)-concentration (table 6 and figure 9) in the post-hydration medium; however, it affords protection when oxygen-concentration is \( \geq 50\% \). Kesavan et al. (1990) have interpreted these data on the basis that at low \( \text{O}_2 \)-concentrations, ascorbic acid possibly does not protect \( \text{RH}^- \) against the development of a "fast component" of the oxic damage.

There are evidences in the literature of barley seed radiobiology to implicate "fast" and "slow" components of reactivity of the radiation-induced oxygen-sensitive sites with \( \text{O}_2 \) or their decay in the absence of \( \text{O}_2 \) (Ahnström and Mikaelson 1968, Ahnström and Sanner 1971). This implies that ascorbic acid donates electron to \( \text{RH}^- \) to protect against not the first "fast" but a later "slow" oxidation of \( \text{RH}^- \) by \( \text{O}_2 \). This "fast" component is not manifested when \( \text{O}_2 \)-concentration is too high to be a limiting factor for the development of the full post-irradiation oxygen effect.
Caffeine potentiates the damage when the $O_2$-concentration in post-hydration medium is $\leq 30\%$ but exerts no effect at $\sim 50\%$ $O_2$-concentration and protects at an oxygen-concentration of $\geq 80\%$.

The protection on the basis of mutual annihilation of oxygen sensitive sites with caffeine and potentiation by binding of potentially lethal "Caffeine- RH' complex" to DNA have been discussed in the previous section III b.

(d) **Influence of wet heat-shock and caffeine on oxic and anoxic damage:**

Both wet heat-shock and caffeine afford significant radioprotection against the post-irradiation oxygen-dependent damage that develops in the seeds of low ($\sim 3.6\%$) but not high ($\sim 11.5\%$) water content (tables 26 and 27). Under the conditions of high seed moisture content and/or the absence of radiation-induced $O_2$-sensitive sites, caffeine acts as a radiosensitizer (Kesavan 1973, Nadkarni and Kesavan 1975). These aspects as also the possible mechanisms thereof have already been discussed (Section III b).

interpreted the radioprotection against oxic damage by heat-shock on the basis of thermal annealment of radiation-induced, $O_2$-sensitive sites. Present result also supports the earlier findings. It might well be that caffeine and heat-shock compete for the same sites since a combination treatment results in greatly reduced protection than that by either of the agents separately (Table 26).

Throughout the present study it was found that seedling injury and peroxidase activity exhibit a parallel trend which is in accordance with the earlier report of Balachandran and Kesavan (1978). Whenever there is protection or potentiation of the damage, the peroxidase activity also decreases or increases respectively. Grosso et al. (1987) have suggested that genetic background and developmental factors may play an important role in the stability of mechanism affecting the expression of peroxidase enzymes.

There are reports in the literature that heat-shock produces heat-shock proteins (HSPs) in a variety of test systems and under different oxidative-stress conditions (Schlesinger et al. 1982, Tanguay 1983, Roop et al. 1983, Nover 1984,

A careful analysis of gel reveals that molecular mechanism of enhancement of the enzyme activity might vary with circumstances. When the seeds of low moisture content (\( \sim 3.6\% \)) were given a post-irradiation hydration in oxic water, the bands are more intense and there is also formation of two new additional bands (Figures 18 and 20). Under these circumstances, heat-shock and caffeine which reduce the peroxidase activity and seedling injury (Kesavan et al. 1973, Balachandran and Kesavan 1978) completely eliminate the two new bands, the intensity of the other peroxidase bands also diminishes. On the other hand, potentiation of seedling injury and peroxidase ascribable to \( O_2 \)-independent component
by caffeine and heat-shock is not accompanied by
the formation of new bands (figure 19). This has
been explained by Singh and Kesavan (1990c) on the
assumption that post-irradiation damage results
from the reaction of $A_n$ sites ($RH^\cdot$, $H^\cdot$, $e^-_{\text{trapped}}$) with
$O_2$. The formation of reduction products of oxygen
such as $RHO_2^\cdot$, $HO_2^\cdot$ and $O_2^\cdot$ could be considered to
provide signal for the concerned genes to transcribe
the two new fractions of peroxidase. Roop et al.
(1983) have also proposed that a product of oxygen
reduction might be involved in the primary signal
for the production of HSPs. However, what is observed
in the present case is that in some circumstances
heat-shock can anneal the $O_2$-sensitive sites and thus
abolish the premise for the generation of the signals.
The peroxidase activity can also be induced to increase
by a pathway not involving the additional bands.
Radiosensitization of $O_2$-independent pathway by
caffeine and heat-shock falls in this category.
This again supports that reduction products of oxygen
in the irradiated seeds are necessary for the formation
of the two new additional bands of peroxidase.
For the same reason, there are no new bands following
oxic post-hydration of the irradiated seeds having
$\geq 10\%$ moisture (figure 19).
The studies undertaken in this thesis have opened new avenues in some ways but have also raised many new questions.