SUMMARY

Dry barley seeds equilibrated with appropriate moisture contents were exposed in vacuo to desired doses of $^{60}$Co-gamma-rays and post-hydrated at 4±1°C or 25±1°C for 8 hrs in oxygen, nitrogen, and nitrous oxide-saturated water. The post-irradiation damage was assessed in terms of seedling injury, peroxidase activity and total peroxide content in the 8-day old seedlings. The effect of different chemicals (known to have high reaction rate constants with electrons and/or hydroxyl radicals) dissolved in the post-hydration media on the magnitude of damage developing under these three different gaseous circumstances was studied. In one set of experiments, the influence of caffeine and ascorbic acid dissolved in the post-hydration media saturated with varying concentrations of oxygen (0-100%) on seedling injury, peroxidase activity and total peroxides was also investigated. The major findings are as follows:

(i) The nitrous oxide (N$_2$O) present during post-hydration at 4°C of dry seeds (≤ 4% moisture) was most radio-protective, surpassing even the N$_2$-saturated post-hydration. This observation that has been reported for the first time is confined to only the dry seeds, as increasing seed moisture results in the disappearance of the protective effect. In the "wet"
seeds (seeds with over 11% seed moisture content), there is absolutely no radioprotective effect of $N_2O$ in comparison with the level of damage observed in $N_2$; again, these results do not support a few of the earlier reports in bacteria and mammalian cells that $N_2O$ causes radiosensitization by converting the hydrated electrons ($e^-_{aq}$) into hydroxyl radicals (OH'). These observations have been interpreted to suggest that a small yield of OH' possibly resulting from the reaction of $N_2O$ with the electrons (H') in the dry seeds is beneficial than harmful. In making this suggestion, the fact that an equilibrium between OH' and H' can result in harmless recombination ($OH' + H' \rightarrow H_2O$) of the free radicals is kept in view.

(ii) Post-irradiation oxygen-saturated hydration induces substantial increase in the seedling injury and peroxidase activity with concomitant reduction in total peroxides. This has been ascribed to the high reactivity of oxygen with radiation-induced, oxygen-sensitive ($A_n$) sites to produce damaging species such as $RHO_2^-$, $HO_2^-$ and $O_2^-$.  

(iii) The initial seed moisture content has considerable influence on post-irradiation oxygen-dependent damage.
It has been shown that post-irradiation oxygen-dependent damage decreases with increasing seed moisture content. However, seed moisture does not have appreciable influence on the post-irradiation oxygen-independent damage.

(iv) The rate of the decay of radiation-induced, oxygen-sensitive \( A_n \) sites in the absence of oxygen is a much slower process than their rate of reaction with oxygen. During the present study in the seeds of \( \sim 3.2 \) per cent moisture, post-hydrated at 4±1°C, the rates of reaction of the radiation-induced, oxygen-sensitive sites with oxygen are \( \sim 8-9 \) times faster than the rates at which these decay in the absence of oxygen.

(v) The chemical modification of radiation-induced damage depends on the concentration of oxygen in the post-hydration medium. The oxygen-dependent damage increases with increase in oxygen-concentration in the post-hydration medium up to 80%; after this a plateau is observed. Ascorbic acid does not exert any effect when oxygen-concentration in the post-hydration medium is \( \leq 30\% \), but affords radioprotection when oxygen-concentration in the post-hydration
medium is \( \geq 50\% \). On the other hand, caffeine potentiates the post-irradiation injury as long as the proportion of oxygen in post-hydration is \( \leq 30\% \), exerts no effect, whatsoever, when the proportion of oxygen is \( \sim 50\% \) and affords radioprotection when proportion of oxygen is \( \geq 80\% \).

(vi) Potassium permanganate affords radioprotection against post-irradiation, oxygen-dependent damage but potentiates the oxygen-independent damage. The radioprotection occurs possibly due to annihilatory reaction of \( \text{MnO}_4^- \) with \( \text{RHO}_2^+ \) and \( \text{HO}_2^- \), resulting in the removal of the damaging species. On the other hand, radiosensitization in the absence of \( \text{O}_2 \) has been explained on the basis of formation of stable lethal products due to the reaction of \( \text{RH}^+ \) with \( \text{MnO}_4^- \), in which \( \text{RH}^+ \) are potentially lethal lesions formed by the direct action of radiation on the target molecule (\( \text{RH}_2 \)). The reaction of \( \text{RH}^+ \) with \( \text{MnO}_4^- \) results in \( \text{RH}^+ \) and \( \text{MnO}_4^{2-} \). \( \text{RH}^+ \) reacts with \( \text{H}_2\text{O} \) to form stable lethal products.

(vii) The radioprotection of oxygen-dependent damage by \( \text{OH}^- \) scavengers like ascorbic acid, \( \text{t}-\text{butyl alcohol} \) and ethanol suggests that there is an "\( \text{OH}^- \) component" within the oxygen-dependent damage in fully oxygenated (\( \sim 10^{-3}\text{M} \)) condition, and the removal of \( \text{OH}^- \) by these
chemicals, therefore, is the basis of radioprotective action. It is noted that in spores, the "OH component" within the oxygen-dependent damage is observed only in the range of oxygen-concentrations of $10^{-5} \text{M} - 10^{-4} \text{M}$. Thus, these data derived from experiments with eucaryotic seeds are at variance with those from procaryotic bacterial spores. There is of course, the other variable, namely that the bacterial spore experiments were carried out with 50 kVp X-rays whereas those reported here employed $^{60}$Co-gamma-rays. Both these differ in their Linear Energy Transfer (LET) by a factor of about 15.

(viii) Caffeine which reacts with both $e^-_{aq}$ and OH' affords radioprotection against oxygen-dependent damage, which is possibly due to mutually annihiliatory reaction of caffeine molecules with radiation-induced, oxygen-sensitive sites. On the other hand, it potentiates the oxygen-independent damage, possibly because of the binding of caffeine with RH' to from "caffeine-RH' complex". This complex possibly interferes with restoration of RH' by charge transfer reaction and/or enzymatic repair.

(ix) Catalase which has been reported to react with both OH' and $e^-_{aq}$ affords significant radioprotection
against oxygen-dependent damage, but potentiates the oxygen-independent damage at 4°C but has no effect at 25°C. The radioprotection against oxic damage is possibly due to partial removal of electrons, hydroxyl radicals and enzymatic decomposition of H₂O₂. The absence of any effect of catalase on the oxygen-independent damage at 25°C may be due to the fact that at higher temperature, the decay of electrons and hydroxyl radicals becomes faster, whereas at low temperature, catalase simulates the action of caffeine to result in "electron sequestration".

Hydrogen peroxide in the concentration range of 1x10⁻⁶ M - 1x10⁻⁴ M affords significant radioprotection against the post-irradiation O₂-dependent damage, but exerts no effect whatever on the O₂-independent damage. As the concentration of H₂O₂ is increased to 1x10⁻³ M, O₂-dependent seedling injury is significantly potentiated. The same is true also for the O₂-independent component of seedling injury. Even 1x10⁻² M of H₂O₂ has no adverse effect on the seedling growth of unirradiated seeds. This means that a general toxicity unrelated to radiation-induced events cannot be invoked. It may be that the potentiation of damage by higher concentration (≥1x10⁻³ M) of H₂O₂ present during oxic
post-hydration is really due to its enhancement of the \( \text{O}_2 \)-independent component of damage. A reduction in the magnitude of oxic damage with concomitant and substantial increase in the anoxic seedling injury ultimately reflects as the potentiation of the former as well. The possible mechanisms could not be considered for want of more experiments both radiobiological and radiation chemical.

(xi) A combination treatment of catalase (300 units/ml) and \( \text{H}_2\text{O}_2 \) (1x10^{-5} M) affords much better radioprotection against the \( \text{O}_2 \)-dependent damage than either of these two additives individually. This observation greatly emphasizes that in addition to the well-known enzymatic decomposition of the \( \text{H}_2\text{O}_2 \) by catalase, there is the physico-chemical pathway of action; the calculations show that a combination of these two additives could result in more effective removal of electrons in competition with oxygen.

(xii) Cysteine and glutathione afford radioprotection against oxygen-dependent damage. Since these two chemicals are well known to react with electrons, the competition of these two chemicals with oxygen for electrons is possibly the pathway of their radioprotection against the oxygen-dependent damage.
(xiii) Superoxide dismutase (SOD) affords radioprotection against oxygen-dependent damage. The indication again is that superoxide anion radicals \( (O_2^-) \) are possibly involved in this pathway of damage and its removal by SOD possibly is the reason for the radioprotection.

(xiv) Wet heat-shock (at 60°C for 90 sec) affords significant radioprotection against oxygen-dependent damage in the seeds of low (~3.6%) but not of high (~11.5%) water content. This has been interpreted on the basis of thermal annealment of radiation-induced, oxygen-sensitive \( (A_n) \) sites. The heat-shock also lowers the protective action of caffeine, which suggests that caffeine and heat-shock compete for the same sites. Further, heat-shock accentuates the sensitizing effect of caffeine on the anoxic component of damage.

(xv) In non-denaturing polyacrylamide gel electrophoretic pattern of the barley peroxidase, there is the appearance of two additional bands when the seeds of low (~3.6%) moisture content are given a post-irradiation hydration in oxygen-saturated water; these two additional bands are not induced in the seeds of high (~11.5%) moisture content. Caffeine and heat-shock eliminate these two new bands induced by \(^{60}\)Co-gamma-rays in the
Dry seeds of ~3.6% moisture. Further, potentiation of O₂-independent component by caffeine and heat-shock is not accompanied by the formation of new bands. This suggests that formation of reduction products of oxygen such as RH₂O⁻, H₂O⁻ and O₂⁻ could be considered to provide the necessary signals for activating the concerned genes to transcribe the two new fractions of peroxidase.