INTRODUCTION

In bacterial as well as mammalian culture cells, radiosensitization by oxygen develops as a function of concentration up to a certain limit beyond which an asymptote plateau is reached (Alper and Howard-Flanders, 1956; Ewing and Powers, 1980). Using 'dry' barley seeds as a test system, it has been shown that radiosensitization by oxygen results from a polyphasic interaction amongst oxygen concentration, radiation dose, seed moisture content and hydration temperature (Kesavan and Ahmad, 1976; Nadkarni and Kesavan, 1975; Afzal and Kesavan, 1979; Donaldson, Nilan and Konzak, 1979 a; b; 1982). Besides this, it is now beginning to be understood that dose-rate also plays a significant role in determining the radiation-induced damage. Albeit, it has already been exhibited in several plant and animal systems (Hall and Bedford, 1964; McCrory and Grun, 1969; Bottino and Sparrow, 1971; Bottino, Sparrow, Schwemmer and Thompson, 1975; Srinivasan and Kesavan, 1979 a; b; Nair, 1980; Hornsey and Alper, 1966; Page, Ainsworth and Leong, 1968; Krebs and Leong, 1970; Hornsey and Bewley, 1971; Puro and Clarke, 1972; Fu, Phillips, Kane and Smith, 1975). The radiation-induced damage in presence of
nitrogen or oxygen has been interpreted in various ways. Alper (1963) designated them as 'N' and 'O' type respectively. Powers (1961) has classified these as Class I, which is independent of oxygen; Class II, i.e. 'immediate' oxygen effect, which probably involves very short-lived radicals, and requires the presence of oxygen during irradiation; and Class III, i.e. post-irradiation oxygen effect which is demonstrable in the dried biological systems post-irradiatively exposed to oxygen. It has, however, been elegantly shown that the so-called 'immediate' oxygen effect (Class II) does not appear as a separate entity in eukaryotic spores and seeds (Dodd and Ebert, 1970; Kesavan and Afzal, 1975; Kesavan and Dodd, 1976; Afzal and Kesavan, 1977; Kesavan, 1979). Presumably, the Class II damage is brought about by the reaction between very reactive form(s) of oxygen-sensitive sites with oxygen; and the Class III damage develops as a result of reaction of oxygen with the same oxygen-sensitive sites which have cascaded to less reactive forms \((A_1 - A_2 - A_3 \ldots A_n; \text{ Dodd and Ebert, 1970})\) in the absence of oxygen. Therefore, the 'immediate' oxygen effect should be regarded as a part and parcel of the Class III damage. Thus, this component has been referred to as
'pseudo-Class II' by Kesavan and Dodd (1976).

A good deal of controversy persists with regard to the mechanism of 'oxygen effect'. One view is that the formation of hydrogen peroxide in a watery milieu is an important pathway of the 'oxygen effect' (Sobels, 1963). An increase in the peroxidase activity in the irradiated biological systems, especially plants, has been shown by several groups of workers (Endo, 1967; Ogawa and Uritani, 1970; Chourey et al., 1973; Warfield et al., 1975). Balachandran and Kesavan (1978) reported that caffeine depresses peroxidase activity in irradiated 8-day old seedlings in the oxygenated condition; but, at the same time, it enhances its activity in oxygen-free condition. These observations are commensurate with its effect on seedling injury. This provided a rationale for the peroxidase estimation in the present study.

Chemical modification of radiation damage particularly in barley, depends on several factors; such as, seed moisture content, hydration temperature, concentration of chemicals and radiation dose. This may be manifested either in terms of decrease (radioprotection) or increase (radiosensitization) depending upon the nature and concentration of chemicals.
A careful survey of the literature reveals only two major lines of interpretation of chemical radioprotection and radiosensitization: (i) biochemical, and (ii) physicochemical. Billen (1963) was one of the first to present a biochemical scheme of modification of radiation damage. He showed in a triple mutant strain of *E. coli* (15T-A-U-) that DNA synthesis, proceeding to completion, with concomitant suspension of RNA and protein syntheses led to increased radioresistance. Kovács, Kari, Nagy and Hernádi (1968) found that L-cysteine exerts its inhibitory effect on protein synthesis prior to RNA synthesis. And this, they suggested, somehow, brought about radioresistance via unbalanced DNA synthesis. Biochemical interpretations of chemical radioprotection, especially with regard to aminothiols, have also been supported by the data of Näslund and Ehrenberg (1978) on *E. coli*. They proposed that aminothiols (cysteamine) afford protection against radiation damage in oxygenated condition via inhibition of RNA synthesis by their autooxidative product, hydrogen peroxide. This has been evidenced by showing removal of radioprotection as a result of (i) depletion of oxygen in growth medium; (ii) removal
of catalytic copper from the growth medium; (iii) addition of catalase in the growth medium. Further support has been extended by restoration of radio-protection by catalase-inhibitors (Boyland and Gallico, 1952; Feinstein and Berliner, 1957) and addition of hydrogen peroxide (no chemical radio-protector aminothiol). Evans (1947) however, found catalase reducing the toxicity of irradiated water to Arbacia sperm. Sodium azide, a potent respiratory-inhibitor, inactivates catalase by binding to its heme group (Balachandran and Kesavan, 1978; Nilan, Kleinhofs and Sander, 1975; Kleinhofs et al., 1974; Wyss et al., 1948). Ascorbate, which was shown to be an efficient scavenger of radiation-induced free radicals (Joshi, Singh, Gopal-Ayengar and Ehrenberg, 1973) has been reported to reverse nearly completely the radioprotective effects of cysteamine (Näslund, Ehrenberg and Djalali-Behzad, 1976). It also needs to be pointed out here that the inhibition of RNA synthesis by any chemical does not always result in enhanced radioresistance. For instance, Pollard and Weller (1969) failed to achieve radioprotection by rifampicin which is known to inactivate DNA-dependent RNA polymerase and thereby inhibit RNA synthesis.
Recently, Eidus, Korystov, Kublik and Vexler (1982) have proposed the existence of a common mechanism for most of the radioprotective compounds, irrespective of their chemical structure. They suggest that any chemical which affords radioprotection does so via enhancement in the repair efficiency of the biological system by depressing their metabolic processes at higher concentrations.

Delay in DNA synthesis leading to radioprotection by MEA via activation of adenyl cyclase, known to enhance the production of cAMP, has been reported by Langendorff and Langendorff (1971), Prasad (1972), and Mitznegg (1973). Oleinik, Brewer and Rustad (1978) found radioprotection by caffeine, independent of any change in the level of cAMP. Pazdernik and Uyeki (1978), however, suggest that any change in the level of cAMP may lead to radioprotection by aminothiols. Näslund and Ehrenberg (1978) also report a similar trend. There are several physicochemical postulates also to explain chemical radioprotection. These may be briefed as (i) anoxia production or anaerobiosis (Patt, 1953; Gray, 1956); (ii) mixed disulphide theory (Pihl and Eldjarn, 1958); (iii) free radical scavenging (Bacq and Alexander, 1961); and (iv) electron donation hypothesis (Löhmann, 1974).
Brown (1967) tried to present a postulate by bridging the biochemical and physicochemical interpretations of chemical radioprotection. He suggested that the chemicals capable of protecting against radiation damage should have certain structural requirements; and that 2-3 carbon containing aminothiols should extend maximum radioprotection. The rationale has not been clearly mentioned. He is of the view that aminothiols bind to certain parts of DNA not covered by histones and thus stabilize its structure. As a result, two things happen: (i) apart from preventing primary lesions due to radiation-induced free radicals, it also helps to prevent the primary lesion to be altered to secondary damage from shortening or chemical alteration by holding the loose ends resulting from single strand rupture. Second, the DNA replication rate is decreased so that the repair process may get enough time to deal with alterations before they are replicated. Binding of this requires that the disulphide form of the chemical radioprotector is the active one and that the disulphide is necessary for ease in removal so that repair and DNA and RNA syntheses may proceed. This, however, does not explain satisfactorily the post-irradiation modification of radiation damage by aminothiols.
As regards the mechanism of radiosensitization, particularly by hypoxic chemical radiosensitizers, there are again both physicochemical as well as biochemical hypotheses. Among the physicochemical interpretations of chemical radiosensitization, the charge separation model of Adams and Dewey (1963) and Adams (1968); target sensitizer interaction theory of Chapman, Reuvers and Greenstock (1973) and the electron sequestration model of Powers (1972) and Powers, Tallentire, Davies and Ebert (1973) are important.

The biochemical interpretation of chemical radiosensitization is largely based on its ability to inhibit repair replication of DNA via complex formation with the substrate or inhibition of one or more of a battery of repair enzymes (Harm, 1967; Bendigkeit and Hanawalt, 1968; Cleaver and Thomas, 1969; Domon and Rauth, 1969; Yamamoto and Yamaguchi, 1969; Gaudin, Gregg and Yielding, 1972; Rommelaere and Errera, 1972; Swietlinska and Žuk, 1974; Kiefer, 1975; Yamaguchi, Tatara and Naito, 1975; Yamaguchi and Tatara, 1977; Apfelzweig and Teplitz, 1979). Painter (1980) has put forward an entirely different mechanism of radiosensitization, especially by caffeine. He has
suggested that caffeine potentiates radiation damage in anoxic condition by triggering off DNA synthesis. According to him, the incipient radiation-induced lesions gradually become fixed when the DNA replication is activated.

It is noteworthy that the above mentioned interpretations of chemical radioprotection and radiosensitization are applicable to only those chemicals which act as either a radioprotector or a radiosensitizer. But, the demonstration of differential modification of the oxic and the anoxic components of radiation damage by one and the same chemical(s) by Kesavan and co-workers (Kesavan, 1973; Kesavan and Ahmad, 1974a,b; Nadkarni and Kesavan, 1975; Kesavan and Afzal, 1975; Kesavan and Dodd, 1976; Kesavan, Sharma and Afzal, 1978; Afzal and Kesavan, 1977, 1979; Sharma, Kesavan and Srivastava, 1982) has warranted an altogether different interpretation. It has been elegantly shown that there exists two major categories of chemicals from the radiobiological point of view. One group which comprises caffeine, WR-2721, N-ethylmaleimide (NEM), hydroxyurea, cycloheximide, dithioerythritol, etc. reduces the magnitude of oxygen-dependent radiation damage; while at the same time they potentiate oxygen-independent radiation damage.
The second group (e.g. ascorbic acid, sodium ascorbate, cysteine, cysteamine, etc.) reduces the oxygen-dependent component, but has no effect whatsoever, on the oxygen-independent component of radiation-damage.

In order to explain these results they proposed a hypothesis which assumes that chemical radioprotection accrues from mutually annihilatory reaction between the chemical molecules and the radiation-induced, oxygen-sensitive sites \( (A_n) \). The hypoxic radiosensitizer chemicals, on the other hand, potentiate radiation damage when they exhibit reactivity towards the oxygen-insensitive sites \( (D_z \) of Dodd and Ebert, 1970).

The entire reactions can be summarized as follows:

(i) Oxygen-sensitive sites \( (A_n) + O_2 \) - Damage enhanced (Potentiation)

(ii) \( A_n \) sites + chemical \[
\text{mutually annihilatory reaction}
\]
Reduction of radiation damage (Protection)

(iii) \( D_z \) sites + chemical \( (\text{Oxygen-insensitive}) \)

Either potentiation of radiation damage or neutral effect, depending upon nature of the chemical.

This hypothesis has been adequately substantiated by several exquisite kinetic studies of Nadkarni and Kesavan (1975) and Afzal and Kesavan (1979).
With this background the present work was undertaken. First, the development of 'oxygen effect' and its modification by caffeine and KMnO$_4$ in 'dry' barley has been studied. Then the influence of dose-rate on the post-irradiation modification of radiation damage by caffeine has been assessed; and finally a critical evaluation of the biochemical (Näslund, Fedorcsák and Ehrenberg, 1976) vis-a-vis physicochemical hypotheses (Kesavan and co-workers) of chemical radioprotection and radiosensitization has been attempted at by performing select experiments.