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MASTER

ENVIRONMENTAL CONDITIONS THAT MODIFY PLANT RESPONSE
TO NEUTRON IRRADIATION*

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Abstract

Various environmental factors that influence response to irradiation in both "wet" and "dry" plant systems are reviewed. The oxygen effect, expressed quantitatively as the oxygen enhancement ratio (OER) is lower for neutrons (1.3-1.9) than for sparsely ionizing radiations. X and γ rays give OER's of 2.4 to 3.4 in wet systems and 10-15 in very dry seeds (2-3% water content). Water appears to regulate the degree to which radiation induced oxygen-sensitive sites or radicals interact with oxygen to produce radiobiological damage. The effect is less with neutrons than with x irradiation. Control over mutagenic or other specific radiobiological responses may lie, not so much in specific reactions with DNA, but more with cellular processes that precede or follow treatment, so that preferential recovery may take place. Examples are given of some environmental perturbations that show promise for altering response to neutron irradiation.

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INTRODUCTION

Experiments aimed at modifying biological responses to irradiation commonly show less influence of environmental perturbations with exposure to radiations of relatively high mean linear energy transfer (LET) than to those of low LET. This paper reviews results of experiments with plant materials which show that environmental variables do indeed affect response to neutrons as well as to other radiations that produce relatively densely ionizing tracks in irradiated tissues. Reference is made to evidence for mutagenic specificity of neutrons; and the prospects for exercising more control over the products recovered from plants exposed to neutron irradiation are discussed.

THE OXYGEN EFFECT (OER) IN "WET" SYSTEMS

An enhancing effect of oxygen on the damage produced by irradiation is one of the best documented environmental influences. This effect is often expressed quantitatively as the oxygen enhancement ratio (OER), which is defined as the ratio between the doses of radiation required to produce equal degrees of damage in anoxic and oxygenated cells.

For convenience, the oxygen effect in plants will be discussed under the species used as experimental test material and under "wet" vs. "dry" systems. Mottram [1] first demonstrated, in 1935, that the effect of γ and x radiations in inhibiting the growth of Vicia faba (broad bean) root tips was materially reduced if the exposure was made under anaerobic conditions. It was not until 1952, however, that oxygen was clearly demonstrated by Read [2], using the reduction in the rate of elongation of the primary bean root as the index of radiosensitivity, to be a dose modifying agent and the OER to be 2.67 for acute exposures to x rays. With this same test system Hall and Cavanagh [3] have shown that the OER is reduced, at 19° C but not at 3° C, for protracted low dose-rate exposures. This is because the aerated dose-response curve varies with dose rate, while the hypoxic dose-response curve is virtually independent of dose rate. It was further shown, using synchronized cell populations of the root tips that the OER did not change significantly with the phase of the mitotic cycle [4].

In the context of this paper results reported with 14 MeV neutrons are of particular interest. Neary et al. [5] reported an OER of 1.4 for reduction in elongation of broad bean roots, and Hall [6] in the same system, obtained the ratio of 1.66 for both 14 MeV neutrons and for californium 252 [7], 63% of the dose of which is contributed by fission spectrum neutrons having an average energy of 2.3 MeV and a modal energy of about 1 MeV. Corresponding measures on barley roots gave an OER of 1.92 for 14 MeV neutrons, compared to 3.4 for x rays [8].

For negative π mesons, Raju et al. [9] have found, with the Vicia faba root test system, OER values of 1.35 and 1.5 at room temperature and at

4°C , respectively. The beam was characterized by π^{-} mesons of about 90 MeV, 80% of the dose was from low ($<100\text{ KeV}/\mu$) LET radiation, and there was contamination with 25% electrons and 10% μ mesons.

The oxygen effect was shown to hold equally well for genetic abnormalities in root tip cells of Vicia faba [10]. However, little or no oxygen effect was observed [11] when α radiation (high LET) was used. Subsequently others [12] have made a study of the relative biological effectiveness of ^{60}Co γ rays and fast neutrons ($\sim 3\text{ MeV}$) under aerobic and anaerobic conditions using the frequency of micronuclei in Vicia faba root tip cells as a measure of chromosome damage. The effect of oxygen was measurable, but less, with the fast neutrons than with γ irradiation. The OER for yield of micronuclei was about 2.4 for γ rays and 1.4 for fast neutrons. Similarly, for chromatid-type aberrations produced by 14 MeV neutrons in meristematic cells of the bean root, Neary and Savage [13] reported an OER of 1.5 for true aberrations and 1.3 for "gaps" (nonstaining chromosome regions).

Another "wet" test system that has been widely used to investigate the oxygen effect in plants is the yield of chromosome aberrations in treated microspores of Tradescantia. Giles and Riley (1949) first demonstrated an effect of oxygen on x-ray induced chromosomal rearrangements in this species [14]. Subsequent experiments [15] demonstrated a measurable oxygen effect with fast (fission) neutrons which was quantitatively intermediate between that of x rays and α particles; i.e. inversely proportional to the relative ion densities. This was interpreted in terms of the different initial spatial distributions, and consequent interactions, in the presence of dissolved oxygen, of the primary radiation products (OH and other free radicals) arising in cells exposed to the various types of radiations. These early studies, as summarized by Swanson [16], support the hypothesis that oxygen tension, in determining the frequency of chromosomal aberrations, does so mainly by modifying the rates of breakage; but rejoining of breaks is also oxygen dependent [17]. The maximum OER with x rays for yield of isochromatid aberrations in pollen tube chromosomes of Tradescantia was 3.4 ± 0.45 [18].

The frequency of induction of somatic mutations in stamen hairs of Tradescantia has been used to study the dependence of OER on neutron energy [19]. Neutron energies of from 0.12 MeV to 14.7 MeV all had OER's below that of ^{60}Co γ (3.27 ± 0.74) or 200 KeV x rays (2.85 ± 0.57). However, there were unexpected features of the results such as the high OER (2.11 ± 0.18) with 0.42 MeV neutrons and the decrease in OER from 2.05 ± 0.13 with 5.15 MeV neutrons to 1.51 ± 0.06 with 14.7 MeV neutrons. These results are somewhat at variance with those on cultured cells of human kidney origin [20,21] where OER values of fast neutrons were found to vary only little with modal energies, ranging from 1.5 ± 0.1 at 1 MeV to 1.6 ± 0.2 at 15 MeV.

INTERRELATION OF MOISTURE AND OXYGEN IN "DRY" SYSTEMS

Another category of tests for studying modification of radiation effects in plant materials utilizes so-called "dry" systems, mainly dry seeds of barley (Hordeum vulgare L.) or spores of bacteria (Bacillus megaterium). Barley seeds are irradiated and the damage is measured by the reduction in some increment of subsequent growth, as seedling height. This is directly correlated with chromosomal damage [22]. An oxygen effect was clearly established for x irradiation when oxygen was applied as a post-exposure treatment [23]. In more recent experiments [24] barley seeds equilibrated to moisture contents ranging from 2% (very dry) to 14% (moist) were irradiated under anaerobic conditions with ^{60}Co γ rays, then hydrated in oxygen or nitrogen saturated water. The OER increased from 1 to 9 as seed water content decreased from 12.7 to 2.5%. In the driest seeds (2.1% water content) the OER was 10 for seedling injury, 15 for chromosome aberrations, and 10 or higher for mutation frequencies.

From these results and others in which Conger et al. [25,26] have explored relationships between water content and oxygen-sensitive sites in barley seeds the following conclusions were reached: 1) Water regulates the degree to which oxygen participates with radiation in the induction of biological effects; 2) Water controls radical-oxygen interactions through its influence on the mobility and activity of the radicals and of oxygen; 3) It is the radical-oxygen interactions, rather than radical-radical interactions, that are mainly responsible for at least a part of the biological damage, and in very dry seeds oxygen-sensitive sites or radicals can remain available to oxygen for a long time; 4) Hydration of macromolecules within the cell may be the rate-determining process in the decay of radiation-induced oxygen-sensitive sites [27].

Failure to observe an oxygen effect following neutron irradiation of dry barley seeds was reported by various workers [23,27,28] probably because the irradiations were carried out in air and interactions between oxygen and radiation-induced oxygen reactive sites had already occurred prior to post-treatment hydration under oxic vs. anoxic conditions. Conger and Constantin [29,30] have now demonstrated an oxygen effect in dry (2.2% water content) barley seeds for fission neutrons by irradiating under strictly controlled anaerobic conditions followed by soaking in oxygen or nitrogen bubbled water at 0° C for 16 hours. The OER was computed to be 1.34 and was independent of dose within the range employed (184 to 938 rads). Similarly, dry rice (Oryza sativa L.) seeds (2.7% water), when irradiated in vacuo then soaked under oxic vs. anoxic conditions, gave an OER of 12.4 for γ rays and 1.44 for fission neutrons [31].

Another dry biological material that has been used in investigations on the causes of radiobiological damage is dry spores of Bacillus megaterium.

Powers [32] demonstrated three classes of radiation damage to the spores: that produced by I) short-lived completely oxygen-independent changes; II) short-lived, oxygen-dependent species (seen only when oxygen is present during irradiation); and III) interactions between radiation-induced free radicals (which may be relatively long lived) and oxygen. To these may be added a fourth class of radiobiological damage, observed in the dry barley seed system [33], which appears to be independent of oxygen and long-lived free radicals and develops very slowly during storage after irradiation.

Relationships between rates of energy loss (LET) and the classes of radiation damage were investigated [34] by exposing dry bacterial spores to stripped atoms accelerated in the Berkeley heavy ion linear accelerator (HILAC), and analyzing curves for survival in an atmosphere of O_2 , N_2 , or H_2S . LET varied from 5 KeV/ μ for ionized deuterons up to 500 KeV/ μ for the Ne^{10+} ions. Class I damage (oxygen independent) increased with increasing LET up to 190 KeV/ μ and remained at a relatively high level, though falling slightly at the highest LET's tested. The oxygen-dependent classes II and III were constant, and accounted for proportionally less damage, up to 190 KeV/ μ ; then decreased to approximately zero at 500 KeV/ μ . In terms of OER this gives a decline in values with increasing LET and is in general agreement with, as well as greatly extending, the results reported with other systems.

EFFECTS OF OTHER CONDITIONS

Soaking of seeds for various periods of time before irradiation provides what may be considered as transitional stages from dry to wet systems. This process is accompanied by increased radiosensitivity. In experiments designed to elucidate factors involved in the enhancement of radiosensitivity, Latterell and Steffensen [35,36] x rayed a genetically marked stock of maize seeds after presoaking at 27° C for periods up to 26 hours. The ratio of somatic mutations induced in the presence of oxygen to those induced in anoxia was 1.2 for dry seeds and seed soaked up to 3 hours. Between 3 and 6 hours the ratio rose to 2.4 and remained relatively constant thereafter. There was an approximate 5-fold increase in radiosensitivity between 16 and 26 hours, which coincided with the major increase in oxygen consumption. This suggested that the most conspicuous rise in radiosensitivity of soaked seeds may be causally related to the associated increase in endogenous respiration and hence to metabolic changes during seed development. Utilizing the same system to compare fission neutrons with x irradiation, Smith and Combatti [37] found an appreciable but lesser enhancement of radiosensitivity with neutrons, so that the relative biological effectiveness (RBE) was reduced from 67 in dry seeds to 5 in seeds soaked for 40 hours at room temperature.

Further experiments were carried out with barley seeds [38], pre-soaked at 1° C to minimize metabolic activity, for from 4 to 36 hours, then irradiated with fission neutrons or 250 kVp x rays. During this period radiosensitivity doubled with neutrons and quadrupled with x rays so that the RBE value was halved. Corresponding findings were reported for 14 MeV neutrons vs. γ rays with presoaked wheat seeds [39]. The enhanced radiosensitivity of barley seeds to neutrons under cold conditions [38] could be accounted for by the increase in nuclear size and hydrogen content. Similar results were obtained by Ahnström and Natarajan [40] who found in addition that caffeine, when present during the earliest period of germination of seeds (<5 hours), enhanced γ -radiation-induced damage by a factor of 2, but had little effect on neutron-irradiated seeds. An explanation offered was based on a differential distribution of lesions after γ ray and neutron irradiation, and a differential mobility of chromosome material in resting and germinating seeds.

Temperature variation has marked effects on the magnitude of response to irradiations; particularly those of lower LET, as noted throughout this review. Following exposure to x rays, a low temperature (-78° C) inhibits the development of damage during storage [41] and post-irradiation heat shocks (60 seconds, 60° C) reduce radiation injury [41,42]. Seeds exposed to neutrons, however, were little affected by post-irradiation heat treatments [41]; furthermore, no increased damage with increased storage time was observed [43]. On the other hand, in spite of this evidence for insensitivity of neutron-irradiated materials to post-treatment environmental perturbations, recent experiences with the IAEA cooperative program on neutron irradiation of seeds suggest that a reexamination of the phenomena may be called for. A genetically uniform stock of barley seeds, grown under nearly identical post-irradiation conditions [44] was used for comparative biodosimetry studies of fission neutron facilities in reactors throughout the world. One test [45] showed that, whereas the Brookhaven Medical Reactor and the Asta Reactor in Seibersdorf (Austria) gave comparable dosimetry for the respective fast neutron facilities, small differences in post-irradiation conditions at the sites caused a 22% difference in D₅₀ for plant height. Tentatively, these minor differences appeared to be in daylength (20 vs. 24 hours), possibly in light intensity (plus or minus 100 foot candles), or quality of light.

PROSPECTS FOR CONTROL OVER RESPONSE TO NEUTRON IRRADIATION

The ultimate in controlling biological effects of radiations would be to be able to produce specific kinds of mutations with one kind of radiation, as neutrons, in comparison to other mutagens. The objective of accomplishing mutagen specificity or directed control of induced mutations has long been a goal in plant genetic research. Little success has been achieved, however, probably because of the nature of DNA in which the coding of genetic information resides in long series of triplet sequences made up of combinations of only four nucleotides.

The best evidence of preferential recovery of specific neutron-induced mutants in higher plants is in the Swedish work on barley. Of 380 eceriferium mutants induced at 44 loci, pronounced locus specificities are found if densely ionizing radiations (protons, neutrons, α rays) and sulfonates are used as mutagens. Neutrons act preferentially on locus i, whereas the sulfonates mutate preferentially loci u, q and c--and produce none at i [46]. Similarly, for erectoides mutants, there is some evidence that the response of different loci to different mutagens is not the same [47]. For example, locus a mutates more frequently after treatment with sparsely ionizing radiations (x and γ rays) compared with densely ionizing radiations (protons and neutrons). For locus c, however, neutrons seem to be equally efficient if not more so than x rays. These apparent specificities could equally well be explained by differential lethal effects with densely vs. sparsely ionizing radiations.

Auerbach [48] has analyzed a case of mutagen specificity in an ad⁻inos strain of Neurospora crassa. X rays and three chemicals were adenine specific, i.e. they produced more adenine than inositol reversions while ultraviolet light and two other chemicals were inositol specific. The degree of specificity could be modified by ancillary conditions such as varied dose rate, post-treatment temperature or genetic background. These observations led Auerbach to the conclusion that most of the observed mutagen specificity in this system is due, not to specific chemical reactions between mutagen and DNA, but to treatment effects on cellular processes that play different roles in the mutational pathways of the two alleles. There are indications that inactivation of a repair enzyme and hastening of inositol-less death may both be involved. Mutational change in DNA, particularly in higher forms, is both preceded and followed by secondary steps that may act as "sieves" which determine whether a change in DNA will take place and whether it will give rise to a recoverable population of mutated cells. It is at the level of these "sieves" that it may be possible to modify the environment in order to exercise greater control over the kinds of mutant products ultimately obtained [49,50].

Three further examples of this kind of modification of radiation effects follow. Seeds of Arabidopsis thaliana were irradiated with ^{60}Co γ rays then quenched of radicals by soaking in water for 1 hour followed by 24 hours on agar at 5° C [51]. Subsequent germination and growth at a constant 27° C reduced survival to 46% from 93% in unirradiated controls. However, when the seeds were germinated during only the first 4 days at 20° C (a more favorable temperature), then grown at 27° C, survival was raised to 77%. A significant difference could also be demonstrated in second generation progeny which exhibited less genetic variance and less sterility in those populations derived from plants recovered in the continuous (unfavorable) higher temperature. The fact that less damage among

survivors is associated with lowered survival indicates that it was the more damaged individuals that were preferentially eliminated from the population during the early stages of growth at 27° C. This particular kind of "sieving" action, or preferential selection among meristematic cells, may be expected a priori to apply equally well following neutron irradiation.

Secondly, Lyman and Haynes [52] observed a marked enhancement of viability in diploid yeast which had been irradiated with x rays or with heavy ions (⁴He, ¹²C, ²⁰Ne) then stored up to 48 hours at 30° C, rather than plated immediately. Several lines of evidence indicated that the recovery was based on enzymatic post-irradiation processes, unrelated to the initial physico-chemical reactions associated with absorption of the radiation, and that these involved a "bypass" rather than a direct repair mechanism.

The last example is from preliminary results of a recent experiment in our laboratory where maize seeds of a stock heterozygous for a leaf color marker gene (Yg₂/yg₂) were soaked, dried back to an original 13% moisture content, then irradiated in air with 10,000 rads of x rays. Presoaking and dryback was at 3° C and at 20° C. Controls were neither soaked nor dried, but were maintained at 13% moisture. The frequency of yellow-green (yg) sectors in the fourth seedling leaf, due mainly to deletion of the dominant Yg₂, was scored as a quantitative measure of genetic damage. The results are shown in Table 1. Presoaking at 3° C, particularly when the preirradiation dryback was at 20° C, caused a marked reduction in radiation-induced genetic damage. For the 3° C presoak and 20° C dryback regime the frequency of yg₂ sectors was reduced to 3 per leaf, from 7.2 in the controls. The "protective" mechanism is unknown. Leaching out of substances, which would otherwise produce mutagenic by-products with irradiation, is one possibility. Another is that the presoak and dryback may cause compaction of chromosomal DNA, which appears to be a factor in the radiosensitivity of maize leaf primordia [53]. Further experiments, including exposure to neutron radiation, are in progress. It should be noted that this is the same maize system in which seeds that are soaked, but not dried back, show a marked increase in yg sector frequency with exposure to either x-ray or neutron radiation [37].

TABLE 1

Effect of temperature during presoak (48 hr) and dryback (to 13% H₂O) on Yg₂/yg₂ maize seeds irradiated with 250 kVp x rays (10,000 r) and scored for yellow-green (yg) sectors on seedling leaf 4.

Presoak temp., C°	Dryback temp., C°	Percent survival		No. <u>yg</u> sectors mean $\pm$ S.E.
		Unirrad. (n=25)	Irradiat. (n=90)	
none	none	95	93	7.2 $\pm$.33
20	3	24	9	6.8 $\pm$.77
20	20	88	79	6.4 $\pm$.29
3	3	56	60	4.0 $\pm$.78
3	20	84	94	3.0 $\pm$.19

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