make excitatory connections with motoneurons, a conclusion with considerable support (*3*). Therefore, facilitation of A-reflexes by C-fibers can be ascribed to spatial facilitation of a subliminal fringe common to both A- and C-fibers rather than to presynaptic hyperpolarization of primary afferent fibers.

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Radiobiological Damage: A New Class Identified in Barley Seeds Stored after Irradiation

Abstract. *A new class of radiobiological damage has been identified in irradiated barley seeds. Consisting of both physiological and genetic damage, it appears to be independent of oxygen and long-lived free radicals, and develops very slowly during storage after irradiation. Increasing the storage temperature accelerates its development.*

Three main classes of radiobiological damage have been recognized in bacterial spores, a dry (dormant, slowly metabolizing) system (*1*, *2*). These include Powers' class I (an "immediate" oxygen-independent portion), class II (an "immediate" oxygen-dependent portion in which oxygen must be present at the time of irradiation in order to be observed), and class III (an oxygen-dependent portion which lasts appreciable lengths of time and which is also termed the free-radical component).

We now report evidence for a new class of radiation damage identified in barley seeds, a dry system, through prolonged storage in a vacuum after irradiation. The damage appears to be initiated by a mechanism which is independent of oxygen and long-lived free radicals.

Seeds of hull-less barley *Hordeum vulgare* var. Himalaya (water content, 10 percent) were exposed in a vacuum to 40 and 25 krad of cobalt-60 gamma rays. Methods for evacuating and irradiating seeds have been described (*3*, *4*). After irradiation, all seeds were stored in a vacuum in sealed glass vials at 40°C for periods up to 6 weeks in the first experiment, and up to 12 weeks in the second experiment, before hydration in oxygenated (oxygen-bubbled) or oxygen-free (nitrogen-bubbled) water at 0°C for a period of 18 hours. After irradiation, the samples were stored at 40°C to accelerate the processes that occur during storage. The water content of the seeds in sealed, evacuated glass vials changes were little, if any, during storage (*3*) after irradiation.

At the end of the hydration period, the seeds were planted and the resultant seedlings were cultured and measured (*5*). The results, from three replications of 50 seedlings each, are reported as seedling injury (percentage of reduction in the mean heights of seedlings of the irradiated seeds compared to the mean heights of seedlings of the nonirradiated controls). Nonirradiated seeds were also stored at 40°C for periods up to 12 weeks, and seeds of each irradiated treatment were compared with the appropriate control.

Shoot tips were collected from some treatments of the second experiment and were cytologically examined for dicentric bridges and acentric fragments in the anaphase stage of the first mitotic division. Twenty cells from each of five seeds from two replications of each treatment (that is, a total of 200 cells for each treatment) were examined by the aceto-orcein smear technique (*3*). Three seeds from each of the three experimental replicates from each storage period were also analyzed for free-radical signals by electron paramagnetic resonance (EPR) (*3*).

Because oxygen was not present during irradiation, Powers' class II damage was not observed in these experiments. However, at least three classes of damage can be recognized from the results of the first experiment (Fig. 1). These include (i) an oxygen-independent portion of about 15 to 20 percent injury observed immediately after irradiation (class I damage); (ii) an oxygen-dependent portion of an additional 60

Table 1. Seedling injury, chromosome aberrations, and signal amplitude obtained from electron paramagnetic resonance (EPR) in barely seeds. Water content of the seeds was 10.1 percent. The seeds were exposed in a vacuum to 25 krad of ^{60}Co gamma rays and then stored for various periods in a vacuum at 40°C before hydration (hyd.) in oxygenated or oxygen-free water at 0°C for 18 hours; the free-radical signal was measured by EPR before and after hydration.

Storage time	Seedling injury reduction		Bridges per 100 cells		Fragments per 100 cells		EPR signal amplitude*
	O_2 hyd. (%)	N_2 hyd. (%)	O_2 hyd.	N_2 hyd.	O_2 hyd.	N_2 hyd.	
None	75.1	6.9	167	21	1060	108	104.3
9 minutes	45.3	6.9	82	38	586	130	77.0
1 hour	14.9	8.1					46.7
7 hours	10.5	6.3	31	31	85	115	27.3
2 days	7.7	6.4	31	39	147	200	10.7
10 days	12.1	10.1					8.7
3 weeks	20.3	18.2	53	54	219	254	7.3
5 weeks	22.2	21.8					6.7
8 weeks	22.6	26.5	48	56	239	254	6.0
12 weeks	38.1	37.6	99	93	351	304	4.7
Control (room temperature)	0.0	0.0	0.0	1.0	1.0	0.0	11.3
Control (12 weeks, 40°C)	0.0	0.0	0.5	0.0	1.5	1.0	4.0

* Arbitrary units.

percent injury (portion between 20 and 80 percent) observed immediately after irradiation—this portion (class III damage) is reduced to the class I level and is no longer oxygen-sensitive after about 7 hours of storage; and (iii) an oxygen-independent portion that develops very slowly during storage after irradiation; at the end of a 6-week storage period the injury level has increased about 30 percent over the initial oxygen-independent (class I) level. This damage is oxygen-independent since the same injury level is observed whether the seeds are hydrated in oxygenated or oxygen-free water after storage from 7 hours up to 6 weeks. The last class of damage has not been previously described.

The data on chromosome aberration, EPR, and seedling injury from the second experiment are presented in Table 1. A lower radiation exposure (25 krad) and longer storage period were used to determine whether the increase in damage would reach a maximum and then level off.

The results for seedling injury are similar to those observed in the first experiment. The level of oxygen-independent (class I) injury remains at about 7 percent immediately after irradiation through the first 2 days of storage. The new class of damage begins to develop after 2 days of storage and increases slowly but steadily during the remainder of the 12-week storage period to about 38 percent. The data on chromosome aberrations, although somewhat more variable, show the same trends as the data on seedling injury. The EPR amplitude, however, decreases during the same storage period. Again, the oxygen independence of the new class of damage is demonstrated by approximately the same extent of injury and chromosome aberrations in seeds hydrated in oxygenated and oxygen-free water after the first 2 days of storage. Furthermore, the increase in damage shows no sign of reaching a plateau.

The decrease in oxygen-dependent (class III) damage, as measured by both seedling injury and chromosome aberrations during the first 2 days of storage, paralleled by a similar decrease in free-radical signal, illustrates the close relation between radiation-induced free radicals and oxygen-dependent damage after irradiation (*2*, *3*). At the end of a 2-day storage period at 40°C after irradiation, the free-radical signal (10.7 mm) has fallen to that for the endogenous signal (11.3 mm) in non-

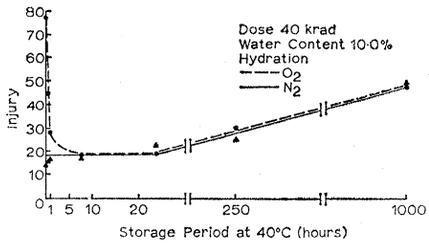

Fig. 1. Injury (percentage of reduction in seedling growth) to barley seeds having a water content of 10.0 percent and exposed in a vacuum to 40 krad of ^{60}Co gamma rays and then stored in a vacuum at 40°C for various periods of time before hydration in oxygenated (dashed line with circles) or oxygen-free water (solid line with triangles) at 0°C for 18 hours.

irradiated seeds stored at room temperature.

The development of the new class of damage also occurs at room temperature, but at a slower rate. An increase in damage was observed when seeds with water content of both 4 and 10 percent were stored in a vacuum at room temperature for 10 weeks after irradiation (*6*). The rate of decrease of oxygen-dependent damage is also much slower if the seeds are stored at room temperature (*3*). Radiation dose may also influence the rate at which the new class of damage develops. The damage appeared to develop at a faster rate in the first experiment in which the seeds were given 40 krad than in the second experiment where the seeds were exposed to 25 krad of ^{60}Co gamma rays.

Two components of damage with respect to storage were observed previously in dry (water content, about 4 percent) irradiated barley seeds (*7*, *8*). However, since availability of oxygen was not controlled during irradiation, the oxygen-dependent portion could not be distinguished and separated from the oxygen-independent portion. Caldecott (*7*) observed an increase in damage during the first 48 hours of storage after irradiating seeds with a 4-percent water content which were stored both aerobically and anaerobically. These results are in contrast to those reported by Conger and co-workers (*3*) who demonstrated that very little increase in damage occurs in seeds (regardless of water content) stored in a vacuum at room temperature for a period up to 2 weeks after irradiation and before hydration in oxygen-free water. A possible explanation for the discrepancy is the lack of precise atmospheric control in the early experiments, and we overcome this deficiency now by storing seeds in a vacuum.

The mechanism (or mechanisms) responsible for the new class of damage appears to be independent of oxygen and also perhaps of radiation-induced, long-lived free radicals, or at least independent of those detectable by electron paramagnetic resonance. Most of the potential for the new class of damage may be eliminated if the seeds are hydrated anaerobically at 0°C for 18 hours and then dried before storage in a vacuum at 50°C. This suggests that the damage may result from an unstable chemical species which may or may not be detectable by EPR but which can be eliminated if water is added to the system.

Undoubtedly, the relative values of the classes of damage will vary with radiation dose, water content of seed, and other conditions. However, the main consideration to be derived from this study is that some biological systems, under certain conditions, may be able to retain the potential for an increase in a given level of radiation damage even though the rate may be very slow. Both physiological and genetic types of damage appear to be involved, which could be significant when one considers the long-range effects of ionizing radiation, especially on dormant, slow-metabolizing biological systems.

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