

After-Effects of Ionizing Radiation in Barley.

I. Modification by Storage of X-Rayed Seeds in Oxygen and Nitrogen

*A Preliminary Report**

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AN "AFTER-EFFECT" of irradiation has been defined as one that continues after exposure to the irradiation. Most previous investigations dealing with such biological "after-effects" have studied these effects a few minutes to several hours after irradiation. The results of many of these investigations have been presented in a recent symposium (Mitchell and Holmes, 1954). These and other studies have also indicated that certain of these "after-effects" of short duration can be modified by various treatments applied immediately after the irradiation. Temperature (Caldecott and Smith, 1952; Kaplan, 1951) and gases (Kaplan, 1953; Hollaender and Stapleton, 1954) have been effective in this regard.

In order to study X-radiation damage such as frequencies of chromosome aberrations and genetic mutations and decrease in viability over a prolonged period (eight weeks) after irradiation, dormant barley seeds may be employed. The use of dormant seeds also makes it possible to study the influence of various environmental factors, such as atmosphere and temperature, on the production of this radiation damage over a similarly prolonged period. The present paper describes the results of initial studies on the "after-effects" of irradiation of dormant barley seeds. This method of approach has evolved in part from brief reports by Tascher (1929) and Gustafsson (1937, 1947), which demonstrated that certain X-ray effects in barley were enhanced through long storage of irradiated seeds.

Material and Methods

Dormant Himalaya barley "seeds" (caryopses), with approximately 8 per cent moisture content, were X-rayed at 7500 *r*. The dosage was measured by a Victoreen *r*-meter. The radiation was obtained from a beryllium window A.E.G. Machlet X-ray tube operated at 34 KVP. and 24 ma. for 13 minutes.

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The gasses used in the experiments were obtained from commercial cylinders and assayed as follows: oxygen (Industrial Air Products), 99.6% O₂ + .4% N₂; water-pumped nitrogen (Industrial Air Products), 99.6% N₂ + .4% O₂ and CO₂.

In 1953, seeds for each of two replications were irradiated separately. The X-rayed seeds were divided into samples for cytological observation. These samples were then tied in cheesecloth bags and stored for two, four, or six weeks at room temperature in glass-stoppered Erlenmeyer flasks containing air, oxygen, or nitrogen.

In 1954, the storage period was extended to eight weeks, and the methods of treatment and storage were altered somewhat. The storage procedure was modified because earlier data indicated that the storage gases were altered by metabolism (respiration) of the seeds, leakage, or both. Within each of two replications, seeds were X-rayed every week for eight weeks. Immediately after X-raying, the seeds were divided into samples for seedling height, germination, and emergence data. These samples were then placed in three mercury-sealed respiration flasks containing air, oxygen, or nitrogen. Irradiated samples were added to each flask weekly, at which time the flasks were refilled with the appropriate gas. Non-irradiated seeds received similar post-irradiation treatments in both 1953 and 1954.

Chromosome bridges were recorded at late anaphase of the first cycle of cell division in shoot-tips of seedlings grown from irradiated seeds. The cytological methods and techniques have been described earlier (Gunthardt, et al., 1953). A total of 6,000 cells in 400 shoots were scored. Each point on the graph (Fig. 1) is a mean from two replications involving 600 cells and 40 shoots.

Seeds were germinated on blotters in glass-covered enamel pans at room temperature under continuous fluorescent light. Seedling height was measured after two weeks. Germinability percentages were obtained from the same material according to the Association of Official Seed Analysts Standards (1949).

Emergence data were determined from seeds that were field planted 6 inches apart in rows. Seedling height and germination data (Figs. 2 and 3) were obtained from 650 seeds in one replication, with each point representing a mean from 50 seeds. The data on emergence as presented in Figure 4 are a summary of two replications involving 17,000 seeds. Each point on the graph represents the mean from 200 to 1,000 seeds.

Regression lines were calculated for each treatment with the no-storage observation as the base point. An analysis of variance of the regression lines tested the significance of difference between treatments.

Experimental Results

The results can be briefly stated as follows: According to the four radiation-damage criteria (Figs. 1-4), postirradiation storage appreciably increased the initial radiation effect. This influence of storage was highly significant

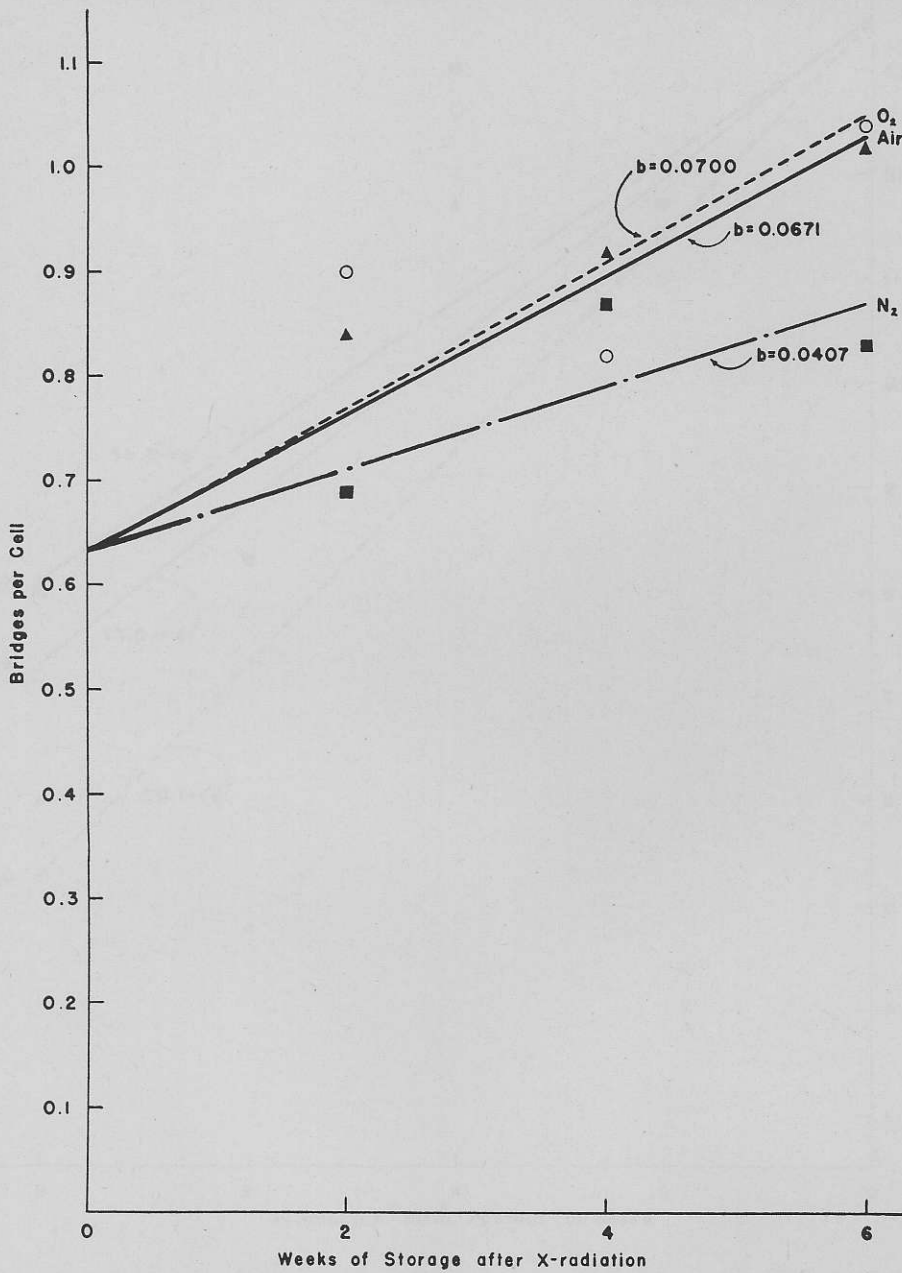


Figure 1. Frequency of chromosome bridges. ○ , Air. ▲ , Oxygen. ■ , Nitrogen.

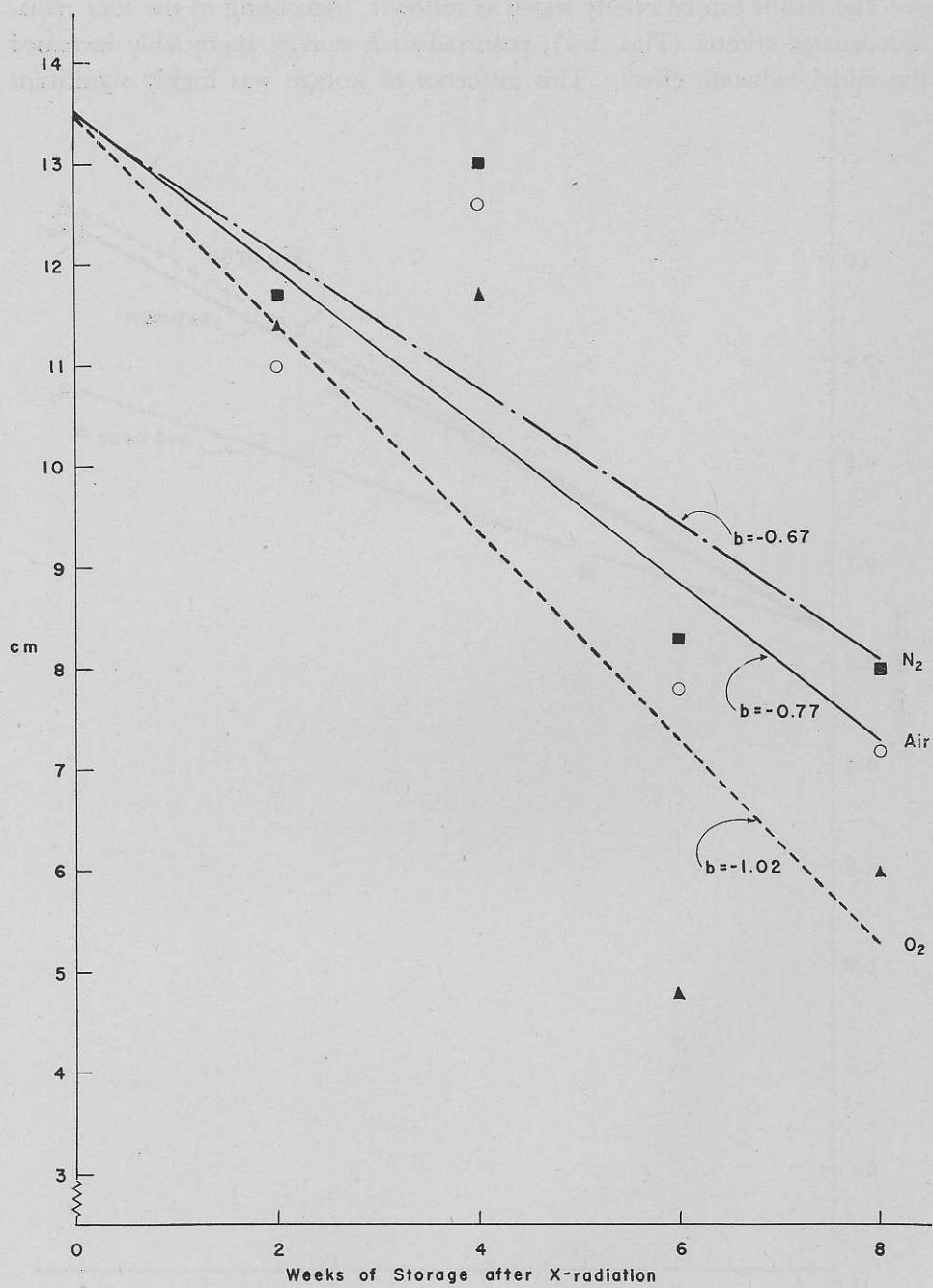


Figure 2. Height of two-week-old seedlings. O , Air. ▲ , Oxygen. ■ , Nitrogen.

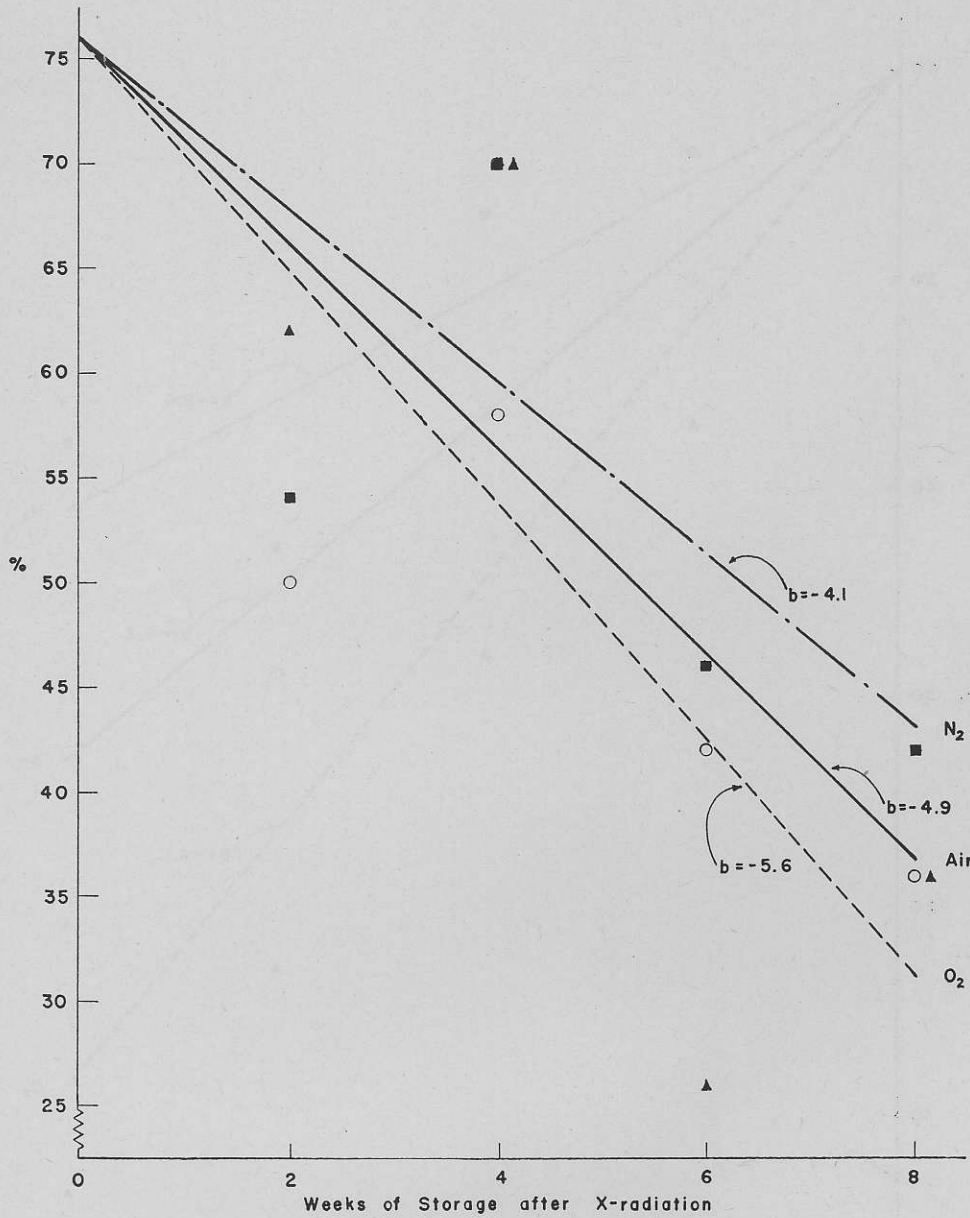


Figure 3. Percentage of germination. \circ , Air. \blacktriangle , Oxygen. \blacksquare , Nitrogen.

according to an analysis of variance of the regression data and is in accord with the findings of Tascher (1929) and Gustafsson (1937, 1947).

There is also evidence that suggests a modification of the storage effect by nitrogen and oxygen. Oxygen enhanced while nitrogen decreased the

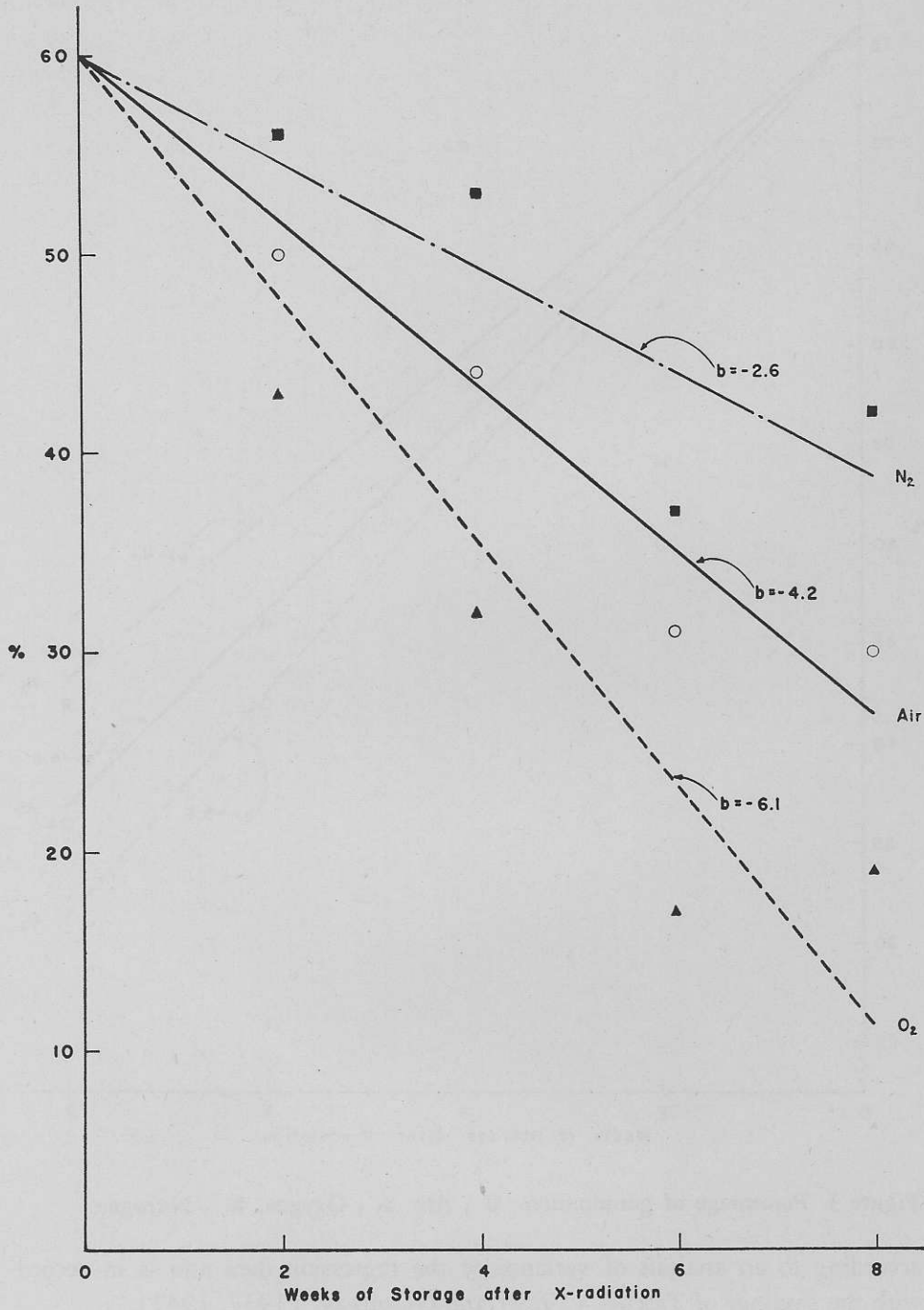


Figure 4. Percentage of emergence in the field. ○ , Air. ▲ , Oxygen. ■ , Nitrogen.

radiation damage during storage. For the emergence data, there was a significant difference at the 5 per cent level between gases according to an analysis of variance. The influence of oxygen on the chromosome-aberration frequency was not significantly different from that of air, whereas the influence of N_2 was significantly different from that of air and O_2 at the 10 per cent level. The lack of influence of O_2 on the chromosome-aberration frequency in 1953 may be a result of the method of storage. The limited seedling height and germination data did not allow for adequate statistical analysis of the apparent differences.

The gases alone did not induce any chromosome aberrations or other damage such as reduction in seedling height, germination, and field emergence.

By connecting the actual points for each gas in Figures 2 and 3, distinctly less damage at four weeks than at two weeks can be observed. This deviation from the general trend would be expected when dealing with small, unreplicated samples. It will also be noted in Figures 2, 3, and 4 that there is a tendency for leveling off between six and eight weeks of storage. Additional data will be gathered to determine the validity of this leveling-off point.

Discussion

Possibly the most significant result from this study is the increase in chromosome-aberration frequency during storage of irradiated seeds. Two possible explanations of this "after-effect" may be considered here.

The first hypothesis is concerned with the restitution of broken chromosomes after irradiation. Kaplan (1951) and others have suggested that chromosome breaks in dormant seeds remain open for a considerable period of time. If such is the case, then conceivably the broken chromosome ends could become altered chemically during the storage period. This then could lead to increased chromosome interchange with resulting bridges at the expense of restitution. Increased oxygen or nitrogen in the cells could enhance or retard this effect through an influence on the chemistry of the broken ends.

The second hypothesis must include some mechanism that results in continuous chromosome breakage during the storage period. The most obvious, if somewhat general, explanation is that chemical products of irradiation are responsible for the "after-effect." These chemicals produced in the cell through the irradiation of water and other cell compounds may be hydrogen peroxide or hydroxyl and perhydroxyl radicals (Ehrenberg, 1954). They might act on latent or potential breaks caused by the direct effects of the radiation, or attack the unaltered chromosome, or both. Such effects may be in part due to these chemicals causing a breakdown of desoxyribonucleic

acid in the chromosomes (Conway, 1954). Adoption or rejection of these or other hypotheses must await further data from the study reported herein and from other studies now in progress.

The present data appear to have some bearing on fractionated X-ray dose studies, particularly where the intervals between partial doses are long. In such studies, it would be difficult to distinguish between the effects of fractionated dose and of storage.

Summary

The data presented herein show that storage of dormant barley seeds after X-radiation increases the amount of radiation damage as measured by frequency of chromosome bridges, seedling height, rate of germination, and emergence. Furthermore, there is evidence that suggests oxygen enhances while nitrogen retards this storage effect.

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