

seven available linkage testers and make identification on the basis of trisomic ratios.

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Inverse Relationship between the Water Content of Seeds and Their Sensitivity to X-rays

It was early established in radiobiological investigations that seeds with a high water content were more susceptible to x-rays than seeds with a low water content (1). A similar relationship has been demonstrated for the ascospores of *Aspergillus terreus* (2). These and other such studies have been taken to support the contention that, in addition to being affected by the direct absorption of energy from ionizing radiations, sensitive sites are affected indirectly through the chemical action of active radicals produced in the presence of water (3).

If the role of water in the sensitizing of a system to x-radiation is in a large part due to chemical events incited through radical production, it would seem reasonable to assume that in any biological system in which the water content could be varied over rather wide limits, the radiosensitivity of the system would increase with increasing water content. This should be true at least until such time as an optimum production of radical species that could contribute to injury had been produced.

To test this assumption (4) seeds were soaked at 3°C for various periods of time from 1½ to 24 hr. Their sensitivity to x-rays, as determined by seedling heights at 7 days, was then compared with that of unsoaked seed. It was found that their water content could be increased from 7 percent of their total weight in the unsoaked seeds to about 20 percent in the soaked seeds before there was an increase in their radiosensi-

tivity. The steeping time required to increase their water content to this level was from 1½ to 3 hr. Increasing the water content of the seeds above 20 percent resulted in a striking increase in radiosensitivity.

These experiments were suggestive of the possibility that water content *per se* may not be responsible for the increased radiosensitivity of seeds with a high water content in comparison with seeds having a low water content. However, it was realized that because of the short periods of soaking required to raise the water content of the seeds to 20 percent, the water may not have been "effectively" distributed in the seed. For this reason it was decided to increase the water content of seeds by permitting them to reach weight equilibrium in desiccators over salt solutions with different vapor pressures. Using this procedure it was possible to obtain the following water contents of different lots of seed: 7, 9, 13, 16, and 24 percent.

When seeds with these water contents were subjected to x-rays it was found that there was an inverse relationship between the water content of the seeds and their radiosensitivity (Fig. 1). This relationship held true for doses of 10,000, 20,000, 30,000, 40,000, and 50,000 r.

It should be pointed out that the seeds with 24 percent water only maintained equal viability to seeds with 7 percent water for a period of about 14 days. However, during the course of these investigations seedling growth studies indicated that the viability of the other seeds used in the study was not affected by the storage conditions.

The question arises whether or not the decreased radiosensitivity that was observed when the water content of the seeds was increased from 7 percent to about 20 percent was due to the physical presence of additional water in the seed or resulted from some other factor(s), possibly those associated with increased metabolic activity. The experimental evidence suggests that the phenomenon observed is directly related to the water content of the seeds, because when seeds with about 16 percent water were desiccated over dry CaCl₂, until they contained about 7 percent water, their radiosensitivity increased.

If the modifications in radiosensitivity reported here can be validly attributed to variations in water con-

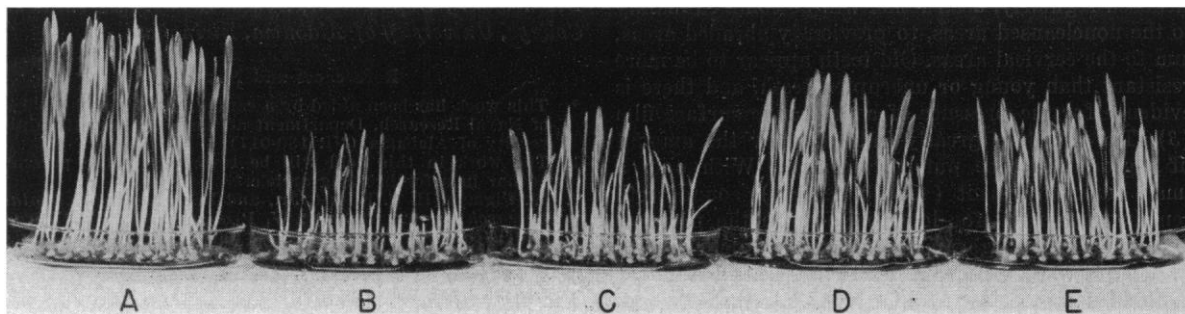


Fig. 1. Seven-day-old seedlings from seeds that reached weight equilibrium at the following relative humidities and were then subjected to 20,000 r of x-rays: (A) Control (no treatment); (B) 0.0; (C) 32.2; (D) 52.0; (E) 75.0.

tent, it is apparent that there is not a 1-to-1 relationship between the water content of seeds and their sensitivity to x-rays. This suggests that reduced seedling height resulting from x-radiation is not simply related to the production of active radicals in the presence of water.

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References and Notes

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 4. This research was carried out at the Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission in cooperation with the U.S. Department of Agriculture.
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In vitro Studies of Dental Decay*

The concept of studying the carious process by using extracted human teeth under *in vitro* conditions was realized and applied early in the development of dental research. E. Magitot (1870) and W. D. Miller (1890) described carious lesions produced in this manner (1).

Recently the value of *in vitro* studies has been increased by the development of the "artificial mouth" (2). For this work, extracted human teeth are mounted singly or in pairs. The mounted teeth are placed in cylindrical funnels, and a bacteriological medium ("artificial saliva") is allowed to flow dropwise over the teeth for several months. At weekly intervals, the teeth are inoculated with pooled samples of human saliva and rapidly become covered with a mass of microorganisms.

Some of the most important factors that have been found to influence the destruction of the teeth are the following. Tooth destruction can be quite general, if localizing factors are not present. If the teeth are cleansed regularly, destruction will be confined mainly to the noncleansed areas, to previously abraded areas, and to the cervical areas. Old teeth appear to be more resistant than young or unerupted teeth, and there is evidence for the presence of a protective surface film (3). The attack is greatly influenced by the amount of D-glucose in the nutrient medium. With only a small amount present (< 0.10 percent), sound teeth remain unchanged for long periods. In the presence of a relatively large amount (for example, 0.5 percent D-glucose), enamel attack and decalcification of exposed dentin proceed rapidly. At intermediate concentrations (0.2 to 0.3 percent), both decalcification

and dentinal matrix destruction occur, so that the entire tooth structure will be destroyed.

More recently, several additional important observations have been made. Because of the considerable delay in publication and a desire to extend this work before publication, a brief summary is presented here.

Reidar F. Sognnaes (Harvard School of Dental Medicine) has examined histologically thin sections from a number of teeth with localized lesions of enamel and of dentin, produced in the "artificial mouth." The sections were found to exhibit a number of features associated with natural lesions. According to Sognnaes, these are the following. (i) Accumulation of plaques containing gram-positive microorganisms has been demonstrated on tooth surfaces subjected to various bacterial substrates in the "artificial mouth." (ii) The primary penetration of the enamel appeared to proceed between the prisms, accompanied by accentuation in the appearance of the prisms, the cross-striations, and the incremental lines, eventually followed by a loss of surface continuity. (iii) Invasion of the dentin occurred along characteristic tracts, indicated by greater permeability to dyes and, eventually, followed by loss of tooth substance and cavity formation. (iv) A predominance of gram-positive spheroid microorganisms could be demonstrated within distended dentinal tubules, eventually invading the ramifications of the tubules and destroying the inter-tubular matrix.

More recently, several experiments have been concluded in which the attack was brought about by single strains of microorganisms. Under conditions such that both decalcification and dentinal matrix destruction would occur with mixed cultures, it was found after 3 mo that an oral *Lactobacillus casei* strain produced decalcification but did not affect the matrix. On the other hand, an oral *Streptococcus salivarius* strain (found later to have become contaminated with a micrococcus) produced decalcification and matrix destruction. The partially attacked dentin was brown and leathery. These experiments are being repeated and extended to other microorganisms. In view of the usually accepted role of lactobacilli in the carious process, these results seem particularly interesting.

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References and Notes

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1. The work in this field will be reviewed by the present author in a forthcoming article.
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