Radiation Damage to Artemia Cysts: Effects of Water Vapor

Abstract. *Water vapor altered the form and greatly increased the rate of decay of the electron-spin resonance pattern of long-lived free radicals obtained upon gamma irradiation of* Artemia salina *cysts (brine shrimp eggs). These results, combined with data on radiation survival, indicate that the water vapor protects the cysts from radiation damage, or heals the damage. They also indicate that water protects the cysts from the effect of oxygen by neutralizing the radiation-induced free radicals before they can interact with oxygen to produce irreversible damage.*

Water, always present in living biological systems, is generally not considered a chemical protector against ionizing radiation. On the contrary, the well known theory of indirect action (*1*) holds that radiation products of water, especially in combination with oxygen, cause a major portion of the biological damage. Nevertheless, in 1954, Caldecott (*2*) showed that dry barley seeds were more sensitive to x-irradiation than were moist seeds and that the sensitivity of the seed varied inversely with water content up to 16 percent. Curtis *et al.* (*3*) showed that this effect of moisture was most evident during the postirradiation period, and that the radiosensitivity was independent of the water content when the seeds were planted immediately after irradiation. Conger (*4*) confirmed these findings and also studied the effects of storage on seeds after irradiation. Engel and Fluke (*5*) recently found that moisture had a similar effect on *Artemia salina* cysts. They showed that the number of *Artemia* cysts hatched after irradiation was significantly greater when the cysts were irradiated in moist rather than dry air. We have confirmed these results and have shown that after irradiation, storage in pure oxygen is much more detrimental to survival than is storage in the normal atmosphere (see Fig. 1).

Figure 2 shows that there is no great difference in the effects of the various environments on survival if the eggs are hatched immediately after irradiation. Thus, environmental gases have the most detrimental effects in the post-irradiation period, probably because they react with long-lived free radicals produced by irradiation. The large amount of water absorbed during the hatching process quickly neutralizes this latent damage. The slow rates at which these damaging reactions proceed in the unhatched *Artemia* cysts make them ideally suited to the study of the mechanisms of radiation damage and protection.

We have found (*6*) that the effects of water vapor on the electron-spin resonance (ESR) signals induced by gamma irradiation of the *Artemia* cysts are different both qualitatively and quantitatively from those induced by other components of the atmosphere, and are different from those induced by H_2S and NO. When the cysts were irradiated after being evacuated by 3 days of pumping, they gave an ESR pattern of an incompletely resolved triplet with a total width of 30 gauss (Fig. 3). Exactly the same type of pattern appeared when the cysts were irradiated in an atmosphere of H_2, N_2, O_2, NO, or H_2S. When they were irradiated in water vapor, however, a singlet pattern with a width of 15 gauss appeared (Fig. 4). When the samples which had been irradiated in vacuum were exposed to water vapor, the triplet pattern was converted to the 15 gauss singlet pattern. The resonance obtained in the presence of water vapor was found to decay much more rapidly than did the triplet in the vacuum or in any of the gases we had tested. Although the ESR pattern had the same form when the cysts were irradiated in O_2 or in NO as in the vacuum, it decayed more rapidly in the O_2 than it did in the vacuum, and still more rapidly in NO (see Fig. 3).

In the unirradiated cysts we observed a singlet resonance which has the same shape and same width as the signal obtained by irradiation of the samples in an atmosphere of water vapor or in moist air (Fig. 4). In the presence of water vapor, the singlet produced by irradiation simply decays to the level of the weaker signal given by these "natural" free radicals in the cysts. We shall not attempt to guess the nature or function of the "natural free radicals" found in these samples.

Conger and Randolph (*7*) observed earlier that the ESR of free radicals induced in seed embryos decays more rapidly in moist air than in dry air, but that the form of the resonance is the same under both conditions. Although our results on the rate of decay of the ESR of irradiated *Artemia* cysts are in agreement with their observations, we found that the ESR pattern has a markedly different width and shape when the cysts are irradiated in moist air or water vapor from that when they are irradiated in a vacuum,

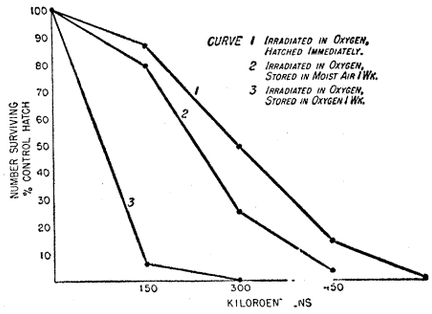

Fig. 1. The effects of storage in moist air and in dry oxygen on the survival of *Artemia* cysts previously irradiated at room temperature with cobalt-60 gamma-rays (200c). The number surviving is given in terms of the percentage of eggs which hatched, normalized to the percentage of unirradiated controls which hatched.

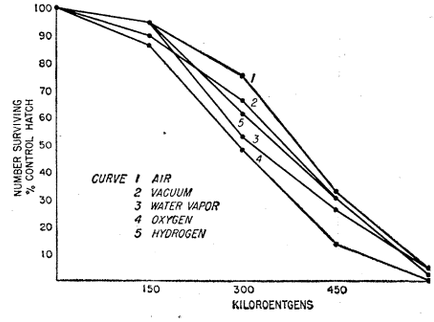

Fig. 2. Curves showing the relative survival of *Artemia* cysts when irradiated at room temperature in different atmospheres and hatched immediately afterward.

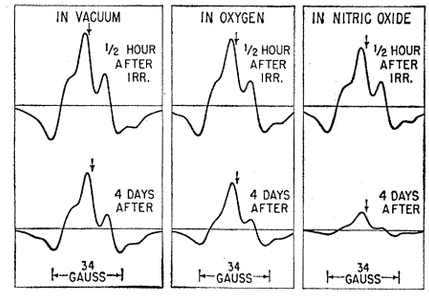

Fig. 3. Electron-spin resonance produced in *Artemia* cysts by exposure to 5×10^6 r of cobalt-60 gamma rays, in vacuum, in O_2, and in NO atmospheres. The observations were made at room temperature at a frequency of 9000 Mc/sec. Arrows indicate the position for the standard DPPH reference signal for which $g = 2.0036$. The curves represent the second derivative of the actual resonance curves.

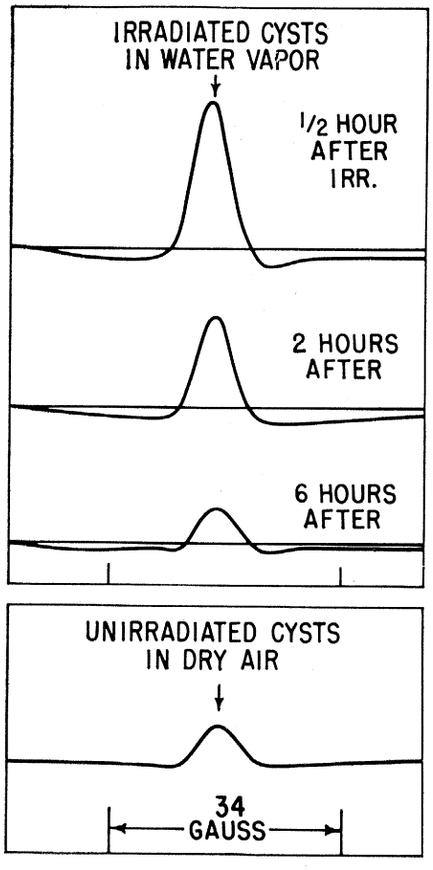

Fig. 4. The top three curves show the electron-spin resonance of *Artemia* cysts irradiated with the same dose of gamma rays and under conditions similar to those described in Fig. 3, except that they were irradiated and stored in an atmosphere of water vapor at room temperature. The bottom curve shows the natural electron-spin resonance of the unirradiated cysts.

dry air, pure O_2, NO, or in any other environment studied.

Shields and Gordy (*8*) found that moisture greatly increases the decay of the ESR patterns of the irradiated nucleic acids DNA and RNA. Furthermore, the shape and width of the DNA and RNA signals, which they obtained when the acids were irradiated in moist air, were similar to those we obtained with cysts irradiated in moist air. This suggests the possibility that the protective or healing effect of water vapor is related to its neutralizing effects on the free radicals produced by irradiation of the nucleic acids. It does not, of course, constitute a proof of such a relationship. Indeed, Shields and Gordy suggested that the water might actually be converting the free radicals to some new stable chemical species which would constitute an irreversible damage. That water vapor apparently heals or prevents biological damage caused by irradiation of the seed and cysts at the same time as the free radical signal decays, indicates the opposite—that is, that the decay in the ESR signals in the irradiated DNA and RNA caused by the moisture may result from a restoration of these acids by the water. The ESR signal in the cysts may arise, of course, neither from the nucleic acid nor from the nucleoproteins. The shells of the cysts constitute a considerable portion of the sample and may contribute to the ESR signal. Also, the healing effect of water may be an indirect one in which the water activates some other healing agent.

A study similar to that reported here has been made on grass seed, *Agrostis stolonifera*, by Sparrman *et al.* (*9*), who obtained evidence, both from ESR signals and survival rates, for the protection of the seeds by NO in water concentrations of less than 12 percent, and found that the radiation resistance of these seeds, when stored in air after irradiation, increased with water content up to about 12 percent. Powers and his associates (*10*) have found that H_2S and NO have radiation protective effects in bacterial spores, and have related these effects to the decay of the ESR patterns of free radicals produced by the irradiation. Reviews of these and related studies have been given by Zimmer (*11*), and by Kirby-Smith and Randolph (*12*).

Thus, from studies of electron-spin resonance in living organisms, evidence is accumulating that much of the biological damage caused by irradiation is associated with radiation-induced free radicals. The extent and nature of the biological damage appears to depend upon the chemicals which happen to react first with those free radicals after they are produced by the irradiation (*13*).

WALLACE C. SNIPES
WALTER GORDY

Department of Physics, Duke University, Durham, North Carolina

References and Notes

1. Z. M. Bacq and P. Alexander, *Fundamentals of Radiology* (Academic Press, New York, 1955).
2. R. S. Caldecott, *Science* **120**, 809 (1954).
3. H. J. Curtis, N. Delihas, R. S. Caldecott, C. F. Konzak, *Radiation Res.* **8**, 526 (1958).
4. A. D. Conger, *J. Cell. Comp. Physiol.* **58**, Suppl. 1, 27 (1961).
5. D. W. Engel and D. J. Fluke, *Radiation Res.* **16**, 173 (1962).
6. These results were reported at the Milwaukee meeting of the Radiation Research Society. See abstract, *Radiation Res.* **19**, 211 (1963).
7. A. D. Conger and M. L. Randolph, *Radiation Res.* **11**, 54 (1959).
8. H. Shields and W. Gordy, *Proc. Natl. Acad. Sci. U.S.* **45**, 269 (1959).
9. B. Sparrman, L. Ehrenberg, A. Ehrenberg, *Acta Chem. Scand.* **13**, 199 (1959).
10. E. L. Powers and B. F. Kaleta, *Science* **132**, 959 (1960); E. L. Powers, C. F. Ehret, B. Smaller, in *Free Radicals in Biological Systems* (Academic Press, New York, 1961), pp. 351–366.
11. K. G. Zimmer, *Studies on Quantitative Radiation Biology* (Oliver and Boyd, Edinburgh, 1961).
12. J. S. Kirby-Smith and M. L. Randolph, *J. Cell. Comp. Physiol.* **58**, Suppl. 1, 1 (1961).
13. We thank Donald J. Fluke for his helpful suggestions. Research was supported by the Air Force Office of Scientific Research, grant No. AF-AFOSR-62-327, and by the Army Research Office (Durham, N.C.).

14 August 1963

Counteracting Effects of Physical Exercises Performed during Prolonged Perceptual Deprivation

Abstract. *Subjects who were required to perform physical exercises during a week's exposure to unpatterned light and white noise showed fewer impairments on 15 behavioral measures than did subjects who were not required to exercise during the same period in an isolation chamber. Furthermore, fewer hallucinatory phenomena and fewer disturbances of the electroencephalogram were observed.*

Research at this laboratory has shown that perceptual deprivation of a week's duration can produce widespread impairments of intellectual and perceptual-motor processes. Hallucinatory phenomena, to a limited degree, are also present. Furthermore, these behavioral deficits are accompanied by significant changes in the electrical activity of the brain (*1, 2*). The purpose of this experiment was to determine whether the introduction of physical exercises during prolonged perceptual deprivation could minimize or even eliminate these behavioral and physiological impairments. Various lines of evidence indicate that physical activity may have beneficial or counteracting effects. Reports of explorers and prisoners of war occasionally mention that performance of calisthenics has proved helpful in combating some of the effects of isolation (*3*). There is also some experimental evidence. Freedman and Greenblatt (*4*), in a review of the literature on sensory deprivation, observed that hallucinatory and delusional experiences seem to occur less frequently during isolation if motor activity is permitted than if it is not.