TABLE 1. The results of aerosol exposed ferrets to challenge with virulent distemper virus.

| No. of ferrets in group | Date of aerosol exposure | No. of days before challenge | Day after challenge | |
|---|---|---|---|---|
| | | | Onset of signs | Death |
| 2 | 8/ 9/53 | 32 | — | — |
| | | | — | — |
| 1 | 8/17/53 | 24 | — | — |
| 1 | 8/25/53 | 16 | — | — |
| 1 | 9/ 1/53 | 9 | — | — |
| 2 | 9/ 3/53 | 7 | — | — |
| | | | — | — |
| 2 | 9/ 5/53 | 5 | — | — |
| | | | — | — |
| 2 | 9/ 6/53 | 4 | — | — |
| | | | 14 | 20 |
| 2 | 9/ 7/53 | 3 | 14 | Recovered |
| | | | 13 | 21 |
| 2 | 9/ 8/53 (A.M.) | 2 | 8 | 12 |
| | | | 9 | 12 |
| 2 | 9/ 8/53 (P.M.) | 1.5 | 8 | 9 |
| | | | 8 | 11 |
| 2 | 9/ 9/53 (A.M.) | 1 | 8 | 10 |
| | | | 9 | 11 |
| 2 | 9/ 9/53 (P.M.) | 0.5 | 8 | 11 |
| | | | 10 | 12 |
| 2 | 9/10/53 | 0* | 8 | 10 |
| | | | 9 | 11 |
| 2 | 9/11/53 | −1 | 8 | 10 |
| | | | 9 | 11 |
| 2 | Nonexposed controls | | 8 | 10 |
| | | | 9 | 11 |

* Simultaneous aerosol exposure and challenge virus.

dihydrostreptomycin sulfate. This dilution, which was used as the aerosol inoculum, was stored at −20° C until used. The inoculum was standardized by a titration, consisting of serial 5-fold dilutions of virus. The 50% infective dose ($ID_{50}$) per 0.1 ml of inoculum in chicken embryos was $10^{3.6}$.

The aerosol was produced by a DeVilbiss 40 type nebulizer operated at 3 lb pressure. The orifice of the nebulizer was fitted into a hole in one side of a chamber which measured 9 × 6 × 6 in. Approximately 0.3 ml of inoculum was nebulized into the chamber during each minute of operation. Young distemper susceptible ferrets were confined to the chamber for 3 min, the first 2 min of which the nebulizer was in operation.

The challenge virus was prepared by inoculating 2 ferrets intramuscularly with the contents of one ampoule of canine distemper vaccine (Distemperoid), Serial 174-A, which was supplied through the courtesy of Fromm Laboratories. The ferrets became moribund on the 11th day following inoculation and were sacrificed. Their spleens were removed and a 20% W/V suspension in nutrient broth was prepared. One ml of the challenge virus was given intramuscularly to each of the ferrets at intervals ranging from −1 to +32 days after exposure to the aerosol inoculum (Table 1). The appearance of conjunctivitis, nasal exudation, dermatitis, or the demonstration of DV inclusion bodies was considered the criterion of infection.

Data obtained from this preliminary experiment indicate that resistance to virulent DV can be stimulated by aerosol exposure to a living egg-adapted virus. Under the conditions of this experiment, the onset of resistance following airborne inoculation of the *Onderstepoort* strain occurred at 5 days. In a previous investigation it was shown that ferrets which were vaccinated intramuscularly with the same strain of egg-adapted virus 2 or more days prior to challenge did not develop distemper (*3*).

### References


1. HITCHNER, S. B., and REISING, G. *Proceedings Am. Vet. Med. Assoc.*, 258, 1952.
2. HAIG, D. A. *Onderstepoort J. Vet. Sci. Animal Ind.* **23**, 149 (1948).
3. BAKER, G. A., LEADER, R. W., and GORHAM, J. R. *Vet. Med.* **47**, 463 (1952).


Manuscript received October 19, 1953.

## Influence of Water-Soluble Vitamin E on Survival Time in Irradiated Mice[1]


Thomas J. Haley, Eve F. McCulloh, and W. G. McCormick[2]

*Division of Pharmacology and Toxicology, Atomic Energy Project, School of Medicine, University of California, Los Angeles*


One of the current theories used in explaining the deleterious effects of ionizing radiation in animals is based upon the formation of peroxide by the action of the radiation on aqueous solutions (*1*). Mead (*2*) has shown that x-irradiation induces organic peroxide formation in linoleic acid, resulting in a chain reaction which destroys this essential fatty acid. More recently, Polister and Mead (*3*) found that *d*-γ-tocopherol was capable of protecting methyl linoleate from radiation-induced autoxidation *in vitro*. If it could be shown that this naturally occurring antioxidant was effective *in vivo*, progress would be made in radiation therapeutics. Furth, Coulter, and Howland (*4*) administered α-tocopherol in oil to rats prior to irradiation without beneficially influencing their survival time. However, Ames, Baxter, and Griffith (*5*) showed that oil-soluble vitamin E (61% α-tocopherol) decreased petechial hemorrhages of the mesentery in rats subjected to injection of radon ointment in the abdomen. These differences in results may have been due to differences in the type of irradiation used (gamma vs alpha) or to the failure of the oil-soluble material to be absorbed and become available as a tissue antioxidant. The tissue availability of the antioxidant is of greatest importance at the time of initial injury and throughout the first two postirradiation weeks if the peroxide chain reaction is to be prevented or stopped. Injections of water-soluble vitamin E would make available to the animal a natural antixodant

[1] Based on work performed under contract No. AT-04-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

[2] We wish to thank Distillation Products Industries for the water-soluble vitamin E used in this investigation.

which should be beneficial in counteracting the deleterious effects of irradiation.

Male CF 1 strain mice, weighing an average of 20 g, were arranged in groups of 20 animals each and maintained under the same conditions used in our previous radiation studies (*6*). The water-soluble vitamin E solutions and control saline were injected intramuscularly in total daily doses of 0.1 ml of the concentrations given in Table 1. Injections were given prior to irradiation and every day thereafter until 90–100% of the animals had died. The 550-r irradiation dose was administered from above and below the mice with two 250 KVP Picker Industrial Units operating simultaneously. The technical factors were: 250 KVP; 15 ma; FOD 100 cm; filters 0.21 mm Cu inherent, 0.5 mm Cu parabolic, and 1.0 mm Al; HVL, 2.02; size of field-total body, r/min measured in air 17.37–17.53. Both units were calibrated prior to the experiment with a Victoreen thimble r-meter. The results obtained were analyzed statistically by the method of Litchfield (*7*).

The results obtained are given in Table 1. It is evident that the lower doses of vitamin E neither significantly increased nor decreased the $ST_{50}$ day or the day of total mortality, whereas the two highest doses significantly decreased both. These results indicate that even when this antioxidant is made available to the animal prior to irradiation and throughout the post-irradiation period, it does not prevent the deleterious effects of ionizing radiation. The doses used should have been adequate, because Polister and Mead (*3*) showed that *in vitro* $2.69 \times 10^{-5}$ *M* tocopherol would prevent radiation-induced oxidation of $2.56 \times 10^{-2}$ *M* methyl linoleate. The fact that the higher doses of vitamin E increased both the rate of mortality and the total mortality would indicate that the vitamin acted synergistically with the radiation. This confirms Mead's (*8*) observation with fat-soluble vitamin E in rats. Such effects indicate that the extensive biochemical derangements which occur in the radiated animal may make otherwise nontoxic doses of essential metabolites a contributing factor in lethality from radiation. It is also possible that even water-soluble vitamin E does not reach the site of peroxide action, and thus the peroxides are not prevented from exerting their deleterious effects. On the other hand, peroxide formation is only one effect produced by radiation, and therapy designed to counteract only one damaging effect might not prevent over-all radiation damage.

TABLE 1. Effect of water-soluble vitamin E in irradiated mice.

| Drug | Total daily dose (mg) | $ST_{50}$* and range (days) | Slope and range | Total mortality (Days) | Total mortality (%) |
|---|---|---|---|---|---|
| Saline 0.9% | — | 8.35 (7.3–9.5) | 1.35 (1.2–1.5) | 12 | 90 |
| B-883 Vitamin E based on 25.5% α-Tocopherol content | 0.5 | 9.4 (8.6–10.3) | 1.23 (1.15–1.31) | 11 | 100 |
| | 1.0 | 7.95 (7.2–8.7) | 1.24 (1.16–1.33) | 11 | 95 |
| | 2.0 | 5.8 (5.5–6.13) | 1.13 (1.08–1.17) | 8 | 100 |
| | 3.18 | 4.21 (3.9–4.5) | 1.19 (1.13–1.26) | 6 | 100 |

* $ST_{50}$ = day on which 50% of animals remain alive. Values are at odds 19/20.


### References

1. DUBOULOZ, P., DUMAS, J., and VIGNE, J. *Compt. rend. soc. biol.* **144**, 1080 (1950).
2. MEAD, J. F. *Science* **115**, 470 (1952).
3. POLISTER, B. H., and MEAD, J. F. *Univ. Calif. Los Angeles Atomic Energy Project Report*, UCLA-253, May 6, 1953.
4. FURTH, F. W., COULTER, M. P., and HOWLAND, J. W. *Univ. Rochester Atomic Energy Project Report*, UR-152, February 1, 1951.
5. AMES, S. R., BAXTER, J. G., and GRIFFITH, J. Q., JR. *Intern. Rev. Vitamin Research* **22**, 401 (1951).
6. HALEY, T. J., and RHODES, B. M. *Science* **117**, 139 (1953).
7. LITCHFIELD, J. T., JR. *J. Pharmacol. Exptl. Therap.* **97**, 399 (1947).
8. MEAD, J. M. Unpublished.


Manuscript received November 9, 1953.

## Root Production in Plants Following Localized Stem Irradiation[1]


Eric Christensen[2]

*Biology Department, Brookhaven National Laboratory, Upton, New York*


This paper reports the induction of adventitious roots in *Xanthium, Nicotiana, Lycopersicum,* and *Phaseolus* following localized irradiation of a small length of the stem of the intact seedlings.

Adventitious roots have been produced artificially in the past in a number of intact plants as a response to various external treatments. The use of auxin pastes is well known. Ethylene, propylene, and acetylene have also been used to produce roots on the stems of *Lycopersicum, Tagetes, Nicotiana, Hydrangea,* and *Coleus* (*1*). Carbon monoxide has produced the same effect in *Cosmos, Impatiens, Amaranthus,* and other plants (*2*). Root development on bean cotyledons followed planting of the seed in soil treated with 2-4-D (*3*). Johnson, using x-rays, reported some increased rooting in *Salix* cuttings but concluded that radiation was harmful to the rooting of herbaceous cuttings (*4*).

The present study was conducted with seedling plants exposed to 250 KVP, 30 ma x-irradiation with 1 mm Al filtration. Lead shielding 2 cm thick was placed so as to protect all plant parts except the short length of stem to be irradiated. The epicotyl and hypocotyl respond similarly to this treatment; no distinction was made between these regions in selecting the site of treatment. Cotyledons were often included in the exposure.


[1] Research carried out at Brookhaven National Laboratory under the auspices of the United States Atomic Energy Commission.

[2] The author wishes to thank Dr. A. H. Sparrow for providing facilities which made the work possible and Dr. Seymour Shapiro for valuable suggestions made in the course of the investigations.