day for 7 days; the control animals were left undisturbed in their home cages. All animals were given free access to food and water during the treatment period.

After treatment all animals (5) were deprived of water for 48 hours and then tested. The test procedure consisted of an 8-hour run with nonshock-reinforced presentations of the clicker every 20 minutes during the session.

As is shown in Table 1, all animals had low suppression ratio values on the last set of conditioning trials, indicating a high degree of acquisition of the "fear" response. On the last extinction session, suppression ratio values for all animals approached 1.00, indicating recovery of lever-pressing during presentations of the clicker stimulus. After the electroconvulsive shock period, however, clear differences are evident between the treated and the control animals on the first trial of the test session (6). Lever responding by all of the control animals was almost completely suppressed in the presence of the auditory stimulus, which was indicated by suppression ratio values ranging from .02 to .06. All treated animals, on the other hand, continued to press the lever during the stimulus presentation, as is reflected in the suppression ratio values which range from .49 to 1.00.

These results are in accord with previous findings (7, 8) which demonstrated that electroconvulsive shock. would virtually eliminate a "fear" response of the type described in the present experiment. In fact, to the extent that the "fear" response is spontaneously recovered during the time interval of the treatment period (and the present findings indicate that this is considerable), this study is essentially similar to the studies of Brady et al. (7) which did not involve an extinction phase prior to administrations of electroconvulsive shock.

The present findings that electroconvulsive shock treatments do not reinstate extinguished "fear" responses appear to be in direct conflict with the findings of Gellhorn. This apparent conflict may result from the possibility that electroconvulsive shock treatments have at least two effects. One effect is to attenuate "emotional" responding. The second effect, suggested by observation of behavior following electroconvulsive shock, is to produce hyperirritability, which is manifested primarily in easily elicited and high-amplitude startle reactions. That this effect of electroconvulsive shock will facilitate avoidance responding of the kind studied by Gellhorn seems to be indicated by the results of a study by Gellhorn and Minatoya (9). These investigators reported: "A partial conditioning leading to an

average of only 20% positive responses in the control group causes 82% conditioned responses in the experimental group subjected to two hypoglycemias during the training period."

In the light of this evidence, and in view of the present findings, it would seem that convulsions do not reinstate extinguished responses in any general sense.

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References and Notes

1. E. Gellhorn, *Ann. N.Y. Acad. Sci.* **56**, 200 (1953).
2. C. L. Hamilton and R. A. Patton, *J. Comp. and Physiol. Psychol.* **45**, 600 (1952).
3. W. J. Griffiths, *Science* **122**, 1267 (1956).
4. W. K. Estes and B. F. Skinner, *J. Exptl. Psychol.* **29**, 390 (1941).
5. Just prior to the electroconvulsive shock period one of the control animals died. During that period two of the experimental animals developed flaccid paraplegia and had to be destroyed.
6. Performance on the first test trial provides the critical data as the extinction procedure of the test session would be expected to attenuate any treatment effect.
7. J. V. Brady and H. F. Hunt, *J. Comp. and Physiol. Psychol.* **44**, 204 (1951).
8. J. V. Brady, H. F. Hunt, I. Geller, *ibid.* **47**, 454 (1954).
9. E. Gellhorn and H. Minatoya, *J. Neurophysiol.* **6**, 161, 171 (1943).
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30 December 1960

Possible Effect of Lethal Visible Light on Year-Class Fluctuations of Aquatic Animals

Abstract. Visible light killed the eggs and developing embryos of brook and rainbow trout. It is hypothesized that light could kill the eggs and embryos of all aquatic vertebrates and invertebrates. Adaptations minimize but do not completely block this lethal effect. Under some circumstances, visible light could cause heavy mortality in a new year-class of an aquatic animal.

Eggs of the brook trout, *Salvelinus fontinalis*, stripped from fish at the New York State hatchery at Cold Spring Harbor in November 1959 and retained in the hatchery troughs, suffered an unusually heavy initial mortality, estimated at over 90 percent, in contrast with a usual mortality for such an operation of about 10 percent. Experiments proved that the visible light from 40-watt cool-white fluorescent bulbs was responsible.

Similar results were reported by Handorf (1) with another species of salmonid, the rainbow trout, *Salmo irideus*. Handorf also found that the various components of white light showed a differential lethal effect on the fertilized eggs and embryos. The violet

and blue bands were highly lethal, while the green, yellow, and orange bands were progressively less lethal and of a much lower level of lethality. A greater resistance to the lethal effect of all bands of visible light was exhibited by the more intensely colored yellow eggs than by the paler eggs.

In nature, salmonid eggs are shielded from the light by the loose gravel of the stream bottom on which they are laid and with which they become covered during the spawning process. Also in nature, salmonid eggs are normally of a more pronounced yellow color than is usual with the eggs of hatchery fish. In the hatchery, it has been proved that direct sunlight kills salmon and trout eggs in a few minutes, while indirect daylight increases the mortality of eggs and embryos of the sockeye salmon (2). Although the lethal effect of direct sunlight might have been due to ultraviolet rays, the subdued indirect light entering a hatchery would be devoid of ultraviolet rays, and the increased mortality of the eggs and embryos must be attributed to natural visible light.

On the basis of these findings, it is hypothesized that visible light is potentially lethal to the fertilized egg and developing embryo of all fishes, other aquatic fishlike vertebrates, and aquatic invertebrates. However, through adaptive changes in the structure of the body and behavior of the adults and in the structure of the fertilized egg and embryo, the lethal effect of visible light has been minimized, at least under normal conditions. Obviously in ovoviviparous forms, the abdominal wall and the often heavily pigmented, coelomic lining shield the fertilized egg and embryo from the deleterious effects of light.

In oviparous forms, the spawning habits of the adults help to protect the eggs and embryos from the lethal effects of light. A large number of fishes and aquatic invertebrates spawn during periods of the year when cloudy, windy, and rainy weather predominates. Such weather conditions, by increasing the turbulence and turbidity of the water, reduce the penetration of light. Also, most fishes and aquatic invertebrates spawn in the littoral and sublittoral zones. Here, submerged and floating algae in salt water, and vascular plants and algae in fresh water, together with suspended matter including phytoplankton, filter out the more lethal violet and blue components of light and permit the greatest penetration of the less lethal green light (3). In the shallower waters of the littoral zone and in some lakes and streams, plankton and organic stains color the water to such an extent that only orange or red light, of low lethality, penetrates.

In addition to this general adaptive

spawning behavior to minimize exposure of the egg and embryo to deleterious light, more specific types of behavior have evolved in some forms for the same purpose. The covering of salmonid eggs with gravel from the stream bottom has already been described. Other fishes, with adhesive, demersal eggs, lay them under projecting stones or in crevices between them, on the stems and leaves of higher aquatic plants, or in clumps of algae. Some fishes build elaborate nests which cover the eggs and embryos. Still others lay their eggs in the empty shells of mollusks and remain coiled about them while they develop. Similar variations in spawning behavior occur in other aquatic animals besides the true fishes.

Aside from this adaptive spawning behavior, the eggs and embryos themselves have also become adapted to help withstand the injurious effect of visible light. Such adaptations would be the development of structures to disperse light or to prevent penetration of lethal rays. In the newly fertilized egg and in the early embryological stages, the small oil globules distributed throughout the yolk could help to disperse light. In later embryological stages the irregular surface of the embryo itself might contribute to this dispersion. The newly fertilized egg and the yolk and embryo of later stages are commonly tinged with yellow. This would act as a filter, permitting mostly the less lethal yellow light to penetrate. In the late stages of embryological development in fishes, large melanophores are common over such sensitive portions of the body as the brain, spinal cord, and abdominal cavity. Large xanthophores are frequently associated with these melanophores. Nicol (4) also reports that the chromatophores in decapod larvae and mysids are organized in definite neural and visceral groups.

If the hypothesis that visible light is potentially lethal to the fertilized egg and developing embryo of oviparous aquatic vertebrates and invertebrates is assumed to be correct, it can be concluded that all adaptations tend to minimize the lethal effect of the light, but do not give complete protection against it. The degree of mortality of the eggs and embryos caused by exposure to deleterious visible light would vary with changes in the environment. Years in which the spawning season extended over a period with clear, sunny weather comparatively free of wind and rain might result in unusually clear water, greater penetration of lethal visible light, and poor survival of the progeny of many aquatic forms. Year-class fluctuations in aquatic organisms have been attributed to changes in a wide variety of biological and physicochemical conditions such as food, predation, disease, temperature, salinity, and currents. Yet frequently such fluctuations have shown either no relationship to or a poor correlation with these factors. It is suggested that in these instances visible light intensity might have been the unknown factor influencing year-class fluctuation.

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References

1. K. Hamdorf, *Physiologie* 42, 525 (1960).
2. E. Leitritz, *Calif. Dept. Fish and Game, Fish Bull. No. 107* (1960).
3. G. L. Clarke, *Elements of Ecology* (Wiley, New York, 1954).
4. J. A. C. Nicol, *The Biology of Marine Animals* (Interscience, New York, 1960).

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Formation of Free Radicals in Tritiated H_2O and D_2O Ice

Abstract. By using tritium as an internal source of radiation, electron spin resonance spectra may be obtained for samples contained in glass without the usual disturbing effects due to irradiated glass. The production of OH and OD radicals in tritiated H_2O and D_2O ice may be readily demonstrated with this technique.

Most of the available data on the production of free radicals in H_2O and D_2O ice have been provided by electron spin resonance studies of samples irradiated by cobalt-60 γ-rays at liquid nitrogen temperature (1, 2).

By using tritium, the effects of much higher linear energy transfer may be investigated. Moreover, a given sample may be irradiated and its electron spin resonance spectrum may be examined,
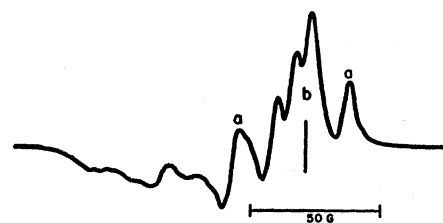

Fig. 1. OH doublet (*a*) and OD triplet (*b*) in electron spin resonance spectrum of tritiated H_2O and D_2O ice. The vertical line indicates the position of DPPH line. Field increases toward the right.

both in the same glass tube; thus one may obtain "pure" spectra of the materials under test without the usual disturbing effects due to irradiated glass.

An example of an electron spin resonance spectrum induced by Tβ-particles is shown in Fig. 1. The spectrum, representing the absorption derivative curve, has been recorded at liquid nitrogen temperature with an X-band Varian ESR spectrometer, model V4500, for a tritiated mixture of activity 1 c/ml, containing 80 percent H_2O and 20 percent D_2O.

The sample was kept over the period of about 2 weeks in liquid nitrogen, and the total dose of energy absorbed was 2.9×10^{20} ev/ml. Between the external peaks (Fig. 1, *a*) of the doublet, with separation approximately 40 gauss and *g*-value about 2.01, is situated a triplet (Fig. 1, *b*) with separation of 6 gauss.

By comparison with the data reported for cobalt-60 γ-rays (2), the doublet and triplet may be interpreted as due to OH and OD radicals, respectively (3, 4).

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References and Notes

1. B. Smaller, M. S. Matheson, E. L. Yasaitis, *Phys. Rev.* 94, 202 (1954); S. M. Matheson and B. Smaller, *J. Chem. Phys.* 23, 521 (1955).
2. S. Siegel, L. H. Baum, S. Skolnik, J. M. Flournoy, *J. Chem. Phys.* 32, 1249 (1960).
3. A full description of these experiments and the production of H and D atoms in tritiated, acidified H_2O and D_2O is in preparation.
4. We are pleased to acknowledge financial assistance from the National Research Council of Canada.

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