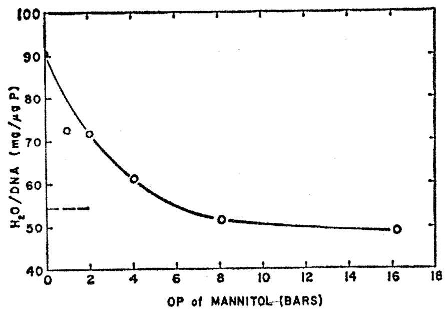

Fig. 3. Ratio of water content to DNA for radish cotyledonary leaves as a function of the osmotic pressure (*OP*) of mannitol in the incubation medium. Each point is the average of two groups of 20 leaves. The dashed line indicates the value of the ratio prior to incubation.

60 percent by just 1 or 2 bars, with a slight additional decrease as the osmotic pressure increased to 16 bars. The uptake of water also declined as the osmotic pressure increased. One bar of osmotic pressure suppressed the uptake by 60 percent, 8 bars, by 95 percent. At 16 bars, the leaf-water content dropped about 2 percent below the amount present before incubation. The nearly complete suppression of water uptake at 8 bars is in agreement with the estimate, based on plasmolysis, of about 8 bars for the osmotic pressure of the cell sap before incubation. This agreement supports the premise that it is primarily the osmotic pressure of the added mannitol rather than the total osmotic pressure of the medium (including the contribution made by the other solutes) that limits the uptake of water. The ratio of leaf-water content to DNA gives a measure of the cell size, and hence, of cell enlargement. Figure 3 shows a graph of this ratio plotted against the osmotic pressure of mannitol in the medium. Cell enlargement at first decreased more gradually than cell division with increasing osmotic pressure. But at 8 bars, enlargement was almost completely prevented whereas DNA was increased by about 14 percent. As a result, the ratio of water to DNA actually fell below the ratio before incubation. Total growth—the product of the two curves (Figs. 2 and 3)—virtually stopped above 8 bars.

It is usually observed that the highest rate of leaf growth occurs during the night when the suction is at a minimum. Thus, one might expect the growth rate to be more closely correlated with the minimum leaf suction than with the average leaf suction. The opposite is the case with transpiration, which is more strongly influenced by the maximum suction near midday when wilting is more probable. The minimum leaf suction, in turn, seems closely related to the average soil suction so that a good correlation between leaf growth rate and average soil suction might be expected.

One reason for much of the confusion concerning water availability is well illustrated by Fig. 1. The leaf suction is relatively unaffected by the soil suction for several days after a soil is irrigated. During this time while the soil suction is low one can reasonably assert that the soil water is, for practical purposes, equally available for growth and transpiration. From day 7 through day 9 in Fig. 1, the increase in soil suction resulted in an increase in leaf suction which reduced growth and, eventually, transpiration. In the case of many soils, the range of water content represented by the range of soil suction from 1 to 15 bars is relatively small compared with the range from 0.2 to 1 bar. In the field, the time during which the soil suction is sufficiently high to reduce growth or transpiration may be short compared with the time during which the soil suction is negligibly low. This fact, coupled with the inherent inhomogeneity of soils and the nonuniformity of the water extraction pattern in the plant root zone, often tends to obscure the very definite effect of soil suction upon transpiration and growth which is observed in the laboratory.

While the percentage at 15 bars may continue to serve a useful purpose in many practical situations, it is increasingly clear that the permanent wilting point does not represent the absolute lower limit of available water. Indeed, no single limit for all plant processes can be defined in any precise way.

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20 January 1964

Radiation-Induced Aversion to Alcohol

Abstract. *Mice genetically susceptible to alcohol orientation were allowed to develop a preference for 10 percent ethanol over tap water. A low dose (12 roentgens per hour for 4 hours) of whole-body gamma radiation was used as an unconditional stimulus to produce alcohol-avoidance behavior. A marked aversion to the alcohol solution occurred but was extinguished within 6 days, owing probably to the very high motivation of the animals to drink alcohol. The study extends the technique of radiation-produced avoidance conditioning to include alcohol consumption as a measure of response in genetically susceptible mice.*

The use of ionizing radiation as an unconditional stimulus in avoidance learning experiments has been shown by Garcia, Kimeldorf, and others (*1*) to be effective with a variety of response measures in several different species. The intake of normally preferred solutions of saccharin by rats and mice or of chocolate-flavored milk by cats can be reduced if the ingestion of these substances is paired in an appropriate temporal arrangement with whole- or part-body exposure to ionizing radiation. That such low-dose radiation effects are not peculiar to the ingestion of foodstuffs or to the activation of taste receptors is revealed by a study in which spatial avoidance behavior was established with the use of x-rays as the aversive stimulus (*1*). Our study represents an attempt to extend the response generality of radiation-produced aversive conditioning by using ingestion of alcohol as a response measure in alcohol-oriented mice. Although the sensory mechanisms through which radiation acts as an unconditional stimulus to produce avoidance behavior are obscure (*2*), the potency of the avoidance response can be demonstrated if it results in the rejection of alcohol solutions by animals whose genetic susceptibility to (*3*) and experiential preference for alcohol has been shown.

Twenty-four male mice of the C57BL/Cum inbred strain were provided only 10 percent ethanol to drink for 14 days during which they were allowed free access to dry food. The ethanol consumption during this period averaged 6.4 ml per day per animal, which equalled their mean daily water intake before the experiment. After the 2-week alcohol orientation period, the mice were placed on a fluid deprivation schedule of 8 hours followed by a 4-hour drinking period. Thus the animals were allowed to drink fluids from 7:30 A.M. to 11:30 A.M., and from 7:30 P.M. to 11:30 P.M.; fluids were withheld at all other times. After 48 hours of habituation to the deprivation schedule, preference trials were conducted during the drinking periods. Each animal was presented with two drinking bottles, one containing 10 percent ethanol and one containing tap water, for each 4-hour drinking period. The two bottles were presented simultaneously to the subject, and the positions of the bottles were varied according to a predetermined random order. At the end of the drinking period, the consumption of each of the solutions was measured. The data so collected are presented as the first ten trials in Fig. 1, where it can be seen that the alcohol solution was strongly preferred to tap water.

The animals were randomly divided into two groups of 12 subjects each. Both groups received four daily habituation trips to a building housing a Co^{60} gamma source, where the morning drinking trial was held. The evening drinking trial was conducted in the home room. On the 5th day of this procedure, the members of the experimental group, deprived of fluids since 11:30 P.M. of the preceding day, were allowed to drink only 10 percent ethanol for the 4-hour period during which they received approximately 48 roentgens whole-body gamma ray exposure. On the 6th day, the control group, similarly deprived, drank alcohol during a sham-irradiation trial.

Preference measures were taken on the evening trials of the radiation and sham-irradiation days, and for nine subsequent 4-hour trials. The results so obtained are shown as the post-irradiation trials in Fig. 1, where it can be seen that the alcohol consumption of the mice in the experimental group, which was irradiated while having access to alcohol, decreases sharply. A compensatory increase in water intake occurs, so that the total fluid intake remains essentially normal. The control group animals, on the other hand,

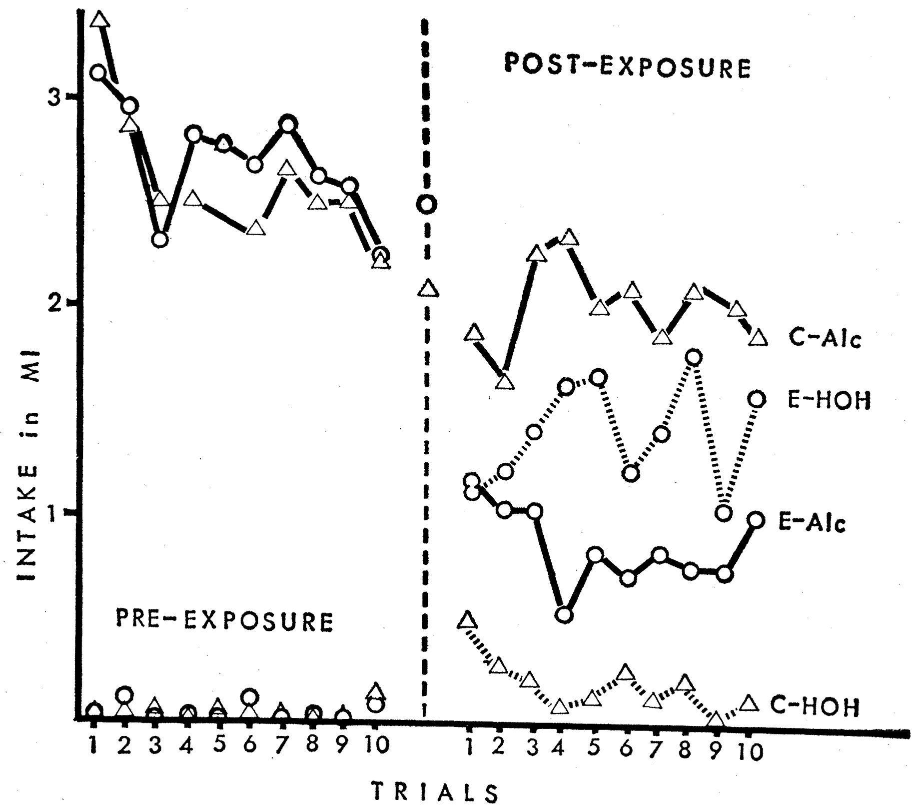

Fig. 1. Fluid intake by mice before and after exposure to radiation or sham-radiation. Solid lines: alcohol intake; dashed lines, water intake; triangles, control group; circles, experimental group. Alcohol intake during radiation or sham-radiation exposure is indicated by points in dotted lines.

show no such shift in their preference for alcohol. An analysis of variance was performed on the alcohol consumption data for the two groups. The difference between the groups in their consumption of alcohol solutions was found to be significant ($p < .001$).

It appears from these data that radiation-produced avoidance conditioning is powerful enough to overcome a strong alcohol preference in mice whose genetic structure makes them prone to alcohol intake. The conditioning effect is not permanent. The total 24-hour intake of alcohol and water since the conclusion of the ten experimental trials has been measured in these mice. Six days after irradiation the preference curves begin to cross and within 12 days total extinction has occurred. Garcia *et al.* (*4*) found that animals which preferred saccharin to tap water (86 percent preference) and which were exposed to 30 or 57 roentgens delivered during a 6-hour period, had a persistent aversion 30 days after irradiation. The more rapid extinction in the alcohol-oriented mice may be a result of either their higher motivation as revealed by the stronger preference shown initially (98.7 percent), the duration of exposure to the aversive stimulus, the radiation dose or dose-rate, or to a species difference. The effects of such variables in producing conditioned aversion to alcohol and in defining the optimal conditions for maximum persistence of the aversion have not yet been determined.

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5. Supported in part by AEC contract AT-(40-1)-2787 and in part by the General Research Fund of the University of Georgia.

3 February 1964