

dered and are particularly useful in differentiating velogenic strains (5). Studies on the genetic stability of certain test characters (6) indicate that, with proper precautions, the method suggested here for strain typing and differentiation can be successfully employed.

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Conditioned Aversion to Saccharin Resulting from Exposure to Gamma Radiation

It was previously observed (1) that the water and food consumption of rats was depressed during exposure to a relatively low dose of low-intensity gamma radiation. The severity of this depression increased with successive exposures to radiation, although consumption between exposures was similar to or exceeded that of nonirradiated controls. It was suggested that the progressive change in consummatory behavior during repetitive exposure may be, in part, a conditioned response in which the avoidance of water and food is strengthened by learning through repeated coupling with the radiation situation.

A clearer demonstration of the learning phenomenon would be provided if the conditioned avoidance of water or food could be elicited in the absence of radiation exposure. This report (2) describes the results of an experiment designed to provide such a test. The water available during irradiation was made discriminative to the animal by the addition of saccharin. Subsequently, the saccharin flavor was employed as a taste stimulus in a postirradiation test for a conditioned aversion to the discriminative fluid.

The animals were Sprague-Dawley stock bred at this laboratory. Each animal was maintained in an individual cage. Two plastic 100-ml drinking bottles with glass nipples were attached to each cage except during specified periods. The difference in weight of each drinking bottle and of its contents before and after a given test period was used as the estimate of fluid consumption.

Eighty male rats from 16 litters were subjected to a preirradiation series of tests to determine their degree of preference of a 0.1-percent saccharin solution in relation to tap water. Both fluids were presented concurrently. Tests were also made to evaluate a possible position preference in drinking in the two-bottle situation. Very few animals exhibited a drinking-bottle position preference strong enough to overcome their preference for saccharin. As a result of these tests, 20 animals with the lowest preference for the saccharin solution and/or the highest position preference were eliminated. The remaining animals were randomly assigned to six groups, with the restriction that each group contain at least one member, and not more than two members, from each litter. The experimental treatment for each group is shown in Table 1.

For radiation exposure, all animals were confined in Lucite boxes and exposed in the gamma field of a 7-c cobalt-60 radiation source. Attached to each box was a single plastic drinking bottle that contained either tap water or saccharin solution, as specified in the experimental design. The 30-r and 57-r exposures were made at radiation intensities of 5.0 r/hr and 9.5 r/hr, respectively. The sham-irradiated groups were placed in the radiation field behind a lead shield. The total exposure period was 6 hr for all groups.

Measurements of fluid consumption

Table 1. Experimental treatment for each group.

Group	No. of animals	Radiation exposure dose (r)	Fluid present during exposure
I	10	0	Tap water
II	10	0	Saccharin solution
III	10	30	Tap water
IV	10	30	Saccharin solution
V	10	57	Tap water
VI	10	57	Saccharin solution

Table 2. The median saccharin solution preference score for each group during the initial 48-hr postirradiation test period

Radiation dose (r)	Groups II, IV, VI (saccharin present during irradiation)	Groups I, III, V (water present during irradiation)
0	88.0 (11.0)*	93.2 (2.3)
30	33.4 (9.8)	94.8 (8.2)
57	3.1 (3.7)	96.8 (1.8)

* Standard error of the median.

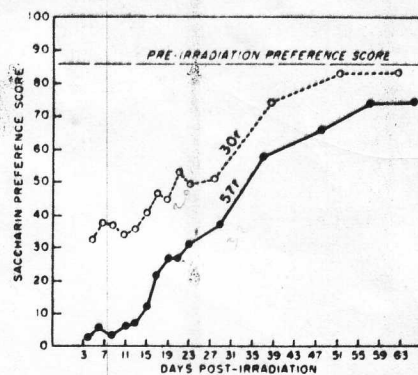


Fig. 1. Median saccharin preference scores for animal groups exposed concurrently to gamma radiation and saccharin-flavored drinking water.

indicated that all animals had tasted the fluid presented to them during radiation exposure. On day 1 postirradiation, the groups that had received water during exposure were presented with the saccharin solution only for 6 hr, and the other groups received water only in order to equalize the experience with saccharin. On day 2 postirradiation, saccharin solution and water were simultaneously available to each animal for a 6-hr period. Beginning with the day 3 postirradiation, both saccharin solution and water were available to each animal on a continuous basis. Measurements of consumption were made at 24-hr intervals.

During the postirradiation preference testing period, the drinking bottles were reversed daily to avoid a stereotyped position response. An effect of position was not detectable when the saccharin preference was distinctively high or low. However, when the scores were in the neutral range of preference, a position effect could be detected. In order to minimize the effect of position on the scores, each consecutive left and right measurement pair are combined for each animal, and 48-hr values are reported.

The saccharin preference score utilized is the quotient for the ratio

$$\frac{\text{Saccharin solution intake (g)}}{\text{Total fluid intake (g)}} \times 100$$

The median preirradiation saccharin score for all animals was observed to be 86.1 (S.E. = 1.0) for two 6-hr test periods; that is, the saccharin solution constituted 86.1 percent of the total fluid consumption. The results of the initial postirradiation preference test period for each group are summarized in Table 2. Animals presented with saccharin during the sham-irradiation (0 r) maintained their preference during the post-irradiation test period. However, animals that were exposed to 30 r or 57 r with saccharin available during irradiation exhibited a marked decrease in their pref-

erence score in the postirradiation test (Table 2). Exposure to 30 r was sufficient to negate the previous preference for saccharin, while exposure to 57 r resulted in a striking aversion to saccharin.

The consumption of saccharin and tap water was determined daily for 20 days and then at intervals of 2 to 5 days during the next 40 days. The saccharin preference scores for groups that had saccharin available during irradiation are summarized graphically in Fig. 1. The conditioned aversion to the discriminant fluid was still present 30 days after irradiation, although some extinction was apparent.

The use of saccharin solution in the present study has made it possible to demonstrate the effectiveness of ionizing radiation to act as an unconditioned stimulus in animal behavior. Rats tend to avoid a taste stimulus that has been associated with radiation exposure, although the stimulus is usually preferred. The conditioned aversion to saccharin is relatively radiosensitive, being effected by a 6-hr coupling with a 30-r dose of low-intensity (5 r/hr) gamma radiation. The conditioning appears to be dose-dependent in terms of the strength of saccharin aversion and in the persistence of this aversion.

Although the conditioned aversion in this study is dependent on taste discrimination, it may be symptomatic of broader behavioral disturbances instigated during radiation exposure. If this is true, then it should be possible to detect avoidance behavior with stimuli other than taste. Such studies are now in progress.

The processes through which radiation is capable of operating as an unconditioned stimulus are unknown. Since consummatory behavior is partially a reflection of gastric function, it is plausible to suspect gastrointestinal disturbances as the physiological events that motivate the animal in the learning situation. Gastrointestinal functions are known to be disturbed during irradiation and are responsive to the same magnitude of radiation dose (3).

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17 March 1955

Intolerance of Dizygotic Human Twins to Reciprocal Skin Homografts

Skin homografts transplanted between two human individuals of ordinary genetic diversity are almost invariably rejected by the host individual (1). When homografts are exchanged between monozygotic, or "identical," human twins, however, they survive permanently (1). With one possible exception (2), the behavior of skin homografts in dizygotic, or "fraternal," human twins has not been reported in the medical literature. Meyer-Burgdorff (2) described the rejection of skin homografts in a pair of twins whom he regarded as monozygotic, but the diagnosis of monozygosity was not based on objective criteria.

Skin homografts exchanged between dizygotic twin cattle frequently survive indefinitely (3) and behave in all respects like autografts. This is in contrast to the rejection of homografts exchanged between ordinary cattle siblings or between dizygotic twin sheep (4). The contrast between cattle and sheep in this respect has been attributed (5) to the interchange of fetal blood in cattle twins, as is evidenced by the occurrence of freemartins in cattle. Furthermore, in cattle each member of a dizygotic twin pair usually has two types of red blood cells, a heterogeneity suggestive of an actual transplantation of blood-forming tissues by way of the fetal vascular anastomoses. Such animals are spoken of as erythrocyte chimeras (3). At least one such chimera has been described in man (6).

The present tests were performed in the hope of determining the zygosity of two pairs of twins that presented difficulty in a twin study on mental deficiency (7). Both pairs of twins had identical blood antigens and very similar dermatoglyphic patterns. Blood of all four twins was examined at the Knickerbocker Foundation, for heterogeneity of the red blood cells in respect to the ABO antigens, with negative results. Since in each case one twin was severely defective, both mentally and physically, ordinary morphological criteria of zygosity could not be relied upon. In one set of twins, 13-year-old girls, the defective member had microcephaly of postnatal origin, retrolental fibroplasia, and a symmetrical growth anomaly of the toes. The other set of twins were 11-year-old boys, of whom one was a mongoloid imbecile; the mongoloid had a moderate amount of brown (superficial) eye pigmentation that was lacking in his brother. A more detailed report of these cases is in preparation.

Full-thickness, circular skin homografts were reciprocally transplanted in corresponding defects made on the volar

surface of the left forearms of the two sets of twins. Follow-up examinations made through the night after transplantation confirmed the survival of the homografts in the twin indicating a probable monozygosity. Between the 18th and postoperative days in the twin, however, a sudden violent rejection of the skin homografts occurred. This was evidenced by a marked induration, redness, vascular thromboses, hemolysis, eventual gangrene, and rapid necrosis of the entire full-thickness of skin on both twins. Except for this relatively violent reaction, the phenomena observed were identical to those associated with the sloughing of skin homografts between two unrelated individuals (1). This result was taken as evidence that the brothers were dizygotic in origin.

The senior author is conducting further studies on the behavior of skin homografts in dizygotic twins.

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7. This work was part of a study of mental deficiency in twins now in progress at the Department of Medical Genetics of the National State Psychiatric Institute. The laboratory is under the joint supervision of Francis J. Man and the Laboratory of Socio-Environmental Studies, Research Branch, National Institute of Mental Health, U.S. Public Health Service. The Psychiatric Institute provided facilities for the skin-grafting operations.

31 March 1955

C¹⁴-Acetate Incorporation into Liver Lipids and Glycogen of Irradiated Rats

In vivo differences in C¹⁴-acetate incorporation into glycogen and lipids in the liver have been studied in normal and irradiated animals (1). Previous investigations into the effects of irradiation have been concerned with either glycogen or fatty acids. Denson *et al.* have reported liver glycogen incorporation in rats starved 24 hr postirradiation. Ross and Ely (3) have shown that glycogen appeared within 3 hr of x-ray exposure. However, Fishel reported diminishing glycogen incorporation after irradiation, and Proctor, studying the later effects, observed no liver glycogens. In fasted irradiated