

Repeated (4 to 5) applications at 5- to 7-day intervals of 100 ppm, beginning at the time of cotyledon expansion and continuing until 4 to 5 true leaves had developed, effectively suppressed staminate flower bud development in many plants. The pistillate flowers on maleic hydrazide-induced male-sterile plants in almost all instances appeared normal and were fertile. When pollinated the fruit developed normally and produced abundant quantities of viable seed. Investigations now in progress suggest that the results reported here for Table Queen squash can be reproduced in many varieties of *C. pepo* and in other cucurbitaceous species and may have widespread utility in making the production of hybrid seed an economic reality.

References and Notes

- * Journal article No. 1656 of the Michigan Agricultural Experiment Station.
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Recovery from the Failure to Eat Produced by Hypothalamic Lesions

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In two recent papers, Anand and Brobeck (1) have reported that bilateral lesions of the lateral hypothalamus can cause rats and cats to refuse food and thus starve to death. The effective lesions in these cases were 1 mm above the floor of the brain and 2 mm off the midline on each side, at the same anterior-posterior setting as the ventromedial lesions that produce overeating (hyperphagia). Two of the cats used in these experiments were kept alive by tube-feeding postoperatively. One cat began eating after 7 days of refusing food, but its intake was always below normal. The second cat refused food for 6 wk of tubing and at this time was sacrificed for histological purposes.

The experiment reported here (2) first of all verifies Anand and Brobeck's findings that lateral hypothalamic lesions can cause rats to refuse to eat and starve to death. Second, the present experiment shows that a recovery of eating behavior can be brought about in such animals.

Bilateral lesions in the lateral hypothalamus were made with the aid of a stereotaxic instrument (3) so as to produce animals that refused laboratory food (Purina Laboratory Chow Meal) and water for at least 5 days postoperatively. These starving animals were then divided into two groups, one of which was maintained with only laboratory food and water and

was thus allowed to starve to death, while the second group was maintained with a nutritive, fluid diet (4) administered by stomach-tube. Within a few days after tubing was begun, these animals were offered a number of special foods in an effort to induce them to eat.

All 40 operated rats showed some failure to eat following the lateral hypothalamic lesions: 9 rats, used in preliminary work, refused to eat for a period of 6 to 9 days postoperatively but then recovered eating behavior spontaneously; 17 rats that were not tubed or offered special food refused to eat laboratory food and to drink for 6 to 15 days and thus starved themselves to death in this postoperative testing period; 14 rats, maintained with tubing and special foods, recovered eating and drinking behavior within 6 to 65 days.

The course of recovery of the eating behavior is illustrated in Fig. 1 by data on one animal. All 14 recovered animals showed the same general course of recovery of eating behavior following lateral hypothalamic lesions. There is an initial period of complete refusal to eat. Following this, animals will accept only evaporated milk or, somewhat less readily, milk chocolate. Only later will they accept water; and only after they have been drinking water will they eat the regular laboratory food. Individual differences in the time spent in each of these stages of recovery are very great, but the sequence of stages is almost invariable.

Tubing the animals may retard the recovery of eating somewhat; yet in a number of cases where tubing was stopped as soon as the animals began to eat the special foods, the final course of recovery was also prolonged. Starvation itself is never a sufficient inducement to eat, for even after animals had been eating evaporated milk and chocolate, they still refused to eat laboratory food when deprived of these special foods, even to the point of great weight loss.

Foods other than evaporated milk or chocolate, of

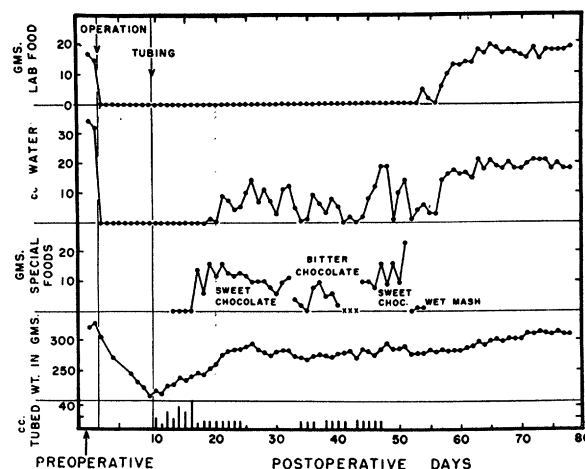


Fig. 1. Course of recovery of eating behavior in one rat, following complete refusal to eat produced by hypothalamic lesions. The x's show when 25 g of evaporated milk was offered and eaten.

course, might elicit eating in the early postoperative periods, but thus far we have had very little success with a number of foods tried: Purina Laboratory Pellets, a mash of 50 percent powdered laboratory food and water by volume, the same mash sweetened with dextrose, straight dextrose, and fresh ground beef. Yet some of our animals would eat bitter baker's chocolate, although in smaller quantities than milk chocolate.

Once rats recover the ability to eat postoperatively, they maintain the same intake of laboratory food and water as comparable normal animals and can regain and hold their preoperative weights. Nevertheless, it seemed worth while to determine more adequately whether or not recovery was complete. Some preliminary comparisons between six normal rats and six recovered rats were made. (i) When fed laboratory powder diluted with nonnutritive cellulose (25 percent by weight), both normal and recovered rats increase their gram intake by about 25 percent, thus maintaining their caloric intake. The recovered animals, however, reduce their intake for the first 3 to 4 days of cellulose-dilution, whereas normal animals make the adjustment within the first day. (ii) Both normal and recovered animals strongly prefer chocolate and evaporated milk when these are offered in addition to laboratory powder and water and reduce their intake of laboratory powder and water by 50 and 75 percent, respectively. (iii) The recovered animals prefer pure corn oil much more than do normals when it is offered in addition to laboratory powder and water. Controls take less and less oil on successive days and the recovered animals take more and more, until, by the end of 3 days, the controls are consuming less than one-half as much oil as the recovered animals.

These findings suggest a number of interpretations. (i) It seems that the lateral hypothalamic lesions impair an excitatory mechanism important in eating. Supporting this idea is the finding of Delgado and Anand (5) that stimulating this region through implanted electrodes can greatly increase eating behavior. (ii) The loss of eating behavior is only temporary. This fact itself is not surprising, for the same recovery of function has also shown up following changes in sleep (6), temperature regulation (6), and emotion (7) induced by lesions of the hypothalamus and related subcortex. The real questions are: What is the nature of the recovery (and therefore the loss)? What is the neural mechanism involved in the recovery? (iii) Some insight into the nature of the loss of function has been gained by plotting the course of its recovery. From our findings with evaporated milk, chocolate, and corn oil, we propose the hypothesis that fat may elicit eating behavior in operated animals sooner and more readily than other foods. Rephrased in more general terms, lesions of the lateral hypothalamus may change the rat's reactions to certain stimulus-aspects of the diet. At first rats will respond to no food stimulus postoperatively. After some recovery, certain food stimuli (provided by fats?) will elicit eating but others still will not. Finally, the recovered rats seem

responsive to enough of the stimuli provided by laboratory food to eat it as normals do, but they still seem to have a heightened fat-appetite.

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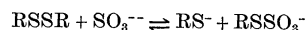
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Amperometric Determination of Disulfides in Intact Proteins

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In studies on disulfide bonds of proteins involved in blood coagulation (1, 2), it was found that the amperometric technic for —SH determinations (3) could be adapted, with modifications, to the measurement of —S—S— also. The basic principle, outlined by Kolthoff and Lingane (4), is the sulfitolysis of the protein —S—S— according to the following reaction:



Thus, 1 mole —S—S— yields 1 mole —SH after reaction with sulfite.

One milliliter of an aqueous protein solution, treated with a minute amount of antifoam emulsion (5), is added to 28 ml of 90 percent ethanol containing sufficient NH_4OH , NH_4NO_3 , and ethylenediamine tetraacetate to make the concentrations 0.25, 0.05, and 3×10^{-5} molar, respectively, in a final volume of 31 ml of reaction mixture; 2 ml of cold saturated Na_2SO_3 then is stirred into the titration mixture, and the titration with $10^{-3}M$ AgNO_3 is started immediately.

Quantitative results are obtained only when the reagents are added in the order given, when 90 percent ethyl alcohol is used, and when an excess of Na_2SO_3 is present. It is essential that the Na_2SO_3 precipitate remain in the titration mixture, since some of the protein is adsorbed on it. This precipitate does not interfere with the titration, provided that the platinum electrode is rotated above the level of the precipitate.