SOME OBSERVATIONS ON THE STARVATION RESULTING FROM LATERAL HYPOTHALAMIC LESIONS[1]

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Several years ago, Anand and Brobeck (1951) showed that bilateral lesions in the lateral hypothalamus produce a complete cessation of eating and death from starvation. This effect also occurred in animals previously made hyperphagic by ventromedial hypothalamic lesions. Teitelbaum and Stellar (1954) confirmed the findings of Anand and Brobeck, and in addition showed that rats with lateral hypothalamic lesions would eventually begin to eat and drink again if they were kept alive long enough by tube-feeding. Their results also indicated that the animals could be induced to eat sooner by offering them highly acceptable foods such as milk or chocolate. The eventual recovery of eating and drinking behavior after lateral hypothalamic lesions has also been demonstrated recently by Morrison and Mayer (1957).

In the present paper, it is shown that some rats with lateral hypothalamic lesions accept a liquid diet immediately after operation, and maintain their weight. However, at the same time they refuse the ordinary diet of lab chow and water and starve to death if these are the only substances offered. This period of dissociation in their eating behavior lasts from two to three weeks, after which time they usually begin to accept the ordinary diet. It was therefore possible to investigate the response to the nutrient content of the liquid diet during a period which would correspond to lateral hypothalamic starvation if the animal were offered ordinary food and water. Since rats with lateral hypothalamic lesions refuse to drink water as well as to eat, methods of forcing rats to drink water were applied. They were used to determine the response of lateral hypothalamic rats to various fluids, and to see the effect of forced drinking on the course of the lateral hypothalamic syndrome.

METHOD

Subjects

Wistar albino female rats were used. They were six to eight months old, weighing approximately 250 gm. In all experiments, four types of rats were used: (*a*) a normal unoperated control group; (*b*) a lateral hypothalamic group, which was composed of rats that had been subjected to lateral hypothalamic lesions, were refusing to eat and drink, and would lose weight steadily when offered the standard lab chow and water; (*c*) a lateral hypothalamic-recovered group, that had initially refused to eat and drink after lateral hypothalamic lesions but had been maintained on the special liquid diet until they began to eat and drink again when offered the standard laboratory diet; and finally, (*d*) a dynamic hyperphagic group that had received ventromedial hypothalamic lesions and had begun to overeat and to gain weight rapidly when offered the standard laboratory diet. Eighteen animals were used in the first experiment, 12 in the second experiment, and 10 in the third experiment, making a total of 40 different animals used.

Hypothalamic Lesions

Bilateral hypothalamic lesions were made with a stereotaxic instrument similar to that described by Stellar and Krause (1954). After the animal was anesthetized with Nembutal injected intraperitoneally, its skull was exposed, and it was placed in the stereotaxic instrument. Two holes were drilled in the skull with a dental trephine. A nichrome anode, 0.25 mm. in diameter and insulated except for 0.5 mm. at the tip, was inserted into the brain and was used to pass direct current. The circuit was completed by means of a rectal cathode. To produce lateral hypothalamic starvation, lesions were made at points $1\frac{3}{4}$ mm. posterior to the bregma, $1\frac{1}{2}$ or $1\frac{3}{4}$ mm. lateral to the midline, and 9.25 mm. below the surface of the cortex, by passing a direct current of 1 ma. for 20 sec. To produce hyperphagia, lesions were made at points $1\frac{1}{2}$ mm. posterior to the bregma, $\frac{3}{4}$ mm. lateral to the midline, and 9.25 mm. below the cortex, by passing a direct current of 1 ma. for 15 sec.

Diet

The liquid diet was prepared by mixing 250 ml. of evaporated milk, 125 ml. of 50% sucrose solution, 150 ml. of whole egg, 30 ml. of Kaopectate (to prevent diarrhea), 3 gm. of iodized salt, and 0.3 ml. of Multi-Vi Drops (White Laboratories, Inc., Kenilworth, New Jersey).

Procedure

The animals were housed in individual living cages and their weight, food intake, and water intake were measured daily. Normally, the animals were maintained on a standard diet of Purina lab chow powder and water. Two days before the experiment was begun, the powdered food was removed and the liquid diet was substituted. This initial preoperative experience seemed to facilitate the immediate postoperative acceptance of the liquid diet. Thus, each rat had

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access to two tubes of fluid 5 in. apart in its cage, one of water and one of the liquid diet. Each fluid was presented in an inverted 100-ml. glass, graduated cylinder, so that the amount consumed during any period of time could be readily measured. From the stopper at the bottom of each cylinder, a curved glass tube projected into the cage approximately $\frac{3}{4}$ in. about the floor of the cage. These drinking tubes were 9 mm. in diameter and were flame-polished at the tip to an aperture of 3 mm.

When the experiment began, the rat's normal daily consumption of the liquid diet and water was measured for three days. Then hypothalamic lesions were produced. For two days after lateral hypothalamic lesions, the liquid diet was removed and the standard diet of Purina powder was offered. If the animal refused to eat the Purina powder and drank no water at all during those two days, this was taken as preliminary evidence of the effectiveness of the lesions, and the animal was classified as a lateral hypothalamic animal. The liquid diet was again substituted for the Purina powder, and the rat's intake of liquid diet and water was measured. Since the liquid diet tended to curdle after standing 15 to 18 hr. at room temperature, food intake was measured every 12 hr. and fresh liquid diet was substituted at those times.

All animals drank from both tubes except the lateral hypothalamic animals. They maintained themselves solely by drinking the liquid diet, being unique in that they never voluntarily drank the tap water, even though the position of the tubes was changed in a random fashion from day to day. The other animals usually drank 5 or 6 ml. of water daily in addition to the liquid diet.

EXPERIMENT I: NUTRIENT INTAKE IN RESPONSE TO CALORIC DILUTION

Caloric Dilution

The caloric content of the liquid diet was halved by diluting the diet with an equal amount of tap water. Each rat's intake was measured for three periods; first for three days on the full-strength diet, then immediately following for four days on the half-strength diet, and finally, on the full-strength diet again for two days. There were six animals in the normal control group, six in the lateral-hypothalamic group, and three animals each in the lateral hypothalamic-recovered group and the hyperphagic group.

Results

Figure 1 shows the effect, on the amount ingested each day, of varying the caloric content of the liquid diet. It is clear that all animals, including animals with lateral hypothalamic lesions, adjusted their volume intake to maintain their nutrient intake at a relatively constant level. That is, they doubled their volume intake when the caloric content was halved, and they decreased their volume intake when the caloric content of the diet was increased again. When the liquid diet was then removed and the animals were offered the standard diet of lab chow and water, all animals except the lateral hypothalamic animals ate and drank readily. The lateral hypothalamic animals refused to eat and drink at all and lost weight steadily. Five of the six animals in this group died of starvation after refusing to eat and drink for 7 to 10 days. The sixth animal refused to eat and drink for six days. On the seventh day it began to drink tap water voluntarily and subsequently recovered normal food and water intake. It is therefore clear that although lateral hypothalamic animals will starve to death when offered Purina chow and water, they may at the same time display an apparently unimpaired ability to adjust their intake to the nutrient content of a diet that they will accept.

Another result which may be seen in Figure 1 is the fact that in general the lateral hypothalamic animals, both starving and recovered, tended to eat more than normal animals and to become obese just as the dynamic hyperphagic animals did. However, despite their overeating, when the liquid diet was removed and the standard diet was substituted, the lateral animals starved to death. The lateral-recovered animals accepted the Purina chow and tap water, continued to overeat, and eventually became quite obese.

EXPERIMENT II: FLUID INTAKE AFTER INJECTION OF HYPERTONIC SALT SOLUTION

Injection of Hypertonic Salt Solution

A procedure was used similar to that described by Adolph, Barker and Hoy (1954). The rats were injected intraperitoneally with a 1 Molar NaCl solution using 4 ml. per 100 gm. of the animal's body weight. For those animals whose body weight had increased due to excessive food intake after hypothalamic lesions, the preoperative weight was used to determine the amount of hypertonic salt solution to be injected. If the obese weight level were used as a reference, it was often found that the increased amount of NaCl solution injected proved lethal. The animals were deprived of all fluid for 4 hr. following

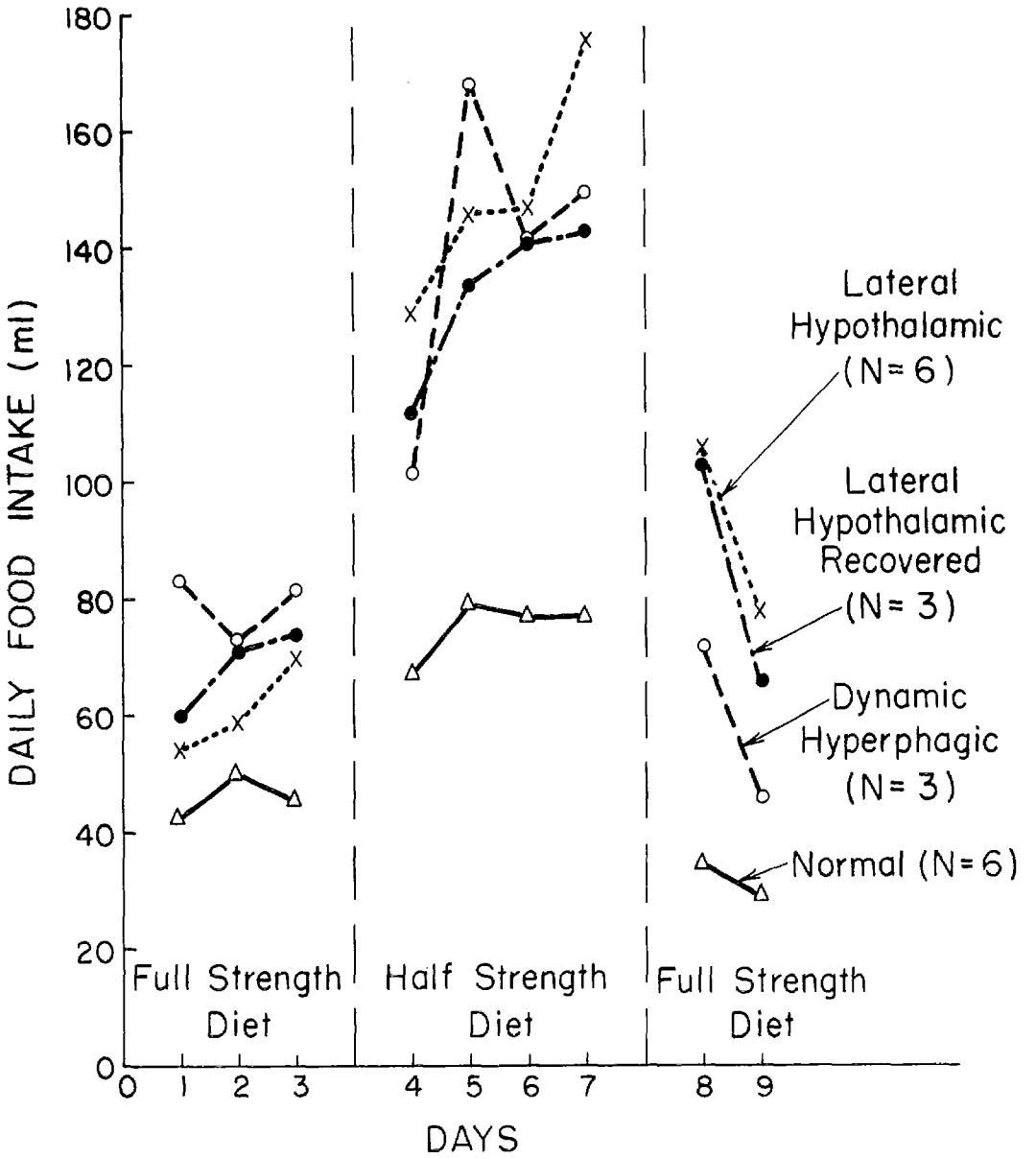

FIG. 1. Mean daily food intake of normal, dynamic hyperphagic, lateral hypothalamic, and lateral hypothalamic-recovered animals in response to different caloric content of a liquid diet.

the intraperitoneal injection. Then they were allowed access to two tubes of fluid, one of water and one of the liquid diet. The amount they consumed of each during the first 15 min. and during the first 2 hr. was recorded. In order to assess the effect of the injection of hypertonic NaCl on fluid intake, a period during the afternoon was chosen when all animals drank very little of the liquid diet (2 or 3 ml.) and usually no water at all.

All animals were subjected once preoperatively to the procedure of hypertonic salt solution injection. In the case of the control unoperated animals, this practice trial took place at least three days before the first experimental test. This was to insure that all animals had had experience drinking fluid in response to the dehydration induced by the hypertonic salt solution. There were three animals in each group, and the experiment

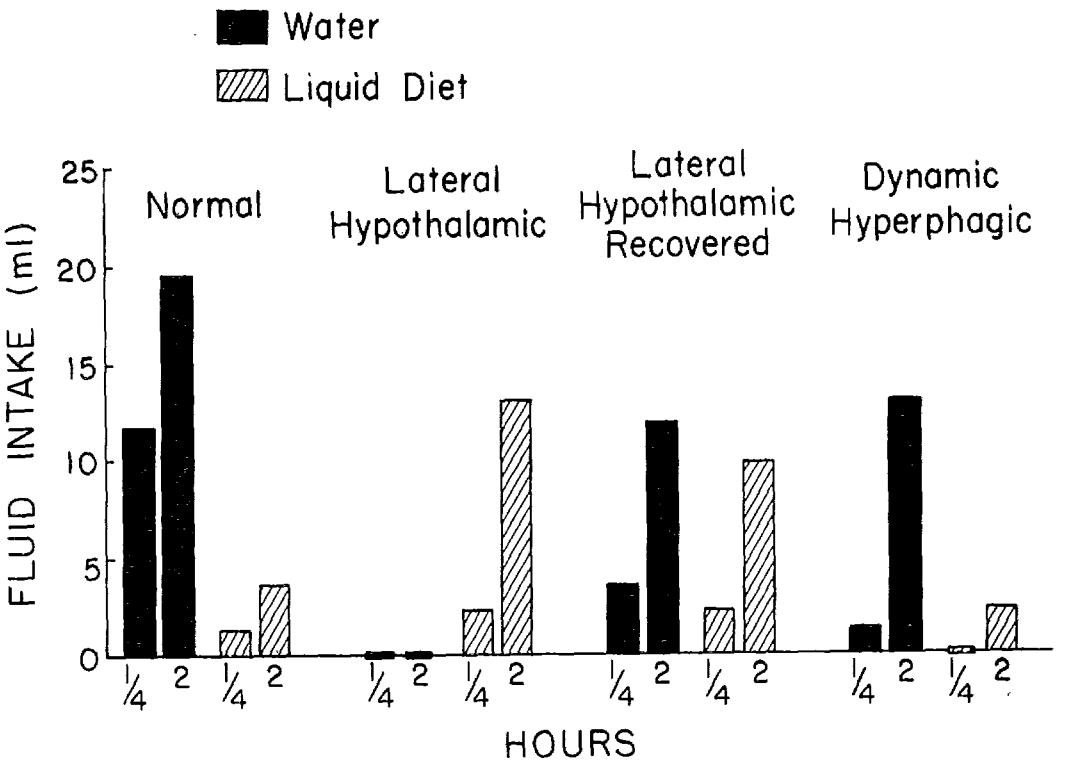

FIG. 2. Mean response of each group to saline injection, showing intake of liquid diet and/or water after ¼ hr. and after 2 hr.

was performed twice on each animal after a three-day interval, making a total of six observations in each group.

Results

Figure 2 shows the amount of fluid that was consumed by each of the four groups of animals in the first 15 min. and during the entire 2 hr. after being deprived of fluid for 4 hr. following the injection of hypertonic salt solution. The normal animals showed an increased intake of fluid after the injection. In achieving this increased intake of fluid, they showed a marked preference for water rather than the liquid diet. The dynamic hyperphagic animals showed a similar preference for water after the injection. The lateral hypothalamic animals responded to the dehydration induced by the hypertonic salt solution by consuming more of the liquid diet, but in no case did a lateral hypothalamic animal drink any water. In several other instances, lateral hypothalamic animals did not drink any fluid at all after the injection. If they did not drink during the period of access to the fluid, they developed signs of being unable to maintain themselves in an upright position, became stuporous, and died within 12 hr. The lateral hypothalamic-recovered animals drank water in response to the injection, but they drank only slightly less of the liquid diet.

After hypertonic salt injection, normal animals took only a little of the liquid diet when it was available as an alternative to water. Since lateral hypothalamic animals do not drink water, it could be said that they have only one choice—whether or not to drink the liquid diet. Therefore, to see the normal animals' performance in this situation, their response to a single tube containing the liquid diet was investigated 4 hr. after hypertonic salt injection. In five naive normal female rats, it was found that no liquid diet was drunk during the 2 hr. of access to the liquid diet. If water was then offered in addition to it, they took approximately 5 ml. of water in the first 15 min. and 10 to 20 ml. during 2 hr. Only after they had drunk an appreciable amount of water would they

begin to drink the liquid diet, and then only in small quantities.

From these results, it seemed clear that the lateral hypothalamic animals differed markedly from the others in that they would not drink water, even in response to the dehydration induced by an injection of hypertonic salt solution. Therefore, a method of forcing rats to drink to escape electric shock was used to see whether lateral hypothalamic animals could be forced to drink water and, if so, whether this would have an effect on the course of the starvation syndrome.

EXPERIMENT III: THE EFFECT OF FORCED DRINKING ON LATERAL HYPOTHALAMIC ANIMALS

Forced Drinking

A technique was used of forcing rats to drink to escape and postpone electric shock. A detailed description of this procedure is given in earlier publications (Teitelbaum & Derks, 1958; Williams & Teitelbaum, 1956). The rat was placed in a cage where intermittent electric shocks were delivered through the bars which made up the floor of the cage. By licking the fluid at the end of a tube, the rat could turn off the train of shock pulses and postpone the onset of shock for a period of time. The licks were detected with a drinkometer (Stellar & Hill, 1952).

The duration of the shock pulse was 92 msec., and shocks were applied at 0.9-sec. intervals if the rat did not respond. The intensity used varied in different experiments ranging from 0.5 ma. to 2 ma., but the usual setting was 0.5 ma.

There were two response-shock postponement periods available to the rat—a short one (1 sec.) for licking once or twice, and a long one (15 sec.) for licking three or more times. When the animal was receiving shock, the first lick following a shock postponed the next shock for 1 sec. Each successive lick resulted in the same short postponement unless three or more licks were made within 1 sec. of each other. When that occurred, the shock was postponed for 15 sec., and each successive lick during this postponement started the long postponement period all over again. If it did not respond for 15 sec., the long postponement period ended, shock came on again, and the rat again had two postponement periods available to it.

All animals were trained preoperatively to drink tap water to postpone shock. After initially training an animal to lick at the tube to turn off shock (Williams & Teitelbaum, 1956), five or six 1-hr. sessions of forced drinking were usually adequate to produce very consistent licking behavior. A cumulative response recorder was used to show graphically the forced drinking to avoid electric shock. As shown in Figure 3, the pen moves upward with each lick and toward the right as time elapses. Each shock period is indicated by a small ($\frac{1}{8}$ in.) downward displacement of the pen. The pen stays down while shock is being received.

Results

As is shown at the top of Figure 3, a normal rat shows a characteristic licking pattern when it is forced to drink water to escape and postpone shock. It licks three or four times after it has received a shock, pauses during the 15-sec. shock postponement period until it receives the next shock, then licks in another burst of three or more licks, and so on. In the course of an hour of forced drinking, it will consume 5 to 6 ml. of water. When the animal is satiated or when the fluid is aversive, the rat changes its licking pattern. It now licks single licks after each shock and postpones shock for only 1 sec. each time it licks (Williams & Teitelbaum, 1956).

Five naive animals that had been trained preoperatively to drink water to escape shock, were forced to drink water after lateral hypothalamic lesions. These animals would freely accept the liquid diet. However, if offered only water and Purina lab chow meal, they refused to drink and to eat. They were kept in their living cages for 23 hr. each day with free access to water and the Purina meal. For 1 hr. each day, they were placed in the forced-drinking situation and were forced to drink water to escape shock. All of them drank at least 2 ml. in an hour, and two of them drank 6 to 7 ml. each hour. But despite the fact that they would drink water when forced, they continued to refuse food and water in the living cage. They lost weight steadily and starved to death in seven to ten days. On each

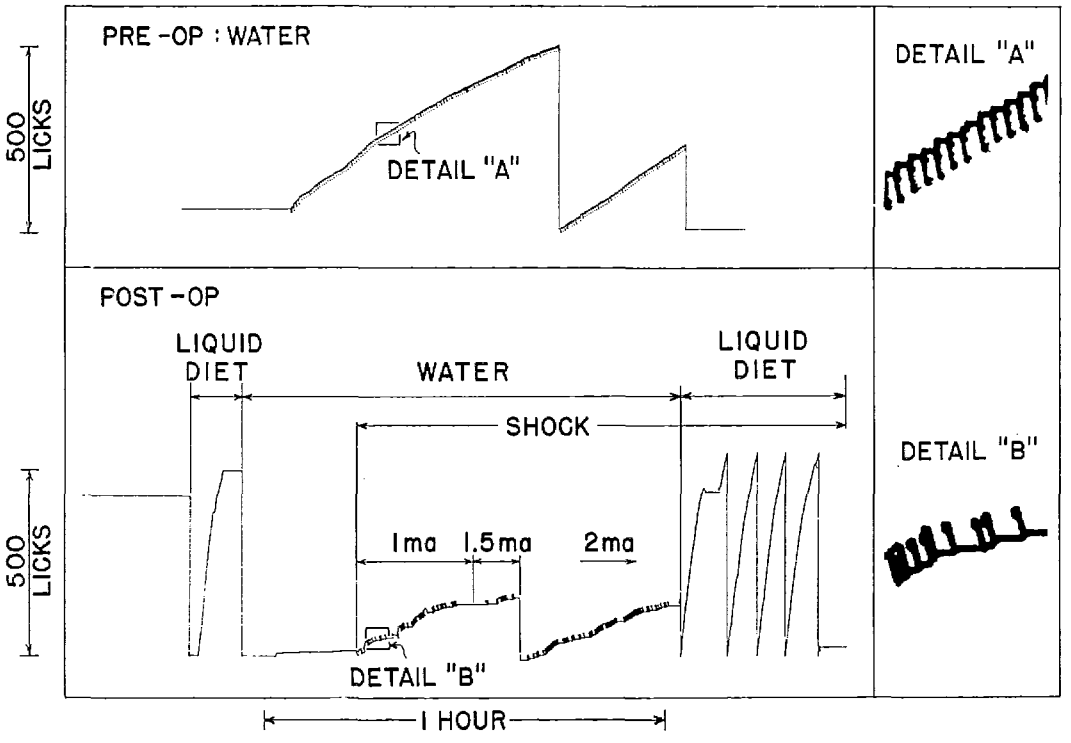

FIG. 3. Cumulative response records showing forced drinking to avoid electric shock. Pre-Op: Normal forced drinking of water, showing several licks after each shock and then the wait for the next shock. Post-Op: The lateral hypothalamic animal drinks the liquid diet freely but shows great reluctance to drink water, even when forced.

day, up to the day before they died, they each ingested water when forced to drink for an hour, but this did not change the course of the starvation syndrome.

All lateral hypothalamic animals showed a greater degree of reluctance to drink water when forced postoperatively than they had shown when they were normal. The bottom half of Figure 3 shows the record of a typical animal which demonstrates this phenomenon. In this experiment, the animal was placed in the forced-drinking cage and it was offered first the liquid diet and then water before the shock was turned on. As can be seen from the cumulative record, the rat freely drank the liquid diet. When water was substituted quickly by switching the tubes in front of the licking aperture, the rat took three or four licks from the water tube and then turned away from the licking aperture. After a few minutes, the shock was turned on and the animal began to drink water to escape the shock. However, as may be seen clearly in Detail *B* of Figure 3, the rat now showed an extreme reluctance to drink water. The pattern of licking resembled that seen when the animal is satiated or the fluid is quite aversive (Williams & Teitelbaum, 1956). The rat licked mostly in single licks and showed many pauses during which it took many shocks but did not drink. On the record during these pauses, the pen stays down, indicating that shock was being received. Since only single licks were seen, and each received only 1-sec. postponement of shock, the individual licks appear as brief, upward $\frac{1}{8}$-in. displacements. Eventually, the rat turned away from the licking aperture and refused to drink entirely, despite the shock. Each time this happened the strength of the shock was increased, and the rat could again be forced to drink water for awhile. Finally, it refused completely, even at a rather strong intensity of shock. At this point, in order to show that the animal would drink if the fluid were acceptable, the liquid diet was

substituted again, and the animal freely drank 10 ml. without the necessity of being shocked.

This reluctance to drink water in the forced-drinking situation was demonstrated in all five lateral hypothalamic animals. It was not seen in normal animals or in the two dynamic hyperphagic animals that were tested. Three rats that had recovered normal food and water intake after lateral hypothalamic lesions showed slightly more than normal reluctance to drink water when forced, but this could be readily overcome by increasing the shock strength.

DISCUSSION

It must be pointed out that the dissociation in eating behavior that is seen immediately after operation and its combination with overeating as described in the present experiment can be demonstrated only under certain circumstances. As Anand and Brobeck (1951) have pointed out, lesions in the appropriate position in the lateral hypothalamus uniformly result in death from starvation. Transient depression of food intake, insufficient to produce death from starvation, may occur when the lesion only impinges on the lateral hypothalamus. But Teitelbaum and Stellar (1954) have shown that lateral hypothalamic lesions that are completely effective if gauged by the criterion of death by starvation may still be compatible with eventual recovery of normal eating behavior if the animals are kept alive for as long as several months if necessary. Most of those animals, with lesions placed 2 mm. lateral to the mid-line, would eat and drink normally when they recovered and would maintain their usual weight. But in isolated instances, these animals would subsequently overeat and would become quite obese. The lesions in the present study were placed somewhat more medially ($1\frac{1}{2}$ and $1\frac{3}{4}$ mm. off the midline) and therefore would be more likely to produce overeating.

Similarly, the intensity and duration of the current used to produce the lesion was carefully chosen. The object was to produce refusal to eat and drink that would last for at least two weeks but not for very much longer. It would therefore be possible to demonstrate death by starvation but would not necessitate a much longer maintenance period before recovery occurred. If larger lesions were made by using 2 ma. for 30 sec., it was not difficult to produce cessation of eating that was complete and which lasted for a long time. However, these animals often showed a profound apathy and refused to accept anything (including the liquid diet) for a long period of time. These animals could be maintained by tube-feeding, and would in most cases eventually show a dissociation in food intake for a variable period of time preceding their full recovery of food and water intake. But the procedure of tube feeding is time consuming and makes it difficult to work with many animals at one time. Therefore, a size and placement of lesion was desired that would produce starvation and yet would allow the immediate demonstration of the dissociation in eating behavior. As has been described, this was accomplished in a number of animals in the present experiment.

From the results of these experiments, it appears that hypothalamic hyperphagia can coexist with lateral hypothalamic starvation. While becoming obese on the liquid diet, lateral hypothalamic animals will starve to death when offered Purina chow meal and water. This fact agrees with the findings of Anand and Brobeck (1951), who found that hyperphagic animals starve to death on lab chow after additional lateral hypothalamic lesions. Also, lateral hypothalamic animals will adjust their intake to the caloric content of an acceptable diet, but will starve to death if offered the standard laboratory diet. It is possible, then, that the refusal to eat that occurs after lateral hypothalamic lesions is not due to a primary disturbance in the regulation of caloric intake.

Prominent in the lateral hypothalamic syndrome is the refusal to drink water. This is clear from the fact that these animals do not drink while starving to death (Morrison & Mayer, 1957; Teitelbaum & Stellar, 1954). It is also shown in the present experiment by the refusal of rats with lateral hypothalamic lesions to drink water in response to the injection of a hypertonic salt solution and their reluctance to drink water even when forced to do so to escape electric shock. In addition, in

our experience, voluntary drinking of water has been an invariable sign that the lateral hypothalamic animal will subsequently display adequate food and water intake and will maintain itself thereafter. These results may be related to those of Stevenson and his coworkers (Montemurro & Stevenson, 1955–1956; Stevenson, Welt, & Orloff, 1950), who showed a decreased water/food intake ratio in hyperphagic rats with lesions just lateral to the ventromedial nuclei. The fact that the animals in the present experiment will drink a liquid diet more readily is of some interest and may be related to the findings of Andersson and McCann (1956) who showed that dogs, with lesions in areas of the hypothalamus which elicited drinking behavior when stimulated, would refuse to drink water but could be induced to accept milk or broth.

The findings in the present paper demonstrate that the refusal to eat and drink after lateral hypothalamic lesions in the rat may include a complex set of phenomena. Thus, under certain conditions, it is possible to show that caloric regulation may still exist in these animals, as well as hypothalamic hyperphagia. In addition, their response to water is markedly aberrant. However, just what it is that prevents the lateral hypothalamic animal from eating and drinking normally is still not clear.

SUMMARY

Rats that were starving to death after lateral hypothalamic lesions would accept a liquid diet in quantities sufficient to maintain their weight. They adjusted their volume intake of this diet according to its nutrient content in the same fashion as normal animals, dynamic hyperphagic, or recovered lateral hypothalamic animals. In consuming the liquid diet, lateral hypothalamic animals showed overeating comparable to hyperphagic animals. However, despite their greatly increased intake and their ability to adjust to the nutrient content of the liquid diet, lateral hypothalamic animals starved to death when the liquid diet was removed and only Purina chow meal and water were available.

Since lateral hypothalamic animals do not drink water voluntarily, attempts were made to force them to drink water. When subjected to dehydration induced by intraperitoneal injection of 1 Molar NaCl solution, all animals except lateral hypothalamic animals drank much water in preference to the liquid diet. Lateral hypothalamic animals drank no water but increased their intake of the liquid diet.

When forced to drink water to avoid electric shock, lateral hypothalamic animals showed great reluctance to drink water, though they would readily drink the liquid diet. Although they drank water when forced for an hour each day, this did not change the course of the starvation syndrome, since they refused to eat Purina chow and to drink water voluntarily, and they starved to death.

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