Regulation of Food Intake During Long-Term Loss of Food from the Intestines of the Rat (40070)

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It has long been established that rats adjust to caloric dilution of their diet by increasing the amount of food eaten. The adjustments have occurred within 1 day when the food was diluted prior to feeding (1, 2) or before injection into the stomach (3, 4). In the present study, rats that lost a substantial portion of their ingested food from the intestines failed to increase their food intake for 5 days. During this period, the amount of food eaten appeared to be regulated by signals that arose in the mouth, throat, stomach, proximal duodenum or the lower intestines.

Surgical preparation. Eight pairs of parabiotic Lewis rats were prepared by uniting the skin and muscle of the abdomen (5). A circular opening with a diameter of 3–4 cm was made in the abdominal wall of each rat of a parabiotic pair to produce a common peritoneal cavity. In four pairs of parabiotic rats, a 30 cm segment of one rat's intestines was exchanged for an equivalent segment of its partner's intestines. This was accomplished by transecting the intestines of each rat about 5 cm below the stomach at midduodenum. The proximal ends of each rat's duodenum were united by 7–0 Connell sutures to the distal ends of its partner's duodenum (see Fig. 1). The intestines were crossed back 30 cm further down the digestive tract at midjejunum. In this surgical preparation the food eaten by one rat arrived in its own stomach, traveled through 5 cm of its own proximal duodenum to the distal duodenum of the other rat. It then traversed 30 cm of the other rat's distal duodenum and proximal jejunum before the remaining unabsorbed food returned to the intestine of the rat that fed. The crossed segment is more than one quarter of the total length of the rat's small intestines (about 110 cm) and is located in the most proximal region. The rats recovered from surgery after a few days and continued to gain weight in the postsurgical period. They were normally vigorous and showed no signs of stress once an adjustment to paired life had been made.

Absorption study. The food eaten by one rat of a crossed intestines pair was absorbed from the intestines into the blood of both rats. The percentage of food absorbed into each rat can be estimated by measuring the relative amounts of a fed radioactive nutrient that has been absorbed into the blood of each rat. Three pairs of each type of rat were included in the study. The rats were deprived of food for 12 hr and then one rat of each pair was injected with a meal at the rat's average feeding rate (9 ml/8 min) through a chronically implanted stomach tube (6). The injected meal consisted of 9 ml of their normal liquid diet, Nutrament (Mead–Johnson Nutritionals) which contained 60% carbohydrate, 29% protein and 11% fat by weight. The liquid diet meal was thoroughly mixed with trace amounts of glucose–^{3}H or amino acid–^{3}H mix (International Chemical and Nuclear Corp.). One-tenth milliliter of blood was withdrawn from each rat's tail just before the injection and every 10 min after the injection began. The blood sample was precipitated in 3 ml of Bray's solution and 1 ml of the fluid was withdrawn, diluted with 20 ml of Bray's solution and counted in a Beckman 250 scintillation counter. Counting efficiency was essentially constant for all of the sample tubes.

Results. As can be seen in Fig. 2, the glucose-^{3}H was rapidly absorbed into the bloodstream of the fed parabiotic rat. Only a small quantity of radioactive label was transferred through the capillaries in the skin and muscle of the parabiotic union to the bloodstream of the unfed parabiotic rat. In contrast, glucose-^{3}H mixed into the diet fed to one of the crossed intestines rats was rapidly absorbed into both rats. There was no significant difference in the blood levels of radioactive glucose in these rats at 10, 30, or 60 min ($F = 0.29$ at 60 min, $P > 0.25$).

Figure 3 presents the same data when

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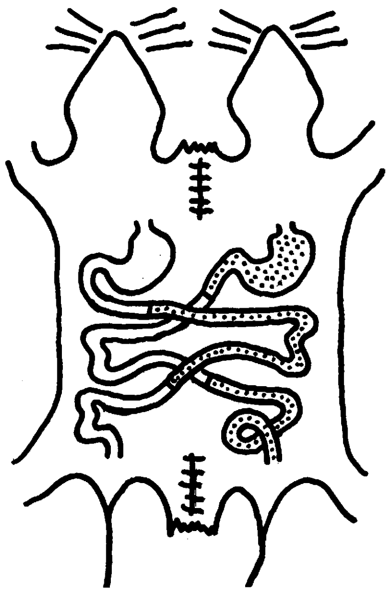

FIG. 1. Diagram of a crossed intestines rat pair. The speckled gastrointestinal tract belongs to one rat and has its blood supply and neural connections intact.

amino acid-^{3}H mix (a typical algal protein hydrolysate with 15 common amino acids) has been added to the diet which was injected into one rat of each pair. Again, approximately half of the radioactive amino acids were absorbed into the bloodstream of each rat. At ten minutes, a significantly greater amount of amino acid had been absorbed into the unfed crossed intestines rat than into the partner rat that was fed ($F = 18.3$, $P < 0.01$) or into the parabiotic control rat that was fed ($F = 29.1$, $P < 0.01$). Since approximately equal amounts of radioactive amino acids were present in the fed rats of each pair ($F = 0.07$, $P > 0.25$), the data suggest that there was initially a more rapid emptying of the stomach contents of the fed crossed intestines rats. These stomach contents passed into the partner rat's intestines and were absorbed there during the next 10–20 min. By 30 and 60 min, the blood levels of the amino acids in each crossed intestines partner were not significantly different ($F = 0.03$ at 60 min, $P > 0.25$) suggesting that the initial rapid rate of stomach emptying had been inhibited by normal physiological processes in the fed crossed intestines rat. Once the initial adjustments after a meal were complete, the radioactive amino acid was divided about equally in the two crossed intestines rats.

Both absorption studies show that approximately half of the free glucose or amino acids present in the food eaten by one crossed intestines rat was absorbed into each rat of the pair. These measurements provide only a rough estimate of the amount of food absorbed into each rat. First, the diet contained complex foods that required enzyme digestion and would not necessarily be absorbed

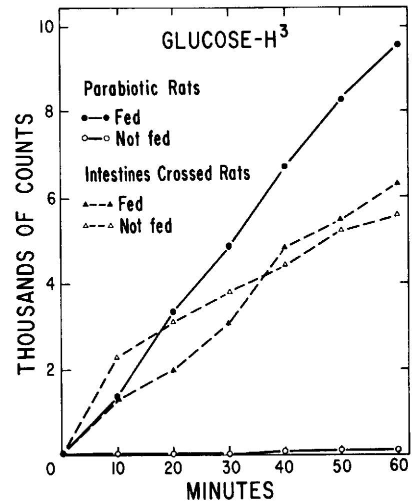

FIG. 2. The number of radioactive counts present in a 0.1 ml sample of blood taken every ten minutes from each rat's tail after one rat in each pair had been infused with its normal diet mixed with trace amounts of glucose-^{3}H. Data is presented for both the fed (solid symbols) and the unfed (open symbols) rats of each type of pair.

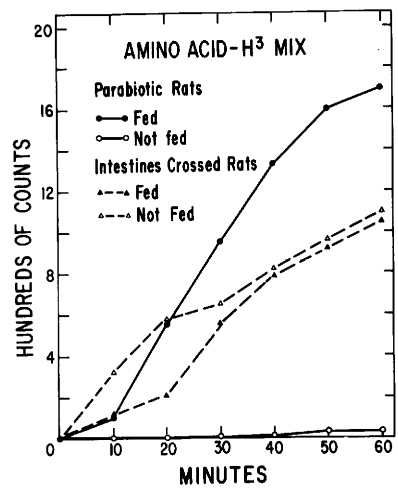

FIG. 3. Same as Fig. 2 except that the infused meal has been mixed with tritium labeled amino acid mix.

at the same rate as free nutrients. Second, the blood levels of radioactive nutrients are influenced by two dynamic processes: their absorption from the gut and their removal from the blood by the body's tissues. If rate of removal differed in the two rats, then the blood levels of the radioactive nutrients would not reflect the absorption rate. Fortunately, the blood levels of the labelled nutrients are approximately the same in the crossed intestines rats, and thus, rate of removal is not likely to be greatly different.

Behavioral study. Both absorption studies show that a substantial proportion of the food eaten by one crossed intestines rat was absorbed into each rat of the pair. Under normal circumstances when both rats were feeding, each rat absorbed the amount of food that it ate. The food lost from the intestines of one rat was gained from the food eaten by its partner. Only when the food intake of one rat was restricted, as in the following behavioral study, did the feeding rat suffer a loss of nutrients.

In the behavioral experiment, the rats were placed on a twelve hour food deprivation schedule. They were allowed to eat during the night from 9:00 PM until 9:00 AM while kept restrained in modified Bollman restraining cages (7). These cages had a plastic partition that prevented the rats from eating each other's food. During the daytime hours, the rats were returned to home cages where they could exercise. They had water freely available at all times.

The results of the experiment are presented in Fig. 4. During the first 5 days, the amount of food eaten by each rat stabilized and the weight of the rat pairs remained constant. On the 6th day the liquid diet, Nutrament, fed to the rats was diluted with an equal quantity of water. As in previous studies (1, 3) the rats increased the volume of food eaten within one day, but they never fully compensated for the caloric dilution. They ate on the average 1.66 times their normal food volume and consequently lost some weight during the three days of food dilution. During days 9–11, the rats were again fed their normal diet and their weight stabilized.

On the 12th day, the critical experiment began. Only one rat of each pair was allowed to feed. For the parabiotic rat pairs, no adjustment in food intake was expected. The parabiotic rat that was allowed to eat would feed itself. A small amount of nutrient in the blood would pass from this rat through the capillary connections in the parabiotic union to the partner rat. However, all blood returning from the partner rat would contain mobilized free fatty acids, amino acids and glucose. Very little net loss of nutrient from the feeding parabiotic partner would be expected. As can be seen in Fig. 4, the fed parabiotic rats did not increase their food intake throughout the five day period as had been expected. ($F = 0.06$, $P \gg 0.25$).

On the other hand, the fed crossed intestines rat would lose a substantial portion of its food to the intestines of its partner (see Figs. 2 and 3). However, no food would enter its own crossed intestines segment because its

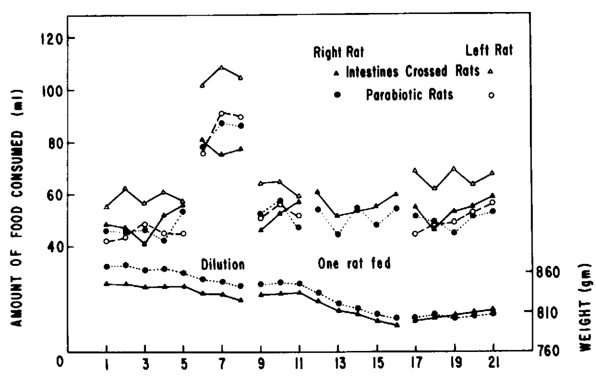

FIG. 4. The daily food intake and body weight for pairs of crossed intestines rats and parabiotic rats. The food intake for the left and right rats in each pair were plotted separately. The left rats of both pair types were not fed on days 12–16.

partner was not fed. If the feeding behavior of the fed crossed intestines rats were affected by physiological signals normally arising in its own crossed intestinal segment or by the total amount of food absorbed, the fed rat should have increased the amount of food eaten to compensate for the food lost to its partner. As can be seen in Fig. 4, the fed crossed intestines rats did not increase their food intake for 5 days. The amount eaten during these days is not significantly different from the amount eaten during the preceding and subsequent control periods ($F = 0.86$, p $\gg 0.25$). The failure of these rats to increase food intake when a substantial portion of the nutrient was lost from the intestines to the partner did not result from the lack of stomach capacity. When the diet was diluted by an equal volume of water, all the rats increased the amount eaten by an average of 26.7 ml, an increase that is statistically highly significant ($F = 175.5$, $P < 0.001$). The stomachs of these rats must have held the increased volume of the diluted diet until the added water had been slowly absorbed. Moreover, the average largest intake on a single day for each of the experimental rats during the control period is 7.3 ml more than the average intake during the intestinal dilution. The rats could have adjusted to loss of nutrient by consistently eating at least an additional 7.3 ml of liquid diet each day, but they did not do so. On days 17–21, food was given to both rats of each type of pair. Food intake of both the fed and previously unfed rats was normal during this period, and the rats slowly gained weight.

Figure 4 shows that the rats ate their usual quantity of food when only one rat of each pair was allowed to feed despite the loss of about 50 g of body weight for both types of rat pair. This loss was approximately 6% of total body weight and comprises about one third of the body fat of normal rats (8). If we assume that the glucose and amino acid absorption curves give a reasonably fair estimate of the amount of food absorbed, then about one third of the body fat was lost in each member of the crossed intestines pairs and about two-thirds of the body fat was lost in the unfed parabiotic rats. This assumption was not tested by carcass analysis in this study because measurements of postdeprivation food intake and return of body weight were desired. Carcass analysis would provide a check on the assumptions about the amount of food absorbed in each crossed intestines rat and has been incorporated in current experiments. In the present study, the deprivation period was restricted to 5 days in order to maintin the unfed parabiotic rats in a healthy condition since rats die after 7–8 days without food (9).

Discussion. Two previous studies have crossed the intestines of rats without crossing them back. In one study, the intestines were crossed just below the stomach (10) and in the other, at midduodenum (11). In both studies, the feeding behavior of the few surviving rats was highly erratic with one rat eating continually while its partner stopped eating at all. This peculiar pattern of eating behavior which I have also observed on similar preparations was probably due to the loss of an important reflex that affects intestinal motility. The gastroileal reflex mediated by the release of gastrin after food enters the stomach, causes food to move from the small to the large intestines (12). When the intestines is crossed in only one direction, the gastrin is released in one rat but the target organ, the terminal ileum, is in the other rat. Thus, feeding does not lead to normal movement of food along the intestines. I have done one short-term food intake experiment on rat pairs which have the intestines crossed over and back (6). In this previous study, the crossing of the intestinal segment occurred 10 cm further down the intestinal tract at mid-jejunum and the crossed segment was only 25 cm long. A glucose-^{3}H absorption study similar to the one reported here has shown that most of the radioactive glucose was absorbed into the fed rat of those crossed-intestines pairs. Since free gluose is absorbed in the upper portions of the digestive tract (13), the glucose-^{3}H absorption study in the previous paper gave an underestimate of the amount of food absorbed. This conclusion was supported by the concurrent measurement of the blood levels of the less rapidly absorbed sugar, 3-O-methyl-*d*-glucose[^{14}C], in a double label experiment. A comparison of the absorption curves of both studies shows that the additional 10 cm of lower duodenum and upper jejunum included in the crossed segment of the present study is important for the absorption of free glucose. This crossed segment is also an important site for the absorption of complex carbohydrate, protein and fat (12). The present study extends the previous results by crossing a longer and more proximal segment of the small intestines and by extending the test feeding period from one hour to 5 days. The results of both studies are consistent: crossing the intestines of rat pairs does not alter food intake during the first hour of feeding or during a 5-day period.

In all studies, rats have adjusted to dilution of their diet by substantially increasing their food intake. Even rats that have been trained to press a lever to deliver food directly to their stomach were able to compensate for changes in their diet (3, 4). When their diet was diluted with an equal volume of water, they doubled the volume of diet delivered to their stomach. When each lever press delivered a smaller amount of food, they increased their lever pressing to compensate for the lost nutrient. In the present study, the rats need only increase the amount of food eaten to compensate for nutrient loss from its intestines. Despite the loss of a substantial portion of the fed nutrient, the rats did not increase their food intake during a 5-day period. Apparently, the rats will compensate for a decrease in food delivered to their stomachs but not for food lost from their intestines. A satiety signal must arise from those sections of the digestive tract that are stimulated by food. In the feeding rat, these sections include the mouth, throat, stomach, proximal duodenum and the lower jejunum and ileum. The signal is either neural or hormonal since it appears to be affected by the amount of food present in the stomach and some sections of the small intestines, but not by the amount of nutrient absorbed. This internal signal continues to control food intake for 5 days even though a substantial amount of the food eaten is lost from the intestines, the metabolism of the rat shifts to a greater dependence on free fatty acids and body weight is reduced by 6%.

Summary. Four pairs of parabiotic rats had a thirty cm segment of each rat's distal duodenum and proximal jejunum exchanged for the same segment of the partner's intestines. The food eaten by one rat of each pair was absorbed in nearly equal amounts into both rats. When the liquid diet was diluted with an equal volume of water, the rats adjusted by nearly doubling their food intake in 1 day. When the rats lost a substantial portion of their ingested food from their intestines, they failed to increase their food intake during 5 days. The amount of food eaten was unaffected by a considerable decrease in the amount of absorbed nutrient and a 6% decrease in body weight.

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