Effects of parabiosis of normal with genetically diabetic mice

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THE MUTATION DIABETES (db) in the mouse resembles maturity-onset diabetes in man in that hyperglycemia, obesity, and insulin resistance are consistent features, ketosis is relatively rare, and the condition responds favorably to food restriction (1, 2). The disease in these mice is caused by a single autosomal recessive gene with complete penetrance allowing matings that will produce predictable numbers of afflicted offspring (11). Continued maintenance of the gene for diabetes (db) in the C57BL/Ks strain of origin greatly facilitates interpretation of biochemical and morphological results as well as allowing transplantation of tissues and parabiosis of normal with diabetic mice.

Previous studies have shown that increased circulating insulin levels are a consistent finding in diabetic mice in all but the last stage of the disease (2). The increase in plasma insulin concentration occurs as early as 2 weeks of age and may be closely associated with the primary action of the gene. Parabiosis was undertaken to ascertain the presence of any circulating factors that might cause either enhancement or inhibition of insulin secretion. An insulin-releasing factor present in the diabetic partner could cause a diabetic syndrome in the normal parabiont or, alternatively, could be destroyed by the normal, thus leading to amelioration of the symptoms in the diabetic partner. On the other hand, a complete or partial cure during parabiotic union might result if a circulating factor that normally inhibits insulin release were transported from the normal to the diabetic parabiont.

MATERIALS AND METHODS

The mice used in this study came from two separate sources: the normal control mice from the C57BL/KsJ inbred strain maintained by the production department of The Jackson Laboratory, and the diabetic db/db mice from the C57BL/KsHu db strain maintained in our research colony. All mice were fed a commercial laboratory chow (Old Guilford Corp., Guilford, Conn.) containing 6% of fat. The diabetic mice are descendents of the original mutants and have been propagated by matings of C57BL/KsHu-db mice heterozygous at the db locus. In order to assess any histocompatibility differences which may have arisen at or since the time of the mutation, control parabiotic unions were made between normal mice of the C57BL/KsHu and the C57BL/KsJ strains.

Parabiosis was not performed on weanling diabetic mice because of the delicate nature of both peritoneal wall and skin. Moreover, older diabetic mice become so grossly obese that parabiosis is technically difficult. Therefore, diabetic mice from 6 to 8 weeks of age that still had reasonably low blood sugar concentrations (usually <250 mg/100 ml) were placed on restricted intake of food for the period of time (25 to 93 days) necessary to reduce their weights to near normal (Table 1, group I). The dietary restriction consisted of feeding for 8 hr on Mondays, Wednesdays, and Fridays, only, while allowing access to water at all times. Blood sugar determinations (fasting levels) were made on Monday morning of each week prior to the first feeding period. Normal mice do not tolerate this severe schedule of food restriction and unrestrained normal mice were used in all experiments.

The technique of parabiosis, carried out under sodium pentobarbital anesthesia (6.2 mg/100 g), included celiotomy as well as anastomosis of the skin from the shoulder to the pelvic girdle. Silk sutures were used to join the peritoneal walls and a stay suture was placed through adjacent scapulas. Wound clips were used to effect skin union. After surgery, each pair of mice was housed separately and fed the laboratory chow ad libitum.

Three types of parabiotic pairs were produced; db/db with +/+ , db/db with db/db , and +/+ with +/+ . Two groups of unparabiosed diabetic mice were maintained as controls; one that remained on the restricted feeding schedule (restricted), and one that was fed ad libitum following a restricted feeding period (restricted refed).

Weights were recorded weekly at the time that blood sugar concentrations were determined on the controls and on each animal in the parabiotic pairs by the micromethod of Folin and Malmros on 50 ml of blood obtained from the orbital sinus (4). Plasma insulin was estimated by an
immunological procedure essentially as described by Hales and Randle (7) using blood obtained from the tail vein, bovine insulin as a standard, and the insulin-125I immunoassay kit supplied by Radiochemical Centre, Amersham, England.

Tissues of mice sacrificed for histological study were fixed and stained as previously described (1).

RESULTS

Effect of restricted feeding. The diabetic mice selected for this study were 6-8 weeks of age and weighed 50-60% more than the normal controls (Table 1). During the initial 2 weeks of restricted feeding, body weights gradually decreased to near normal, thereafter remaining stable as long as the feeding regimen was maintained. After 6 weeks of food restriction, fasting blood sugar concentrations had decreased from the initial average of 149 mg/100 ml to a significantly different value (P < .01) of 110 mg/100 ml but, even so, seldom reached the low levels observed in fasted normal mice. Rather surprisingly, the average fed blood sugar concentration (104 mg/100 ml), measured 2 hours after the initiation of the Monday morning feeding period, was not significantly different from the average fasted concentration (110 mg/100 ml) in restricted diabetic mice. In contrast, the average fed blood sugar concentration (144 mg/100 ml) in normal mice was double the average for those fasted for 24 hr (71.5 mg/100 ml). This difference is possibly explained by the marked increase (from 109 to 886 μU/ml) in average plasma insulin concentration that accompanied feeding in restricted diabetics in contrast to the small increase (17.4 to 54.1 μU/ml) between fasting and fed normal mice (Table 1).

On reinstitution of feeding ad libitum the body weight of the diabetic mice doubled in about 3 weeks (Table 2, group 3). Serum insulin stabilized at elevated levels (888 μU/ml) and the blood sugar concentration increased, gradually reaching hyperglycemic levels within 3 weeks after feeding ad libitum was resumed. The degree of hyperglycemia attained, however, never became as great as that seen in diabetic mice fed ad libitum throughout life.

Seven diabetic mice from the control group that had been on the restricted feeding program for 3-6 months were sacrificed for histological observation. At this time they were 5-9 months old, weighed from 20 to 38 g, and were hyperglycemic or euglycemic with fasted blood sugars ranging from 36 to 105 mg/100 ml. Although these mice had had no food available for from 16 to 40 hr prior to sacrifice, their stomachs contained stored food masses and it should be emphasized that they were in no sense starved. The pancreatic islets were numerous and were mostly small and medium in size. All showed slight to moderate degranulation of β-cells (Fig. 1A) but not the marked degranulation of unrestricted diabetics (1). Also few islets showed the other changes characteristic of untreated diabetics of this age range such as the inclusion of acinar cells and epithelial-lined ducts within their borders (1, 11). The pancreatic acinar cells were filled with fine cosinophilic granules interspersed with droplets which in some areas appeared to have coalesced to form conspicuous vacuoles.

Four diabetic mice from the control group that had been restricted for 10 weeks and subsequently allowed food ad libitum for 22 days were sacrificed when 4-5 months old. All were obese, weighing from 53 to 57 g, and all were hyperglycemic with blood sugar concentrations of 243-482 mg/100 ml. Histologically, the pancreatic islets were not greatly different from those of the restricted diabetics. There were, however, more large islets, and degranulation of β-cells was more marked (Fig. 1B). The acinar cells also differed in appearance, having coarser granules and no vacuoles.

Parabiosis studies. Over a 3-month period, 18 diabetic mice after 25-93 days (average duration 45 days) of restricted feeding were placed in parabiosis with normal control mice that had never been subjected to food restriction. Of these 18 pairs, 12 died 8-49 days after surgery (median survival time 23 days), 2 died after 187 and 189 days, and 4 other pairs were sacrificed for histological examination; 2 pairs when the normal partner looked moribund, one after 9 days when the normal partner contracted an infection of the wound site, and the other while both partners appeared healthy. Of the 12 pairs dying before 50 days, the normal partner died first in 11 cases. In these, and in the four pairs sacrificed, the progression was similar in that after a 1-week period of moderate hypoglycemia in both partners, the average blood sugar concentration of the diabetic partner increased to normal levels while the normal partner remained hypoglycemic (107 mg/100 ml) (Table 2, group 1). Blood sugar concentrations continued to decline in the normal partners until in those pairs that survived more than 2 weeks of parabiosis, the normal partners had an average blood sugar concentration of 74.8 mg/100 ml (range 56-106 mg/100 ml) compared with an average of 162 mg/100 ml (range 121-258 mg/100 ml) for the diabetic partners. Death of the normal partner usually occurred a few days after the blood sugar concentration decreased below

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age</th>
<th>Weight</th>
<th>Blood Sugar Concentration</th>
<th>Plasmatic Immuno-reactive Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fed</td>
<td>Fasted</td>
</tr>
<tr>
<td>1) db/db</td>
<td>weeks</td>
<td>g</td>
<td>mg/100 ml</td>
<td></td>
</tr>
<tr>
<td>(before food restriction)</td>
<td>6-8</td>
<td>30.0</td>
<td>±0.74</td>
<td>293</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(99)</td>
<td></td>
<td>(15)</td>
</tr>
<tr>
<td>2) db/db</td>
<td>12-14</td>
<td>26.1</td>
<td>±0.55</td>
<td>104</td>
</tr>
<tr>
<td>(after 6 weeks' food restriction)</td>
<td></td>
<td>(16)</td>
<td></td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td>6-8</td>
<td>23.5</td>
<td>±0.57</td>
<td>144</td>
</tr>
<tr>
<td>3) +/+ (no food restriction)</td>
<td></td>
<td>(7)</td>
<td></td>
<td>(7)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Number of mice on which determinations were made is in parentheses. * Values are significantly different from fed values (P < .01).
sis for 9, 15, 15, and 21 days were sacrificed for histological 

60 mg/100 ml. Pronounced hyperglycemia in the diabetic partner was observed only in those four pairs that survived longer than 4 weeks. Plasma insulin concentrations were determined in only six pairs (four pairs at the time of sacrifice plus 2 additional pairs between the 2nd and 3rd week of parabiosis) because the loss of blood (0.5 ml) required for an accurate assay is not well tolerated by diabetic mice. Plasma insulin concentrations averaged 356 μU/ml (range 265–450 μU/ml) in the diabetic and only 20 μU/ml (range 13–30 μU/ml) in the normal partners (Table 2).

The reason for the prolonged survival of two parabiont pairs (data not included in Table 2) is unknown. In each case the normal partner retained near normal blood sugar concentrations and only became severely hypoglycemic 3 to 4 weeks prior to its death. In one case the diabetic partner had malocclusion, unnoticed before parabiosis, and was unable to eat adequately for 2 weeks until this defect was recognized by weight loss in both partners. Thereafter the teeth were clipped weekly, allowing a more nearly normal feeding pattern to be resumed. However, some difficulty in eating remained, thus restricting the food intake and perhaps forcing the normal partner to eat more to compensate for, or, at least tending to equalize the amounts of food consumed by each.

In addition to blood congestion in the livers, lungs, spleens, and kidneys of the normal partners (always apparent in the parabiont partner that dies first), autopsies revealed small pancreases, extremely small livers, little or no adipose tissue, and very little food in the stomachs of the normal parabionts. Their body weights had decreased (24%) from averages of 23.5 to 17.9 g. In contrast, the diabetic partners had very large pale livers, extremely large stores of adipose tissue (6–8 g), and stomachs bulging with food. Body weights had increased nearly 46% from averages of 26.1–37.0 g (Table 2). These observations suggest that the normal partners die of inanition.

Four pairs, one of males and three of females, in parabiosis for 9, 15, 15, and 21 days were sacrificed for histological study. There was little difference in number and size of islets, in cell degeneration, or in size and appearance of acinar cells between members of the pair of females in parabiosis for 9 days. There were, however, quite conspicuous differences in some or all of these features between the members of the other three pairs. In each case, the pancreas of the diabetic (Fig. 2B) resembled those of the restricted-refed controls (Fig. 1B) with respect to number, size, and morphology of islets; to β-cell degranulation; and to size and appearance of acinar cells. The numerous islets varied in size from very small to quite large, and cells were large and degranulated, some markedly.

Pancreases of the normal partners varied individually with their physiological states, but each had few and small islets with small fairly well granulated β-cells, and small acinar cells (Fig. 2B). Figure 2, A and B are photomicrographs of pancreases of a normal and diabetic female pair in parabiosis for 15 days during which time the diabetic had gained 8 g and the normal had lost 2 g. The diabetic had a blood sugar concentration of 257 mg/100 ml and the normal a concentration of 128 mg/100 ml and both appeared to be healthy. The striking differences are in β-cell degranulation and in the appearance of the acinar cells. The acinar cells of the diabetic partner (Fig. 2B) are large and the granules are limited to distal parts of the cells superior to the nuclei. Those of the normal partner (Fig. 2A) are smaller and are almost completely filled with fine cosinophilic granules.

Atrophy of acinar cells is the most conspicuous feature of the pancreas of the normal partner of another pair of females also in parabiotic union for 15 days. During this time the diabetic gained 7 g while the normal lost 12 g and became definitely hypoglycemic with a blood sugar concentration of 55 mg/100 ml. The normal partner was emaciated and at the point of death when the pair was sacrificed. The acinar cells (Fig. 3A) are much smaller than those of the diabetic partner (Fig. 3B) and have densely staining basal and sparse, foamy, distal cytoplasm. A similar picture of acinar atrophy is seen in the pancreas of a C57BL/KaJ female starved (no...
TABLE 2. Effect of parabiosis on body weight and on blood sugar and plasma insulin concentrations in diabetic (db/db) and normal (+/+ ) mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
<th>Weight, g</th>
<th>Blood Sugar Concentration, mg/100 ml</th>
<th>Immuno-reactive Insulin, μU/ml</th>
<th>Median Survival, days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Starting</td>
<td>At Death</td>
<td>Starting</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1) db/db with +/+ 16 pair</td>
<td>26.1±0.55</td>
<td>23.5±0.57</td>
<td>37.0±1.51</td>
<td>65±4.5</td>
<td>128±9.9</td>
</tr>
<tr>
<td>2) db/db with db/db 4 pair</td>
<td>25.5±0.57</td>
<td>17.9±0.57</td>
<td>114±6.8</td>
<td>196±3.4</td>
<td>118±3.1</td>
</tr>
<tr>
<td>3) +/+ with +/+ 4 pair</td>
<td>22.5±0.42</td>
<td>23.5±0.87</td>
<td>121±14.2</td>
<td>196±57.4</td>
<td>188±26.8</td>
</tr>
<tr>
<td>4) db/db (single) 6</td>
<td>22.8±1.58</td>
<td>24.2±1.03</td>
<td>144±5.4</td>
<td>144±5.4</td>
<td>103±10.0</td>
</tr>
</tbody>
</table>

Values are means ± se. * Diabetic mice in this table were all fed ad lib. after having been on the food restriction regimen (see text) for 25–93 days. † Significantly different from starting values (P < .01).

FIG. 2. Photomicrographs ×240. Aldehyde fuchsin stain. Acinar cells (ac) of the exocrine pancreas surrounding islets of Langerhans. Granules in the islet β-cells are deeply stained. A pair (db/db with +/+ ) in parabiosis for 15 days, both healthy at sacrifice. A: pancreas of the +/+ parabiont which weighed 19 g (loss = 2 g), had a blood sugar concentration of 128 mg/100 ml, and a plasma insulin concentration of 128 mg/100 ml, and a plasma insulin concentration of 128 mg/100 ml. Arrows point to some of the few granulated β-cells.

food but water ad libitum) for 13 days before sacrifice (Fig. 3C). This animal was also emaciated, moribund, and hypoglycemic with a blood sugar concentration of 52 mg/100 ml when sacrificed. The similarities in pancreatic histology of the two normal mice, one in parabiosis with a hyperphagic diabetic partner and the other deprived of food to the point of death, suggest severe inanition in both cases.

Of the 4 parabiotic unions between restricted diabetics (db/db with db/db), one died after 24 days, one after 52 days, and the other two were still alive after 60 days (Table 2, group 2). The pair dying early (24 days) were poorly matched with regard to the severity of diabetes (initial blood sugars 105 and 355 mg/100 ml) and this may have contributed to their early death. The pair surviving 52 days was found dead on its back on Monday morning and could have been without food or water for up to 48 hr. This difficulty in turning over, once placed or after falling on their backs, is a common hazard even in single diabetics that are grossly obese. Plasma insulin was not determined in this group because of the limited number of pairs. These mice remained euglycemic during the first 2 weeks after parabiosis (Table 2, group 2) in contrast to restricted-refed controls (group 4) and only became hyperglycemic after 5–6 weeks of parabiosis. The more nearly normal blood sugar concentrations observed may reflect the decreased food intake seen in mice in parabiosis when compared with single animals.

Each partner of the four control parabiotic unions (+/+ with +/+ ) maintained normal blood sugar and normal plasma insulin concentrations throughout parabiosis. A slight tendency toward hypoglycemia was observed but after the 1st week was no longer statistically significant (Table 2, group 3). Also, some loss of weight occurred initially but this was rapidly regained and these normal pairs continued to thrive until sacrificed 4 months after surgery for histological examination of tissues. Parabiosis appeared to have no effect on the pancreatic morphology. Islets were...
Acinar cells surrounding islets of Langerhans in which β-cell granules have not been stained. A second db/db with +/+ pair in parabiosis for 15 days, and an unpaired +/+ starved for 13 days. The +/+ partner and unpaired +/+ were moribund when sacrificed. A: pancreas of the +/+ parabiont which weighed 15 g (loss = 12 g), had a blood sugar concentration of 55 mg/100 ml and a plasma insulin concentration of 20 μU/ml. Both islet and acinar cells are observable in the numbers and size typical of normal C57BL/KsJ mice and none showed degranulation of β-cells. Acinar cells were normal in size and appearance. No evidence of graft vs. host reaction or other suggestions of histocompatibility differences were seen.

**Food consumption.** Determination of food consumption of each individual in a parabiotic pair, by means of partitioned cages or other restraining devices as described by Han et al. (8), was found impractical with these mice. Under restraining conditions the mice struggled continuously, refused to eat, and eventually tore the skin in the region of the anastomosis. Such tears occur very readily in the thin skin of the diabetic mice. Therefore, total food consumption for parabionts was obtained by leaving the mice in the home cage and feeding daily an excess but preweighed amount of food and recording the weight of that which remained after each 24-hr period. Food consumption and body-weight gains were recorded for at least three parabiont pairs of each type, as well as on pairs of single mice not in parabiosis (Table 3). The period of observation was the interval between the 1st and 2nd weeks after surgery, i.e., after the wound had healed and while both animals in the pairs were still healthy. Normal mice in parabiosis consumed an amount of food nearly identical to that eaten by two normal mice not in parabiosis. In contrast, diabetic parabiont pairs (db/db with +/+) consumed only 65% of the amount eaten by two single diabetic mice of similar age. The rate of weight gain was less than 60% of that seen for unparabiosed diabetics. Parabiosis of diabetic with normal mice resulted in a decreased food intake much more severe than seen in either of the other types of parabiosis. Food consumption averaged 3.67 g for this combination compared to 7.16 g for diabetic parabionts and 4.68 g for normal parabions. It can be calculated by extrapolation from the data for single animals (Table 3, lines 1 and 2) that an ideal pair, eating normally, should eat 7.9 g/day. If allowance is made for the decrease in food consumption noted for diabetic mice in parabiosis (line 5), the calculated food consumption should be at least 5.92 g. It is of interest that the total food eaten per day (3.67 g) by these diabetic-normal pairs was only slightly higher than one-half the amount (3.58 g) consumed by diabetic-diabetic pairs. These data demonstrate that this type of parabiosis resulted in marked undernutrition of the pair and support the contention that the normal mouse ate very little, a supposition which was based previously on indirect observations at autopsy.

Further evidence that the normal partner ate very little, if at all, comes from direct observation of four parabiont pairs (db/db with +/) which were trained to eat their food in the 2-hr period between 9:00 and 11:00 AM. Under these conditions the diabetic parabiont was observed to eat continuously while the normal partner showed no apparent interest in the readily accessible food. Total food consumption in these trained pairs averaged 3.20 g/day after 7 days of adaptation to this schedule. This value is 87% of that observed for similar parabions fed ad libitum which suggests that the total food consumption was not unduly curtailed under these unnatural conditions.
DISCUSSION

Parabiosis of diabetic with normal mice produced only a slight amelioration of the disease. Hyperglycemia was delayed in parabiotic diabetics (whether in parabiosis with normal or with other diabetic mice) until 4-6 weeks after return of feeding ad libitum compared with the 3-week delay observed in the restricted-refed diabetic controls. This delay in regaining elevated blood sugar concentrations is most likely explained by the shock of the surgery, rather than by production of any alleviating factor by the normal partner. Transfer of insulin releasing factors from diabetic to normal, or of inhibiting factors from normal to diabetic, seems unlikely in the light of both histological and biochemical evidence.

The maintenance of high levels of circulating insulin and glucose in the diabetic and low levels in the normal partner in the presence of a common blood circulation is difficult to explain. However, the rate of plasma exchange in most parabiosis studies has been shown to be about 1% min⁻¹ (9, 10). Huff et al. (10) have shown that in parabiotic rats with exchange rates of this order substances with a rapid metabolic turnover, such as testosterone and sodium barbiturate, when injected into one partner produce little or no change in the other. It seems reasonable to assume that in our studies with parabiotic mice the small amount of insulin that would cross from diabetic to normal would be rapidly metabolized in the normal mouse by the usual biochemical mechanisms. Fleming and Nugent (3) have shown that even marked disturbances in blood glucose concentration in one partner did not affect the blood sugar concentration of the other. Thus, in our studies, glucose entering the bloodstream of the normal from the diabetic partner would be rapidly utilized and blood sugar concentrations would be maintained independently.

The lack of food in the stomach at autopsy and the atrophic condition of the acinar cells of the pancreas of the normal parabiont suggest that these mice were themselves eating little. The data on total food consumption for the pair support this contention. It can be calculated that the small rate of glucose transfer would not sustain life if for some reason the normal partner refused to eat.

If hyperphagia is secondary to hypoglycemia induced by the oversecretion of insulin in diabetic mice as suggested by earlier studies (2), it is of interest that the normal parabiont does not respond to the insulin coming from the diabetic partner by overeating but, instead, stops eating and apparently starves to death. It is doubtful whether sufficient insulin crosses via the common circulation to produce hypoglycemia and resultant hyperphagia and the explanation of the normal partner's failure to eat may be that, when a normal animal overeats, a substance is released which affects the satiety centers regulating food consumption. This substance may be released by, but be ineffective in, the diabetic mouse. In the parabiotic situation it may be transported to the normal partner where it could effectively shut off the eating drive. Support for such a satiety factor comes from the results of experiments in which genetically obese mice (ob/ob) were placed in parabiosis with normal mice (6), and others in which rats, made hyperphagic by hypothalamic lesions, were placed in parabiosis with normal rats (9). In each of these experiments, a marked loss in body weight and indications of starvation were observed in the normal partner while, at the same time, the obese or lesioned animal remained hyperphagic and showed a marked increase in body weight. In the mouse experiment, failure of the normal mouse to eat was not observed and the loss of weight of the lean partner was attributed to parasitization of the normal by the genetically obese partner. In our experiments, parasitization by the diabetic partner seems unlikely since the flow of nutrients, although small in actual amount, should be from diabetic to normal and under these conditions it is difficult to visualize any mechanism which would be effective in draining nutrients from the normal animal.

In Hervey's study (9) with lesioned rats a reduction in food intake by the normal parabiont was observed and this failure to eat was attributed to a signal from the intact hypothalamic centers of the normal partner in response to some circulating satiety factor originating in the lesioned rat. In our experiments with diabetic mice, the evidence points to a similar cause for the failure of the normal to eat. On the other hand, Han et al. (8), in experiments similar to those of Hervey, were unable to find any evidence for a satiety factor which was effective under conditions of parabiosis. However, their parabionts were maintained in restraining cages during a 16-hr feeding period with the result that food consumption was insufficient to allow the rats to gain weight. In our studies and those of Hervey, it was only when the mutant or lesioned animal gained weight rapidly that the presence of the postulated satiety factor was manifested by the failure of the normal to eat.

Other interpretations are possible to explain why the normal mice in parabiosis with diabetic mice starve to death. In some cases some of the gut and adhering fat from the diabetic was found within the peritoneal cavity of the normal partner. The presence of this extra bulk in the peritoneal cavity of the normal may have contributed to a feeling of satiety and thus could have caused undernutrition of the normal. However, Hervey (9) found no difference between those pairs in which celioanastomosis was performed and those in which it was not, suggesting that the presence of some gut from one animal in the peritoneal cavity of another was not related to the cause of death.

The explanation that a defective hypothalamus in the diabetic mouse causes death by starvation of the normal parabiont is supported by other characteristic features of diabetic mice such as the hypogonadal condition and hyperinsulinemia. High levels of circulating insulin, characteristic of diabetic mice from 2 weeks of age until the terminal stage of the disease (2), are a known consequence of ventromedial hypothalamic lesions in rats (5). However attractive the concept of defective hypothalamic regulation seems, one cannot rule out a physiological defect in the endocrine pancreas, as yet undetected, nor abnormal effectiveness of insulin, believed of importance in the pathogenesis of the diabetic syndrome in the sand rat (12).
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