A Study of Glucose Tolerance and Insulin Response in Partially Depancreatized Dogs

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SUMMARY

The effects of a standard glucose tolerance test (GTT) and of pretreatment with aminophylline (AM) were examined in dogs with a reduced reserve of islet function prepared by partial removal of the pancreas and in normal intact dogs. Serum glucose, free fatty acid and insulin levels were measured. No significant differences were found before and after partial pancreatectomy when less than 50 per cent of the pancreas was removed or after sham operation. When AM was infused prior to the GTT, serum insulin levels were elevated to almost twice those obtained during a standard GTT. Dogs with less than 50 per cent of the pancreas remaining showed deterioration in GTT two weeks after operation, although the fasting blood sugar was normal except in one animal. The deterioration in GTT response progressed with time. Maintaining the animal on a high protein diet improved the GTT response. In chow-fed dogs with less than 50 per cent of the pancreas but no glucosuria or fasting hyperglycemia, prior AM infusion significantly improved the GTT and increased insulin secretion. In one dog with fasting hyperglycemia, AM did not cause improvement. DIABETES *23*:424-32, May, 1974.

A number of groups have brought forward evidence indicating that hyperglycemia exhibited by diabetic patients is due to an inadequate insulin supply.[1-3] Whether or not tolerance for glucose is directly related to islet tissue mass has not been determined. Others have reported that prediabetics have a sluggish insulin response to a glucose challenge and an abnormal tolerance for glucose.[4-6] In these prediabetics the poor insulin response was improved by aminophylline (AM) treatment prior to glucose infusion.[7]

Experiments outlined here were initiated in the hope of demonstrating whether changes in islet function are directly related to a reduction in islet mass produced by removing increasing amounts of pancreas. Changes of blood glucose, serum insulin and free fatty acid (FFA) levels in response to a standard glucose load were followed, and an attempt was made to relate these alterations to the amount of pancreas (and presumably islet mass) remaining.

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MATERIALS AND METHODS

Thirteen normal dogs were used in this experiment. The body weights of the dogs ranged from 12 to 20 kg. Sham operations were performed on three dogs, and in the other ten, a portion of the pancreas was removed. The dogs were trained to stand in slings and tests were done without anesthesia. Unless specifically described, the animals were fed with standard dog chow (Purina). After fasting for sixteen to eighteen hours, the dogs were given an intravenous glucose tolerance test (GTT) with a 25 per cent glucose solution in 0.9 per cent saline injected in four minutes. The amount of glucose administered was 1 gm./kg. [0.7, 8] The results were plotted and areas under the glucose and insulin curves were measured using a planimeter.

The method used for AM infusion prior to the GTT was an adaptation of the procedure of Cerasi and Luft.[7] A dose of 10 mg. per kilogram body weight of AM was injected initially and this was followed by AM infusion for one hour at a rate of 10 mg. per kilogram body weight per hour.

Serum glucose was determined by the glucose oxidase method,[9] and FFA in plasma was assayed by a modification of the method of Dole and Meinertz.[10] Insulin in the serum was determined by the immunoassay of Hales and Randle.[11] Each sample was assayed in triplicate.

A technic of partial pancreatectomy was employed whereby pieces of pancreas of nearly equal size were removed from both the splenic and duodenal parts of the pancreas of the dogs. This procedure was adopted because of the differences in insulin concentration between the splenic end, the attached duodenal portion and the free duodenal end of the pancreas.[12]

Dog pancreatic tissue was fixed in Bouin's fluid and embedded in paraffin. Sections of 6μ thickness were cut and stained with Gomori's aldehyde fuchsin stain.

RESULTS

In table 1 are included the values for the insulin contents of the remnants and of the parts removed, the insulin concentrations in the remnants and in the parts removed, the insulin of the remnants as a percentage of the total pancreatic insulin, and the insulin of the remnants in units per kilogram body weight. Figure 1 shows the remnant weight as a percentage of the total pancreas plotted against the insulin of the remnant as a percentage of the total pancreatic insulin. A straight-line relationship is observed, the line crossing the per cent remnant axis at about 13 per cent.

Glucose tolerance tests were carried out in the dogs before and after partial pancreatectomy. Glucose tolerance curves, changes in plasma free fatty acids, and changes in serum insulin levels in response to a glucose load were all examined.

Two tests were done before the partial pancreatectomy (one was done two weeks before and the other one week before), and the data were pooled. Two tests were done at the second week and the sixth week, *after* the sham operation, and the data were pooled. Data were pooled because no significant difference in the two sets of data (either before or after the operations) was observed. For the partially depancreatized dogs, additional tests were carried out according to the experimental designs.

An indication of changes in the tolerance for glucose and in the insulin response was obtained by measuring the areas under the glucose and insulin curves. When the areas under the glucose curves were plotted against the per cent of pancreas remaining in the animal (figure 2) it became evident that the glucose area did not change greatly until the pancreatic remnant was less than 50 per cent of the pancreas, at which point the glucose areas started to become greater. At about the same point, the insulin areas become smaller. These results indicate that it would be reasonable to make further comparisons between animals having more than 50 per cent of the pancreas

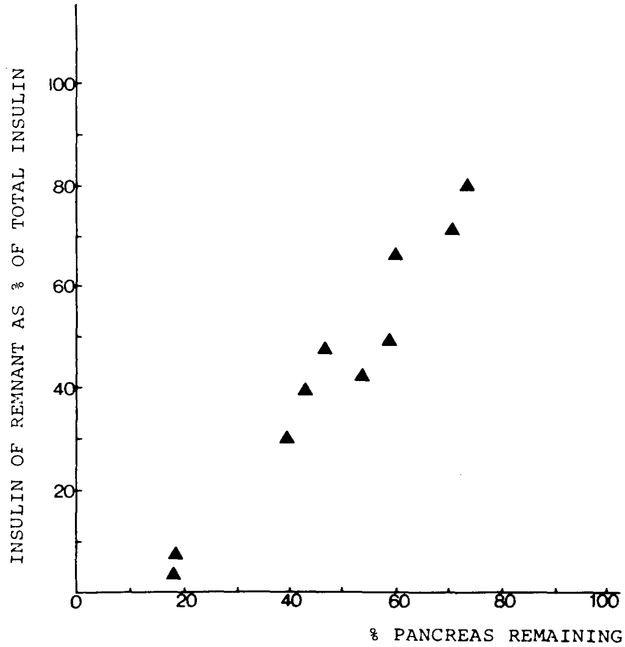

FIG. 1. Relationship between remnant weight and insulin content of the remnant.

TABLE 1

Insulin measurements in pancreas sections from dogs

Dog. no.	Dog wt. (kg.)	Total insulin in part removed (U.)	Mean conc. insulin in part removed U./gm.	Total insulin in remnant (U.)	Mean conc. insulin in remnant U./gm.	Insulin of remnant as % of total insulin	Insulin of remnant (U.)/kg. body wt.
1	19	0	0	100.21	3.10	100	5.27
2	14	0	0	104.61	5.06	100	7.47
3	17	0	0	95.67	3.04	100	5.63
4	20	18.67	2.44	72.69	3.46	80	3.63
5	17	31.32	2.80	76.12	2.81	71	4.47
6	14	25.15	2.25	49.82	2.98	66	3.56
7	20	76.51	5.09	72.40	3.38	49	3.62
8	15	40.64	2.95	29.48	1.85	42	1.97
9	12	30.21	2.41	27.03	2.26	47	2.25
10	18	31.46	1.62	20.50	1.41	39	1.14
11	16	63.41	4.47	26.71	2.87	30	1.67
12	15	33.46	1.77	2.29	0.52	7	0.15
13	18	79.74	3.46	2.81	0.73	3	0.15

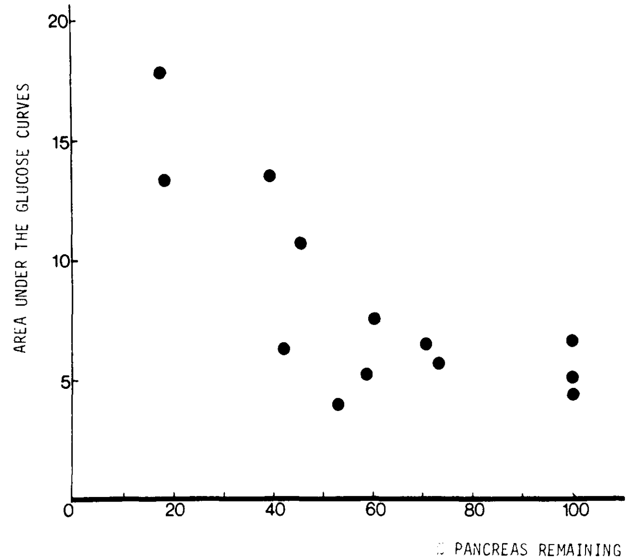

FIG. 2. Areas under the glucose concentration curves for zero to 120 minutes, compared with the percentage of pancreas remaining in the animal. The areas were measured using a planimeter, and the unit is arbitrary.

remaining and those having less than 50 per cent of the pancreas remaining.

Figures 3 to 5 show changes in blood levels of glucose, FFA and insulin after a glucose load, in three groups of dogs, namely sham-operated dogs, dogs with remnants of pancreas greater than 50 per cent and dogs with pancreas remnants of less than 50 per cent. Areas under glucose and insulin response curves are given in table 2 for these same groups. In addition the influence of AM on the response is shown in figures 3 and 4, and the effect of a protein dietary supplement is illustrated in figure 4.

Sham operation. The results in the dogs that had sham operations are given in table 2 and figure 3. The maximum glucose concentration was found in the first sample five minutes after the glucose infusion was completed. The blood glucose levels returned to initial values in sixty minutes. FFA values went down immediately after the glucose infusion and decreased to about half their original values at fifteen to thirty minutes. In response to this glucose infusion, serum insulin levels increased rapidly to about 70 μU./ml. between five to fifteen minutes after the glucose infusion was stopped, and then declined to the resting level at sixty minutes. Glucose tolerance in these dogs was not significantly different after sham operation than before, and when the areas under the glucose or insulin curves were measured with a planimeter, no statistically significant differences in the areas were

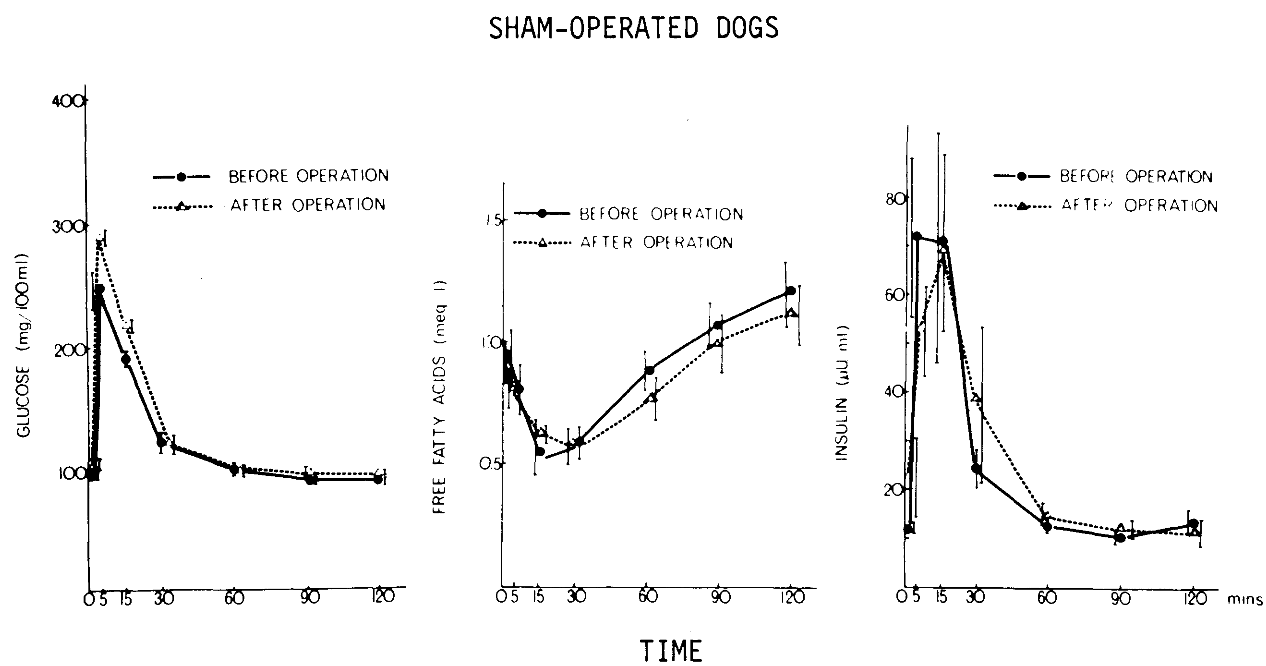

FIG. 3. Levels of blood glucose, plasma free fatty acids and serum insulin during intravenous glucose tolerance tests in three dogs after sham operations. Each point represents the mean of six observations. Vertical lines are S.E.

observed (table 2). This indicates that the variation in GTT in specific dogs over a period of time was minimal, and that the operative procedures were without significant effects.

Remnants greater than 50 per cent of the pancreas. When more than 50 per cent of the pancreas remained in the animal, no significant changes were observed in the GTT at successive times, before and after partial pancreatectomy. This indicates that dogs with more than half of the pancreatic tissues remaining had sufficient islets of Langerhans and, therefore, a relatively normal response to a glucose challenge. The blood glucose levels, FFA levels and serum insulin levels at successive times after intravenous injection of glucose were not significantly different before and after partial pancreatectomy.

The partially depancreatized dogs were kept on a standard chow diet for five to six months after the operation, and at the end of this period, additional tests were performed. The levels of serum glucose, FFA and insulin accompanying intravenous GTT were not significantly altered as compared to measurements obtained before the operation (figure 4). The areas under the glucose and insulin curves were not significantly different before and after partial pancreatectomy (table 2).

Experiments with AM infusion before the intravenous GTT were carried out two or three weeks after the last standard GTT. Following AM, the disappearance of plasma glucose was accelerated and the blood sugar values returned to initial levels thirty minutes following the glucose infusion; the reappearance of FFA was also faster, the plasma FFA levels returning to their original values within sixty minutes, as compared to ninety minutes in the standard GTT. Also, following aminophylline serum insulin levels were elevated from 70 μU./ml. (obtained during a standard GTT) to about 100 μU./ml. in the first fifteen minutes. Comparison of the areas under the glucose curves (in the GTT) in the same dogs five to six months after partial pancreatectomy and immediately after aminophylline treatment shows that tolerance is significantly improved ($p < 0.05$) after AM treatment (table 2).

Remnants less than 50 per cent of the pancreas. When the pancreas was reduced to less than 50 per cent, fasting blood sugar levels remained normal, but tolerance to intravenous glucose was decreased both two weeks and six to eight weeks after the operation (figure 5). The insulin levels in these animals were reduced significantly five and fifteen minutes ($p < 0.05$) after glucose (figure 5), and the areas under the insulin curves were significantly less ($p < 0.05$) than those obtained before operation (table 2).

The dogs were kept on the chow diet for another three to four months. Fasting blood glucose levels remained normal, but glucose tolerance deteriorated further, and very little insulin was released in response to glucose infusion. Significant differences were observed in the areas under the glucose curves ($p < 0.05$) and insulin curves ($p < 0.025$), when compared with the tests performed two to eight weeks after operation (table 2).

To test the effect of dietary regimen on glucose tolerance, the animals with pancreatic remnants of less than 50 per cent and on chow diets for five to six months, were given a high protein diet (chow plus meat) for approximately two months, when similar GTT's were conducted. It was interesting to find that glucose tolerance improved. Both the lowering of blood glucose levels and the restoration of FFA levels accelerated. The plasma levels of insulin at five, fifteen and thirty minutes after glucose infusion were elevated (figure 5). The areas under the glucose curves were significantly reduced ($p < 0.05$), and the areas under the insulin curves significantly increased ($p < 0.025$) after the period on the protein supplemented diet (table 2). These dogs were then put back on a chow diet. In about five weeks, one of the five dogs became diabetic, showing glucosuria and fasting hyperglycemia.

After six to eight weeks the effect of AM infusion prior to GTT on these dogs was tested (figure 5). The results revealed that in the dogs without glucosuria or fasting hyperglycemia, insulin secretion still occurred in response to glucose, indicating that the remaining islets were still functioning. Histological study showed that β cells of islets were still well granulated, even in the dog with only 18 per cent of pancreas remaining, though some islet cells showed reduced granulation or even "hydropic" change. In the dog with fasting hyperglycemia, the insulin release in response to glucose was very low, even in the presence of aminophylline. In this dog, no normal islet tissue was observed in the pancreatic remnant. These results suggest that, in dogs with less than 50 per cent of the pancreas and deteriorated glucose and insulin responses, the islets of Langerhans still function, except in the frankly diabetic animals.

DISCUSSION

It is evident that in partially depancreatized dogs a linear relationship exists between the percentage of

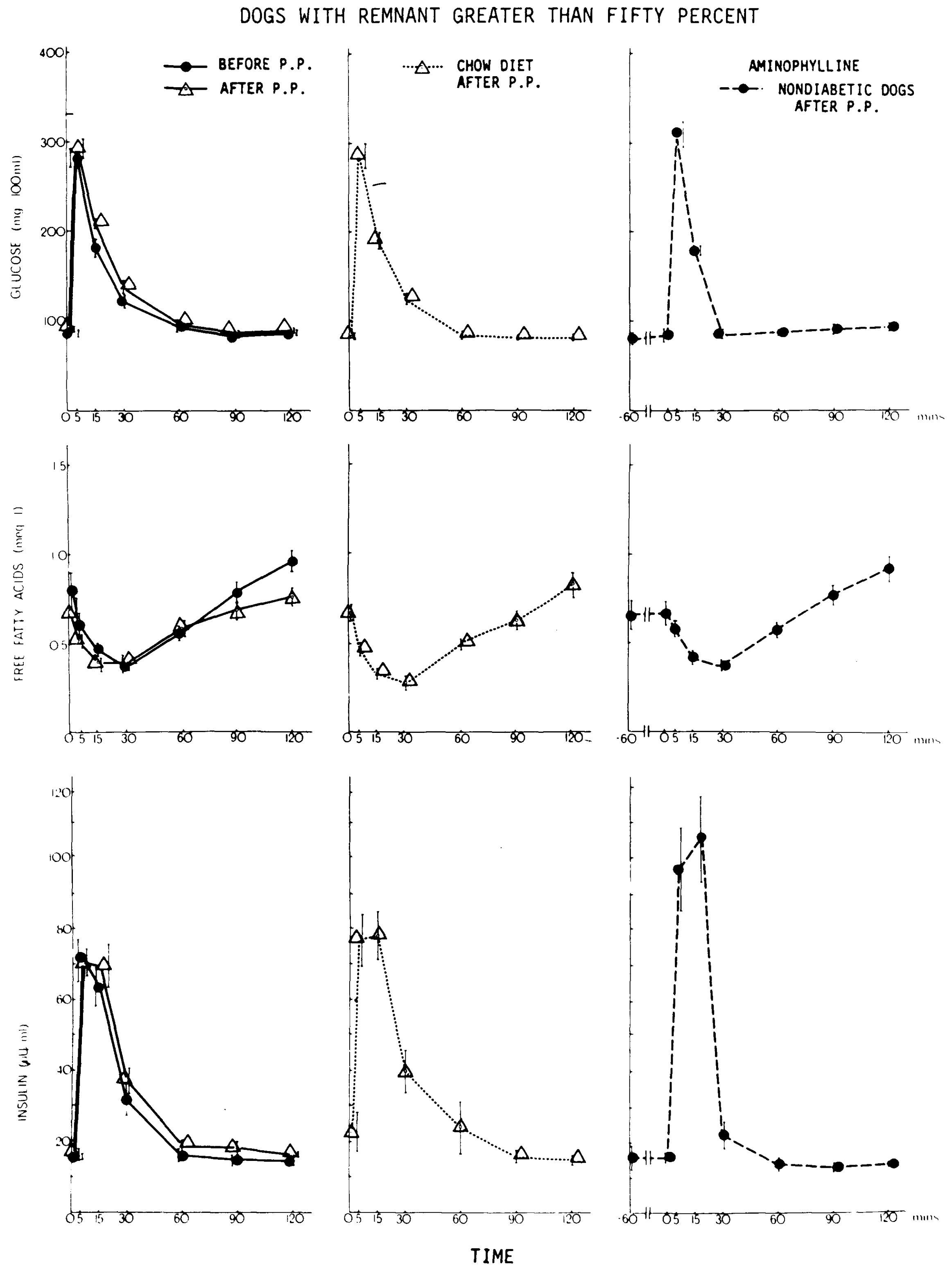

FIG. 4. Levels of blood glucose, plasma free fatty acids and serum insulin during intravenous glucose tolerance tests in five dogs with more than 50 per cent of the pancreas remaining. Left column: the results before and after partial pancreatectomy. Center column: the results after the dogs were kept on a standard chow diet for five to six months after the operation. Right column: the effect of aminophylline infusion prior to glucose tolerance tests.

pancreas remaining and the percentage of total insulin remaining. It is also seen that when the remnant is less than 13 per cent of the pancreas, the insulin of the remnant becomes zero, in other words the animal is frankly diabetic. The fact that the line cuts the base line to the right of zero indicates that the *concentration* of insulin in the pancreas gradually becomes lower. Histologically, granulation of many beta cells was maintained except in the dog that became frankly diabetic. Since the insulin concentration in the pancreas and the degree of granulation of the beta cells run roughly parallel,[13,14] it would appear that the actual insulin concentration within some islet cells may not be drastically reduced. The initial blood sugar, FFA and insulin levels, as well as glucose tolerance of the dogs, correspond well with established values.[15-17] The tolerance for glucose, as indicated by the areas under the glucose curves, remained relatively normal, until the remnant was reduced to less than 50 per cent of the pancreas. With less than 50 per cent of the pancreas remaining, the glucose areas increased, indicating that the tolerance for glucose was reduced. This implies that the rate of insulin secretion is able to meet the challenge of a large glucose load in dogs retaining more than half of the pancreas. When more than about 50 per cent of the pancreas was removed, glucose tolerance deteriorated. At the same time the insulin secretory response to glucose was reduced, indicating that the blood glucose and insulin responses were closely related. In all but one of these dogs, the islets still showed many well granulated beta cells and the fasting blood sugar levels were normal. In one frankly diabetic animal, no normal β cells were found. With this one exception, under resting conditions, sufficient insulin apparently was being supplied by the islets of the remnant.

One must conclude that the altered blood sugar response and the reduced insulin secretion that occurred when a glucose load was given to dogs having less than 50 per cent of the pancreas remaining were not consequences of exhaustion of remaining islets, or necessarily of a defect in the insulin-releasing mechanism in individual β cells,[4] but rather that not enough insulin entered the general circulation because not enough islet cells were functioning. The amount of insulin secreted from the islets in the remnant apparently was not able to take care of the sudden increase in glucose load during the GTT and, therefore, tolerance was abnormal. The total blood flow through islet tissue was probably also reduced. The stimulus for extra insulin secretion under the conditions of the GTT is presumably the increase in the level of glucose reaching the beta cells, and it is a reasonable assumption that the total insulin output of the remnant will be related to the number of beta cells responding, as well as to the blood glucose level. This response could be increased by the prior infusion of aminophylline, which would increase stimulatory effectiveness of a given level of glucose on secretion by beta cells. Aminophylline and its analogues have been reported to potentiate the release of insulin induced by glucose, especially from the fast releasing compartment.[18,19] This occurs in vivo and in vitro.[20-23] Also, aminophylline in the presence of glucose greatly increases blood flow to the pancreas (Rappaport —personal communication). This latter action of aminophylline might contribute to an improvement in insulin secretion and help to support synthesis.

Protein can be changed to carbohydrate in the body. Nonetheless, when dogs with remnants less than 50 per cent of the pancreas and maintained on chow diet for six months were given a high protein feedings, the glucose tolerance was significantly improved, the insulin release during the GTT enhanced and the FFA changes brought back toward normal. The explanation might lie in the effects of amino acids on insulin synthesis and insulin secretion. Amino acids are essential for protein synthesis and some amino acids reportedly stimulate insulin release.[24-26] It is conceivable that partially depancreatized dogs fed high protein meals may have more stored insulin in their pool and, upon stimulation, more insulin might be released. Also the insulin secretory response of the islets to a given level of glucose may be improved by a high protein dietary regimen.

While there may be other changes which would lead to an altered insulin output in response to glucose stimulation, such as an altered responsiveness of islet cells to a given stimulus or the secretion of an abnormal insulin, it has nevertheless been demonstrated by the present study that a reduction in the mass of normally functioning islet tissue by partial removal of the pancreas can lead to a reduction in the insulin secretory response to a glucose load, even though the fasting blood sugar is maintained at a normal level. These animals with reduced pancreas showed some progressive deterioration in glucose tolerance with time, and in one instance frank diabetes developed. In the earlier phases, therefore, a state of "prediabetes" was present.[27,28] The glucose tolerance and insulin response in such animals was improved by a diet supplemented with protein.

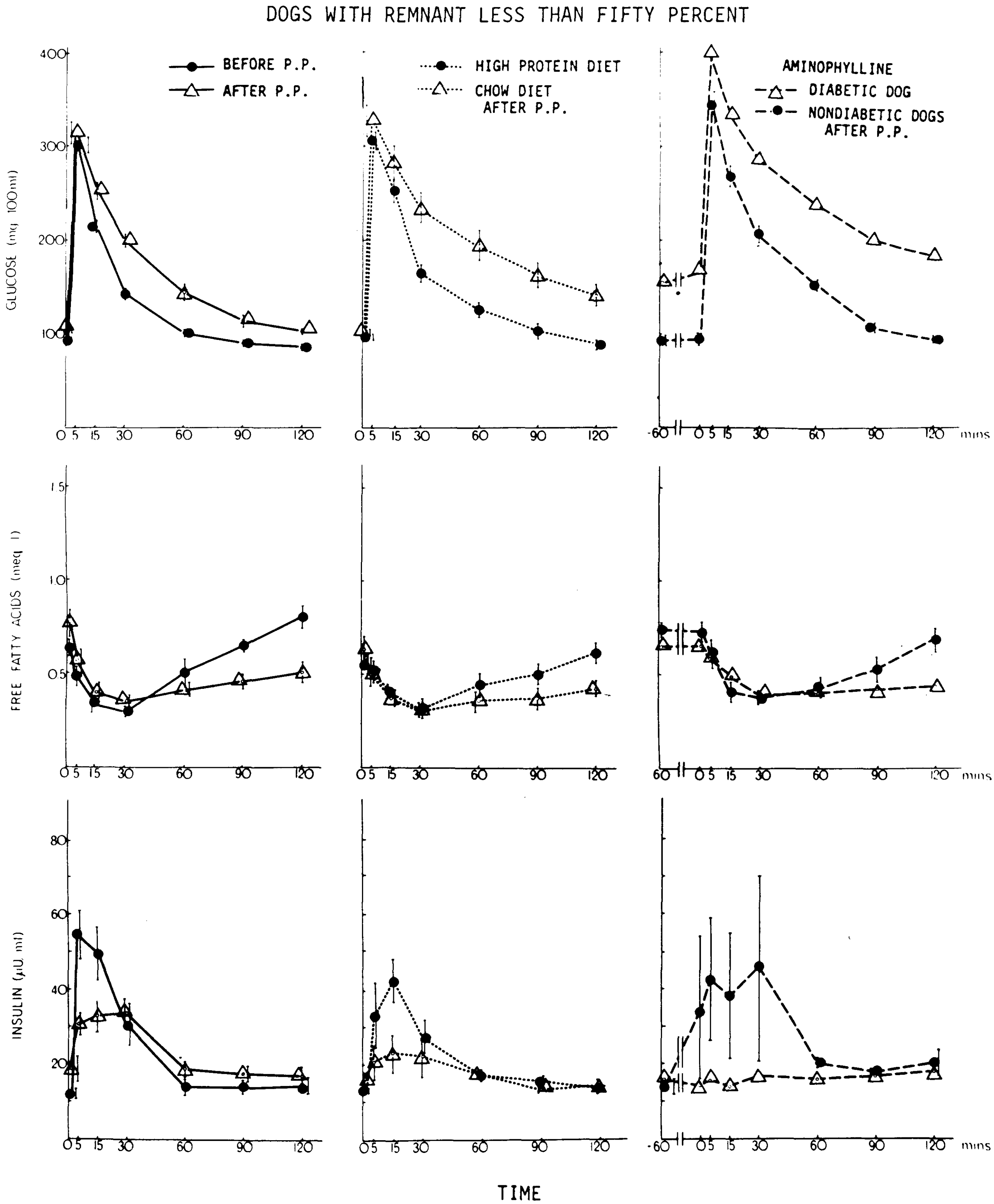

FIG. 5. Levels of blood glucose, plasma free fatty acids and serum insulin during intravenous glucose tolerance tests in five dogs with *less than 50 per cent of the pancreas remaining. Left column: the results before and after partial pancreatectomy. Center column:* the results after the dogs were kept on either a standard chow diet for three to four months or a high protein diet for two months after the operation. Right column: the effect of aminophylline infusion prior to glucose tolerance tests (chow diet).

TABLE 2

Mean areas under the glucose and insulin curves associated with glucose tolerance tests in dogs before and at various times after sham operation or partial pancreatectomy

A. Mean plasma glucose response area under curves (arbitrary units)
Mean ± S.E.

Group	A Before	B After (2-8 weeks)	C Chow diet (5-6 months)	D Aminophylline (5-6 months)	E High protein diet (7-8 months)	F Aminophylline (8-9 months)
Sham Operation	4.36 ± 0.37	5.30 ± 0.68				
Remnant > 50%	5.48 ± 0.83	5.82 ± 0.59	6.04 ± 0.54	4.52 ± 0.33		
Remnant < 50%	5.92 ± 0.39	8.16 ± 1.13	12.26 ± 1.90		8.90 ± 1.12	10.65 ± 0.88 17.1*

*Diabetic dog.
Sham: P (A vs. B) < 0.40
Remnant > 50: P (A vs. B) > 0.50; P (B vs. C) > 0.50; P (C vs. D) < 0.05.
Remnant < 50: P (A vs. B) < 0.025; P (B vs. C) < 0.05; P (C vs. E) < 0.05.

B. Mean insulin response area under curves (arbitrary units)
Mean ± S.E.

Group	A Before	B After (2-8 weeks)	C Chow diet (5-6 months)	D Aminophylline (5-6 months)	E High protein diet (7-8 months)	F Aminophylline (8-9 months)
Sham Operation	3.36 ± 0.21	3.20 ± 0.75				
Remnant > 50%	3.60 ± 0.45	4.32 ± 0.51	4.30 ± 0.38	5.36 ± 0.60		
Remnant < 50%:	3.34 ± 0.06	2.10 ± 0.30	0.94 ± 0.47		2.68 ± 1.16	3.07 ± 1.05 0*

*Diabetic dog.
Sham: P (A vs. B) > 0.50.
Remnant > 50: P (A vs. B) < 0.40; P (B vs. C) > 0.50; P (C vs. D) < 0.10.
Remnant < 50: P (A vs. B) < 0.05; P (B vs. C) < 0.025; P (C vs. E) < 0.025.

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