Effects of Sudden Deprivation and Restoration of Insulin Secretion on Glucose Metabolism in Dogs

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SUMMARY

Fourteen subtotally depancreatized aglycosuric dogs had the remaining pedunculated uncinate process enclosed in plastic casing and grafted subcutaneously. After one week the pedicle of the autograft was clamped for thirty to sixty minutes. In seven dogs given intravenous glucose at the time of clamping, diabetes-like changes in glucose tolerance occurred which increased in magnitude with increasing duration of deprivation of exogenous insulin. In seven fasting dogs the blood glucose level was rising in the four- to ten-minute interval after clamping. In three such dogs (one under local and two under Nembutal anesthesia) the method of successive measured injections of tracer (C-14-glucose U.L.) showed that the rate of glucose appearance had doubled and the rate of its disappearance was decreased to one third at one to thirteen minutes after clamping, resulting in high rates of accumulation of body glucose. The initial increase in the rate of glucose production appears to result from glycogenolysis.

Restoration of blood flow through the autograft (unclamping) caused a prompt decrease in rate of appearance of unlabeled glucose and increase in its rate of disappearance, resulting in restoration of these rates to their preclamping values within fifteen hours, and to a restored tolerance for intravenous glucose. The rapidity of these rate changes at clamping and unclamping demonstrates the importance of the continuous secretion of native insulin to prevent glucose accumulation in the partially depancreatized dog. DIABETES *14*:689-95, November 1965.

In an earlier paper[1] the method of Successive Measured Injections of Tracer (SMIT) was used to determine the changes which occur in the rates of glucose production, accumulation, and utilization in the dog following pancreatectomy. Designed to follow events extending over long periods of time, the above tracer method has limited effectiveness in demonstrating rapid transient changes in rates.[2] Furthermore, while the Hédon-type of partially depancreatized dog used in the above study permitted a fairly rapid total pancreatectomy, the process was irreversible and entailed some avoidable surgical handling prior to total pancreatectomy.

In the studies to be presented, use was made of a modified Hédon preparation.[3,4] In this adaptation of the method, described briefly under Materials and Methods, the pancreatic remnant and its pedicle were not allowed to become attached to surrounding tissues. In addition the length of the vascular pedicle to the pancreatic remnant was increased. Using dogs surgically prepared in this manner, and allowing a week for recovery, studies were made on the very early effects on glucose tolerance of suddenly removing and restoring the source of secreted insulin by clamping and unclamping the pedicle, respectively. In addition, tracer studies were made on the rapidity with which changes in the rates of production, accumulation and utilization occurred following both clamping and unclamping.

While tracer method SMIT was used, nonessential assumptions have been employed in specific parts of the study to permit observations to be made on rapid transient changes in the glucose transfer rates. The nature of these assumptions will be indicated.

Answers were sought to the following questions: (a) How soon following total pancreatectomy does a significant change occur in the tolerance of the unanesthetized dog for intravenous glucose? (b) What are the earliest changes following pancreatectomy in the rates of endogenous production and utilization of glucose, and what are their relative contributions to the rate of glucose accumulation which occurs? (c) What are the effects on these rates and on the tolerance for intravenous glucose of the reinclusion into the circulation of the pancreatic remnant by unclamping its pedicle?

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MATERIALS AND METHODS

Twenty-one dogs, unselected as to breed and sex, and weighing from 9 to 21 kg., were subtotally depancreatized. The remaining pedunculated uncinate process was transplanted into a subcutaneous pocket of the lower abdomen, its blood supply from the inferior pancreaticoduodenal artery being kept intact. The removed pancreas was weighed, and the mass of the remaining autotransplant was estimated by weighing another piece of comparable size. This weight amounted to 27 ± 1 per cent (Mean ± S.E.M.) of the total pancreatic weight. Two dogs died because of necrosis of the duodenum, and a third during anesthesia. Four dogs developed glycosuria. The results to be presented are based on the remaining fourteen aglycosuric dogs.

The autograft was isolated from the surrounding body tissue by a plastic jacket, through which the external pancreatic secretion was allowed to drain to the exterior. A long vascular pedicle suitable for clamping and unclamping was formed, and the pedicle passing through the incised abdominal wall was isolated by packing around it gauze saturated with petroleum jelly.[3,4]

The operated dogs were kept in metabolism cages. Food was provided according to appetite (200-250 gm./day of Purina Dog Chow) together with 150 to 200 gm. of raw beef pancreas. Daily urine was collected quantitatively, and tested for glucose and ketone bodies.

One week after the operation the pancreatic graft was exposed in the aglycosuric dogs by partially reopening the skin incision ("handling") under either local or Nembutal anesthesia. The gauze soaked in petroleum jelly was removed and the vessels in the free pedicle were infiltrated with Novocaine to eliminate pain and reactions via the sympathetic system during handling. After handling, a period of adjustment of twenty to forty-five minutes was allowed. For a measured period of time ranging from thirty to sixty minutes a bulldog clamp was then placed across the pedicle to determine the effects of the sudden temporary but total stoppage of delivery of the internal pancreatic secretion into the circulation. After removal of the clamp, the graft without the plastic casing was replaced in the subcutaneous pocket.

The tolerance for intravenous glucose was studied in *six* such dogs under local, and in one dog under Nembutal anesthesia. Immediately before clamping the pedicle, 1 gm./kg. body weight of glucose was injected intravenously as a 50 per cent glucose solution. Blood glucose concentrations were determined by the Folin-Wu method[5] prior to and for as long as three to four hours after administering the glucose load. The effects on the glucose tolerance tests of the exclusion of the isolated pancreatic remnant for thirty, forty-five and sixty minutes were studied. In each dog three glucose tolerance tests were performed: the first at one day before clamping, the second beginning at time of clamping and the third one day after unclamping the pedicle supplying the pancreatic graft.

Absolute rates of appearance (production), disappearance (utilization *plus* excretion), and accumulation of glucose as well as the intermixing amount of body glucose were determined at either two or three points in time before clamping, once during clamping, and twice after unclamping the pedicle of the graft in three dogs. Effects of the re-inclusion of the pancreatic remnant into the circulatory system were studied soon after unclamping the pedicle and again seven to twenty hours thereafter.

The dogs were fed twenty hours before the first tracer injection and were fasted thereafter. Blood samples were obtained from the right atrium through an indwelling catheter. Plasma glucose concentrations were determined by a glucose oxidase method.[6] Glucose was separated from plasma by paper chromatography and the chromatograms were eluted quantitatively.[7] The radioactive strengths of C-14 glucose were determined in the first tracer experiment by an automatic windowless flow counter (Tracerlab, Boston) and subsequently by a liquid scintillation system (Nuclear Chicago Corporation-series 720). Single exponential functions were fitted to sets of values of the specific activity and time by an IBM 7090 computer following the same procedure which gave valid rates of glucose production in dogs.[8]

The dogs on which the tracer studies were performed were killed with an overdose of Nembutal immediately after the experiment; the other dogs were kept under observation for various periods of time. An autopsy was performed on each dog that died or was killed. A careful search for any remaining pancreatic tissue in the abdomen was carried out and the histology of the abdominal organs including the autograft and its pedicle was studied.

RESULTS

Effects on the tolerance for intravenous glucose of depriving the partially depancreatized dog of his pancreatic remnant by clamping are shown in figure 1 for

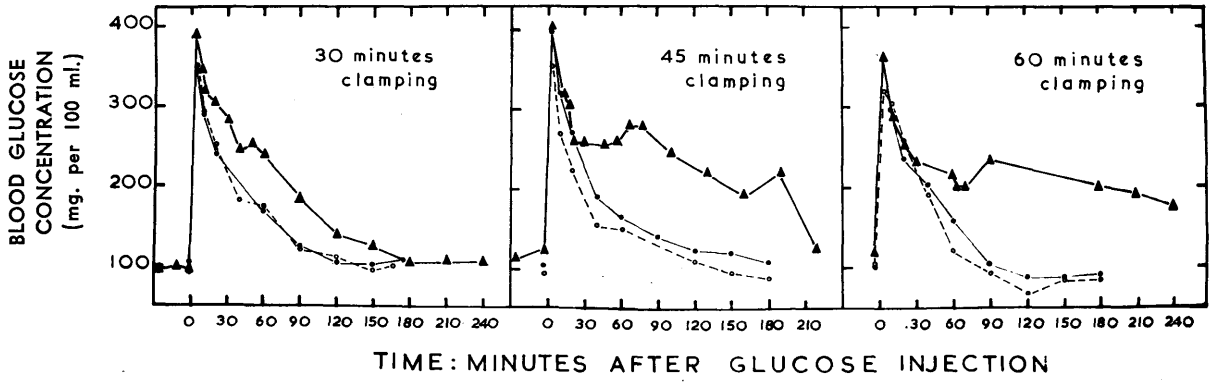

FIG. 1. Effects of Intravenous Glucose Tolerance Test (IGT) of clamping the vascular pedicle for thirty, forty-five, and sixty minutes in the transplanted pedunculated isolated pancreatic remnant in three dogs. Clamping was done at zero time when glucose was injected (1 gm./kg.). IGT with clamping (— ▲ — ▲ —); day before clamping (— ● — ● —); day after clamping (O — O —).

three of the seven experiments performed. While these experiments did not reveal distinct early effects of clamping, they show clearly that the temporary deprivation of endogenous insulin produced diabetes-like effects on the glucose tolerance which increased in magnitude as the time of deprivation was increased. The close similarity of the glucose tolerance test, made in each dog on the day before and the day following the test with clamping, indicates that the temporary isolation of the pancreatic remnant did not reduce its ability to secrete insulin.

The fasting blood glucose level was rising soon after clamping the pedicle to the pancreatic autograft in all but one dog in which it started to rise at about thirteen minutes after clamping. In the other dogs the rates of increase between four and ten minutes after clamping were: 16, 35, 49, 52, 108 and 132 mg. per 100 ml. per hour. These rates of increase were dependent on whether or not handling had induced hyperglycemia prior to clamping (figures 2 and 3). In one of the tracer-injected dogs (figure 4) the plasma glucose concentration had started to increase within two minutes following clamping, the average rate of increase being 108 mg. per 100 ml. per hour during the thirty-five-minute period that the pancreatic autograft was isolated from the dog by clamping.

Changes in the rates of production and utilization of glucose following clamping were at least as prompt in this dog. When a measured injection of tracer was administered *one minute* after clamping, the endogenous rate of glucose production *at that time* was 3.0 gm./hour in contrast to the two preclamping values of 1.7 gm./hour (figure 4 and table 1). Assuming that clamping was not accompanied by an early change in the

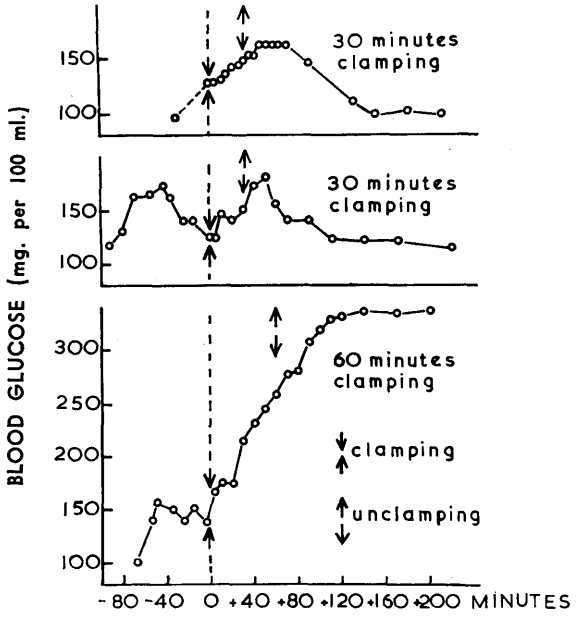

FIG. 2. Effects on fasting blood glucose level in three dogs (a) of exposure of the subcutaneous pancreatic autograft (minus times); (b) of transient pancreatectomy by clamping; (c) after unclamping.

apparent glucose space, the rate of accumulation of glucose at time of tracer injection amounted to 2.1 gm./hour. Thus the rate of utilization of glucose had decreased to 3.0-2.1 = 0.9 gm./hour from its preclamping equivalence with the rate of glucose production (1.7 gm./hour). In the other two tracer experiments of this type the above-mentioned glucose transfer rates were determined at ten and thirteen minutes after clamping (figures 5 and 6, respectively) but the changes in rates followed the same pattern as is described above at one minute after clamping (table 1).

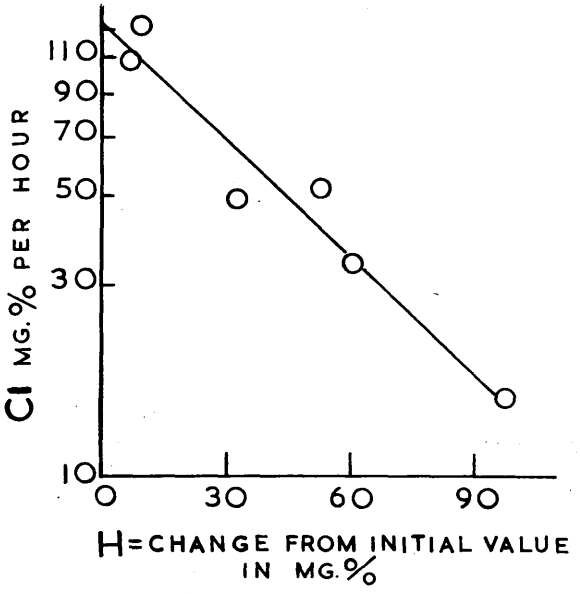

FIG. 3. Negative correlation between the maximum "handling" rise, H, in the plasma glucose level before clamping and the rate of rise, CI, in blood glucose level with pancreatic pedicle clamped off.

Early and late effects of restoring blood flow through the autograft by unclamping the pedicle can be seen in figures 4 to 6. In view of the linearity of all other graphs of specific activity *versus* time in these figures, the abrupt flattening of this plot in figures 4 and 5 at times of unclamping can only be interpreted as a corresponding decrease in rate of production of unlabeled glucose. The slow progressive decrease in plasma glucose level following unclamping in each case indicates that the rate of glucose disappearance had increased sufficiently to exceed the production rate. These conclusions were confirmed in one experiment by a tracer injection made eleven minutes after unclamping (T_4 in figure 6). The restoration to *pre*clamping levels, not only of the fasting plasma glucose concentration but also of the rate of glucose production, was complete in all subjects by the following morning (T_5 in figures 4, 5 and 6).

A thorough search in the abdomen at autopsy, including the examination of thin slices of the duodenum, revealed no residual pancreatic tissue in any of the dogs.

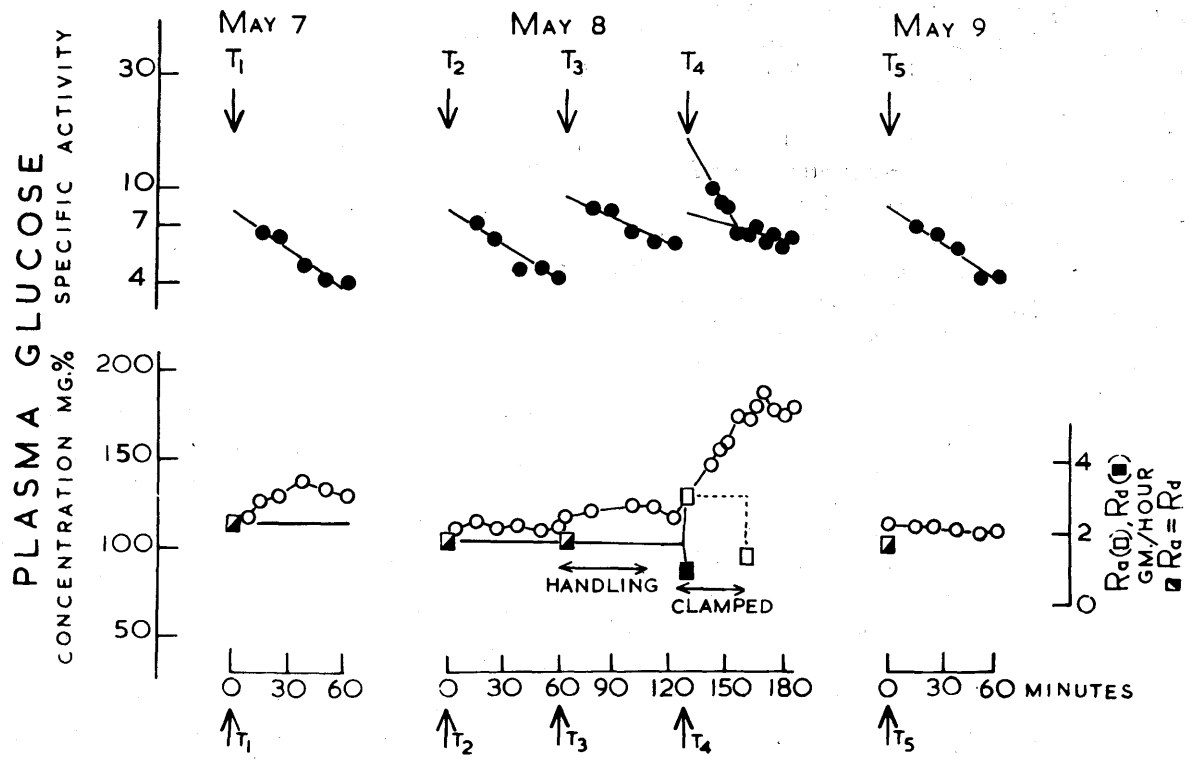

FIG. 4. Course of tracer experiment on dog No. 1 of table 1. Times of intravenous injection of C-14 glucose are shown as T_1 — T_5. Rates of glucose appearance R_a (□) and disappearance R_d (■), shown as rectangles, refer to scale at lower right. When $R_a = R_d$ a half-open square is used. Plasma glucose levels, shown as open circles (O), refer to scale at lower left.

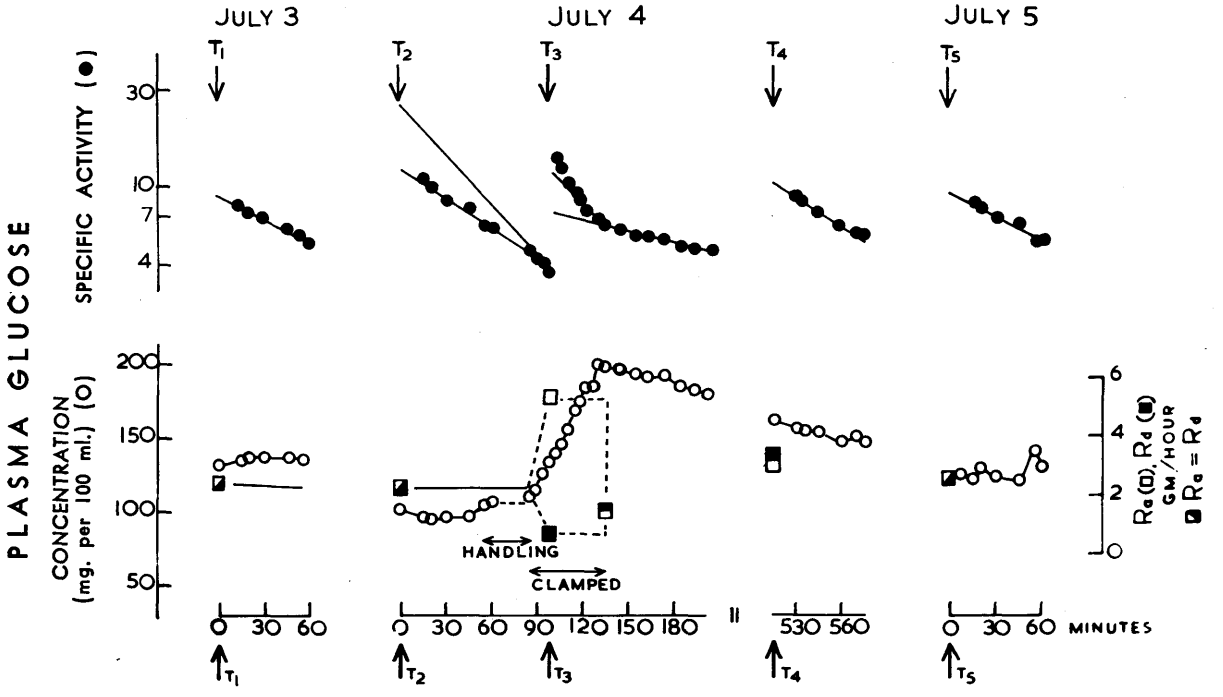

FIG. 5. Course of tracer experiment on dog No. 2 of table 1. Meanings of symbols are indicated under figure 4.

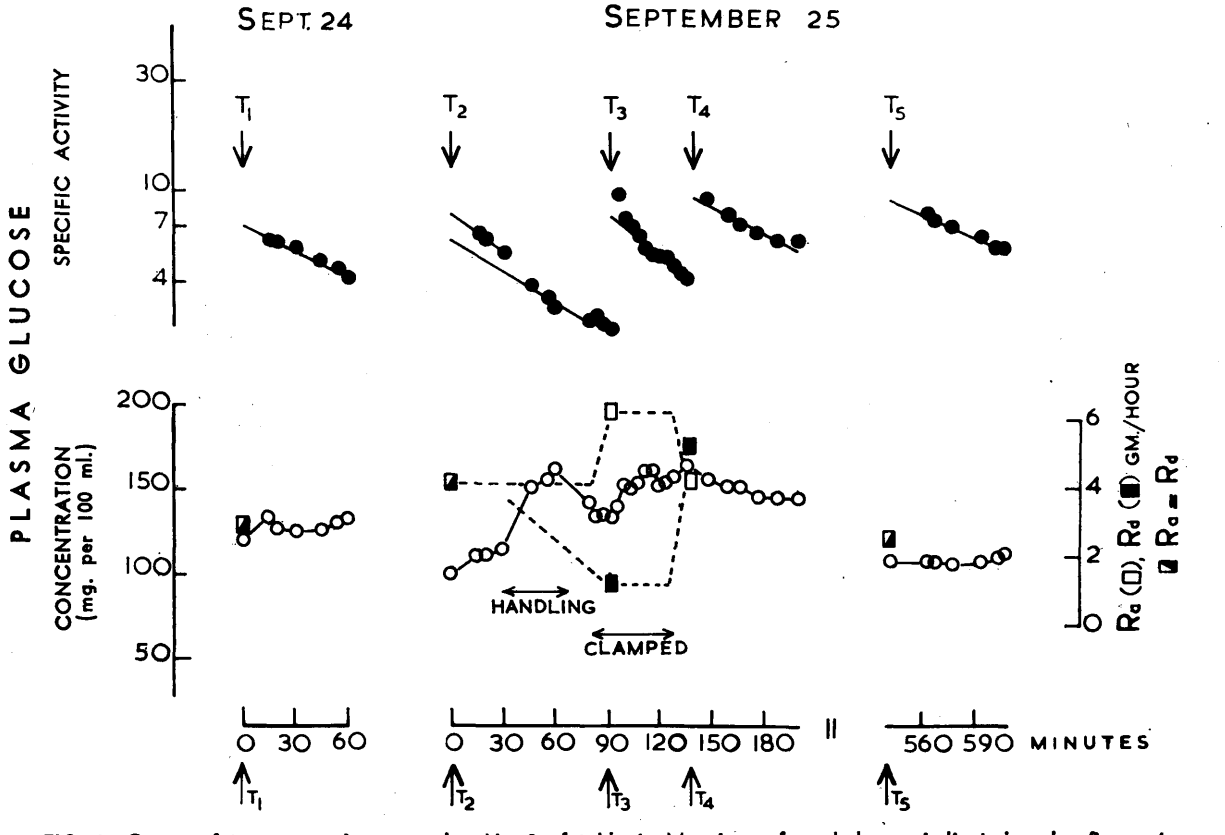

FIG. 6. Course of tracer experiment on dog No. 3 of table 1. Meanings of symbols are indicated under figure 4.

Clamping up to fifty minutes did not visibly alter the histological structure or staining reactions of either the exocrine or endocrine tissue in the pancreatic autograft, and both the remaining artery and vein in the pedicle to the graft were patent. While no recognizable histological changes were observed soon after clamping, both islet and acinar tissue atrophied within five months, leaving only scar tissue and resulting in fatal diabetes, even though small blood vessels in the pedicle had remained patent.[4]

DISCUSSION

The results of the experiments described above indicate that distinct very early effects of depriving the partially depancreatized dog of native insulin are both a marked increase in the rate of glucose appearance and a marked decrease in its rate of disappearance. By "very early" is meant: at times mainly within the four- to ten-minute range, but including as well values from individual experiments as long as thirteen and as short as one minute following total pancreatectomy. The earlier finding of an abrupt and very large decrease in the rate of glucose disappearance following pancreatectomy[1] has been confirmed and now has been observed as early as one minute following pancreatectomy.

These conclusions correspond well with those of Franckson et al.,[9] who studied the causes of the hyperglycemia produced in five out of seven normal anesthetized dogs following the intravenous injection of guinea pig anti-insulin serum. The reversals of the effects on rates of glucose production and utilization observed to follow unclamping of the pancreatic pedicle in our experiments again may be comparable to those which Franckson et al. observed following the intravenous injection of insulin to their anti-insulin serum treated dogs.

The marked increase in the absolute rate of appearance of glucose very soon after pancreatectomy has been observed in each of the three tracer experiments (table 1). A statistically significant correlation was observed between the maximum rise (H) in the fasting plasma glucose level caused by "handling" and the average rate of rise (Cl) in the plasma glucose concentration during the four- to ten-minute interval after clamping the pedicle (figure 3). While this effect of handling was very small in two of the tracer experiments (figures 4 and 5) it was present to a marked degree in the third. In this case (figure 6) it will be noted that the hyperglycemia of handling was accompanied by a significant depression in the corresponding specific activity of the plasma glucose. This depression indicates that the dog mobilized a quantity of unlabeled glucose during the interval when the hyperglycemia of handling appeared.

Such an abrupt brief increase in the rate of glucose appearance is indicative of glycogenolysis by the liver. In terms of this hypothesis, there would be a greater reserve for such glucose release following clamping in normoglycemic dogs than in those where handling caused hyperglycemia. This interpretation finds positive support in the significant *negative* correlation found in figure 3.

The above evidences of rapid insulin deprivation following total pancreatectomy find support in the comments of Izzo et al.[10] He has noted 95 per cent of iodinated plasma insulin in rats is removed, mainly by the liver, within eight minutes of its intravenous injection in physiologic amounts. Similarly, Soeldner[11] has noted that by *sixty seconds* after the end of an intravenous infusion of glucose immuno-reactive insulin has reached its maximum concentration in most normal human individuals.

A prompt transient increase in rate of glucose production following a single intravenous injection of an effective dose of guinea pig anti-insulin serum into the dog has been reported by Altszuler et al.[12] Wright[13] has suggested that this might be due to the remainder in the circulation of glucagon following the insulin inactivation. In this connection it is noted that the islets of Langerhans in the uncinate process of the dog are

TABLE 1

Tracer-determined rates of glucose appearance (R_a), accumulation (R_{acc}) and disappearance (R_d) in the fasting dog before and during insulin deprivation by clamping off the pancreatic autograft

Dog Number	Weight (kg.)	Time after clamp (min.)	R_a: (gm. glucose/hr.) Preclamp	R_a: (gm. glucose/hr.) Clamp	R_{acc}: (gm. glucose/hr.) Preclamp	R_{acc}: (gm. glucose/hr.) Clamp	R_d: (gm. glucose/hr.) Preclamp	R_d: (gm. glucose/hr.) Clamp	Plasma glucose level when clamped (mg./100 ml.)
1	7.2	1	1.7	3.0	+0.19	+2.1	1.5	0.9	123
2	15.7	13	2.3	5.3	−0.05	+4.4	2.4	0.9	110
3	19.8	10	4.2	6.3	−0.03	+5.1	4.2	1.2	140

said to be devoid of alpha cells.[14,15] From this it appears that the similar transient increase in the rate of glucose appearance which follows clamping in our experiments resulted specifically from the deprivation of insulin, and not from a residuum of circulating glucagon.

The immediate effects of *un*clamping the pedicle of the pancreatic autograft on subsequent insulin secretion would be complicated in these experiments if there were any changes in the insulin and insulin secreting mechanism caused by absence of circulation in the tissues of the autograft during the period of clamping. However, the experimental evidence of a prompt *reversal* upon unclamping of the changes caused by clamping in the rates of glucose appearance, accumulation and disappearance, together with lack of histological evidence of damage to the beta cells caused by the clamping, indicates that such damage was neither extensive nor permanent in these experiments. The *long*-term experimental findings are definite in that unclamping led after one day to restoration of the preclamping glucose tolerance. Within fifteen hours there was a restoration of the initial plasma glucose levels, and the rates of glucose appearance and disappearance had reverted to their common value observed prior to clamping. That there is a limit to the length of time during which the pancreas can be clamped without causing irreversible damage is illustrated in figure 1. Here a sixty-minute isolation resulted in diabetes while similar isolations for thirty-minute periods did not (figure 2).

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