Importance of Hepatic Portal Circulation for Insulin Action in Streptozotocin-Diabetic Rats Transplanted with Fetal Pancreases

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ABSTRACT The importance of the hepatic portal circulation in the response to insulin was assessed in streptozotocin-diabetic rats transplanted with syngeneic fetal pancreases. Partial reversal of diabetes was accomplished by transplantation of two or three fetal pancreases beneath the capsule of the kidney; complete reversal followed shunting of the venous drainage from the transplants to the liver. Plasma glucose after streptozotocin of 509±31 mg/dl (mean±SEM) fell after transplantation to 395±23 and after the shunt to 143±5 mg/dl. Urine volume fell from 84±4 to 50±5 ml/d and then to normal (17±1 ml/d) after the shunt. Glucose excretion which was 8.1±0.3 g/d after streptozotocin fell after transplantation to 4.8±0.3 g/d and after the shunt completely disappeared from the urine. The disappearance rate of glucose injected into the circulation, which was 0.50±0.07%/min in untreated diabetes, increased to 1.39±0.38%/min after transplantation and to 2.52±0.31%/min after the shunt, not different from normal controls (2.79±0.25). Plasma immunoreactive insulin (IRI) was below normal (25–35 μU/ml) and unresponsive to glucose in untreated diabetic rats. After transplantation IRI levels ranged from 73–223 μU/ml and there was no rise after glucose injection. After the shunt both the basal IRI (36±5 μU/ml) and the peak response to glucose at 10 min (58±7 μU/ml) were the same as in normal controls (42±4 and 62±7 μU/ml, respectively). The fall in IRI after the shunt is explained by increased extraction of insulin passing into the liver and also diminished secretion. After removal of the transplants plasma glucose and urine values returned almost to pretransplant levels.

Secretion of insulin by transplanted pancreases into the liver enhances the effectiveness probably by increased extraction and action and reveals the importance of the normal route for insulin delivery.

INTRODUCTION

Insulin released from the pancreas passes via the portal vein directly into the liver, which is a major site for control of blood glucose concentration. In the treatment of human diabetics insulin injected in the subcutaneous tissues of the extremities or the abdominal wall enters the general circulation and reaches the liver only after dilution in most of the blood volume. This raises the question of the effectiveness of insulin entering the circulation by these different routes. Attempts to compare the metabolic effects of insulin administered directly into the portal vein or into the peripheral blood in normal and diabetic humans and experimental animals have not clarified this question.

In considering the optimal site for implantation of fetal pancreases for reversal of streptozotocin-induced diabetes mellitus in the rat the venous drainage from the transplanted tissue becomes important. Transplantation of whole fetal rat pancreases beneath the kidney capsule of normal and diabetic rats has proved to be an excellent site for growth and development of the fetal organ because of rapid vascularization and easy accessability for implantation and removal (1, 2). Transplanted fetal pancreases rapidly develop into a relatively pure endocrine organ and under optimal conditions a single transplanted pancreas can reverse the diabetic state (3). The venous drainage from the transplants is mainly into the renal vein and thus into the inferior vena cava bypassing the liver (as confirmed

A preliminary report of some of these observations was presented at the 36th Annual Meeting of the American Diabetes Association in San Francisco, Calif., June 1967 and published in abstract form. 1976. *Diabetes.* 25(Suppl. 1): 338.

Received for publication 9 March 1979 and in revised form 20 August 1979.

by visual observation after injection of cardiac green dye into the renal artery [unpublished observation]). Response of the diabetic state in transplanted rats before and after diversion of the renal venous flow into the hepatic portal vein provided an opportunity to assess the importance of the portal route of insulin delivery under chronic conditions. Dramatic improvement in the diabetes after establishment of a renal-portal shunt supports the importance of the portal venous route for insulin delivery.

METHODS

Adult male Lewis rats weighing 250–350 g (Microbiological Associates, Walkersville, Md.) were made diabetic by rapid intravenous injection of streptozotocin (supplied by E. Dublin, Upjohn Co., Kalamazoo, Mich.) at a dose of 40–60 mg/kg body wt. Throughout the experimental period all urine was collected and measured daily and glucose content determined on a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, Calif.) (glucose oxidase). Weekly tail-blood glucose concentrations were measured by the same procedure.

Experimental design. The experimental plan was designed to provide data on the severity of the diabetic state during four phases of the experiment. The diabetic status was monitored by measurement of plasma glucose (9:00 a.m.), urine volume, urine glucose content, and body weight. At key periods the rate of disappearance from the blood of injected glucose and the plasma immunoreactive insulin response were measured. The duration of each phase was designed to permit stabilization of the diabetic state.

Phase 1, lasting about 15 d (range, 4–26), was the period from injection of streptozotocin until transplantation. Animals selected as fetal transplant recipients were those with plasma glucose greater than 460 mg/dl and urine volume greater than 70 mg/d. Transplantation of two or three fetal pancreases was designed to partially reverse the diabetic state (2). Phase 2, lasting an average of 32 d (range, 24–43), was the period after transplantation before the renal vein-portal vein shunt operation. This phase was continued until the diabetes achieved a stable state. Glucose disappearance rate and plasma insulin response to glucose injected via a tail vein was determined at the end of this period.

Phase 3, lasting ~41 d (range, 28–62), was the period after the shunt procedure until removal of the transplanted fetal pancreases. The second measurement of glucose disappearance rate and plasma insulin response to glucose injection using a tail vein was carried out after stabilization of the diabetes near the end of this period. For comparison the glucose injection study was repeated under nonstress conditions after 7 d (range, 4–14) using a cannula implanted the previous day into a carotid artery. The transplants were removed immediately thereafter.

Phase 4, lasting about 44 d (range, 36–53), was the period of observation of recurrence of diabetes after removal of the transplants until death of the rat or sacrifice.

Glucose disappearance rate and insulin response. Rats were fasted overnight, weighed, and restrained in a towel wrap. Blood samples were collected from a tail vein in 250 μl heparinized capillary tubes before, and at 10, 20, 30, and 40 min after rapid injection of D-glucose into a tail vein in a dose of 0.5 g/kg body wt. After separation of the plasma from the blood, glucose content was determined as above and the remaining plasma frozen for radioimmunoassay of insulin content using a rat insulin standard (Novo Industri, Copenhagen, Denmark). The carotid artery technique for determination of glucose disappearance rate and insulin response, without anesthesia or restraint, previously described (2), employs a polyethylene cannula implanted into a carotid artery under pentobarbital anesthesia the day before the study. Glucose is injected into the cannula and the blood samples obtained are identical to the tail vein method except for the blood vessel employed.

Transplantation of fetal pancreases and renal-portal shunt procedure. After streptozotocin injection and establishment of diabetes the rats were transplanted with pancreases removed from syngeneic fetal rats of 16.5 to 17.5 d gestation. Two to three fetal organs were placed beneath the capsule of the right kidney to partially reverse the diabetes (1). After stabilization of the diabetic state the renal-portal shunt was carried out.[1] In this procedure the end of the right renal vein is connected to the side of the portal vein lengthened by an interposed segment of vein from a syngeneic animal.

Controls. Rats of the same age and sex were made diabetic by injection of streptozotocin and tail vein glucose injection studies were carried out 30–45 d later. Normal rats of the same age and sex were subjected to the glucose injection study. The renal-portal shunt procedure was carried out on two severely diabetic rats. Mild diabetes was produced in four rats by injection of streptozotocin in a dose of 40–50 mg/kg. A glucose disappearance study was performed after 50 d and the renal portal shunt carried out within 2–5 d thereafter. After 30–40 d of observation a second glucose injection study was carried out.

RESULTS

Metabolic response to transplantation and the renal-portal shunt.

The response of plasma glucose, urine volume, and urine glucose content in one diabetic rat during the four phases of the experiment is shown in Fig. 1. During the 10-day period after injection of streptozotocin (50 mg/kg body wt) plasma glucose was 550 mg/dl (day 7), mean urine volume 105±4 ml/d (±SEM), and urine glucose excretion 8.3±0.7 g/d. After transplantation of three fetal pancreases beneath the capsule of the right kidney neutral protamine Hagedorn insulin was injected daily for 8 d in a dose of 2 U/d resulting in a transient response of the diabetic state. During the following 36-d stabilization period, before the shunt procedure, plasma glucose measured weekly was 458±17 mg/dl. The volume of urine decreased ($P < 0.01$) to 61±3 ml/d and urine glucose fell ($P < 0.01$) to 5.6±0.5 g/d. After the shunt the mean plasma glucose fell to normal (162±5 mg/dl) as did the urine volume (19±1 ml/d) and glucose completely disappeared from the urine. Removal of the transplants was followed by prompt recurrence of the diabetic state with values for plasma glucose (512±21 mg/dl), urine volume (94±4 ml/d), and urine glucose (7.6±0.9 g/d) reaching pretransplant levels.

The response of the diabetic state in eight rats as measured by plasma glucose, urine volume, and glucose excretion in the urine are shown in Fig. 2. Plasma

[1] Mullen, Y. Manuscript in preparation.

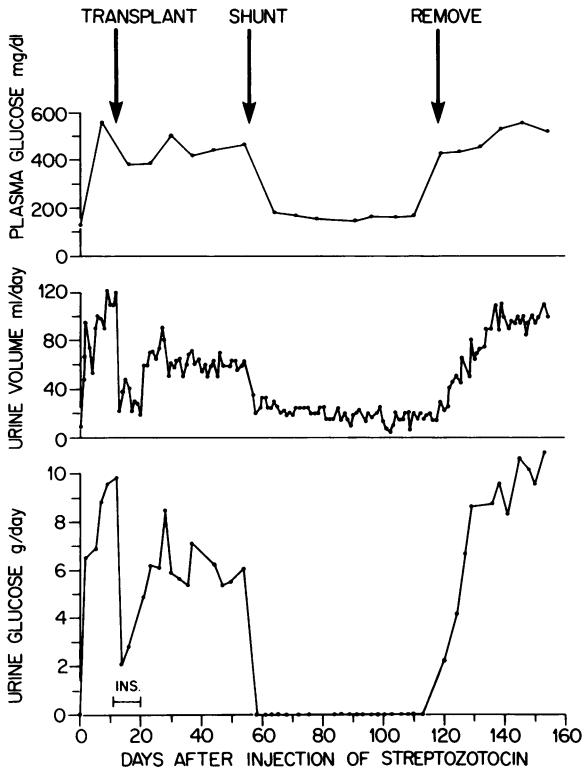

FIGURE 1 Response of a representative diabetic rat, 12 d after streptozotocin, to transplantation with three fetal pancreases followed by injection of neutral protamine Hagedorn insulin 2 U/d for 8 d. At 44 d after transplantation a renal-portal vein shunt was constructed. The transplants were removed 62 d later.

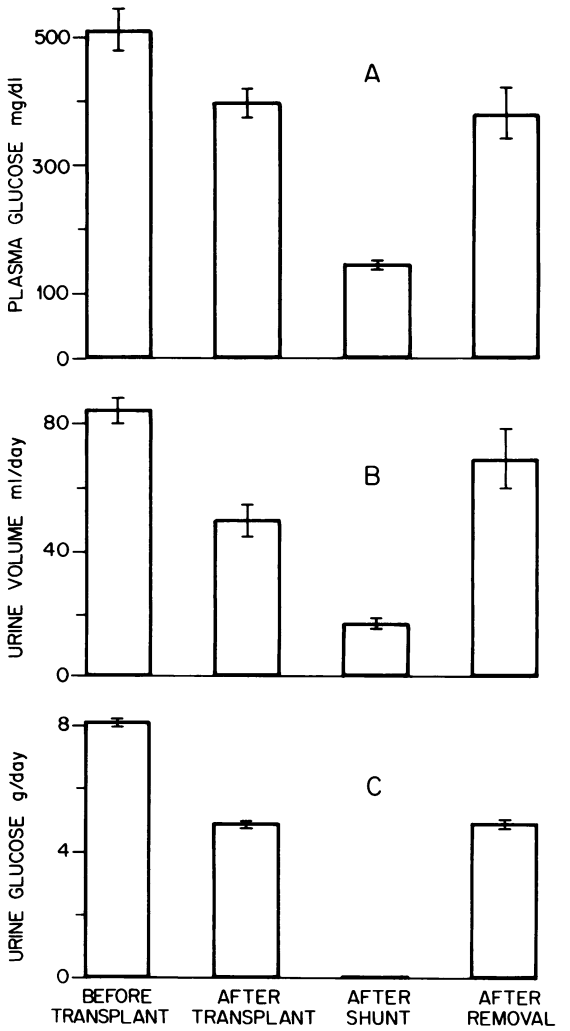

FIGURE 2 Plasma glucose (A), urine volume (B), and urine glucose (C) of eight streptozotocin diabetic rats. Mean (±SEM) values are given during four phases of the experiment: (phase 1) before transplantation of two to three fetal pancreases, (phase 2) after transplantation before the renal-portal vein shunt, (phase 3) after the shunt, and (phase 4) after removal of the transplants.

glucose measured at 9:00 a.m. on ad lib diet was greatly elevated before transplantation and fell ($P < 0.01$) gradually after transplantation of two or three fetal pancreases but remained above normal. The response to the renal-portal venous shunt was rapid and dramatic; mean plasma glucose fell to a normal level between 2 and 3 wk after the shunt. After removal of the transplants hyperglycemia reappeared in all rats and the blood glucose reached a mean value of 378±41 mg/dl, below ($P < 0.05$) the pre-transplant level (509±31 mg/dl).

Urine volume fell ($P < 0.01$) after transplantation of two to three fetal pancreases but remained threefold elevated. After the renal-portal shunt urine volume fell to normal within 2–4 d in most rats. The longest time required to reach a normal urine volume was 17 d. After removal of the transplants urine volume increased within 1–3 wk to a level not significantly different from the pre-transplant level. Urine glucose, which was 8.1 ±0.3 g/d after streptozotocin injection fell ($P < 0.01$) to approximately one-half after transplantation and completely disappeared from the urine after the shunt procedure in all rats. In six rats glucose disappeared from the urine in 2–4 d, but required 15 d in one rat and 20 d in another. After removal of the transplants glucose reappeared in the urine on the 2nd or 3rd d after removal, which was the 1st d of urine collection. Urine glucose reached a plateau within 3–6 d in most rats, and by 12 d in all, reaching a mean value of 4.9±1.2 g/d, not returning ($P < 0.05$) to the pre-transplant level (8.1±0.3 g/d).

Body weight during phase 2, the interval from transplantation to the shunt procedure, increased 0.58±0.13 g/d and during phase 3 after the shunt procedure 0.89 ±0.15 g/d. After removal of the transplants there was a loss in weight of 0.31±0.25 g/d.

Glucose disappearance rate. The fasting plasma glucose concentration in tail vein blood before and

after glucose injection in a group of four diabetic controls and eight rats after transplantation and again after the renal-portal shunt and in four normal controls are given in Table I. Compared with the diabetic controls the fasting and post-injection blood glucose levels of the transplanted rats are much lower ($P < 0.01$). Similarly the disappearance rate of glucose (Table I) after transplantation (1.39±0.38%/min) is significantly improved ($P < 0.02$) over the diabetic controls (0.50 ±0.07%/min), but remains below ($P < 0.01$) the normal value. The shunt procedure is followed by completely normal fasting and post-injection glucose levels and a significant improvement ($P < 0.05$) in the disappearance rate of glucose from blood (2.52±0.31%/min), which is not different from the rate in normal control rats. The glucose levels in the same eight transplanted rats using the carotid artery technique and performed after the post-shunt tail vein study are shown for comparison because this technique does not require restraint of the rat. There is no difference in the fasting glucose levels in the blood nor in the disappearance rate of glucose when the tail vein and carotid artery samples are compared and the values do not differ from those obtained in normal rats by the tail vein route.

Immunoreactive insulin levels (IRI)[2] in blood. The IRI content of blood samples obtained before and after glucose injection are given in Table II. Blood obtained from diabetic control rats contained IRI in the low normal range despite plasma glucose of nearly 500 mg/dl. After glucose injection, which raised plasma glucose to over 600 mg/dl, there was no rise in plasma IRI content. In contrast the mean basal IRI content in the blood of fasted transplanted rats was almost 10 times as high as the diabetic control animals but again there was no rise after glucose injection; the level fluctuated widely but remained elevated throughout the 40-min collection period. A repeat tail vein study on the same rats after the shunt procedure and normalization of glucose values revealed a dramatic fall in both basal and stimulated plasma IRI levels to those characteristic of normal controls. There is no difference between the post-shunt IRI levels in the diabetic rats compared to normal rats when measured by the tail vein method. By comparison, the IRI concentration in blood obtained from the carotid artery of shunted rats 10 min after glucose injection (93±10 μU/ml) is significantly higher ($P < 0.02$) than the tail veil level (58±7 μU/ml) but the IRI values are not significantly different before glucose injection and after the 10 min sample.

Renal-portal shunt in diabetic controls. The shunt procedure did not affect the diabetic state in two severely diabetic rats or in four mild diabetic rats observed for 50 d before and 30–40 d after the shunt. In the latter animals the disappearance rate of glucose was the same before (1.35±0.31%/min) as 30–40 d after (1.03±0.23%/min) the shunt and the IRI response was not different in the two studies.

DISCUSSION

These studies demonstrate that shunting the venous drainage of pancreases transplanted under the kidney capsule from the inferior vena cava to the hepatic portal vein reverses to normal all measured parameters of the diabetic state. These include plasma glucose, the rate of glucose disappearance from blood after injection, and the IRI levels in blood before and after glucose

[2] *Abbreviation used in this paper*: IRI immunoreactive insulin.

TABLE I

Plasma Glucose and Disappearance Rate after Injection in Streptozotocin Diabetic Rats Compared with Normal Controls

	Diabetic				Normal control
	Tail vein			Carotid	
After glucose injection	Control ($n = 4$)	After transplant ($n = 8$)	After shunt ($n = 8$)	After shunt ($n = 8$)	Tail vein ($n = 4$)
min			*mg/dl**		
0	471±5	136±12	107±25	120±5	106±7
10	621±18	324±19	253±31	244±16	259±10
20	580±9	283±25	186±31	177±15	169±4
30	560±7	247±29	151±25	146±10	151±2
40	539±7	220±25	139±19	129±7	133±10
disappearance rate, *%/min*	0.5±0.07	1.39±0.38‡	2.52±0.31§	2.36±0.16	2.79±0.25

* Glucose, mean±SEM.

‡ Significantly different from diabetic control ($P < 0.02$).

§ Significantly different from diabetic controls ($P < 0.01$), and from results before shunt ($P < 0.05$).

TABLE II

Plasma Immunoreactive Insulin in Streptozotocin Diabetic and Normal Rats before and after Injection of Glucose

After glucose injection	Diabetic: Tail vein: Control (n = 4)	Diabetic: Tail vein: After transplant (n = 8)	Diabetic: Tail vein: After shunt (n = 8)	Diabetic: Carotid: After shunt (n = 8)	Normal control: Tail vein (n = 4)
min			*μU/ml**		
0	27±6	223±68	36±5	54±9	43±4
10	31±4	91±16	58±7	93±10‡	62±7
20	35±8	152±65	55±4	56±10	54±9
30	30±5	73±21	49±5	57±7	48±7
40	29±5	90±19	51±6	41±8	39±6

* Immunoreactive insulin, mean±SEM.

‡ Significantly different from 58±7 ($P < 0.02$).

injection. Urine volume and glucose content became normal and body weight gain increased. Since the rats were maintained in a stable diabetic state for an average of 32 d before the shunt procedure, it seems reasonable that insulin synthesis and secretion were stable before the shunt. The shunt procedure did not alter the diabetic state in nontransplanted severely or mildly diabetic rats and did not change the glucose disappearance rate or insulin response in the mild group. Insulin entering the portal vein after the shunt is diluted in a smaller volume of blood and thus reaches the liver in a higher concentration. Because the liver extracts 40–50% of insulin reaching the organ in each circuit (4), it follows that more insulin is extracted by the liver after the shunt procedure.

A comparison of the effects of long-term secretion of insulin into the peripheral vs. the portal circulation has not previously been made. Short-term experiments by Madison and Unger over 20 yr ago comparing the effects of equal amounts of insulin injected into the portal vein or a peripheral vein of anesthetized dogs revealed a similar fall in arterial glucose concentration (5). Because peripheral insulin injection resulted in a greater arterio-venous glucose gradient across the lower limb than intraportal insulin, it was concluded that intraportal insulin produced a greater effect on hepatic glucose release but a smaller effect on peripheral glucose uptake. Support for this thesis derived from their observations that in rats 51% (6) and in humans 54% (7) of ^{131}I-labeled insulin injected into the portal vein was extracted by the liver after a single transhepatic circuit compared to only 27% in rats after peripheral injection. Variation of the rate and dose of peripheral administration of insulin suggested that the liver is more sensitive to insulin than the peripheral tissues (7).

Similar conclusions were drawn by Cheng and Kalant (8) from studies in normal human subjects using a constant infusion technique of [6-^{14}C]glucose. It was observed that insulin injected intravenously at a dose below 0.05 μU/kg body wt caused a lowering of arterial blood glucose by decreasing the inflow of glucose from the liver into the blood but did not affect outflow of glucose from blood. At higher doses insulin caused hypoglycemia by both effects; decrease in the rate of inflow from the liver and an increase in outflow from the blood into the tissues. These studies suggested to them that the balance of glucose across the liver is sensitive to small concentrations of insulin that do not affect glucose uptake by peripheral tissues. Support for this thesis was provided by Felig and Wahren (9) who found that infusion of glucose at 2 mg/kg per min into normal subjects resulted in an 85% reduction in splanchnic glucose production without a significant increment in peripheral glucose use despite a doubling in mean peripheral insulin concentration. However Felig (10) concludes from these studies that the apparent greater responsiveness of the liver to small changes in insulin may not reflect an inherently greater sensitivity of the liver cell but a consequence of the higher level of endogenous insulin in the portal circulation. In human subjects Blackard and Nelson (11) found that the ratio of portal to peripheral IRI levels was 2 in the basal state and rose to 10 immediately after injection of glucose exposing the liver cells to much more insulin than is apparent from peripheral concentrations.

IRI was not completely absent from the blood of the untreated diabetic rats in the present study but the level was fixed and unresponsive to glucose injection suggesting that in the diabetic rat a small amount of remaining beta cells were functioning at a maximal rate in response to markedly elevated blood glucose values. Previous measurements of the IRI content of rat pancreases 28 d after injection of streptozotocin in

the dose used here revealed only 2.5–10% of the normal content and plasma IRI levels similar to those found here (12). After transplantation of fetal pancreases and amelioration of the diabetic state, the IRI content of tail vein blood was approximately fivefold higher than normal but did not rise after glucose injection. The level fluctuated widely during the 40 min after glucose injection but remained two to three times above the IRI concentration in the tail vein blood of normal rats. An explanation for only partial reversal of the diabetes despite elevated peripheral IRI levels is provided by comparision with IRI levels in the portal vein of normal rats. Measurement by Misbin et al. (13) of the concentration of IRI in the portal vein of 14 rats in the post-absorbtive state was 115 μU/ml and the levels rise ~10-fold after glucose infusion. Thus the liver of normal rats is perfused in the fed state with blood containing more insulin that the livers of these transplanted rats before the shunt procedure.

After the shunt procedure and a period of stabilization at normal blood glucose levels the basal IRI content of tail vein blood fell to normal, and there was a normal IRI response to glucose stimulation consistent with the normal fasting glucose concentration and glucose clearing rate. In considering the explanation for the decrease in basal and glucose-stimulated IRI content of blood after the shunt procedure, increased extraction of insulin by the liver must be considered. Calculation of this effect using the model proposed by Bucolo (14) and the blood flow data of Lucas et al. (15) will account for only a 53% fall in peripheral (tail vein) insulin concentration, far less than the observed change. It seems likely that the fall in IRI level after the shunt procedure is mainly caused by decreased insulin release subsequent to a fall in blood glucose to normal. The normal rise in IRI after glucose injection suggests a reserve storage of insulin in the beta cells in contrast to the absence of a rise before the shunt.

Among the other hormones secreted by the pancreas, glucagon must be considered because alpha cells are present in the fetal pancreas transplanted beneath the kidney capsule (2). Glucagon was not measured in the studies reported here but McEvoy and Hegre (16) using the same system found plasma glucagon to return to normal in diabetic rats after reversal of diabetes. It seems unlikely that the transplanted tissue releases a significant amount of glucagon since alpha cell mass in the transplant declines from 46% of the total cell mass in the fetal organ to 9% at 8 wk after transplantation and increases only fourfold in total mass. Beta cells become predominant during this growth period increasing 60-fold and occupy 89% of the total cell mass. The insulin content of the transplant increases 560-fold in contrast to only an 18-fold increase in glucagon. A similar relative decline in D cell mass from 8 to 0.9% of the total was observed during 8 wk of growth, the D cell mass increasing only fivefold in contrast to the large increase in beta cells.

To determine if the restraint necessary to obtain blood samples and inject glucose via the tail vein affected the glucose or IRI values a non-stress technique (carotid artery cannula) was performed in all transplanted animals. It has been demonstrated that restraint inhibits the rise in insulin and the rate of clearance of glucose after injection (17) and this effect of stress appears to be greater in rats bearing denervated transplanted islets (18). Because the clearance rate of glucose was the same in the study performed with restraint and that performed without restraint, the stress of the towel-wrap did not affect glucose clearance. However the peak level of IRI reached at 10 min after glucose injection in the nonstressed group was significantly higher than the restrained (stressed) group. This difference may be attributed to the alpha adrenergic inhibition of insulin secretion by circulating catecholamines (18).

After removal of the transplants, diabetes promptly recurred in all animals. The severity of the diabetic state did not return completely to the pre-transplant level although the differences are of borderline significance. Partial recovery of beta cell function in islets not totally destroyed by streptozotocin after a period of amelioration of diabetes from transplantation has been recently demonstrated (19) and could explain these findings.

These observations emphasize the important role of the hepatic portal circulation for delivery of insulin to the liver and the key role of the liver in control of the blood sugar. It is not now possible to replace insulin in human beings with insulin-deficient diabetes by this route and insulin injections may result in chronic exposure of peripheral tissues to excess insulin. In considering alternative methods for replacement of insulin in diabetic patients such as transplantation or artificial devices, attention to the site of insulin delivery is important.

ACKNOWLEDGMENTS

The technical assistance of Jean Slucher and secretarial support of Pam Meyers are appreciated. James S. Whiting, Ph.D. kindly provided the model and calculations for hepatic insulin extraction after the shunt procedure.

Grant support was provided by the National Institutes of Health (AM 17980 and AM 20827), The American Diabetes Association, and the Kroc Foundation.

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