Syngeneic Transplantation of Fetal Rat Pancreas

I. Effect of Insulin Treatment on the Reversal of Alloxan Diabetes

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SUMMARY

Sixty-nine alloxan-diabetic male Fischer rats received syngeneic transplants of eight 18-days-postcoitum fetal pancreases at the renal subcapsular site. One half of the recipients were given 2 to 4 U. protamine-zinc insulin for seven days immediately after transplantation. This insulin-treatment regimen effectively normalized blood glucose rapidly. Forty-seven transplant recipients survived, and diabetes was reversed in all. Insulin treatment had no effect on recovery time or glucose tolerance. Those animals requiring longer periods to reach normoglycemia had impaired glucose tolerance. Some insulin-treated recipients returned to normoglycemia rapidly while others required an extended period. Those animals that showed rapid reversal exhibited elevated concentrations of plasma insulin both in the fasting state and during glucose tolerance tests. No pretransplant parameters could be identified as predictors of rapid reversal. DIABETES 27:982-87, October, 1978.

INTRODUCTION

Although pancreatic transplantation to diabetics was suggested as a possible cure in 1902,[1] only in the last few years has transplantation of the endocrine pancreas successfully reversed experimental diabetes. Because of the significant immunologic problems of rejection, long-term reversal of experimental diabetes by transplantation has only been possible in highly inbred, isogenic strains of laboratory rodents. Many different techniques, tissue preparations, and sites have been used; these have been reviewed in detail recently.[2,3] Transplantation of fetal pancreas at the renal subcapsular site has successfully ameliorated experimental diabetes.[4-7] These reports have established that fetal pancreas is an excellent source of donor tissue. The technique has proved to be a valuable research tool for several reasons. First, only small amounts of fetal pancreas are required. It has been presumed that the inherent growth potential of the fetal tissue allows proliferation of the beta cells with eventual amelioration of the diabetes. Secondly, no pretreatment of the tissue or islet isolation is required. It has been demonstrated in organ culture of fetal pancreas that the exocrine tissue degenerates and the islet portion of the pancreas persists and in some cases proliferates.[8,9] Lastly, the kidney site is readily accessible for both the initial surgery and the subsequent removal of the entire mass of donor tissue. Easy removal of the donor tissue by unilateral nephrectomy allows confirmation of the efficacy of the transplanted tissue in the reversal of the diabetes. Removal of the entire mass of transplanted tissue also permits morphometric and hormonal analysis of the tissue to assess growth and differentiation of the islet cells during the transplant period. The venous drainage from the kidney site is into the systemic circulation, while that of the pancreas is into the liver via the portal vein. In spite of the nonorthotopic hormonal secretion, other studies have demonstrated that pancreatic transplants at the kidney are capable of maintaining normal glucose homeostasis.[5-7]

The present study details the effect of isogenic fetal pancreatic transplantation on reversal of alloxan-induced diabetes in the rat with or without an initial posttransplant period of insulin therapy. Subsequently, morphologic and hormonal analysis of the

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transplanted donor pancreas will be reported. Preliminary results of this work have been published.[7]

MATERIALS AND METHODS

Inbred Fischer-344 rats (ARS/Sprague-Dawley) were used. All transplant recipients were male rats made diabetic by a single intravenous injection of alloxan (2 per cent aqueous solution, 32 mg. per kilogram body weight) at about six weeks of age. Diabetes (blood sugar, 350 mg. per deciliter) was established for at least six weeks before transplantation. Donor pancreas was obtained aseptically from 18-days-postcoitum fetuses. The age of the fetuses was determined from the time of witnessed matings; the day after mating was designated day 1 postcoitum. The left renal subcapsular site was used for transplantation. Eight whole fetal pancreases were implanted in each of the recipients without prior segmentation. An 18-day fetal pancreas has a wet weight of about 1 mg.

Transplant recipients were randomly separated into two groups. The first group received no treatment (control). The second group received seven daily injections of 2 to 4 U. protamine-zinc insulin (Lilly) beginning the day after transplantation (insulin treated).

All transplanted rats were followed by weekly determinations of body weight, blood glucose, and urine glucose. Reversal of the diabetic state was arbitrarily defined as a postprandial blood glucose less than 100 mg. per deciliter. This was accompanied by aglycosuria and was always preceded by significant weight gain. Four to six weeks after the establishment of such normoglycemia, each rat was subjected to an intravenous glucose tolerance test (3 gm. per kilogram body weight). Samples were obtained for blood glucose and plasma insulin determinations from unanesthetized animals via tail vein–bleeding according to a previously established protocol.[10] The glucose tolerance was expressed as the index of diabetes (I_D)[11]

$$I_D = \frac{\text{blood glucose (60 min.)}}{\text{mean normal blood glucose (60 min.)}} \times \frac{\text{blood glucose (120 min.)}}{\text{mean normal blood glucose (120 min.)}}$$

(Normal range = 0.5 to 2.0).

Assays

Blood glucose was estimated by the method of Hoffman.[12] Urine glucose was measured by the method of Somogyi.[13] Plasma insulin was determined by the two-antibody method of Morgan and Lazarow[14] using guinea pig anti-insulin antiserum, crystalline rat insulin standards (Novo), and ^{125}I-porcine insulin (New England Nuclear). Precipitating antibody (goat anti–guinea pig gamma globulin serum) was purchased from Antibodies, Inc. All samples were diluted to that portion of the standard curve where crystalline rat insulin standards and pancreatic tissue extracts give comparable results.[8,15]

RESULTS

The effects of transplantation of fetal pancreas on reversal of alloxan diabetes are summarized in table 1. Five of 35 animals in the control group died before amelioration: Four of the five died within 48 hours of surgery; the fifth animal, although gaining weight, died apparently from a respiratory infection four weeks after transplantation. One of 34 animals treated with insulin died of undetermined cause within 24 hours after surgery. Eight animals in each of the two groups were killed 15 days after transplantation for morphologic study of the transplanted tissue. None of these 16 animals was normoglycemic at the time of killing. All the remaining animals in both groups eventually returned to normoglycemia. Thus, diabetes was reversed in 100 per cent of the animals regardless of control or insulin treatment.

TABLE 1

Summary of experimental groups

Group	Number of animals			
	Total	Reversed	Killed before reversal	Died
Control	35	22	8	5
Insulin-treated	34	25	8	1

The regimen used for insulin treatment was effective in establishing and maintaining normoglycemia during the treatment period. As shown in figure 1, the blood glucose decreased to within the normal range after the second injection and remained normal for the seven-day period. As illustrated in the insert, the blood glucose was maintained within a narrow range throughout the entire day. All animals were aglycosuric after two days of insulin treatment.

Table 2 summarizes clinical parameters from the animals before transplantation. There were no statistical differences in any of these parameters between the control and insulin-treated groups before transplantation. On the basis of the period of time to reversal, the insulin-treated group tended to separate into two subgroups: those animals that reached normoglycemia

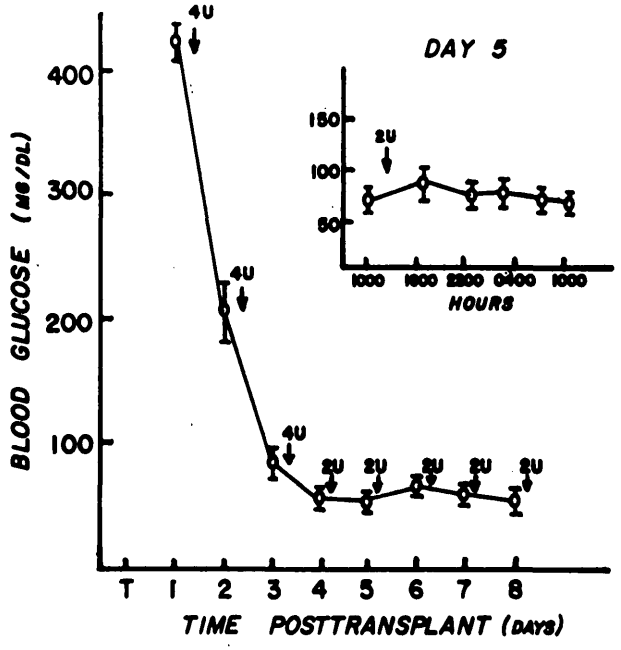

FIG. 1. Normalization of blood glucose in transplant recipients (N = 20) during the seven-day period of insulin treatment. Blood glucose returned to the normal range after two daily insulin injections were given, and it remained normal for the rest of the period. The insert illustrates the control of blood glucose during the fifth day of insulin treatment (N = 5). The blood glucose was stable over the 24-hour observation period.

within four weeks posttransplant (rapid response) and those that required a longer period (8 to 42 weeks—slow response). The animals that responded more rapidly to the transplant had a significantly greater pretransplant body weight and showed a slight increase in weight between alloxanization and transplantation. In addition, they exhibited a slightly greater degree of glycosuria than did the slow-response animals before transplantation. The rapid-response animals gained weight more rapidly than did the slow-response animals (figure 2) and reached normal weight for age by three months posttransplantation. The slow-response animals never reached normal weight.

No clearly temporal dividing line was apparent for the control-transplanted animals. For the purpose of comparison, the group was arbitrarily divided into those animals that responded in a shorter time than the mean and those that required a longer time to reach normoglycemia; there were no differences in any pretransplant parameter between these two subgroups. As a whole group, the control animals exhibited a steady weight gain (figure 2), reaching normal weight for age by about seven months after transplant.

To assess the degree of normalization of glucose homeostatic regulation after transplant, all reversed animals were given an intravenous glucose tolerance test (3 gm. glucose per kilogram body weight); the results are summarized in table 3. Again, no significant differences related to length of time required for reversal were detected within the control transplant group. Although the disposal rate of glucose as estimated by the index of diabetes was similar between the control and insulin-treated groups, those animals treated with insulin showed significant hyperinsulinemia both in the fasting state and at the peak concentration of insulin measured during the glucose challenge. After separation of the insulin-treated animals by length of time to recovery, it was seen that only the animals that ameliorated more rapidly exhibited this exaggerated insulin response to glucose challenge. The slow-response animals had a signifi-

TABLE 2

Summary of pretransplant parameters of glucose homeostasis in transplant animals

Group	N	Weeks after alloxan	Blood glucose (mg./dl.)	Urine glucose (gm./24 hr.)	Body wt. (gm.)	Weight change after alloxan (gm.)	Weeks to reversal
Control							
All	22	8.1 ± 0.3‡	396 ± 12	7.1 ± 0.2	170 ± 5	0 ± 4	11.4 ± 0.9
Rapid response*	14	8.0 ± 0.5	393 ± 18	7.1 ± 0.3	174 ± 6	+2 ± 4	8.6 ± 0.7//
Slow response	8	8.2 ± 0.5	400 ± 16	7.2 ± 0.3	164 ± 8	−6 ± 5	15.3 ± 0.8
Insulin-treated							
All	25	7.7 ± 0.5	424 ± 12	6.9 ± 0.3	170 ± 6	+2 ± 5	13.1 ± 3.6
Rapid response†	10	7.0 ± 0.6	420 ± 21	7.8 ± 0.3§	193 ± 10//	+12 ± 6§	1.9 ± 0.4//
Slow response	15	8.9 ± 0.9	426 ± 21	6.3 ± 0.4	154 ± 4	−5 ± 5	21.0 ± 3.1

*Rapid response indicates those animals that ameliorated more quickly than the mean; slow response took a longer time than the mean.
†Rapid response indicates those animals that ameliorated in less than four weeks; slow response took more than eight weeks.
‡Mean ± S.E.M.
Statistical analysis: Control (all) versus insulin treated (all), not significantly different $2p > 0.05$. Rapid versus slow response, §$2p < 0.05$, //$2p < 0.001$. All others not significantly different (Student's t-test).

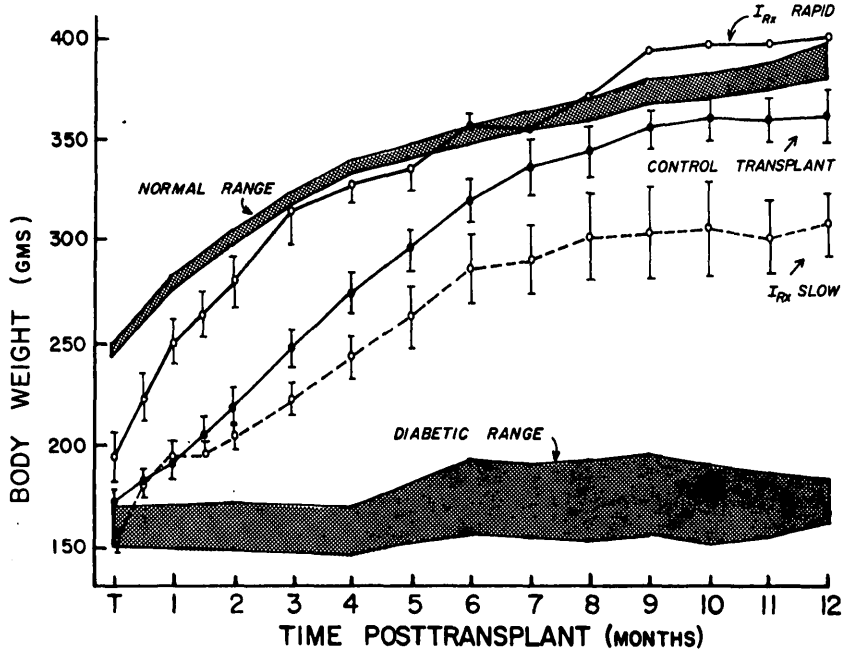

FIGURE 2

The pattern of weight gain of the transplant recipients compared with that of normal and diabetic animals. The rapid-response, insulin-treated animals reached normal weight for age by three months posttransplant. The control recipients required seven months to reach normal weight. The slow-response, insulin-treated animals never reached the normal weight for age.

cantly higher index of diabetes compared with the rapid-response animals.

In both the transplant groups there was a correlation between the length of time to recovery and the glucose tolerance (correlation coefficient r = 0.79). There was no significant difference between the control and insulin-treated groups in this regard. As illustrated in figure 3, those animals that required longer than 14 to 18 weeks to reach normoglycemia tended to have an index of diabetes in the subdiabetic range (I_D = 2.0 to 5.0), while those animals that ameliorated more rapidly tolerated the glucose challenge normally (some of these animals actually responded better than normal).

DISCUSSION

Fetal pancreas transplanted to the renal subcapsular site was effective in restoration of normoglycemia in moderately to severely diabetic rats. There was no difference between the control and insulin-treated groups (100 per cent reversal in both groups). All

TABLE 3
Summary of glucose tolerance in transplant animals after reversal of diabetes

Group	N	Glucose tolerance: Fasting blood glucose (mg./dl.)	Index of diabetes	Fasting plasma insulin (μU./ml.)	Peak insulin during GTT (μU./ml.)
Control transplants					
All	22	69 ± 3‡	2.2 ± 0.4	19 ± 2	64 ± 7
Rapid response*	14	68 ± 3	1.8 ± 0.4	21 ± 3	72 ± 9
Slow response	8	74 ± 4	3.2 ± 0.6	17 ± 1	49 ± 8
Insulin-treated transplants					
All	25	70 ± 3	1.9 ± 0.5	28 ± 4§	179 ± 45//
Rapid response†	10	70 ± 4	0.5 ± 0.1††	32 ± 5**	292 ± 68††
Slow response	15	71 ± 4	3.7 ± 0.8	17 ± 2	39 ± 8
Normal	6	66 ± 4	0.5–2.0	21 ± 7	78 ± 10
Diabetic	10	399 ± 29	7.4 ± 1.2	6 ± 3	20 ± 9

*Rapid response indicates those animals that reversed more quickly than the mean; slow response took longer than the mean.
†Rapid response indicates those animals that reversed in less than four weeks; slow response took more than eight weeks.
‡Mean ± S.E.M.
Statistical analysis: Control (all) versus insulin treated (all), §2p < 0.05, //2p < 0.02. Rapid versus slow response, **2p < 0.01, ††2p < 0.001. All others not significantly different 2p > 0.05 (Student's *t*-test).

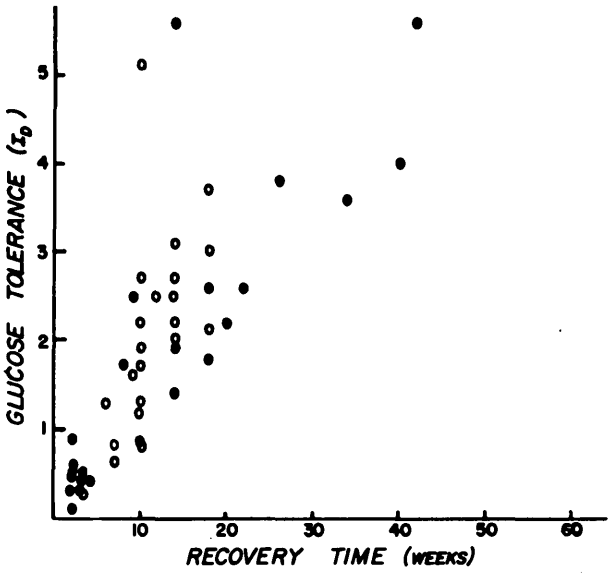

FIG. 3. Correlation between recovery time and glucose tolerance as measured by the index of diabetes (I_D). There was no effect of insulin treatment. The animals that required longer than 14 to 18 weeks to reach normoglycemia had subdiabetic glucose tolerance curves (correlation coefficient r = 0.79; N = 47). Open circles: control recipients; closed circles: insulin-treated recipients.

animals that underwent confirmatory nephrectomy rapidly returned to hyperglycemia after removal of the graft-bearing kidney. Certain differences between these experimental groups could be noted, however: The control group was homogenous, with relatively little variation in time to recovery and glucose tolerance within the group. Fasting insulin and insulin response to glucose load were normal. Insulin treatment, in contrast, resulted in two clearly distinct subgroups. The first showed rapid return to normoglycemia and normal glucose tolerance. In fact, six of these animals never returned to hyperglycemia after the seven days of insulin treatment. As a group, these animals showed catch-up weight gain and, by three months after transplantation, their body weight was similar to age-matched normal rats. The normal control of glucose homeostasis was associated with elevated plasma insulin concentrations, particularly the maximum plasma insulin during the glucose tolerance test. The nonorthotopic venous drainage from the transplant site could account for, at least, part of the elevation in plasma insulin. Human beings who have undergone portacaval vascular diversion for hypercholesterolemia exhibit slight elevation of plasma insulin, particularly during glucose tolerance tests.[16] It has also been noted that rats receiving intraperitoneal transplantation of isolated islets showed hyperinsulinemia (and hyperglucagonemia) after amelioration of their diabetes.[17] Alternatively, the hyperinsulinemia of the rapid-response, insulin-treated animals may have been due to inappropriate secretion, although it was not associated with hypoglycemia if this was the case.

The slow-response, insulin-treated animals achieved normoglycemia but never attained normal weight. Although there was considerable variability, their glucose tolerance tended to be in the subdiabetic range and their insulin response to glucose was diminished compared with normal animals or with the control-transplanted animals.

The differences in response between the two insulin-treated subgroups cannot be explained adequately with the available data. The rapid-response animals had a greater pretransplant body weight and gained weight between alloxanization and transplantation. These factors would indicate that the severity of their diabetes was less than that of the slow-response animals. The similar blood glucose and the elevated level of glycosuria in the rapid-response group would not support that hypothesis. There was also no difference in the control of blood glucose during the seven-day period of insulin treatment. Although some undetected variability in the quality of fetal tissue might account for differences, the same variability should have been seen in the noninsulin-treated control group but was not.

In summary, experimental diabetes in the rat can be reversed readily by transplantation of fetal pancreas. Insulin treatment during the initial week after transplantation did not affect the aggregate success rate and it increased the variability of response by the diabetic recipients. However, insulin treatment did result in a rapid reversal in some animals, and this beneficial effect has become a focus of our continuing interest in pancreatic transplantation.

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