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Research Article

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Blood Flow Measurements in Autotransplanted Pancreatic Islets of the Rat

Impairment of the Blood Perfusion of the Graft during Hyperglycemia

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Abstract

No information is available on the rate of blood flow in transplanted islets. In this study, adult rats were partially depancreatized, and islets from the excised pancreas were then isolated, maintained for 7 d in tissue culture, and subsequently transplanted back to the animal, beneath the renal capsule. Some rats were rendered diabetic with streptozotocin before transplantation. A month after transplantation the blood flow of the grafts was measured by a microsphere technique. Autotransplantation to streptozotocin–diabetic rats of ~ 500 islets did not revert the hyperglycemia, and the blood flow of these grafts was ~ 25% of that in the normoglycemic–transplanted rats. However, in insulin-treated diabetic rats the blood flow of the pancreatic graft was similar to that in the normoglycemic rats. The present results suggest that the blood flow in transplanted islets is markedly diminished by hyperglycemia and that this can be enhanced by insulin administration.

Introduction

Since the first report in 1972 on transplantation of isolated pancreatic islets (1), a large number of reports have confirmed that this is an efficient method of curing experimental diabetes (for a review see Sutherland [2]). Since the implanted islets have been found to revert the hyperglycemia of diabetic animals within a few days and then maintain normoglycemia, it has been assumed that they are rapidly vascularized upon implantation. Although it is likely that revascularization of the grafted islets is of crucial importance for their long-term function in the recipient, there is no information on the blood flow through transplanted islets. Likewise, the possible effects of the diabetic state on the blood supply to the grafted islets remain unknown. The aim of the present study was to measure the rate of blood flow in pancreatic islets transplanted to a site beneath the left renal capsule in normoglycemic and in insulin-treated and non–treated streptozotocin–diabetic recipients. For this purpose a partial pancreatectomy was performed in rats, and islets were isolated from the excised pancreatic specimen and autotransplanted after culture. To have access to normoglycemic control animals it was not the aim of the partial pancreatectomy to induce hyperglycemia. By the same token, the autotransplantation of 500 islets did not cure the streptozotocin-induced diabetes. 1 or 3 mo after transplantation the blood flow in the grafted islets and in the remaining pancreas was measured by a microsphere technique (3). Also, the insulin response to a glucose load was measured in the effluent from the grafted islets and in the systemic circulation.

Methods

Animals. Non–inbred male Sprague-Dawley rats, which were 3 mo old and weighed 350–400 g, were obtained from Anticimex AB (Sollentuna, Sweden). The animals were housed one per cage and had free access to tap water and standard pelleted food (type R3; Anticimex, Södertälje, Sweden) throughout the experiments.

Surgical treatment, islet isolation, and culture. The rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (Mebumal; 40 mg/kg body wt, ACO Läkemedel, Stockholm, Sweden); if necessary this anesthesia was maintained by ether during the operation. The animals also received 0.05 mg/kg body wt atropine (ACO Läkemedel) intraperitoneally before the operation. The pancreas was exposed through a transverse incision in the upper left part of the abdomen and the portion adjacent to the spleen and stomach was mobilized, gently dissected free, and removed. Thus, the part of the pancreas in close proximity to the duodenum was left intact. By this procedure, ~ 0.9–1.0 g of pancreatic tissue was removed from each animal, which corresponded to ~ two-thirds of the total pancreatic mass or ~ 1.5 g at this age (4), before the peritoneum and skin were closed by sutures.

The excised portion of the gland was distended by multiple injections of Hanks' solution (Statens Bakteriologiska Laboratorium, Stockholm, Sweden) into the parenchyma and cut into ~ 30 to 40 pieces. The tissue pieces were distributed to two sterilized glass vials each containing 25 mg of collagenase from *Clostridium histolyticum* (Boehringer Mannheim GmbH, Mannheim, Federal Republic of Germany) dissolved in 8 ml of Hanks' solution. The vials were rapidly shaken in a 37°C water bath for 15–20 min until the tissue had disintegrated, as determined by visual inspection. The digest was sedimented three times in 20 ml of Hanks' solution (24°C), and pancreatic islets were handpicked by using braking pipettes while viewing through a stereomicroscope at 30× magnification. To increase the contrast between the rat islets and the acinar tissue, the dish with the pancreatic digest was placed on a dark support soaked with Hanks' solution. By this procedure we were consistently able to pick 500 to 700 islets from the excised part of the rat pancreas.

Groups of ~ 125 islets each were cultured free-floating at 37°C in 5 ml of medium RPMI 1640 (Flow Laboratories, Irvine, Ayrshire, United Kingdom) containing 100 U/ml benzylpenicillin (Astra Läkemedel, Södertälje, Sweden), 0.1 mg/ml streptomycin (Glaxo Laboratories Ltd., Greenford, United Kingdom), and 10% calf serum (Statens Bakteriologiska Laboratorium) according to Andersson (5). The atmosphere was humidified air plus 5% CO_2. The culture medium was changed every second day.

2 d after the partial pancreatectomy and while the islets were maintained in culture, some rats were given an intravenous injection of streptozotocin (40 mg/kg body wt) (U-9889, lot 2408A, kindly provided by

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Dr. W. E. Dulin, Upjohn Co., Kalamazoo, MI) to induce a manifest diabetic state.

1 wk after the partial pancreatectomy, the animals were autotransplanted with ~ 500 cultured islets each. For this purpose, an incision was made in the left renal capsule of the animals while under ether anesthesia and the islets, collected in a braking pipette, were deposited beneath the capsule. The transplanted streptozotocin–diabetic rats were subdivided into two groups, one treated with subcutaneous injections of insulin (4 IU Ultralente; Novo Research Institute, Copenhagen, Denmark), whereas the other remained untreated.

Measurements of blood flows. The method for measuring pancreatic and islet blood flow by using non–radioactive microspheres has been described in detail (3, 4). Briefly, the transplanted rats were anesthetized intraperitoneally with 130 mg/kg body wt sodium thiobutabarbital (Inactin; Byk Gulden, Konstanz, Federal Republic of Germany) and heparinized. Polyethylene catheters were inserted into the left ventricle of the heart via the right common carotid artery and into the abdominal aorta via the left femoral artery. After 5 min, non–radioactive microspheres (New England Nuclear, Boston, MA) with a diameter of 10.2±0.6 μm (mean±SD) that were suspended in 0.3 ml of 0.9% saline and 0.002% Tween 80 (Sigma Chemical Co., St. Louis, MO) were injected through the intracardiac catheter. The catheter was immediately flushed with 0.3 ml of saline. This solution contained ~ 1.0–1.5 × 10^5 microspheres. Simultaneously, the catheter in the abdominal aorta was used to obtain an arterial blood reference sample by using a peristaltic pump (Minipuls II; Gilson, Villiers-le-Bel, France) set at a withdrawal rate of 0.6 ml/min for 90 s. The accuracy of this rate was confirmed in each experiment by weighing the sample. When the animals had been killed, the graft-bearing kidney, the pancreatic remnant, and adrenal glands were removed. The kidney was fixed in Bouin's solution, and the pancreatic and adrenal tissue was blotted, weighed, and treated for visualization of the islets by using a freeze–thawing technique (4).

After the pancreatic preparations had been thawed, their microsphere content was determined with the aid of a stereomicroscope (M3; Wild Heerbrugg, Heerbrugg, Switzerland) that had both dark and bright field illumination. The microsphere content of the adrenal glands was estimated similarly, whereas that of the reference blood sample was calculated by transferring the sample to 2.5-cm microfiber filters (GF/A; Whatman Ltd., London, United Kingdom) with a pore size of 0.2 μm and then counting the microspheres illuminated by transmitted light while viewing through a stereomicroscope. Before embedding, the islet graft was localized as a distinct whitish spot and cut out with an ~ 4-mm wide margin. The fixed islet graft with surrounding kidney tissue was subsequently processed for light microscopy by serial sectioning into 7-μm thick sections, which were stained with hematoxylin and eosin. The total areas of the islet grafts were measured in the consecutive sections by using a computer system for morphometry (MOP-Videoplan; Zeiss, Svenska AB, Stockholm, Sweden), and the total graft volume in each animal was calculated as the total area times the section thickness. The microsphere content of the entire graft was then counted by using a light microscope. The dark microspheres were easily recognized at low power magnification (× 100) (Fig. 1) and their identity was then confirmed at higher magnification (× 400) (Fig. 2). Because of their physical properties, the microspheres were not cut with the microtome knife; thus, they were present in only one section and could therefore be counted only once. In some initial experiments the freeze–thawing technique was adopted for visualization of the islets in the pancreas (4) on preparations from the graft-bearing part of the kidney, but with this procedure the islet cells could not be distinguished from the kidney cells.

The blood flow of the transplanted islets and of the pancreatic remnant was calculated according to the formula: $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$, where Q_{org} is the organ blood flow (milliliters per minute); Q_{ref} is the rate of withdrawal of the reference blood sample (milliliters per minute); N_{org} is the number of microspheres present in the organ; and N_{ref} is the number of microspheres present in the reference sample. The blood flow values based on the microsphere content in the adrenal glands were used to confirm that the microspheres were adequately mixed in the circulation. A difference of < 10% (expressed as milliliters per minute times gram) between the glands was taken to indicate sufficient mixing and all animals met this requirement. In some of the organs the number of observed microspheres was fairly low (~ 25), but we have previously shown that even such a small number of microspheres will give a reliable blood flow estimate (6).

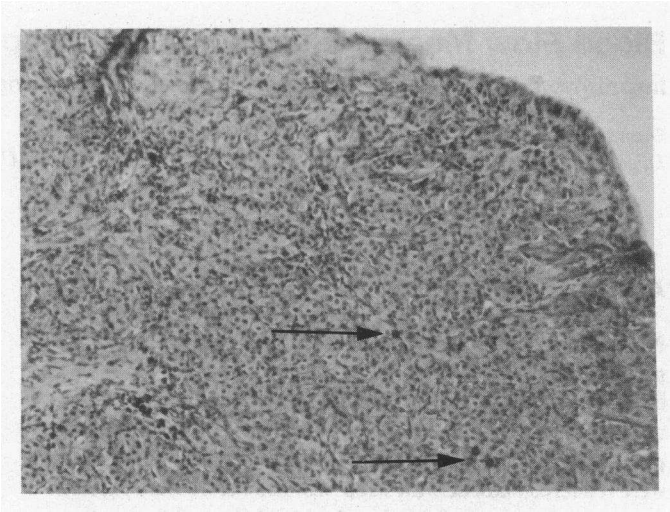

Figure 1. Light micrograph of an islet graft beneath the renal capsule in a normoglycemic rat killed 1 mo after autotransplantation. Normal kidney parenchyma can be seen adjacent to the graft. Three microspheres can be seen within the graft (*arrows*). Hematoxylin-eosin. × 125.

Insulin response to a glucose injection. Immediately after the microsphere injection, the abdomen was opened and catheters were inserted by direct puncture into the portal vein and left renal vein. 1 ml of a 30% (wt/vol) glucose solution was subsequently infused into the left femoral vein. Blood samples (0.2 ml) were taken before the glucose infusion and after 2, 5, and 10 min, and the animals were then killed by cervical dislocation. Serum insulin concentrations in the samples were measured by radioimmunoassay (RIA) (7) by using mouse crystalline insulin (Novo) as standard and ^{125}I-insulin (Novo) as tracer.

Measurements of body weight and serum glucose concentrations. The rats were weighed before the experiment, on the day of transplantation, and before injections of microspheres. Blood samples of non–fasted animals were taken without anesthesia from the tail veins before the experiments, then at weekly intervals, and finally before injection of microspheres. This latter blood sample, however, was collected through an arterial catheter (see above) while animals were under anesthesia. The serum glucose concentrations in the samples were assayed by a semiautomatic glucose-oxidase method (Glucose Analyzer 2; Beckmam Instruments, Inc., Fullerton, CA).

Experimental groups. The design of this study yielded four experimental groups, namely: normoglycemic rats killed 1 mo after transplantation (N1);[1] normoglycemic rats killed 3 mo after transplantation (N3); streptozotocin–diabetic rats killed 1 mo after transplantation (D1); and insulin-treated streptozotocin–diabetic rats killed 1 mo after transplantation (ID1).

Statistical analyses. The results are expressed as means±SEM. Groups of data were compared by means of Student's unpaired *t* test.

Results

General characteristics of the transplanted rats. Before the start of the experiments there was no difference in body weight be-

1. *Abbreviations used in this paper:* D1, streptozotocin–diabetic rats killed 1 mo after transplantation; ID1, insulin-treated streptozotocin–diabetic rats killed 1 mo after transplantation; N1, normoglycemic rats killed 1 mo after transplantation; N3, normoglycemic rats killed 3 mo after transplantation.

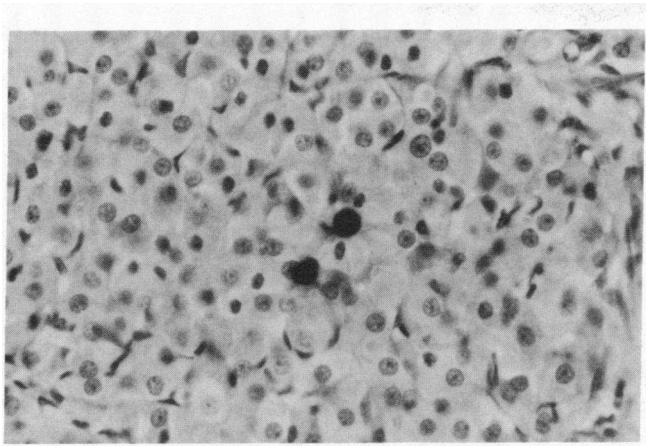

Figure 2. Two of the same microspheres as in Fig. 1 observed in higher magnification. Hematoxylin-eosin. × 500.

tween the experimental groups of rats (data not shown). However, 1 wk after the partial pancreatectomy (i.e., on the day of transplantation), the streptozotocin-treated animals (experimental groups D1 and ID1) had lost weight as compared with the N1 group (Table I). 1 mo after transplantation the weight of ID1 rats was similar to that of N1 rats, whereas the body weight of D1 rats was ~ 30% lower than that of N1 rats. N3 rats (6-mo old) had further increased their body weight as compared with N1 rats (4-mo old). The weight of the pancreatic remnant at killing was ~ 900 mg in all the experimental groups (Table I). The serum glucose concentrations at the time of transplantation were markedly increased in the streptozotocin-treated animals (Table I). The insulin treatment promptly normalized the hyperglycemia and the animals remained normoglycemic during the month of insulin administration (data not shown), until the day of killing (Table I). It should be emphasized that neither did partial pancreatectomy induce overt hyperglycemia or impaired glucose tolerance (data not shown), nor did autotransplantation of 500 islets reverse the diabetes of the streptozotocin-treated animals. When the animals were killed, the serum insulin concentrations in the blood samples taken through the abdominal arterial catheter appeared to be higher in N3 than in N1 rats, although this difference was not statistically significant ($P > 0.05$).

Pancreatic and islet blood flows in the autotransplanted rats. The blood flow in the pancreatic remnant was the same in all four experimental groups (Table II). A few islets could still be observed in the frozen–thawed preparations of the pancreas in the streptozotocin-treated rats and this allowed for the calculation of a profound diminuition of the islet blood flow. In contrast, the islet blood flow was high in the pancreatic remnant of N1 rats; and when expressed as percentage of the total pancreatic blood flow in these animals. This latter figure was further increased in N3 rats.

In all animals, irrespective of their treatment, distinct islet grafts could be identified and no significant differences in the volume of the grafts were found (Table II). The blood flow per islet-graft volume was the same in the two normoglycemic groups of rats and in ID1 rats (Table II). However, the blood flow of the grafted-islet mass in D1 rats was reduced to < 25% that in N1 rats. Note that actual measurements of single islet blood flow were not possible in the grafted islets since individual islets could not be distinguished as separate structures in the sectioned grafts (Fig. 1). In control experiments the identity of the apparent grafts has been confirmed as being mainly composed of insulin-positive cells by immunocytochemical staining. However, it was not feasible to use immunocytochemical stained sections when counting the dark microspheres.

Insulin secretion in vivo. When the serum insulin concentrations were measured in vivo through catheters inserted in the portal vein and left renal vein, before any glucose had been infused, it was found that the portal and renal vein serum concentrations of insulin were higher in N3 than in N1 rats (Table III). D1 rats showed reduced insulin concentrations both in the portal and renal vein as compared with N1 rats. In D1 rats, glucose administration did not result in a significantly increased serum insulin concentration either in the portal or the renal venous blood. N3 rats also failed to increase their serum insulin concentration in either vein in response to glucose. N1 rats responded with elevated serum insulin levels both in the portal ($P < 0.001$) and renal vein ($P < 0.01$) within 2 min after glucose injection as compared with time zero. Before the glucose load, ID1 rats exhibited elevated insulin concentrations in the renal

Table I. Characterization of Rats Submitted to Autotransplantation

	N1 rats	N3 rats	D1 rats	ID1 rats
n	8	7	6	6
Body weight at transplantation (*g*)	389±17	392±8	306±6*	333±12‡
Body weight at killing (*g*)	467±18	536±11	332±107§	446±15
Weight of pancreatic remnant (*mg*)	964±44	984±23	934±59	869±46
Serum glucose at transplantation (*mM*)	8.6±1.2	7.6±0.2	38.7±0.2§	24.5±1.2§
Serum glucose at killing (*mM*)	10.8±0.3	12.4±0.7‡	34.9±1.9§	11.8±2.7
Serum insulin at killing (*ng/ml*)	1.58±0.30	4.98±2.0	1.12±0.33	1.10±0.06

A partial pancreatectomy was performed on anesthetized rats and islets were isolated from the excised specimen and cultured. After 1 wk, groups of 500 cultured islets were transplanted back to the same animal, beneath the renal capsule, under brief ether anesthesia. Some animals were given streptozotocin 2 d after the partial pancreatectomy to induce diabetes. The serum glucose concentration at transplantation was measured in blood samples from a tail vein in non-anesthetized rats, whereas the samples for serum glucose and serum insulin before killing were collected from an arterial catheter in anesthetized rats. Values are means±SEM for *n*, number of rats. *, ‡, and § denote $P < 0.01$, $P < 0.05$, and $P < 0.001$, respectively, as compared with N1 rats (Student's unpaired *t* test).

Table II. Pancreatic and Islet Blood Flow and Graft Volume in Rats Submitted to Islet Autotransplantation Measured by a Microsphere Technique

	N1 rats	N3 rats	D1 rats	ID1 rats
n	8	7	6	6
Pancreatic blood flow (*ml/min × g pancreas*)	1.28±0.24	1.08±0.21	1.24±0.19	1.14±0.11
Islet blood flow (*μl/min × g pancreas*)	245±41	335±66	12±3*	13±5*
Islet blood flow (percent of pancreatic blood flow)	19.3±2.6	32±2.5‡	0.8±0.2*	1.3±0.5‡
Volume of graft (*nl*)	446±98	376±71	313±81	470±96
Graft blood flow (*nl/nl graft x min*)	34.9±9.9	41.5±16	8.2±2.6§	30.3±13

The different groups of rats had been treated as described in Table I. Organ blood flows were measured in anesthetized animals by using a microsphere technique. The graft volume was calculated with the aid of a computer system for morphometry. Values are means±SEM for *n*, number of rats. *, ‡, and § denote $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively, as compared with N1 rats (unpaired Student's *t* test).

but not in the portal vein as compared with N1 rats. 2 min after glucose administration the insulin-treated rats had higher insulin concentrations in the renal than in the portal vein ($P < 0.05$).

Discussion

Using the microsphere technique it was found possible in this investigation to measure the blood flow of transplanted islets in rats. Since the mean volume of a single islet in situ in the rat pancreas has been calculated to ~ 1 nl (8), the expected volume of an islet graft consisting of 500 islets would then be ~ 500 nl. The measured graft volumes in this study were 313–470 nl in different groups of rats, which is in good accordance with the theoretical estimate. The observed blood flow rates in the grafts in the normoglycemic and insulin-treated diabetic rats corresponded to a single islet blood flow of ~ 35 nl/islet × min for an islet volume of 1 nl. The blood flow rates found in this study are thus in excellent agreement with values reported for a single islet blood flow in the pancreas of normal rats (20 nl/min) (8). There was no difference in the graft blood flow values in the normoglycemic rats 1 and 3 mo after transplantation, which suggests that the vascularization of the grafts was already completed after 1 mo. In line with this observation Griffith et al. (9) found in a morphologic study that rat islets transplanted intrahepatically were fully vascularized after 14 d. Moreover, Brown et al. (10) observed in the light microscope that a prominent vascular supply appeared a few days after transplantation of fetal rat pancreas to a site beneath the renal capsule.

Although the blood flow in the grafts of the non–insulin-treated diabetic rats showed large variations, it was much reduced compared with that in the control group (N1 rats). This may indicate that the diabetic state impaired the vascularization of the grafted islets, especially if the insulin production was insufficient to reverse the hyperglycemia. This notion is supported by the finding that insulin-treated rats had a blood flow in the transplanted islets that was similar to that in the normoglycemic rats. The possibility cannot be ruled out, however, that it was the more normal insulin concentrations or the production of angiogenic factors, rather than the normoglycemia per se, that promoted the development of higher blood flow rates in the insulin-treated diabetic animals. The findings nevertheless suggest that intensive insulin treatment may be beneficial for the vascularization of grafted isolated islets.

The difference in insulin concentrations in vivo in serum from the portal and left renal vein after glucose injection indicates

Table III. Serum Insulin Concentrations in the Portal and Left Renal Vein in Rats Submitted to Autotransplantation before and 2.5 and 10 min after an Intravenous Glucose Injection

	N1 rats	N3 rats	D1 rats	ID1 rats
n	8	6	6	6
Serum insulin (*ng/ml*)				
Portal vein				
0 (*min*)	1.70±0.20	9.06±2.52*	0.36±0.08*	1.40±0.31
2 (*min*)	3.73±0.40	10.0±3.07‡	0.45±0.08§	1.72±0.53*
5 (*min*)	3.46±0.48	9.08±2.89‡	0.47±0.10§	1.39±0.45*
10 (*min*)	3.74±0.52	11.6±3.97‡	0.40±0.10§	1.40±0.36*
Renal vein				
0 (*min*)	1.38±0.24	6.38±2.23‡	0.47±0.11*	2.79±0.60‡
2 (*min*)	2.95±0.40	7.53±2.63	0.75±0.22§	3.14±0.32
5 (*min*)	2.68±0.24	7.97±2.42‡	0.55±0.13*	2.39±0.32
10 (*min*)	2.45±0.46	8.04±2.42‡	0.58±0.13*	3.52±0.98

The different groups of rats had been treated as described in Table I. Glucose (1 ml 30% glucose wt/vol) was infused into the left femoral vein in anesthetized rats and blood samples were taken through catheters inserted in the portal vein and left renal vein. Insulin concentrations were measured by RIA. Values are means±SEM for *n*, number of rats. *, ‡, and § denote $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, as compared with N1 rats (Student's unpaired *t* test).

that the grafts of the insulin-treated rats secreted insulin, whereas those of the manifest diabetic rats did not function significantly. The normoglycemic rats tested 1 mo after transplantation exhibited increases in serum insulin concentrations both in the portal and renal vein. It is possible, however, that this increase in the renal vein was due to insulin secretion from the pancreas. In the 6-mo-old normoglycemic rats, highly elevated concentrations of insulin were noted in both veins before glucose administration. This observation was unexpected and might reflect a combined effect from the regenerating pancreas and the transplanted islets.

The pancreatic remnant weighed ~ 900 mg in all four groups of rats, which shows that the pancreatic glands had almost doubled their weight after the partial pancreatectomy. Bonner-Weir et al. (11) observed similarly that the pancreatic remnant had more than doubled in weight 8–10 wk after partial pancreatectomy. The pancreatic regeneration was accompanied by higher total pancreatic blood flows than have previously been observed in normal rats with intact glands (3, 12). Also, the islet blood flow in the pancreases of the normoglycemic rats was much increased and represented a fraction of not < 20–30% of the total pancreatic blood flow. The mechanism underlying the markedly increased islet blood flow in the regenerating pancreas is at present unclear. It could be assumed that an increase in insulin secretion is parallelled by increased islet blood flow (compared with the 6-mo-old rats). On the other hand, we have previously shown that there is in some situations a dissociation between islet blood flow and insulin release in vivo (13). Finally, note in this context that the vascularization and the blood flow rates of the transplanted islets may have been influenced by the regenerating pancreas. However, it appears less likely that such influence would account for the observed differences in the blood flow of the transplanted islets between the experimental groups in the present investigation.

In conclusion, this study has shown that transplanted islets are vascularized within a month after implantation and that the blood flow rates in these islets in normoglycemic rats are comparable to those in islets located in the pancreas. The vascularization of grafted islets seems to be diminished by hyperglycemia; however, this can be normalized by insulin treatment.

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