Growth of Neonatal Islet Transplants in the Spontaneously Diabetic BB/Wor Rat

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We have previously shown that culture-isolated neonatal islets are able to survive both rejection and the recurrence of autoimmunity in the spontaneously diabetic BB/Wor rat. In trials designed to demonstrate the MHC restriction of the autoimmune response in this model, we discovered that neonatal islet grafts from diabetic BB rats appeared larger than grafts from nondiabetic controls. This study was undertaken to quantify the mass difference seen in this original study and to determine the characteristics of graft growth in more highly controlled trials. Grafts from diabetic animals in the original study were significantly larger than those from nondiabetic animals (81 ± 36 vs. 238 ± 216 µg, P = 0.01). These findings were supported by results from a second series of experiments, in which the mean growth index of grafts from diabetic animals was 7.25 ± 4.91, whereas that from nondiabetic animals was 2.5 ± 1.15 (P = 0.011). Three animals in this study were reversed of hyperglycemia: two had normal and one had a subdiabetic ip GTTs. These three rats received 97, 317, and 408 µg of islet tissue that increased in mass to 1790, 3270, and 4107 µg, respectively. Nuclear/total cell area percentages were the same in diabetic and nondiabetic grafts (P = 0.76), suggesting that the increase in mass was attributable primarily to proliferation rather than hypertrophy. Limited studies that use BrDU incorporation support this conclusion. High glucose levels have been shown to stimulate β-cell replication in fetal, neonatal, and adult islets and may be the stimulus for enhanced graft growth in this model. This study shows that small, immunomodulated neonatal grafts placed in the diabetic environment of the spontaneously diabetic BB/Wor rat can increase in mass >10-fold and can ameliorate the symptoms of diabetes. Diabetes 41:1122–29, 1992

If islet transplantation is to become a feasible therapeutic method for the treatment of diabetes, enhanced methods must be developed for the delivery of adequate islet mass. This would be facilitated by the growth of the islet graft in the recipient. The endocrine components of the fetal pancreas have been found to increase in mass after syngeneic transplantation and, if the initial transplant mass is large enough, diabetes can be reversed over time (1–4). These fetal studies have been extended to allografts (5) and porcine xenografts (6). In addition, Wohlrab et al. (7) have shown that fetal β-cells and pancreatic ductular elements are capable of proliferating in situ after transplantation into a chronically hyperglycemic environment. In trials using adult islets, Andersson et al. (8) and Swenne et al. (9) have demonstrated that islet transplants can increase in mass and manifest a higher autoradiographic labeling index if maintained in a hyperglycemic environment rather than a normoglycemic one. They found that this capacity for proliferation decreases as islet donor age increases from 1 mo through 9 mo. Thus, islet grafts appear capable of growth after transplantation under appropriate conditions that may be related to hyperglycemia or the diabetic state. This growth potential apparently decreases as the age of the donor increases.

With some exceptions (5,6), the studies summarized above have tested syngeneic islet transplants in chemically diabetic animals. In this study, we examined the ability of highly purified neonatal rat islets from MHC-matched and mismatched donors to increase in mass after transplant to the spontaneously diabetic BB/Wor rat.
where the islet transplants are subjected to metabolic and immune conditions similar to those found in human IDDM. Islets used in this study were isolated by a nonenzymic method and cultured 10 days before transplant (10). This culture-isolation procedure protects against rejection by eliminating the class II-bearing pas-
senger leukocytes in the islets (11-13). In addition, both MHC-matched and mismatched islets are afforded a degree of protection from autoimmune destruction in the BB/Wor environment by a mechanism as yet undefined (14). Therefore, we were able to study the growth in both syngeneic and allogeneic combinations in the presence of autoimmune disorder using this model. Finally, these transplants can be used to test the ability of the differen-
tiated β-cells to proliferate after transplant because the grafts are highly purified and free of ductular elements (except those few cells that may be present inside the islets), thus reducing the possibility that mass increases are due to ductular proliferation and differentiation.

This study began during the analysis of results from an MHC-restriction study in BB/Wor rats (14). In these trials, Wistar-Furth (WF, Rt1\(^*\)) and Fisher-344 (FSH, Rt1\(^{1\#}\)) islet grafts were compared for their ability to serve as targets for autoimmune destruction in the BB/Wor rat (Rt1\(^{1\#}\)). Some 40 grafts from diabetic and nondiabetic BB/Wor rats were analyzed, and only two grafts (from WF donors) were found to be destroyed by autoimmunity. Upon reexamination of the remaining 38 grafts, it became apparent that grafts from diabetic animals were signifi-
cantly larger than grafts from nondiabetic controls. This study was undertaken to quantify the mass difference seen in the original study by use of computer-assisted image analysis. In addition, we extended this investiga-
tion to trials designed to provide better quantification of graft growth—for measuring the graft mass before and after transplant. We also used two approaches to deter-
mine whether the increase in mass was caused by hypertrphy or hyperplasia. We compared nuclear/total cell area ratios in grafts from diabetic and nondiabetic BB/Wor recipients, and we compared the rate of cell division, measured by nucleotide uptake, in the grafts of the two groups. In addition, to determine whether growth continued throughout the entire experimental period, we inves-
tigated whether graft size was linearly correlated with the number of days of engraftment. Finally, we tested the ability of small islet grafts to grow large enough to ameliorate IDDM symptoms and produce normal glucose tolerance in the diabetic BB/Wor rat.

**RESEARCH DESIGN AND METHODS**

This work was conducted in two series. Series 1 was the original study, designed to test MHC restriction. The methods for this series have been described previously (14) and are briefly summarized herein. Series 2 was the subsequent study, designed to investigate graft growth.

Recipient animals were young (27-70 days old) male and female diabetes-prone BB/Wor rats (Rt1\(^{1\#}\)) obtained from the colony in the Department of Pathology, University of Massachusetts (Worcester, MA). Animals were housed in laminar flow-protected environments and re-
ceived sterile food and water. In series 1, donor animals were 3-4 day neonatal FSH (Rt1\(^{1\#}\)) and WF (Rt1\(^{1\#}\)). In series 2, donor animals were neonatal FSH.

**Islet isolation.** The method of islet isolation and culture has been described previously (10). Briefly, pancreas from 10-20 neonatal rats were pooled and minced. Tissue was cultured for 7 days, with one media change, in Ham's F12 medium supplemented with 5% FCS and 1% PSF at 37°C in 5% CO\(_2\) in air. On day 7, the tissue was detached from the culture dishes, subcultured in fresh medium, and incubated for an additional 24 h. Islets were then hand-picked to ensure purity and returned to culture for an additional 2 days in medium supplemented with 25% equine serum and 1% PSF. Islets were washed in HBSS before transplantation.

**Transplantation.** Renal subcapsular transplantation was performed, as described previously (15). Recipients were transplanted before the onset of diabetes (27-70 days old).

**Series 1.** Because series 1 was not designed to test islet growth, the number of islets transplanted was simply estimated by counting. Islets were counted individually when they were put into subculture. At transplant, the islets were divided such that each recipient received approximately equal numbers of islets. The number of islets per transplant was estimated based on the total number of islets available divided by the number of transplants performed. Computer-assisted image analysis of 7207 islets from 35 sets of FSH neonatal donors revealed that the average FSH neonatal islet mass is 1.02 ± 0.30 μg/islet. Analysis of 3822 islets from 21 sets of WF neonatal donors yielded an average islet mass of 1.17 ± 0.20 μg/islet. Therefore, an approximate mass was calculated based on the number of islets trans-
planted. Although this method of quantification yields only approximate values, it is unlikely that the diabetic animals consistently received larger grafts than nondiabetics, especially considering we had no way of knowing at the time of transplant which animals would become diabetic and which would remain normal. In this series, 10 BB/Wor male recipients received 100-200 FSH islets, 10 BB/Wor males received 100-200 UF islets, and 10 BB/Wor females received 100-200 UF islets at the upper pole of the kidney and 100-200 UF islets at the lower pole of the same kidney.

**Series 2.** In series 2, BB/Wor rats received transplants of 100, 200, or 400 neonatal FSH islets. Except where noted, the mass of each transplant was quantified by computer-assisted image analysis (see below).

**Physiological monitoring and sacrifice.** Plasma glu-
cose levels were determined by glucose oxidase method using a Beckman Glucose Analyzer II. Urine glucose was

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were then captured by a video camera and transferred
with its center of gravity at the lowest point. Total trans-

xylidine to demonstrate the presence of p-cells  and
were stained with aldehyde fuchsin and Ponceau de
Volume of each islet was determined by
The shortest radius also was used as an estimate of the
eter was photographed for calibration. Individual graft

GTTs. Ip GTTs were performed on all normoglycemic
transplant recipients in series 2 at 38–164 days post-
Elegant WF and nondiabetic BB transplant
recipients served as controls. Animals were fasted over-
night, and at time zero a blood sample was drawn from
the tail vein before glucose injection. A 30% glucose
solution was injected at a dose of 1 ml/100 g ip, and
animals were bled at 10, 20, 40, 60, 120, 180, and 270
minutes after injection. The K value (% of glucose load
cleared/min) was calculated as described by Seltzer

Histology. Grafts were removed at the time of graftec-
tomy or death and were fixed in Bouin’s and prepared for
routine paraffin histology. The entire graft area was
sectioned at 4 μm, and every 10th section (40 μm intervals)
was mounted for quantitative analysis. Slides were
stained with aldehyde fuchsin and Ponceau de
xylidine to demonstrate the presence of β-cells and
permit definition of the graft site. Slides prepared from
adjacent sections were stained with hematoxylin and
eosin to permit determination of nuclear/total cell area
ratio.

Quantification of islet mass before transplant. Islets
were divided for transplant, and each islet group was
photographed individually at 4x magnification using an
inverted compound microscope. A 2 mm stage microm-
eter was photographed for calibration. Individual graft
montages were constructed from the resulting pictures.
From the photographs, the outline of each islet was
hand-traced onto a transparency to ensure accuracy in
the case of touching or overlapping islets. The outlines
were then captured by a video camera and transferred
directly into a Macintosh Image 1.29 image analysis
system. The best fitting ellipse for each islet was deter-
mined, as were the minor and major radii in micrometers.
Volume of each islet was determined by
Vol = 4/3 π (minor radius)² (major radius)
The shortest radius also was used as an estimate of the height
of the islet because it is most likely to come to rest
with its center of gravity at the lowest point. Total trans-
planted islet mass was determined by adding individual
islet volumes and converting volume to mass, assuming
the density of islets to be 1 g/cm³. This analysis also
yielded a count of the number of islets transplanted.

Quantification of graft mass after transplant. Graft
mass from animals in series 1 was quantified by drawing
the graft region on every 10th section using a camera
lucida. To obtain the area of each section |xm², images
were outlined using a digitizing pad and entered into a
locally written image analysis program on a Hewlett-
Packard computer. The section volume was calculated
assuming a section thickness of 4 μm. The volume of
intervening sections and total graft volume was calcu-
lated as described below for series 2.

For series 2 animals, after calibration using a stage
micrometer, the graft region on every 10th tissue section
was identified and entered directly into a Macintosh
Image 1.29 using a compound microscope. The graft
outline was digitized and an area (μm²) was determined.
Each section’s volume was calculated assuming a thick-
ness of 4 μm. The volume of intervening sections was
predicted by first determining the increment of increase
(II) for each pair of adjacent sections as follows

\[
II = \frac{\text{volume of section}_{n+1} - \text{volume of section}_n}{9}
\]

Then, the volume from section_1 to section_{n+1} was deter-
mined as

\[
\begin{align*}
V_n &\rightarrow V_{n+1} = V_n + (V_n + II) + (V_n + 2II) \\
&+ (V_n + 3II) + (V_n + 4II) \ldots (V_n + 9II) \\
or \\
V_n &\rightarrow V_{n+1} = 10V_n + 45II
\end{align*}
\]

The total graft mass then was calculated by summing all
the section volumes.

Correlation between mass calculations using living
and fixed dehydrated tissue. In addition to comparing
diabetic and nondiabetic animals in this study, we
wanted to quantify, as accurately as possible, the in-
crease in mass size in both environments. Because the
mass of islets before transplant is measured on living
islets in culture, whereas the mass of the graft is mea-
sured using processed tissue sections, we had to deter-
mine how these methods of estimating mass compare.
We therefore quantified the mass of eight syngeneic islet
grafts to normal recipients before transplant and after 3
days of engraftment before any significant growth would
have occurred. It was determined that the graft mass
after processing was 0.37 ± 0.08 times the original trans-
planted mass. We assumed the differences in graft mass
were due, in minor part, to cell death during the engraft-
ment process, and, in major part, to tissue shrinkage
during processing for histology. Therefore, graft sizes are
multiplied by 2.7 to reflect as accurately as possible the
equivalent mass of living, nonprocessed tissue.

Determination of nuclear/total cell area ratios within
graft. Nuclear/total cell area ratios were calculated in
series 1 on the largest grafts from four diabetic and four
nondiabetic animals. All grafts in series 2 were analyzed.
Endogenous pancreatic islets from five WF neonatal rats
were analyzed as controls. Ratios were determined on
hematoxylin- and eosin-stained sections using an Image
1.29 program. Approximately 50 cells per grafts were
analyzed from 4 to 5 randomly chosen microscope fields
at 100x magnification. After calibration with a stage
micrometer, images were entered into the program di-
rectly from slides, and the nuclear and cellular areas of
selected cells were determined. Cells were chosen if they
were morphologically endocrine, located in the center of the graft where the β-cells overwhelmingly predominate, and the nucleus and cytoplasm were clearly delineated.

**BrDU incorporation trials.** To determine whether the diabetic state stimulated cell division in islet grafts, we measured in vivo incorporation of a DNA nucleotide, BrDU. Animals in this trial received two grafts: one larger graft quantified before and after transplant (as described above) and a smaller graft of 50 islets on the same kidney used for BrDU incorporation studies. At 57 or 80 days posttransplant and 7 days before graftectomy, pellets containing 50 mg of BrDU (Boehringer Mannheim, Mannheim, Germany) were placed subcutaneously on the back of the recipient at a dose of ~1 mg/g body wt. These pellets were dissolved completely in 24 h; 7 days after pellet delivery, animals were graftectomized. The large graft was taken for image analysis (as described above); the small graft was fixed in 70% ethanol at 4°C overnight, then prepared for routine paraffin histology. The entire graft area was sectioned at 4 μm and every 3rd section was mounted. Slides were rehydrated to 70% ethanol, left in cold PBS for 15 min and incubated in 0.05% protease in PBS for 5 min at 37°C to expose BrDU sites. Slides then were incubated at room temperature in 1 N HCl for 30 min and overnight in mouse anti-BrDU (1:25) (Becton Dickinson, San Jose, CA) at 4°C. Slides then were incubated with fluorescein-conjugated goat anti-mouse IgG (1:25) (Jackson ImmunoResearch Labs, West Grove, PA) for 1 h at 4°C, and coverslipped in PBS-glycerine. Slides were washed between steps in PBS. Positive cells were identified and counted using fluorescence microscopy. After fluorescent analysis, the mass of the graft was determined (as described above) to allow standardization of positive cells by graft mass.

**Statistical analysis.** All statistical tests are two-tailed Student’s t tests unless otherwise indicated.

**RESULTS**

**SERIES 1**

Results from series 1 are summarized in Fig. 1. Ten animals remained nondiabetic as determined by plasma glucose analysis and pancreatic islet morphology; they had mean plasma glucose values of 6.44 ± 0.78 mM on the day of death. The 14 grafts removed from these animals had a mean mass of 81 ± 36 μg. This graft size is reasonable considering that ~100–200 μg of tissue was transplanted, but it does not represent significant growth.

Twenty animals in series 1 had manifest insulitis or end-stage islet destruction and a mean plasma glucose level of 23 ± 12 mM at the time of sacrifice. Among these animals, 24 grafts survived rejection and disease recurrence and had a mean mass of 238 ± 216 μg. The grafts in the diabetic animals were significantly larger than the grafts in nondiabetics (P = 0.0059, by Mann-Whitney U test). In addition, although the time in situ (19–84 days) had no effect on the size of the grafts from nondiabetic animals (P = 0.78, by linear regression analysis), graft size was found to be linearly correlated with length of time in situ diabetic animals (P = 0.047, by linear regression analysis). Thus, in series 1, the grafts in diabetic animals increased in mass relative to grafts in nondiabetic animals and appeared to do so in a time-dependent fashion, suggesting that growth continued over the entire experiment period.

**SERIES 2**

**Analysis of graft growth.** Table 1 summarizes the growth data from series 2. Twenty animals received islet grafts in series 2, and the mass of the islets before transplant was quantified in 14 of these animals. In the remaining 6 animals, because of photographic technical failure, islet mass was estimated based on the counting method outlined for series 1 and the assumption that islet mass was 1.02 μg/islet. The growth index for each graft was calculated by dividing the mass of the graft after transplant (adjusted for the shrinkage produced by tissue processing) by the mass of the islets before transplant.
In this portion of the study, 13 of 20 transplant recipients became diabetic. The mean growth index in diabetic recipients was significantly greater than in nondiabetic recipients (P = 0.011, by one-tailed Student's t test). The mean growth index in diabetic animals was 7.25 ± 4.91, whereas that in nondiabetic animals was 2.5 ± 1.15. The statistical significance is maintained even if the 6 animals with estimated transplant mass are eliminated from the data set (P = 0.03).

**Correlation between graft size and length of time in situ.** We found no correlation between the growth indexes in nondiabetic animals and the length of time in situ (P = 0.51, by linear regression analysis). Growth indexes in diabetic animals showed a positive correlation with length of time in situ; however, correlation was not found to be statistically significant (P = 0.16). Given the significant correlation found in series 1 between graft size and days in situ and the relatively low P value found in series 2, it appears that there may have been continued growth of the neonatal islet grafts in diabetic recipients throughout the experiment period of up to 168 days. Graft growth is highly variable, and the large variability in this response may be masking a time-dependent increase in graft size.

**Amelioration of the diabetic symptoms by graft growth.** Of the 13 animals that became diabetic in series 2, 3 (V-2, V-8, and V-11) maintained normal or near-normal plasma glucose levels throughout the experiment period but became hyperglycemic upon graftectomy (Fig. 2). These animals had three of the four highest growth indexes and posttransplant graft masses (Table 1). Ip GTTs were performed on these 3 animals 118–164 days posttransplant (Fig. 3). Normal controls for the GTTs were age-matched nondiabetic BB/Wor animals bearing

**TABLE 1**
Growth index and metabolic outcome for BB/Wor recipients of cultured, neonatal FSH islets transplanted before the onset of disease.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Diabetic state</th>
<th>Days of engraftment</th>
<th>Number of islets transplanted</th>
<th>Mass transplanted (μg)</th>
<th>Mass after transplant (μg)*</th>
<th>Growth index†</th>
<th>Plasma glucose range after disease onset (mM)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>V-1</td>
<td>D</td>
<td>168</td>
<td>200§</td>
<td>204</td>
<td>2363</td>
<td>11.58</td>
<td>22.7–38.3</td>
</tr>
<tr>
<td>V-2</td>
<td>D</td>
<td>169</td>
<td>400§</td>
<td>408</td>
<td>4107</td>
<td>10.07</td>
<td>6.8–9.6</td>
</tr>
<tr>
<td>V-3</td>
<td>D</td>
<td>168</td>
<td>350§</td>
<td>357</td>
<td>1004</td>
<td>2.81</td>
<td>25.0–38.6</td>
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<tr>
<td>V-7</td>
<td>D</td>
<td>134</td>
<td>76</td>
<td>84</td>
<td>759</td>
<td>9.04</td>
<td>23.5–33.2</td>
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<tr>
<td>V-8</td>
<td>D</td>
<td>137</td>
<td>308</td>
<td>317</td>
<td>3270</td>
<td>10.32</td>
<td>7.1–18.7†</td>
</tr>
<tr>
<td>V-11</td>
<td>D</td>
<td>144</td>
<td>188</td>
<td>97</td>
<td>1790</td>
<td>18.45</td>
<td>9.7–12.2†</td>
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<tr>
<td>VII-3</td>
<td>D</td>
<td>102</td>
<td>90</td>
<td>186</td>
<td>640</td>
<td>3.44</td>
<td>24.0–38.6</td>
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<td>D</td>
<td>102</td>
<td>314</td>
<td>362</td>
<td>1137</td>
<td>3.14</td>
<td>25.5–38.8</td>
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<td>D</td>
<td>102</td>
<td>192</td>
<td>227</td>
<td>1696</td>
<td>7.47</td>
<td>19.7–32.8</td>
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<tr>
<td>VII-7</td>
<td>D</td>
<td>102</td>
<td>418</td>
<td>372</td>
<td>500</td>
<td>1.34</td>
<td>26.1–43.6</td>
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<tr>
<td>VII-12</td>
<td>D</td>
<td>39</td>
<td>400§</td>
<td>408</td>
<td>497</td>
<td>1.22</td>
<td>12.5–19.5</td>
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<td>D</td>
<td>32</td>
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<td>100</td>
<td>851</td>
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<td>D</td>
<td>80</td>
<td>173</td>
<td>148</td>
<td>1021</td>
<td>6.90</td>
<td>21.9–36.5†</td>
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<td>V-9</td>
<td>ND</td>
<td>144</td>
<td>293</td>
<td>266</td>
<td>718</td>
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<td>V-12</td>
<td>ND</td>
<td>144</td>
<td>267</td>
<td>195</td>
<td>972</td>
<td>4.98</td>
<td>7.7–9.8</td>
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<td>ND</td>
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<td>219</td>
<td>213</td>
<td>375</td>
<td>1.76</td>
<td>5.6–7.0</td>
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<tr>
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<td>ND</td>
<td>102</td>
<td>94</td>
<td>141</td>
<td>321</td>
<td>2.28</td>
<td>6.4–7.8</td>
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<tr>
<td>VII-10</td>
<td>ND</td>
<td>87</td>
<td>100§</td>
<td>102</td>
<td>208</td>
<td>2.04</td>
<td>6.1–6.6</td>
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<tr>
<td>VII-11</td>
<td>ND</td>
<td>87</td>
<td>200§</td>
<td>204</td>
<td>329</td>
<td>1.61</td>
<td>6.8–7.1</td>
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<tr>
<td>VII-18</td>
<td>ND</td>
<td>87</td>
<td>381</td>
<td>433</td>
<td>929</td>
<td>2.15</td>
<td>6.8–7.4</td>
</tr>
</tbody>
</table>

(D), diabetic; (ND), nondiabetic.
*Calculated mass multiplied by 2.7 to allow correlation with mass before transplant determined using living islets in tissue culture.
†Growth index is mass after transplant divided by mass before transplant.
‡When onset could not be determined because no hyperglycemia occurred, glucose values are those recorded after 120 days of age when most BB/Wor diabetic rats will have manifested the disease.
§Number of islets estimated by counting before transplant as in Series 1.
|M| Mass not quantified by image analysis, rather, estimated based on average islet mass of 1.02 μg/islet.
| | Animals were normoglycemic due to transplant.

**FIG. 2.** Nonfasting plasma glucose levels for 3 diabetic BB/Wor recipients in series 2 that were reversed of their diabetic symptoms by FSH neonatal islet grafts. (A—A), V-2: 320 μg In, 4107 μg out, growth index 12.83; (B—B), V-8: 317 μg In, 3270 μg out, growth index 10.32; (C—C), V-11: 97 μg In, 1790 μg out, growth index 18.45; (D), day of graftectomy; (horizontal line), mean ± SD for nondiabetic BB/Wor.
FSH transplants of approximately the same islet mass, nondiabetic BB/Wor animals without transplants, and a normal WF. A hyperglycemic BB/Wor rat bearing an FSH islet transplant also was used as a control in one trial. Essentially normal glucose tolerance profiles were seen in animals V-2 and V-8. These animals had the third and fourth largest growth indexes at 10.07 and 10.32, respectively, and the two largest grafts after transplant. These two animals compared very favorably with the normal controls in the percent of glucose load (K) removed per minute. Animal V-2 had a K of 3.3%/min, whereas its normal control had a K of 0.92%/min. Animal V-8 had a K of 2.04%/min compared with 2.17%/min for the nondiabetic BB/Wor and 1.54%/min for the normal WF rat. Mean K values for normal WF and normal FSH rats are 2.06 ± 0.93 and 2.97 ± 0.75%/min, respectively. Thus, the GTT results for these two rats were well within the normal range for nondiabetic rats.

Animal V-11 gave a subdiabetic GTT profile with K of 0.86%/min compared with 1.31%/min for the nondiabetic BB/Wor control with an FSH graft and 0.59%/min for the BB/Wor control without a graft. This animal had the fourth largest graft after transplant at 1790 µg and, having started with a very small graft of 97 µg, had the highest growth index at 18.45. Although this graft mass was sufficient to produce a near-normal nonfasting plasma glucose profile, the animal remained subdiabetic, but not frankly diabetic, by GTT analysis. It appears, therefore, that these very small grafts (97–408 µg) increased in size in the diabetic environment of the BB/Wor rat sufficiently to ameliorate diabetes symptoms.

Nuclear/total cell area ratios. To provide insight into whether the increase in graft mass in this study was caused by hypertrophy, hyperplasia, or both, we calculated the percentage of the cells' area occupied by the nucleus in grafts from diabetic and nondiabetic BB/Wor rats and in the normal neonatal pancreas. In series 1, nuclear/total cell area ratios were calculated for four of the largest grafts from each group of diabetic and nondiabetic recipients. The nucleus was found to occupy 38.5 ± 6.3% of the cellular area in diabetic and 37.4 ± 3.5% in nondiabetic animals. This difference was not statistically significant (P = 0.76).

In series 2, we measured the nuclear area percentage for all grafts from diabetic and nondiabetic recipients and, again, found no difference, with means of 36.3 ± 4.2% for 13 diabetic and 35.8 ± 3.4% for 7 nondiabetic animals (P = 0.76). The mean nuclear percentage for endogenous neonatal islets was 39.4 ± 1.7%, which appears to be slightly larger than grafts from both diabetic (P = 0.125) and nondiabetic (P = 0.045) recipients, although only marginally so by statistical analysis. Therefore, these findings support the hypothesis that the increase in graft size seen in this study is attributable primarily to hyperplasia rather than hypertrophy; however, very moderate hypertrophy may also be present in the grafts of both diabetic and nondiabetic recipients.

BrDU incorporation studies. We were able to collect BrDU incorporation data from four animals—three nondiabetic and one diabetic. The mean incorporation index for nondiabetic rats was 1.51 ± 0.69 positive cells/µg of graft and the incorporation index for the diabetic animals was 10.2 cells/µg (P = 0.004, by one-tailed Student's t test). Careful analysis of positive cells using phase contrast microscopy was performed to ensure that counted cells had an endocrine morphology with secretory granulation. Although the number of animals analyzed is very small and the data must be regarded as preliminary...
rather than conclusive, the BrDU data supports the nuclear percentage results and suggests that growth in grafts from diabetic animals is attributable primarily to proliferation.

DISCUSSION

This study suggests that culture-isolated, neonatal islet transplants can be induced to grow in situ by factors associated with the diabetic state in the BB/Wor rat. Although growth of the neonatal islets appears to occur in the normal BB/Wor rat as well, growth is significantly greater in the diabetic animals. Limited trials using nucleotide incorporation and nuclear/total cell area ratios both support the hypothesis that this growth is caused by proliferation of cells within the islet, and not cellular hyperplasia. Our experience in trying to reverse manifestly diabetic BB/Wor rats has shown that it takes 1500–5000 µg of islet tissue. The higher range represents animals that needed multiple transplants for reversal because of poor engraftment in the diabetic environment. However, in animals that received one transplant that appeared to engraft well, 1500 µg was necessary. In this study, using small grafts into prediabetic rats, 97–436 µg of islet tissue increased in mass to 1790–4107 µg, a mass sufficient to reverse or nearly reverse diabetic symptoms.

Although the metabolic environment of the spontaneously diabetic BB rat varies from the normal condition in several ways (17), it is likely that elevated plasma glucose levels provided an important stimulus enhancing islet growth in the diabetic BB rats. Elevated plasma glucose levels have been shown to be associated with β-cell hyperplasia in numerous experimental models. Several genetic models in the rodent for NIDDM or other disorders are characterized by moderate hyperglycemia and also are associated with islet hyperplasia (18–22). In addition, steroid-induced diabetes in several species has been shown to be associated with islet hyperplasia (23,24). In vitro studies indicate that corticosteroids inhibit adult islet cell proliferation, thus suggesting that the islet hypertrophy seen in models of steroid diabetes is induced by the associated hyperglycemia rather than by direct steroid action (25,26).

Several investigators have tested the effect of elevated plasma glucose levels on the development of adult islet hyperplasia by more direct methods. In these studies, hyperglycemia was produced by glucose infusion (27–29) or partial pancreatectomy (30). These investigators found a significant increase (50–100%) in β-cell mass, as determined by morphometric analysis and severalfold increases in mitotic index and cellular birth rate for β-cells from hyperglycemic animals when compared with normoglycemic controls. Thus, adult rodent β-cells appear able to proliferate in response to moderately elevated glucose levels.

In vitro evidence for this ability comes from several studies using fetal (31,32), neonatal (25,33), and adult (26,29,33) rodent islets. In these studies, when glucose concentrations were increased from the normoglycemic rat range of 2.8–3.9 mM to the hyperglycemic range of 16.8–25.2 mM, tritiated thymidine incorporation in β-cells increased two- to eightfold. By synchronizing the cell cycle before performing tritiated thymidine incorporation studies, Swenne (33) found a decrease in the ability of islets to proliferate in response to elevated glucose levels with age. He attributed this decrease to a diminishing pool of β-cells that could proliferate in response to elevated glucose levels. However, 2.6% of the β-cells in 3-mo-old adult islets were still able to proliferate in response to elevated glucose levels. The cellular birth rate (the production of new cells/24 h) for this adult tissue was 4.0%, which would allow a doubling of islet mass in 18 days if proliferation continued unabated.

Although many investigators have found that elevated glucose levels stimulate islet proliferation, several groups have reported that hyperglycemia is detrimental to islet function, particularly glucose-stimulated insulin release (34–36). Irreversible damage and loss of islet mass in response to persistent hyperglycemia also have been reported (37). Although Korsgren et al. (38) have proposed that this damage may be caused by an impairment in β-cell glucose oxidation, they also have shown that irreversible damage and loss of mass is dependent on the genetic background of the islets. In this study, we tested only WF and FSH islet grafts. Other strains of islets may be more sensitive to damage by chronic hyperglycemia. Also, especially in neonatal tissue, it is likely that the detrimental effects of hyperglycemia can be overridden by its stimulatory effect operating through mechanisms that allow long-term compensation for β-cell loss using glucose elevation as a signal for β-cell proliferation.

Although hyperglycemia appears to be a well-documented stimulus for β-cell growth, two of the reversed diabetics in series 2 did not go through a period of hyperglycemia. In fact, these animals were thought to be nondiabetic controls until nephrectomy produced hyperglycemia. Similarly, in series 1, five diabetic animals were only marginally hyperglycemic at the time of death with plasma glucose values ranging from 8.06 to 10.7 mM. The grafts for these animals were among the largest in the study. In light of these findings, it may be possible that other factors present in the diabetic BB/Wor rat, such as an altered endocrine profile, might be providing the stimulus for growth. However, it is also possible that very moderately elevated plasma glucose levels, below those thought to be clinically significant, are sufficient to stimulate growth.

The results presented herein provide compelling evidence for the growth of pure neonatal islet tissue in response to developing hyperglycemia. The only ductal elements that may have been present in these transplants are cryptic ductal cells within differentiated islets. Therefore, the increase in mass during this study probably was attributable to the proliferative response of differentiated β-cells.

Our study suggests that treatment of diabetes-prone BB animals early in life—before overt symptoms of the disease—with small cultured neonatal islet transplants, may permit the continued growth of transplanted islet tissue in response to moderate hyperglycemia resulting...
from loss of endogenous islet cells. Thus, small amounts of initial islet graft may be able to respond to the presence of the autoimmune process that destroyed tissue from young donors, far less transplant mass was required for reversal. These smaller grafts were more easily obtained from donor sources and were more amenable to immunomodulation.

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