Reduced beta-cell glucose transporter in new onset diabetic BB rats.

L Orci, … , H F Lodish, B Thorens

J Clin Invest. 1990;86(5):1615-1622. https://doi.org/10.1172/JCI114883.

Research Article

Previous studies from our laboratories have suggested a defect in glucose transport in islets isolated from BB rats on the first day of overt diabetes. To quantitate by immunostaining the glucose transporter of beta-cells (GLUT-2) before and at the onset of autoimmune diabetes we employed an antibody to its COOH-terminal octapeptide. On the first day of overt diabetes, defined as the day the daily blood glucose first reached 200 mg/dl, the volume density ratio of GLUT-2-positive to insulin-positive beta-cells was only 0.48 +/- 0.06, compared to 0.91 +/- 0.02 in age-matched nondiabetic diabetes-resistant controls (P less than 0.001). In age-matched nondiabetic diabetes-prone rats, most of which would have become diabetic, the ratio was 0.85 +/- 0.02, also less than the controls (P less than 0.05). Protein A-gold labeling of GLUT-2 in beta-cells of day 1 diabetic rats revealed 2.17 +/- 0.16 gold particles per micrometer length of microvillar plasma membranes compared to 3.91 +/- 0.14 in controls (P less than 0.001) and 2.87 +/- 0.24 in the nondiabetic diabetes-prone rats (P less than 0.02). Reduction in GLUT-2 correlates temporally with and may contribute to the loss of glucose-stimulated insulin secretion that precedes profound beta-cell depletion of autoimmune diabetes.

Reduced β-Cell Glucose Transporter in New Onset Diabetic BB Rats

L. Orci,‡ R. H. Unger,* M. Ravazzola,‡ A. Ogawa,* I. Komiya,* Dany Baetens,‡ H. F. Lodish,§ and B. Thorens§

**Center for Diabetes Research, Gifford Laboratories, Department of Internal Medicine, University of Texas Southwestern Medical Center and Department of Veterans Affairs Medical Center, Dallas, Texas 75235; ‡Department of Morphology, University of Geneva Medical School, Geneva, Switzerland; and §Whitehead Institute for Biomedical Research and the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142*

Abstract

Previous studies from our laboratories have suggested a defect in glucose transport in islets isolated from BB rats on the first day of overt diabetes. To quantitate by immunostaining the glucose transporter of β-cells (GLUT-2) before and at the onset of autoimmune diabetes we employed an antibody to its COOH-terminal octapeptide. On the first day of overt diabetes, defined as the day the daily blood glucose first reached 200 mg/dl, the volume density ratio of GLUT-2-positive to insulin-positive β-cells was only 0.48±0.06, compared to 0.91±0.02 in age-matched nondiabetic diabetes-resistant controls ($P < 0.001$). In age-matched nondiabetic diabetes-prone rats, most of which would have become diabetic, the ratio was 0.85±0.02, also less than the controls ($P < 0.05$). Protein A–gold labeling of GLUT-2 in β-cells of day 1 diabetic rats revealed 2.17±0.16 gold particles per micrometer length of microvillar plasma membranes compared to 3.91±0.14 in controls ($P < 0.001$) and 2.87±0.24 in the nondiabetic diabetes-prone rats ($P < 0.02$). Reduction in GLUT-2 correlates temporally with and may contribute to the loss of glucose-stimulated insulin secretion that precedes profound β-cell depletion of autoimmune diabetes. (*J. Clin. Invest.* 1990. 86:1615–1622.) Key words: homeostasis • glucose-stimulated insulin secretion • glucose transporter

Introduction

Normal blood glucose homeostasis depends in large part on the integrity of glucose-stimulated insulin secretion by the pancreatic β-cells. In the preovert phase of autoimmune diabetes both in man (1) and in BB/Wor rats (2) glucose-stimulated insulin secretion wanes progressively and is absent at the onset of fasting hyperglycemia, at which point the diagnosis of overt diabetes is made. The loss of glucose-stimulated insulin secretion generally precedes the loss of the response to nonglucose secretagogues, which indicates the presence of viable β-cells (2) and suggests the possibility of early involvement of a glucose-specific component of the glucose stimulating pathway of β-cells (3). Our demonstration of a profound reduction in the rate of glucose transport in islets isolated from BB/Wor rats on the first day of overt diabetes (2) pointed to the β-cell glucose transporter as a possible site of a lesion.

The demonstration that the rat hepatocyte glucose transporter is identical in primary structure to the glucose transporter of β-cells (4, 5), and that antibodies to it react specifically with β-cells (4, 6) made possible the quantitation of this transporter in β-cells and the testing of the foregoing hypothesis. The results demonstrate that, at the onset of autoimmune diabetes, immunodetectable glucose transporters of β-cells (GLUT-2),[1] synonomously referred to as the "liver-type" glucose transporter (4) GLUT-2 (7) or as the L-type transporter (8), are reduced to a degree that could contribute to the β-cell glucose insensitivity that constitutes the earliest known functional derangement of autoimmune diabetes. It has been shown in a rodent model of noninsulin-dependent diabetes that the insulin response to glucose is absent when < 60% of β-cells display immunocytochemically detectable GLUT-2 (9).

Methods

Male BB/Wor rats were employed as models of autoimmune diabetes. All diabetes-prone rats were kept in metabolic cages with free access to standard laboratory chow. Blood glucose levels were determined daily from tail blood samples and urinary glucose was measured daily using Ketodiastix. Because fasting hyperglycemia below 200 mg/dl may be transient (2), diabetes was diagnosed only when the blood glucose reached that level. At least 20% of β-cells are present on the first day of diabetes in BB rats, but virtually all β-cells have been destroyed by the 14th day (2). Therefore, to study adequate numbers of β-cells after the onset of overt diabetes it was necessary to obtain pancreata within 24 h of the diagnosis. These were compared with pancreata from age-matched diabetes-prone rats that had not yet become diabetic and from age-matched diabetes-resistant controls that never develope diabetes. Pancreata were isolated and perfused by the method of Grodsky and Fanska (10) as modified previously (11). The insulin response to 20 mM glucose and to 10 mM arginine were determined on pancreatic effluent collected at 1 min intervals. Insulin was assayed by the method of Yalow and Berson (12) as modified by Herbert et al. (13).

In another group of rats a 40% pancreatectomy was performed by the method of Foglia (14). 3 wk later an indwelling catheter was inserted in the superior jugular vein and 50% glucose was infused continuously for 14 d at a variable rate designed to maintain daily fasting glucose levels > 250 mg/dl. The method used has been described in detail elsewhere (15). The purpose of the partial pancreatectomy was to facilitate the maintenance of hyperglycemia by reducing the β-cell mass.

Immunocytochemical studies of the glucose transporter were carried out with an antibody raised against the COOH-terminal domain of the rat hepatocyte glucose transporter (4). Antibodies to this region of the hepatic glucose transporter have been used previously for im-

Address reprint requests to Roger H. Unger, M.D., University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235.

Received for publication 21 March 1990 and in revised form 21 May 1990.

J. Clin. Invest.

0021-9738/90/11/1615/08 $2.00
Volume 86, November 1990, 1615–1622

1. *Abbreviation used in this paper:* GLUT-2, glucose transporter of B-cells.

munocytochemical studies of normal β-cells at both the light and electron microscopic levels (4, 6). The antibody recognizes a single 55-kD band on immunoblots of membrane preparations of islets and liver (4). Immunofluorescence is blocked by preincubation of the antibody with the synthetic antigen (6). Pancreata were fixed in Bouin's solution and processed for paraffin embedding. Immunofluorescent staining of the β-cell for glucose transporter and for insulin were carried out as described previously (6). The juxtasplenic region of the pancreas was used (16), except in the partially depancreatized rats. Quantitation (volume density) of cells expressing glucose transporter and insulin was determined on consecutive serial sections by the method of point-counting (17). Five different islets were studied per pancreas. The volume density of GLUT-2 (*Vv* GLUT-2) was obtained according to the formula *Vv* GLUT-2 = P GLUT-2/P islets, where P GLUT-2 is the number of points of the morphometric lattice falling upon GLUT-2 positive cells (irrespective of the magnitude of the fluorescent signal encircling the cell, see Fig. 1) and P islets is the number of points of the lattice over total islet tissue. For the determination of insulin volume density (*Vv* insulin), the initial formula was modified to correct the overestimation due to the bright insulin immunofluorescence (18). The formula used was:

$$Vv\ \text{insulin} = \frac{\text{P cells}}{\text{P islets}} - \frac{\text{I cells}}{\text{P islets}} \times \frac{T}{4d},$$

where P cells = points of lattice upon fluorescent cells; P islets = points of the lattice upon islets; I cells = intersection of each horizontal and vertical line of the lattice with fluorescent cells; T = thickness of the section; d = distance between horizontal and vertical lines of the lattice. Immunodetection of glucose transporter at the electronmicroscopic level was performed by the protein A–gold method (19). The animals were fixed by vascular perfusion with 1% glutaraldehyde in 0.1 M phosphate buffer pH 7.4. The pancreas was dissected, minced, and processed for Lowicryl embedding at low temperature (20). Thin sections containing islets of Langerhans were collected on nickel grids and incubated with affinity purified anti-GLUT-2 COOH-terminal decapeptide (1:20) for 3 h at room temperature followed by washing with PBS (0.01 M phosphate buffer, 0.15 M NaCl), pH 7.4 and exposure to protein A gold (gold size : ~ 15 nm) for 1 h. Grids were washed again and counterstained with uranyl acetate and lead citrate before examination in the electron microscope.

The specificity of the immunolabeling was assessed by incubating the tissue with the anti-GLUT-2 antiserum previously adsorbed with an excess of the immunizing antigen or by omitting the first layer antibody.

Results

Glucose-stimulated insulin secretion

As reported previously in a larger group of animals (2), glucose-stimulated insulin secretion was completely absent in perfused rat pancreata from new onset diabetic rats (n = 3). The insulin response to glucose averaged 0.99±0.41 mU/10 min in nondiabetic diabetes-prone rats and 0.8±0.26 mU/10 min in nondiabetic diabetes-resistant rats. Arginine-stimulated insulin secretion by pancreata of new-onset diabetic rats averaged 0.83±0.13 mU/10 min, confirming the antecedence of the loss of glucose-stimulated insulin secretion and indicating that arginine-responsive β-cells were present at the time blood glucose first reached 200 mg/dl. Arginine-stimulated insulin secretion averaged 4.2±0.55 mU/10 min in the nondiabetic diabetes-prone rats and 5.9±0.66 mU/10 min in the nondiabetic diabetes-resistant group.

Immunocytochemical studies

Immunofluorescence. Immunostaining for GLUT-2 was significantly reduced in β-cells of new onset diabetic rats (Fig. 1 and Table I). In diabetes-resistant controls, the volume density of glucose transporter-positive cells was approximately equal to the volume density of the insulin-positive cells (ratio > 0.90); in the day-1 diabetic rats, however, this ratio averaged 0.48±0.06. This indicates that approximately half of the surviving β-cells appeared to be negative for GLUT-2. In the nondiabetic diabetes-prone rats, the ratio of GLUT-2-positive cells to insulin-positive cells was also slightly reduced (0.85±0.02). There was no immunofluorescence staining of pancreas section using an antibody against the human red cell transporter, GLUT-1 (21).

Immunogold. Because the foregoing "all or none" method of recording this signal (see Material and Methods) could have scored as negative β-cells expressing very low levels of GLUT-2, we performed a detailed electron microscopic analy-

Table I. Volume Density (Vv) of GLUT-2 and Insulin Immunofluorescent Cells in Islets of BB/Wor Rats (A–C) (Nondiabetic Diabetes-resistant, Nondiabetic Diabetes-prone and Diabetic on First Day of Diabetes) and 40% Pancreatectomized Nondiabetic Wistar Rats (D) after Chronic Infusion (14 d) with 5 or 50% Glucose

	Vv GLUT-2 (×10³)	*Vv* insulin (×10³)	Ratio (*Vv* GLUT-2/*Vv* insulin)
A Nondiabetic diabetes-resistant BB/Wor (n = 10)	480.11±14.02	532.16±18.44	0.91±0.02
B Nondiabetic diabetes-prone BB/Wor (n = 10)	461.73±25.67	549.65±33.75	0.85±0.02*
C Diabetic BB/Wor (n = 7)	138.69±41.09	279.41±60.52	0.48±0.06‡
D 40% pancreatectomized nondiabetic Wistar§			
5% glucose (n = 2)	416.69±22.01	454.78±3.90	0.92±0.05
50% glucose (n = 2)	450.52±60.37	451.47±60.93	1.00±0.01

n, No. of animals. * P value < 0.05 between *A* and *B*. ‡ P value < 0.001 between *A* and *C* and *B* and *C*. § The ratio *Vv* GLUT-2/*Vv* insulin of the resected portion of the pancreas averaged 0.95±0.01 in the four rats subjected to the glucose perfusion studies.

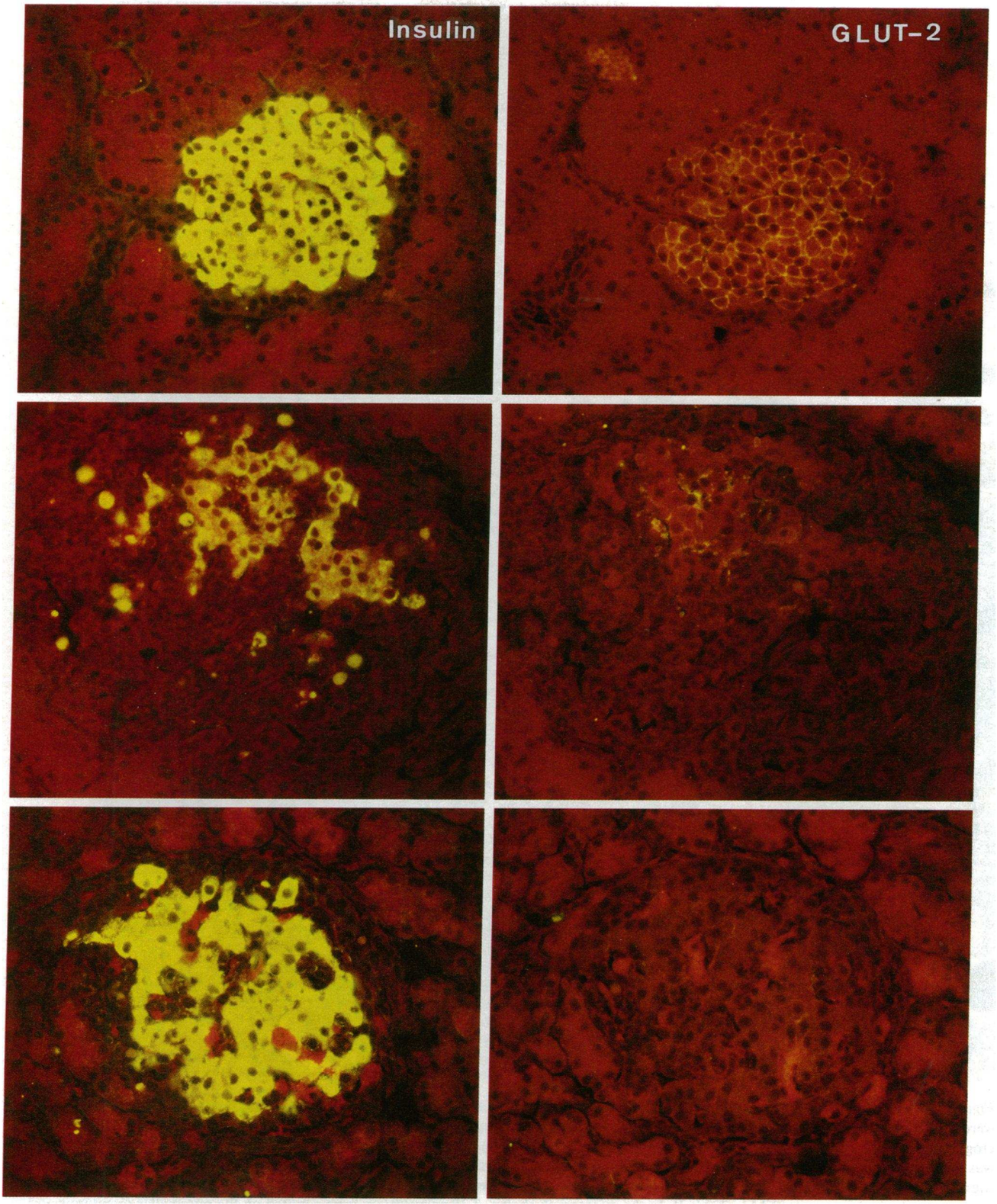

Figure 1. Pairs of consecutive serial sections of BB/Wor rats pancreas stained by immunofluorescence with antibodies to insulin and GLUT-2. The upper row is from a diabetes-resistant animal: left panel shows the brightly stained insulin-cell mass and, to the right in a consecutive section, a comparable number of cells with the characteristic peripheral rim of GLUT-2 staining. The middle row is from a 1-d diabetic: it reveals a reduced number of insulin cells and still fewer GLUT-2 immunofluorescent cells on the consecutive section. The bottom row is also from a 1-d diabetic animal and illustrates a less frequent pattern of immunostaining in this experimental group: rather abundant insulin-positive cells, with profound reduction of GLUT-2 labeling in the consecutive section. The quantitative evaluation of these immunofluorescent patterns was expressed as the ratio of GLUT-2-positive cells to insulin-positive cells on pairs of consecutive sections and is shown in Table I. Magnification, 320.

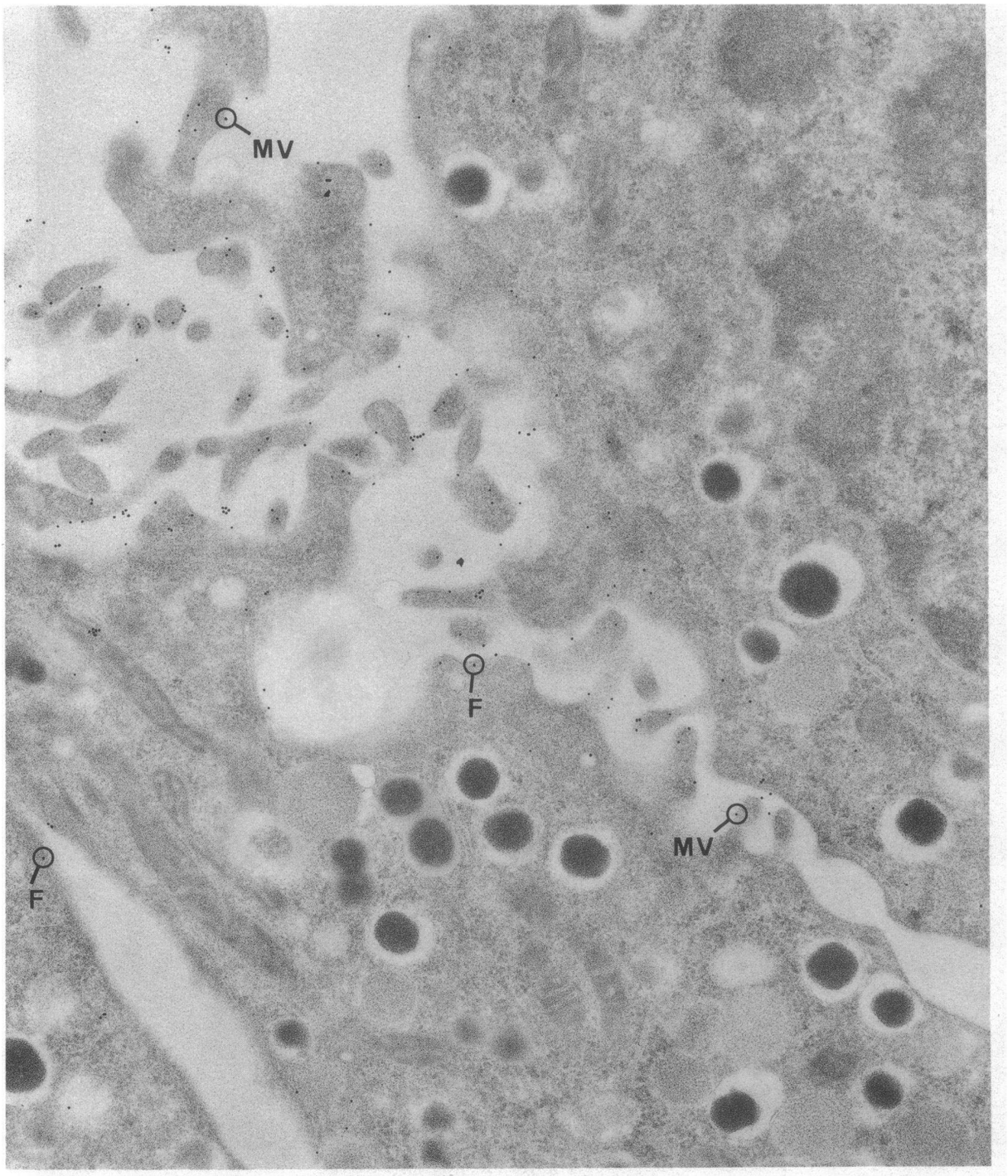

Figure 2. (*A*) Electron micrograph showing parts of three β-cells in a diabetes-resistant animal. 72 such micrographs at the same magnification were used to quantitate GLUT-2 immunogold labeling on β-cell plasma membranes in this group (four animals, three islets per animal, six micrographs per islet). In each picture, the respective length (in micrometers) of the flat (*F*) or microvillar (*MV*) regions of the plasma membrane was measured with an electronic pen and the number of immunogold particles (*black dots*) in each region was recorded. The number of immunogold particles per micrometer length of membrane is expressed as frequency histograms for microvillar or flat membrane in Fig. 3, *A* and *B*, respectively, and as mean±SEM in Table II. In the micrograph shown, 3.36 particles/μm were counted on the microvillar membrane and 0.39 particles/μm on the flat membrane. This micrograph thus belongs to the class 3–3.99 and 0–0.49 of the respective frequency histograms (Fig. 3, *A* and *B*). Circles outline immunogold particles associated, respectively, with the flat (*F*) or microvillar (*MV*) membrane. Magnification, 34,500. (*B*) Electron micrograph showing parts of two β-cells in a 1-d diabetic animal. 72 such micrographs at the magnification shown were used to quantitate GLUT-2 immunogold labeling on β-cell plasma membrane in this group (four animals, three islets per animal, six micrographs per islet) (cf. Table II). In the micrograph shown, 0.41 particles/μm were counted on the microvillar membrane and 0.14 particles/μm on the flat membrane. This micrograph thus belongs to the class 0–0.99 and 0–0.49 of the respective frequency histograms (Fig. 3, *A* and *B*).

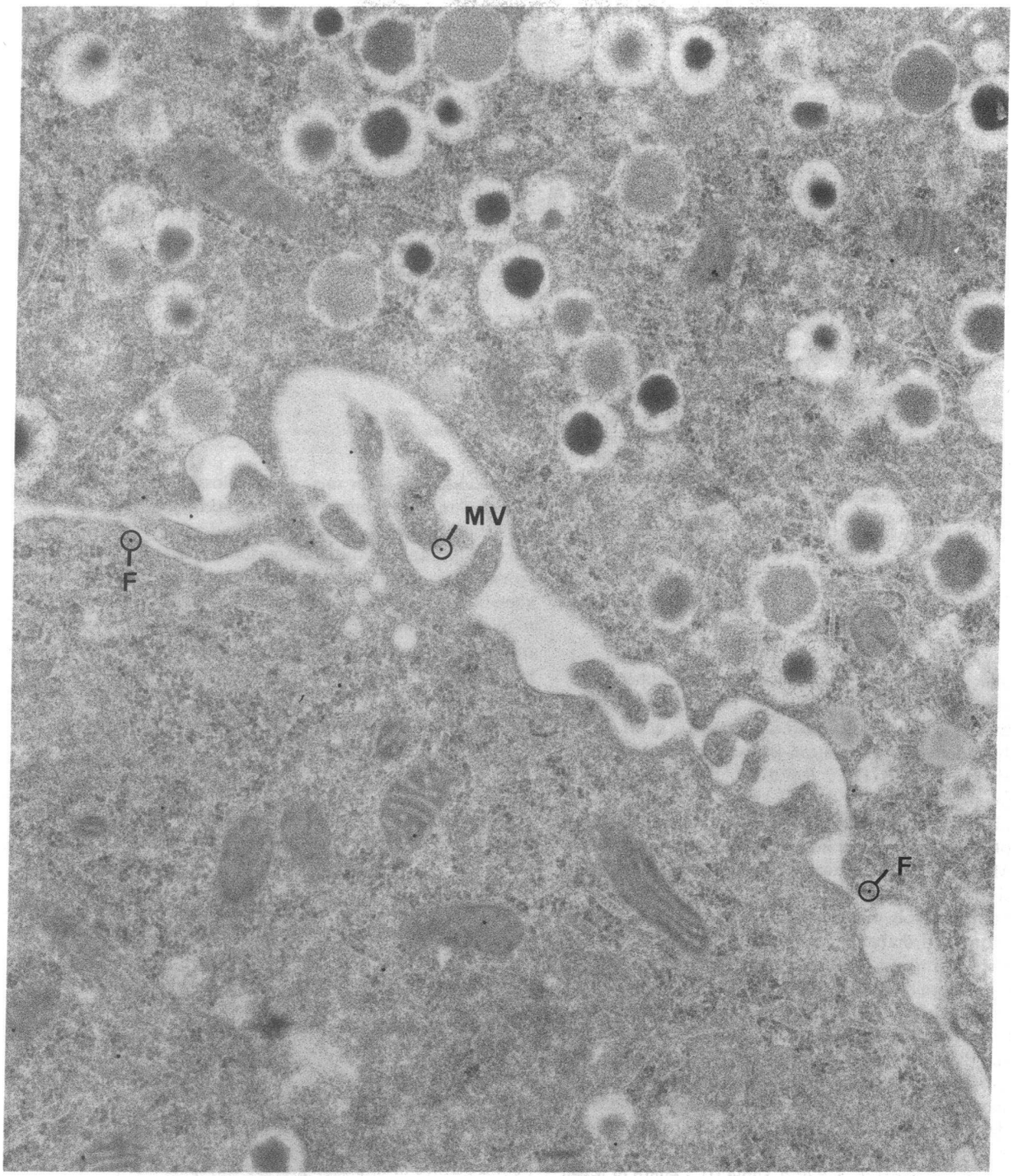

Figure 2 (Continued)

sis of GLUT-2 immunolabeling using the protein A–gold method (immunogold). GLUT-2 immunoreactivity was preferentially expressed on domains of the β-cell plasma membrane that contain microvilli, while the flat portion of the membrane was less intensely labeled (Fig. 2 *A* and Table II). This inhomogeneous distribution of the transporter is consistent with our previous studies of normal rat islets (6). In diabetes-prone animals immunolabeling of β-cells in some islets

Circles outline immunogold particles associated, respectively, with the flat (*F*) or microvillar (*MV*) membrane. The same method of quantitation was applied to the diabetes-prone group (three animals, three islets per animal, six pictures per islet = 54 micrographs) and the data are also shown in Fig. 3, *A* and *B*, and in Table II. Magnification, 34,500.

Table II. Protein A–Gold Labeling of GLUT-2 on the β-Cell Plasma Membrane in BB/Wor Rats (Nondiabetic Diabetes-resistant, Nondiabetic Diabetes-prone and Diabetic on First Day of Diabetes)

	GLUT-2 density*	
	Flat membrane	Microvilli
A Nondiabetic diabetes-resistant BB/Wor $n = 72$	1.01±0.06‡ (849)	3.91±0.14§ (1445)
B Nondiabetic diabetes-prone BB/Wor $n = 54$	0.82±0.08 (744)	2.87±0.24‖ (906)
C Diabetic BB/Wor $n = 72$	0.78±0.06 (946)	2.17±0.16 (1022)

* Densities are expressed as the number (mean±SEM) of gold particles per unit length (1 μm) of membrane. Immunogold particles were counted on pairs of β-cell membranes delimiting the intercellular space (cf. Fig. 2, *A* and *B*). *n*, number of pictures. In parentheses is the micrometer length of plasma membrane evaluated. The quantitative data were evaluated on four rats for *A* and *C* and three rats for *B*. In each animal three islets from the juxtasplenic part of the pancreas (six randomly-taken pictures per islet at a calibrated magnification of 34,500) were used. The labeling density on the plasma membrane of exocrine acinar cells was 0.10±0.02 (358 μm evaluated).
‡ $P < 0.01$ between *A* and *C*.
§ $P < 0.001$ between *A* and *B* and between *A* and *C*.
‖ $P < 0.02$ between *B* and *C*.

did not differ markedly from controls, whereas in other islets GLUT-2 labeling of β-cells was reduced. In diabetic animals, β-cells of most islets displayed an overall reduction of GLUT-2 labeling, but there was a variation in the degree of labeling among individual β-cells (Fig. 2 *B* and Table II). This variability was quantitated in each animal group by arranging the number of immunogold particles over microvillar and flat portions of β-cell membranes into frequency classes (Fig. 3, *A* and *B*). Although some immunolabeling values in diabetes-prone and diabetic animals were comparable to those of diabetes-resistant controls, on the microvillar membrane of the diabetes-prone and diabetic groups, a distinct shift toward a low number of immunogold particles per micrometer of membrane was apparent (Fig. 3 *A*). Pooling of all individual data of GLUT-2 labeling of microvilli of β-cells in each group (Table II), reveals a 27% decrease in immunolabeling in the nondiabetic diabetes-prone group and a 45% decrease in the diabetic group compared to diabetes-resistant controls. The level of labeling present on the flat membrane of β-cells is slightly decreased in diabetes-prone and diabetic animals compared to controls; the decrease reaches significance, however, only for diabetes (Fig. 3 *B* and Table II). Length of β-cell plasma membrane occupied by microvilli was 63% in the islets of a diabetes-resistant control, 54% in a diabetes-prone but nondiabetic BB rat and 51% in a diabetic animal. Thus electronmicroscopic analysis reveals a significant overall decrease of GLUT-2 transporters in surviving β-cells on the first day of overt diabetes with a spectrum of labeling among individual cells.

Effect of hyperglycemic clamping

To determine if the reduction in β-cell glucose transporter was secondary to the brief period of antecedent hyperglycemia, we studied the effects of exposure to high glucose concentration upon GLUT-2 immunoreactivity in normal β-cells. Normal Wistar rats were subjected to 40% subtotal pancreatectomy 3 wk earlier so as to reduce the compensatory capacity of their β-cell mass, thereby facilitating the maintenance of blood glucose levels at or above 250 mg/dl for 14 d by means of a constant infusion of 50% glucose. At the end of the 2-wk period of the hyperglycemic clamp, at which time the mean insulin response to 20 mM glucose was 0.95±0.1 mU/10 min (vs. 0.83±0.13 in controls), the ratio of glucose transporter-positive to insulin-positive cells was 1.0±0.1 (see Table I). Thus it appears that in normal rats hyperglycemia by itself will not abolish or reduce the percentage of insulin-positive cells that express glucose transporter. Therefore it is unlikely that the reduction observed in the new onset diabetic rats was secondary to the briefer period of less severe hyperglycemia.

Discussion

This study in BB/Wor rats reveals a loss of immunostainable GLUT-2 in approximately half of β-cells that have survived until the first day of overt diabetes and an overall 45% reduction in immunoreactive transporters per length of β-cell microvillar membrane at the electronmicroscopic level. In nondiabetic, diabetes-prone, age-matched animals there was a 27% reduction in GLUT-2 below controls. This relative scarcity of immunodetectable glucose transporters in the 20% of β-cells still present on the first day of the disease may contribute to the > 90% reduction of glucose transport rates reported previously in islets of new onset BB rats (2), particularly if some immunodetectable transporters are nonfunctional. In addition, the 50% inhibition of 3-O-methyl-glucose uptake caused by incubation of normal islets with immunoglobulins from new onset type 1 diabetic patients (3) has also been observed with serum of new onset diabetic rats (Johnson, J. H., B. Crider, and R. H. Unger, unpublished data); thus transport via a reduced number of functioning transporters on a depleted β-cell population would be still further impaired. The complete loss of glucose-stimulated insulin release at a time when the response to arginine is still present may be the consequence of the fact that < 60% of β-cells displayed immunodetectable GLUT-2 (9). In addition glucose transport inhibition by immunoglobulins bound to a profoundly reduced number of β-cells may have further contributed to the functional loss observed here. Because the insulin response to a high glucose concentration is required to maintain normal blood glucose homeostasis, the initial hyperglycemia early in type 1 diabetes before profound autoimmune depletion of β-cells could be, at least in part, a consequence of impaired glucose transport into β-cells.

The reduction in GLUT-2 observed here may well represent a nonspecific consequence of an autoimmune injury that similarly affects expression of other β-cell surface proteins not involved in the insulin response to glucose. A reduction in glucagon-stimulated insulin secretion has been reported to parallel the reduction in the response to glucose in type 1 diabetic patients (22). On the other hand, reduction in immunodetectable GLUT-2 could represent a relatively selective β-cell response to autoimmune injury; indeed, low levels of GLUT-2 were noted occasionally in islets that otherwise appeared to be relatively normal.

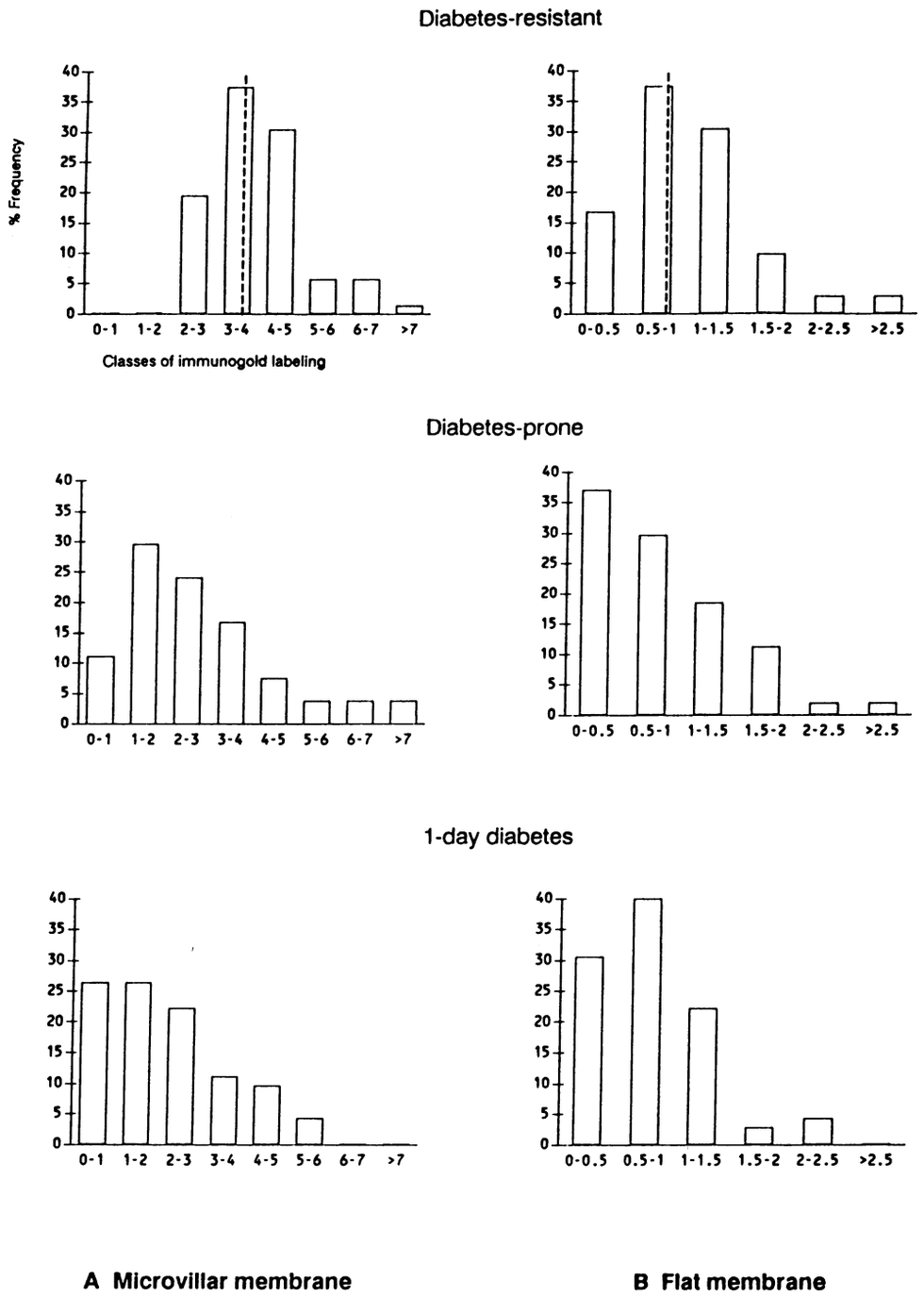

Figure 3. Frequency of GLUT-2 immunogold labeling of β-cell plasma membranes. The number of gold particles per micrometer length of membrane are arranged in classes of increasing magnitude (0 to > 7 in *A;* 0 to > 2.5 in *B*). The height of each bar indicates the percent frequency in each class = (No. of micrographs in the class/total no. of micrographs) × 100. In upper panels, the dotted vertical line shows the median number of immunogold particles per micrometer length of membrane in diabetes-resistant control animals. (*A*) Microvillar regions of plasma membranes showing the shift towards fewer particles in diabetes-prone and diabetic groups. (*B*) Flat regions of plasma membranes.

Acknowledgments

Supported by the Swiss National Science Foundation (grant No. 31-26625.89), National Institutes of Health (grant No. DK02700-30, GM40916, and HL41484), Veterans Administration Institutional Research Support (grant No. 549-8000), Diabetes Research and Education Foundation, and Juvenile Diabetes Foundation.

References

1. Srikanta, S., O. P. Ganda, G. S. Eisenbarth, and J. S. Soeldner. 1983. Islet-cell antibodies and β-cell function in monozygotic triplets and twins initially discordant for type 1 diabetes mellitus. *N. Engl. J. Med.* 308:322–325.

2. Tominaga, M., I. Komiya, J. H. Johnson, L. Inman, T. Alam, J. Moltz, B. Crider, Y. Stefan, D. Baetens, K. McCorkle, L. Orci, and R. H. Unger. 1986. Loss of insulin response to glucose but not arginine during the development of autoimmune diabetes in BB/W rats: relationships to islet volume and glucose transport rate. *Proc. Natl. Acad. Sci. USA.* 83:9749–9753.

3. Johnson, J. H., B. P. Crider, K. McCorkle, M. Alford, and R. H. Unger. 1990. Inhibition of glucose transport into rat islet cells by immunoglobulins from patients with new-onset insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 322:653–659.

4. Thorens, B., H. K. Sarkar, H. R. Kaback, and H. F. Lodish. 1988. Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney and β-pancreatic islet cells. *Cell.* 55:281–290.

5. Johnson, J. H., C. B. Newgard, J. L. Milburn, H. F. Lodish, and B. Thorens. 1990. The high km glucose transporter of islets of Langerhans is structurally identical and functionally similar to the high km glucose transporter of liver. *J. Biol. Chem.* 265:6548–6551.

6. Orci, L., B. Thorens, M. Ravazzola, and H. F. Lodish. 1989. Localization of the pancreatic beta cell glucose transporter to specific plasma membrane domains. *Science (Wash. DC).* 245:295–297.

7. Fukumota, H., S. Seino, H. Imura, Y. Seino, R. L. Eddy, Y. Fukushima, M. G. Byers, T. B. Shows, and G. I. Bell. 1988. Sequence, tissue distribution, and chromosomal localization of mRNA encoding a human glucose transporter-like protein. *Proc. Natl. Acad. Sci. USA.* 85:5434–5438.

8. Pilch, P. F. 1990. Editorial; Glucose transporters: what's in a name? *Endocrinology.* 126:3–5.

9. Johnson, J. H., A. Ogawa, L. Chen, L. Orci, C. B. Newgard, T. Alam, and R. H. Unger. 1990. Underexpression of high K_m glucose transporters in β-cells: role in noninsulin-dependent diabetes. *Science (Wash. DC).* In press.

10. Grodsky, G. M., and R. E. Fanska. 1975. The *in vitro* perfused pancreas. *Methods Enzymol.* 39:364–372.

11. Hisatomi, A. H., H. Maruyama, L. Orci, M. Vasko, and R. H. Unger. 1985. Adrenergically-mediated intrapancreatic control of the glucagon response to glycopenia in the isolated rat pancreas. *J. Clin. Invest.* 75:420–426.

12. Yalow, R. S., and S. A. Berson. 1960. Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* 39:1157–1175.

13. Herbert, V., K. S. Lau, C. W. Gottlieb, and S. J. Bleicher. 1965. Coated charcoal immunoassay of Insulin. *J. Clin. Endocrinol. & Metab.* 25:1375–1384.

14. Foglia, V. G. 1944. Caracteristica de la diabetes en la rata. *Rev. Soc. Argent. Biol.* 20:21–37.

15. Komiya, I., D. Baetens, M. Kuwajima, L. Orci, and R. H. Unger. 1990. Compensatory capability of islets of BB/Wor rats exposed to sustained hyperglycemia. *Metabolism.* 39:614–618.

16. Orci, L. 1982. Macro- and micro-domains in the endocrine pancreas. *Diabetes.* 31:538–565.

17. Weibel, E. R. 1979. Practical methods for biological morphometry. *In* Stereological Methods. Vol. 1. Academic Press, Inc., London. 101–161.

18. Baetens, D., Y. Stefan, M. Ravazzola, F. Malaisse-Lagae, D. L. Coleman, and L. Orci. 1978. Alteration of islet cell populations in spontaneously diabetic mice. *Diabetes.* 27:1–7.

19. Roth, J., M. Bendayan, and L. Orci. 1978. Ultrastructural localization of intracellular antigens by the use of protein A-gold complex. *J. Histochem. Cytochem.* 26:1074–1081.

20. Armbruster, B. L., E. Carlemalm, R. Chiovetti, R. M. Garavito, J. A. Hobot, E. Kellenberger, and W. Villiger. 1982. Specimen preparation for electron microscopy using low temperature embedding resins. *J. Microsc.* 126:77–85.

21. Wheeler, T. J., I. A. Simpson, D. C. Sagin, P. C. Hinkle, and S. W. Cushman. 1982. Detection of the rat adipose cell glucose transporter with antibody against the human red cell glucose transporter. *Biochem. Biophys. Res. Commun.* 105:89–95.

22. Heaton, D. A., N. R. Lazarus, D. A. Pyke, and R. D. G. Leslie. 1989. β-Cell responses to intravenous glucose and glucagon in nondiabetic twins of patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia.* 32:814–817.