Intestinal Adaptation to Diabetes

Altered Na-dependent Nutrient Absorption In Streptozocin-treated Chronically Diabetic Rats

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Abstract

To examine the pattern and mechanism of enhanced intestinal nutrient absorption in diabetes, we measured intestinal transport of 3-*O*-methylglucose (3OMG), *l*-alanine (ALA), and SO_4 in male Lewis rats made diabetic with streptozocin. Diabetes enhanced 3OMG absorption fivefold in ileum and threefold in jejunum; ALA absorption increased twofold in ileum but not at all in jejunum; ileal SO_4 transport was unaffected. Increases in 3OMG and ALA transport were due solely to increases in maximum velocity. The enhancement of ileal glucose absorption was half-maximal in 40–45 d, could be reversed by 10 d of treatment with insulin and did not result from adrenergic denervation. The density of glucose carriers per milligram brush border protein (measured as [^{3}H]phlorizin binding sites) was not altered but there was a sixfold increase in the number of glucose-inhibitable [^{3}H]phlorizin-binding sites in the intact epithelium. Generalized mucosal hypertrophy accounted for < 30% of this increase. We conclude that the intestine adapts to streptozocin-induced diabetes through recruitment of additional brush border carriers for sugar, probably in the midvillus–to–crypt region.

Introduction

Alterations in the intestinal absorption of both nutrients and inorganic ions can occur in response to dietary availability, body stores, nutrient requirements, and surgery. In human, poorly controlled insulin-dependent diabetic subjects, increased jejunal *d*-glucose absorption has been reported (1). However, a separate study found no change in the absorption of a nonmetabolized glucose analogue (2). Experimental diabetes in rats has been found to enhance diaccharidase activity but not to increase microvillus membrane protein (3). Enhanced absorption of amino acids, glucose, bile acids, phosphate, cholesterol, fatty acids, and fatty alcohols have also been reported (4–10). These enhancements of absorption have been variously attributed to increased maximal transport capacity (6, 11), increased passive permeability (11), alterations in unstirred water layers (11), and increased mucosal mass (12–14). The mechanisms responsible for these adaptive changes remain elusive.

In the present study we have investigated the changes in Na-coupled nutrient absorptive processes that occur in the small intestine of streptozocin-treated rats. We first measured transport of glucose, alanine, and SO_4 in jejunum and ileum and then measured the change in ileal sugar transport in more detail. We determined its time course, its reversibility with insulin, and its relationships to the number of phlorizin-binding sites on the brush border and to overall mucosal hypertrophy and adrenergic denervation.

Methods

Materials

Streptozocin was obtained from UpJohn Co. (Kalamozoo, MI). Intermediate-acting beef/pork insulin (4 mg/IU) was obtained from Eli Lilly Co. (Indianapolis, IN). [^{3}H]PEG (mol wt 900, 5 mCi/mmol), [^{3}H]-3-ortho-methyl-*d*-glucose (60 Ci/mmol), [^{14}C]-*l*-alanine (150 mCi/mmol), $Na^{35}SO_4$ (113 mCi/mmol), and [^{3}H]phlorizin (55 Ci/mmol) were obtained from New England Nuclear, Boston, MA.

Hepes was obtained from Sigma Chemical Co., St. Louis, MO. The remainder of the chemicals (reagent grade) were also obtained from Sigma Chemical Co.

Animal preparation

Induction of diabetes. Male Lewis rats (250–300 g; Harlan Sprague Dawley, Inc., M. A. Laboratory Animals and Teklad Diets, Indianapolis, IN) were made diabetic with streptozocin (50 mg/kg) administered via dorsal tail vein. Diabetic and age-matched control animals were allowed access to standard rat chow and water ad lib. and housed in a light cycled animal care facility. Blood glucose concentration was determined periodically on a drop of blood obtained from the dorsal tail vein. A reflectance photometer and glucose reagent strips (Ames Division, Miles Laboratories, Inc., Elkhart, IN) were used for these determinations. Total glycosylated hemoglobin was measured by the thiobarbituric acid method (15) on blood obtained by cardiac puncture immediately after animals were sacrificed. Chronic diabetic rats (CD)[1] were those with persistent hyperglycemia (> 325 mg/dl) for 90–120 d.

Insulin administration. Insulin-treated rats were given intermediate-acting insulin (4–6 U subcutaneously) each day at 0800 h for 10 consecutive days to maintain euglycemia. Blood glucose in each of these animals was determined daily immediately before insulin injection. Mean blood glucose was 445±26 mg/dl before insulin treatment and decreased to 151±95 mg/dl during the insulin treatment period. Mean blood glucose concentration in age-matched nondiabetic rats was 129±15 mg/dl.

Sympathectomy using 6-hydroxydopamine. Using a modification of the method of Chang et al. (16), we gave normal littermates of the chronic diabetic group a single dose of 6-hydroxydopamine (6-OHDA), 50 mg/kg dissolved in 0.9% NaCl and 1% ascorbic acid, through the dorsal tail vein. 6-OHDA selectively destroys postsynaptic adrenergic neurons within the intestine (17). 3-*O*-Methylglucose (3OMG) transmural transport was

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1. *Abbreviations used in this paper:* ALA, *l*-alanine; BBM, brush border membrane; CD, chronic diabetic rat; Isc, short-circuit current; m, mucosa; 6-OHDA, 6-hydroxydopamine; 3OMG, 3-*O*-methylglucose; PD, potential difference; R, electrical resistance; s, serosa.

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measured as described below at 14 and 30 d post 6-OHDA injection. The degree of sympathectomy induced by 6-OHDA was assessed as previously described (16). Briefly, mucosal norepinephrine stores were estimated by the short-circuit current (Isc) response to the serosal addition of 10 μM tyramine which, at that concentration, effectively releases 80% of all stored norepinephrine (18).

Experimental preparations. Rats were sacrificed with diethyl ether overdose. 30 cm of distal ileum, ending 2 cm from the ileal cecal valve, and 30 cm of proximal jejunum, beginning just distal to the ligament of Trietz, were quickly excised, opened along the mesenteric border, and rinsed with ice-cold normal Ringer. The serosa and outer muscle layer was then removed by dissection. Before use, all tissues were maintained in ice-cold normal Ringer solution gassed with 5% CO_2 in O_2. Tissues were then clamped in Ussing-type chambers and bathed on both sides by normal Ringer (in mmol/liter: Na, 144; K, 5; Ca, 1.25; Mg, 1.1; Cl, 117.5; HCO_3, 25; H_2PO_4, 0.35; HPO_4, 1.65), gassed with 5% CO_2 in O_2 (pH 7.4), and maintained at 37°C. Transepithelial electric potential difference (PD), electrical resistance (R), and Isc were determined as previously described (19). A Cl- and HCO_3-free Ringer solution, in which these ions were replaced by gluconate and 10 mM Tris/Hepes (pH 7.4), gassed with 100% O_2, was used to determine the anion dependency of glucose-induced changes in short-circuit current. In all experiments, 40 mM fructose was added to the serosal and mucosal bathing medium to ensure sufficient substrate for energy metabolism.

Intact tissue experiments

Transmural solute flux measurements. Transmural fluxes of radioactively labeled 3OMG, *l*-alanine (ALA), and SO_4 were determined in duplicate in tissues taken from adjacent gut segments. Tissues were not used for paired analyses if their electrical resistances differed by > 25%. 20 min after tissues were mounted in chambers, [^{3}H]3OMG (0.25 μCi/ml), [^{14}C]ALA (0.15 μCi/ml), and $^{35}SO_4$ (0.1 μCi/ml) were added to one side and unidirectional transmural fluxes from mucosa (m)-to-serosa (s) and from s-to-m (J_{ms} and J_{sm}, respectively) were determined as previously described (20) during a 20-min flux period following a 25-min equilibration period. In preliminary experiments, serial flux measurements established that steady-state rates of radioisotope transfer of each of these solutes from m-to-s and from s-to-m were present within 15 min of the addition of radioisotope (data not shown).

Morphological techniques. Jejunal and ileal mucosal sheets from control and CD animals were fixed while mounted in Ussing chambers with 1% glutaraldehyde. This initial fixation, which ensured that the tissues maintained the shape they held under chamber conditions, was followed by fixation for 2 h at 4°C in a solution of 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. After washing in cacodylate buffer, tissues were postfixed in 1% osmium tetroxide, washed again, dehydrated, and embedded in epoxy resin. To ensure that differences in structural appearance of chamber-mounted control vs. CD intestine were present before chamber mounting, transmural sections of jejunum and ileum from these animals were also fixed, as outlined above, immediately upon removing the intestine from the animals. Oriented 1-μm sections were obtained from multiple blocks of each tissue source with glass knives and oriented representative thin sections were obtained with diamond knives.

To compare mucosal surface lengths per unit intestinal length between control and CD animals, 12 1-μm sections of ileal mucosa, each containing at least six oriented villi, were photographed and photomicrographs were printed at final magnifications of 450 ×. Morphometric analysis of these micrographs was performed using a Zeiss Videoplan Morphometry Unit. The surface length of the mucosa from each block was traced with the magnetic stylus, expressed as units of surface length per unit muscularis mucosa length, and data obtained from each block was treated as an individual measurement.

Because no discernable differences were detected by electron microscopy between either the length or density of microvilli in control as compared with CD-absorptive cells, we arbitrarily selected oriented absorptive cells in the midvillus zone to quantitate microvillus height, width, and density, i.e., parameters that relate to surface area expansion of the absorptive cell apical membrane by microvillus projections. Because microvillus heights are extremely uniform in individual absorptive cells, microvillus height for each cell was determined by direct measurement of one microvillus from an electron photomicrograph printed at 15,000 ×. Only microvilli cut in a central plane parallel to their axis were selected for measurement. Totals of 47 and 62 measurements were taken from control and diabetic rats, respectively. Widths of these microvilli were also obtained with the use of a calibrated ocular micrometer and microvillus densities were assessed by counting the number of microvilli per 4 μm of absorptive cell width in each block at the midvillus site.

Phlorizin binding to intact tissue. Binding of [^{3}H]phlorizin to intact tissue was measured at 37°C in modified influx chambers (21, 22) following a 20-min equilibration period. The luminal surface was bathed in normal Ringer solution containing either 40 mM *d*-glucose or 40 mM fructose for 2 min before addition of [^{3}H]phlorizin (0.89 μM, 50 μCi/ml). The serosal surface was bathed in normal Ringer solution containing 40 mM fructose. 2 min following addition of phlorizin, the phlorizin-containing solution was removed and the tissue surface washed with 5 ml of ice-cold 300 mM mannitol. The tissue was then punched out, blotted on absorbant paper, and placed in 1 ml of 10% perchloric acid. After 24 h the samples were vortexed and the supernatant was dissolved in 4 ml scintillation fluid and assayed for radioactivity. Specific binding was determined from the difference between binding in the presence of fructose, i.e., total binding, and binding in the presence of glucose, i.e., nonspecific binding. Nonspecific binding never exceeded 20% of total binding.

Membrane vesicle experiments

Brush border membrane preparation. 30 cm of ileum beginning just proximal to the ileo-cecal valve was quickly excised, rinsed with ice-cold normal Ringer solution, and opened along its mesenteric border. The mucosa was then scraped from underlying muscle with a glass slide and used for membrane isolation. Two rats were usually required for one vesicle preparation. Light microscopy revealed that mucosal scraping removed the upper half of the villus, leaving the remainder of the lower villus-crypt structure attached to underlying muscle and connective tissue (data not shown). These results of mucosal scraping are similar to others (14). Brush border membrane (BBM) vesicles were freshly prepared on the day of experiment according to the procedure of Kessler et al. (23). Purification of BBMs was assessed by measurement of sucrase and alkaline phosphatase activities, expressed as μmol/mg protein/h (24). Alkaline phosphatase was, on the average, enriched 14-fold in the BBM fraction, (64.8±4.8 and 80.2±3.6) compared with whole homogenate (4.2±0.4 and 4.9±0.3) in control and CD, respectively. Sucrase was, on the average, enriched 17-fold in the BBM fraction, (37.8±3.0 and 47.4±3.6) compared with whole homogenate (2.3±0.2 and 2.8±0.3) in control and CD, respectively. Enrichments were the same for CD and control rats but enzyme-specific activities were slightly higher in CD rats. Protein was estimated according to Lowry et al. (25) using bovine serum albumin as standard.

Phlorizin binding to brush border membrane vesicles. Binding of [^{3}H]phlorizin to BBM was carried out at room temperature as described by Toggenburger et al. (26). Briefly, 10 μl of vesicles were placed at the bottom of a microfuge tube. 10 μl of incubation medium containing radiolabeled phlorizin was positioned on the side of the tube. At time zero the two drops were rapidly mixed by tapping the bottom of the microfuge tube. The composition of the combined media was in mol/liter: mannitol, 0.1; NaSCN 0.1; Tris-base, 0.02, buffered to pH 7.2 with 1 N HCl; *d*-glucose or fructose, 0.025; and [^{3}H]phlorizin, 2×10^{-7} to 10^{-4} (12 μCi/ml). Binding was carried out in the presence of an inwardly directed 100 mM NaSCN gradient since Tannenbaum et al. (27) have shown that a transmembrane potential (inside negative) is required for binding of phlorizin to the glucose cotransporter. Because binding of phlorizin to the Na-dependent glucose carrier is virtually complete in 2 s (26), and because nonspecific binding becomes excessive after this time period (26), phlorizin binding studies were stopped at 2 s with 1 ml ice-cold 150 mM NaCl. The sample was then rapidly filtered through a 0.45-μm nitrocellulose filter (Schleicher & Schuell, Inc., Keene NH) and

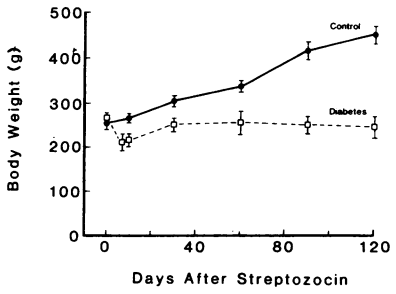

Figure 1. Body weight following administration of streptozocin. Results for chronic diabetes (- - □ - -) are compared with age-matched controls (— ● —). Points are means±SEM for $n \geq 6$ rats.

washed with 5 ml ice-cold 150 mM NaCl. The time required for wash and filtrations was 8 s. The filters were then dried at 50°C for 60 min, dissolved in scintillation fluid and counted. Total phlorizin binding was determined by incubation in the presence of 25 mM fructose. Nonspecific phlorizin binding was determined in parallel incubations with 25 mM D-glucose. Nonspecific binding never exceeded 20% of total binding.

Statistical analysis. Results are presented as means±SEM. Two-tailed Student's *t* test of unpaired data was used to analyze differences between diabetes and age-matched controls.

Results

Animal groups. Streptozocin-treated rats were compared with age-matched untreated littermates. Following streptozocin injection blood glucose determinations were > 325 mg/dl within 24 h. Unlike nondiabetic control rats, diabetic rats failed to gain weight after induction of diabetes (Fig. 1). Blood glucose glycosylated hemoglobin and body weight of CD animals, 120 d following streptozocin injection, and age-matched control animals are shown in Table I. It can be seen that CD animals weighed less and had higher blood glucose and glycosylated hemoglobin concentrations than did control animals. Periodic measurements of serum electrolytes and blood urea nitrogen showed no differences between the two groups of animals.

Na-dependent solute transport across jejunum and ileum in vitro. Unidirectional and net fluxes of 3OMG, ALA, and SO_4, measured at medium concentrations of 20, 5, and 0.2 mM, respectively, are shown in Table II. All three solutes are absorbed by Na-dependent cotransport systems. In control rats, net transmural fluxes of 3OMG across jejunum and ileum were similar in magnitude. In CD rats, the net flux of 3OMG was increased 3.1-fold in jejunum and 5.1-fold in ileum. Phlorizin, a nontransported ligand of the Na-glucose cotransporter (27), completely blocked net transmural 3OMG flux in both control and CD ileum. The phlorizin effect was exerted only on the m-to-s unidirectional fluxes; the s-to-m fluxes remained unchanged. Furthermore, the phlorizin concentration required to inhibit 50% of the increase in Isc induced by 20 mM 3OMG was similar in ileum from control (2.3±0.4 μM) and CD (3.0±0.6 μM) rats (Fig. 2). Thus the stimulation of glucose absorption that develops in the diabetic state is not due to induction of a phlorizin-independent carrier or of one less sensitive to phlorizin.

The net transmural flux of ALA was larger in the ileum of control rats than in the jejunum. In the CD rat, the net flux of ALA was increased 2.2-fold in ileum, but was unchanged in jejunum. The observed increases in net flux were due mainly, if not exclusively, to increases in m-to-s unidirectional fluxes. In both control and CD rats, SO_4 absorption was detected only in ileum, consistent with previous observations (28). Ileal SO_4 transport was the same in CD and control rats. Therefore, there did not appear to be a uniform alteration of Na-coupled transport pathways in CD rats.

Isc changes produced by 3OMG also proved to be independent of the anions in solution. Thus glucose-induced Isc changes in diabetics and controls were the same in magnitude when the Ringer solution contained Cl and HCO_3 as when these anions were replaced with gluconate and Hepes, respectively (data not shown).

Kinetic analysis. To assess whether the enhanced absorptions of 3OMG and ALA observed in the CD rats were due to changes in maximal transport capacity (V_{max}), changes in carrier affinity ($K_{0.5}$), or both, we determined Isc responses to varying concentrations of the two nutrients (concentration ranges 3OMG, 5–120 mM; ALA 0.5–64 mM). Measurements were made in jejunum and ileum of both CD and control rats. Results, plotted as rate of 3OMG- or ALA-stimulated Na transport ($\Delta Isc/cm^2$) vs. concentration of 3OMG or ALA, can be fit by a rectangular hyperbola for each solute. Eadie-Hofstee plots of the data for 3OMG are shown in Fig. 3 *A* and for ALA in Fig. 3 *B*. As shown in Table III, CD increased the V_{max} for the 3OMG-induced change in Isc 8.3-fold in ileum and 2.4-fold in jejunum. In control rats and in contrast to the results for 3OMG, the V_{max} for the ALA-stimulated change in Isc was larger in the ileum than in the jejunum. CD increased this V_{max} 1.6-fold in the ileum but had no effect in the jejunum. The relative changes in V_{max} for 3OMG and ALA-stimulated changes in Isc, are similar to the relative changes in transmural fluxes of 3OMG and ALA shown in Table II.

CD did not significantly affect affinity for transport of either 3OMG or ALA (Fig. 3 and Table III). $K_{0.5}$ values for 3OMG in jejunum and ileum of CD and control rats did not differ significantly. Enhanced absorption of 3OMG and ALA in CD rats is not due to changes in carrier affinity, therefore, but to an increase in the number of carriers or their rate of turnover or to an increase in the electrochemical gradient for net uptake across the brush border.

Time course of enhanced glucose transport in chronic diabetes. To examine the development of enhanced glucose transport over time and to assess the role mucosal hypertrophy may play, ileal 3OMG fluxes (Fig. 4 *A*) and ileal dry weight/cm^2 (Fig.

Table I. Profiles of Animal Groups

Group (*n*)	Injection weight	Sacrifice weight	Blood glucose	Glycosylated hemoglobin
	g	*g*	*mg/dl*	%
Chronic diabetes (12)	300±3	251±20*	343±16*	10.0±0.09*
Age-matched control (12)	312±4	452±12	129±15	5.3±0.10

Values are means±SEM for *n* rats. Chronic diabetes was induced by streptozocin (50 mg/kg) injected 120 d before sacrifice. * Different from control, $P < 0.001$.

Table II. Glucose, Alanine, and Sulfate Transport in Chronic Diabetes and Age-matched Controls

	Control			*Chronic diabetes*		
	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}
n						
Jejunum *μmol/cm²/h*						
3OMG (7)	0.85±0.10	0.59±0.10	0.26±0.02	1.23±0.09*	0.42±0.03	0.81±0.10*
ALA (7)	1.00±0.12	0.77±0.11	0.23±0.05	1.29±0.17	1.04±0.14	0.25±0.10
Jejunum *nmol/cm²/h*						
SO_4 (8)	9.0±1.0	15.0±1.0	−6.0±1.0	4.0±1.0	20.0±2.0	−16.0±1.0
Ileum *μmol/cm²/h*						
3OMG (6)	0.90±0.06	0.70±0.09	0.20±0.05	1.62±0.12*	0.60±0.15	1.02±0.16*
3OMG + Phz (4)	0.33±0.03‡	0.54±0.02	−0.21±0.01‡	0.24±0.01‡	0.39±0.02	−0.15±0.02‡
ALA (7)	1.60±0.19	1.13±0.21	0.47±0.08	2.17±0.28*	1.15±0.19	1.02±0.19*
Ileum *nmol/cm²/h*						
SO_4 (9)	72.0±7.0	7.0±1.0	66.0±10.0	82.0±9.0	8.0±1.0	75.0±10.0

Fluxes±SEM of 3OMG (20 mM), ALA (5 mM), and SO_4 (0.2 mM) for *n* experiments. Where phlorizin (Phz, 200 μM) was used, it was added to the mucosal bathing solution just before 3OMG addition. Results for each animal are averages of duplicate determinations. * Different from control, $P < 0.02$. ‡ Different from non-Phz-treated, $P < 0.001$.

4 *B*) were measured on the same tissues at various times following injection of streptozocin. Results were compared to age-matched controls. Enhanced glucose absorption was evident by 30 d after induction of diabetes, reached a maximum by 60 d, and remained constant for the next 60 d. Maximal enhancement of glucose transport in CD was seen well before the development of significant changes in ileal dry weight. In addition, the maximal increase in dry weight was 1.5-fold, whereas the maximum enhancement of 3OMG absorption was 5.1-fold. Age-matched controls maintained constant net transmural 3OMG flux and ileal dry weight over the entire 120-d period.

Effect of insulin treatment on glucose transport. To determine if in vivo insulin administration could reverse the observed enhancement of intestinal glucose transport in CD rats, insulin was given to some of the CD rats daily for 10 d. Results for these rats were compared with untreated CD rats and age-matched controls. As shown in Table IV, 3OMG-stimulated Isc responses were significantly higher in non-insulin-treated CD animals than in age-matched controls. Correction of hyperglycemia for 10 d with insulin completely reversed the change in Isc response to glucose observed in CD rats. This difference could only be produced in vivo. When added to ileal mucosa in vitro, 100 μM insulin did not alter Isc responses to glucose in either control or diabetic rat ileum when these were tested 60 min after insulin addition (data not shown).

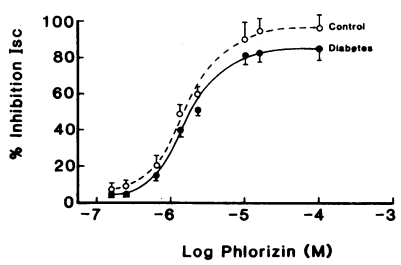

Figure 2. Dose-response curve of phlorizin inhibition of 3OMG (20 mM) stimulated Isc in ileum from chronic diabetic and age-matched control rats. Chronic diabetes (— • —); IC_{50} = 3.0±0.6 μM. Control (— ○ —); IC_{50} = 2.3±0.4 μM. Points represent means±SEM of duplicate determinations in four animals.

Effect of chemical sympathectomy with 6-hydroxydopamine. Altered fluid and electrolyte transport in ileum and colon of CD rats has been shown to be due to impaired alpha-2 adrenergic innervation of enterocytes (18, 29). Rats treated with 6-OHDA develop both alpha-2 adrenergic denervation and impaired intestinal fluid absorption similar to the changes observed in CD (16). Accordingly, we investigated whether sympathetic denervation with 6-OHDA would alter intestinal glucose absorption in nondiabetic rats.

Similar degrees of sympathetic denervation, as assessed by responses to tyramine (see Methods), were achieved in 6-OHDA–treated control rats and in non-6-OHDA–treated CD rats (responses to tyramine were 13±3 and 12±6 μA/cm², respectively). These represent 50% of control (27±3 μA/cm²) tyramine responses. However, net transmural flux of 20 mM 3OMG in animals treated with 6-OHDA for 14 or 30 d was not enhanced. Mean glucose absorption was in fact slightly, though not significantly, lower in 6-OHDA–treated rats than in controls (J_{net} in control rats was 0.64±0.20; J_{net} in 6-OHDA–treated rats was 0.45±.09 at 14 d and 0.46±.03 at 30 d following injection). In addition, administration of 6-OHDA to rats previously made diabetic with streptozocin enhanced the degree of sympathectomy (response to tyramine: 2±1 μA/cm²) but did not alter 3OMG transport when compared with those treated with streptozocin alone (data not shown). We conclude that adrenergic denervation does not enhance intestinal glucose absorption.

Morphometric analysis. Jejunal and ileal mucosa, removed from CD and control animals and directly placed into fixative, had normal structural appearances. Mucosal preparations first mounted in chambers and equilibrated there for 30–40 min prior to fixation, maintained epithelial integrity but showed the elements of the lamina propria to be separated from one another more than they were in the freshly fixed tissues, giving the lamina propria a lucent appearance and slightly widening the villi (Fig. 5). In our experience these features are commonly found in absorbing chamber-mounted intestinal epithelia and are likely due to the absence of an intact vasculature with a resulting accumulation of subepithelial fluid. Morphometric analysis of

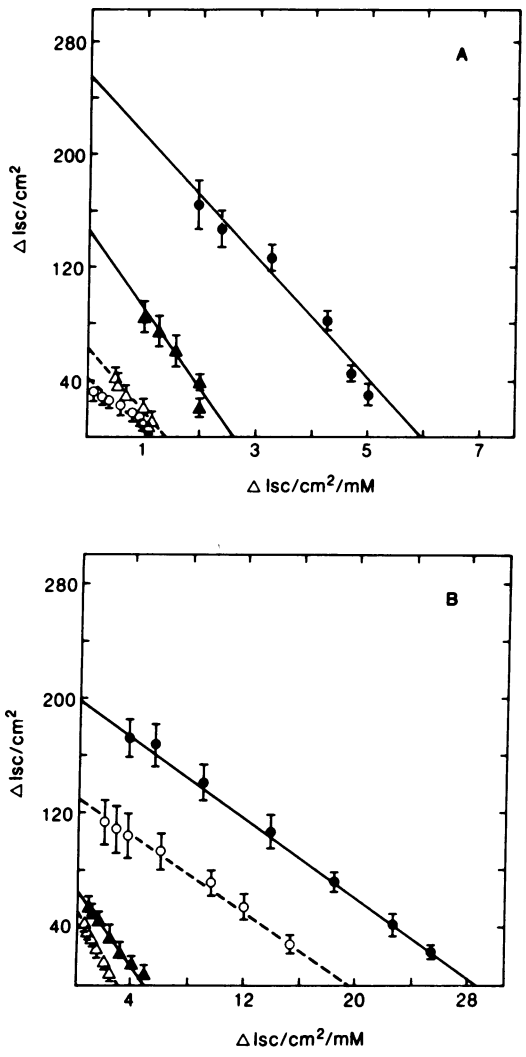

Figure 3. Eadie-Hofstee plots of effects of different 3OMG and ALA concentrations on Isc. Lines and drawn by linear regression analysis. (*Circles*) Results for ileum; (*triangles*) results for jejunum. Solid symbols and continuous lines refer to the diabetic state, and open symbols and dashed lines refer to age-matched controls. Values are means±SEM for six experiments, each performed in duplicate. (*A*) 3OMG effects. (*B*) ALA effects.

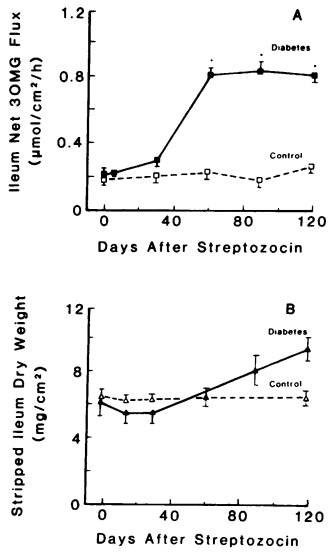

Figure 4. Time course of changes in net transmural flux of 3OMG and in dry weight of rat ileum stripped of its serosa and outer muscle layer. (*A*) Net transmural fluxes of 3OMG (20 mM) across chronically diabetic (— ■ —) and control (- - □ - -) ileum are shown. (*B*) Intestinal dry weight of chronically diabetic (— ▲ —) and control (- - △ - -) rats was assessed on tissues from the Ussing chamber following completion of flux measurements. Points are means±SEM of duplicate experiments for six animals. * Different from control, $P < 0.01$.

chamber-mounted ileal mucosa revealed a 1.5-fold greater villus height in CD animals than in controls. The mucosal surface length was 3.5±0.2 times the muscularis mucosal length in diabetic animals but only 2.3±0.2 times the muscularis mucosal length in controls, $P < 0.02$ (Fig. 5).

Electron microscopic analysis of the microvilli in a midvillus ileal enterocyte revealed that the microvilli expand intestinal surface area to a similar degree in CD and control rats (Fig. 6). Morphometric analysis revealed that microvillus height was similar in both animal groups (1.05±0.03 μM and 1.06±0.03 μM, control and CD, respectively). Similarly, microvillus densities (7.11±0.34 microvilli per μM absorptive cell width in controls and 7.06±0.46 in CD rats) and microvillus widths (0.11±0.1 and 0.10±0.02 μM in control and CD rats, respectively) were the same for the two animal groups.

[^3H]Phlorizin binding to isolated brush border membranes. The possibility that diabetes increases intestinal glucose absorption by increasing the number of carriers in the brush border membrane was explored by first quantitating the number of Na-dependent glucose carriers per milligram brush border protein using [^{3}H]phlorizin. Fig. 7 compares specific phlorizin binding to ileal BBM prepared from CD rats and age-matched controls.

Table III. Kinetic Constants for Effects of 3OMG and ALA on Isc in Ileum from Rats with CD and Age-matched Controls

	Jejunum		Ileum	
(*n*)	V_{max}	$K_{0.5}$	V_{max}	$K_{0.5}$
	μA/cm²	mM	μA/cm²	mM
Diabetes (6)				
3OMG	141±22.6*	46.2±3.1	256.1±29.1*	43.8±4.3
ALA	62.7±3.1	12.9±4.6	196.4±17.5*	6.2±0.1
Control (6)				
3OMG	61.3±4.3	41.4±4.4	31.0±6.9	23.0±4.1
ALA	55.1±5.4	21.9±3.4	124.7±17.3	6.8±0.4

Values are means±SEM for six experiments, each done in duplicate. Kinetic constants were derived from the Eadie-Hofstee plots shown in Fig. 6. The statistical analysis is described in Methods. * Different than corresponding value in controls, $P < 0.02$.

Table IV. Effect of Insulin Treatment on Glucose Transport

Group	(*n*)	Isc
		$\mu A/cm^2$
Control	(4)	28.4±1.7
Non-insulin-treated CD	(4)	42.8±3.5*
Insulin-treated CD	(4)	30.3±3.9‡

Results from *n* rats are changes±SEM in ileal Isc following addition of 3OMG (20 mM). Age-matched controls are compared with non-insulin-treated CD and CD given insulin for 10 d. Results for each animal are the averages of duplicate determinations.
* Different from control, $P < 0.01$.
‡ Different from non-insulin-treated group, $P < 0.05$.

Comparison of phlorizin-bound sites by Scatchard analysis in control (11.9±1.2 pmol/mg protein) and CD (11.4±0.9 pmol/mg protein) membranes indicates the same number of Na-dependent glucose carriers per milligram of brush border protein. Fig. 7 also demonstrates an unchanged affinity for phlorizin in control (1.0±0.2 μM) and CD (1.3±0.1 μM) membranes. The number of [^{3}H]phlorizin binding sites and their affinity for phlorizin are similar to those obtained in rat BBM by Semenza et al. (30, 31).

[^{3}H]Phlorizin binding to intact epithelium. Because the phlorizin-binding properties of BBM derived from the villus tip region were similar for CD and control animals, we measured [^{3}H]phlorizin binding to intact mucosa to determine the total number of phlorizin-inhibitable glucose carriers present in the brush border membrane of the epithelium. Many of these carriers could be present in the midvillus region below the region used to prepare BBM (see Methods). Sampling of the serosal bathing solution for [^{3}H]phlorizin after 20 min of exposure demonstrated no accumulation, indicating that phlorizin is not transported across the epithelium. In addition, the total amounts of [^{3}H]phlorizin bound were the same at 2 and 20 min after introducing the radioisotope, indicating no transport into the cellular compartment. Specific binding of [^{3}H]phlorizin to intact sheets of ileal mucosa was determined in CD and age-matched control

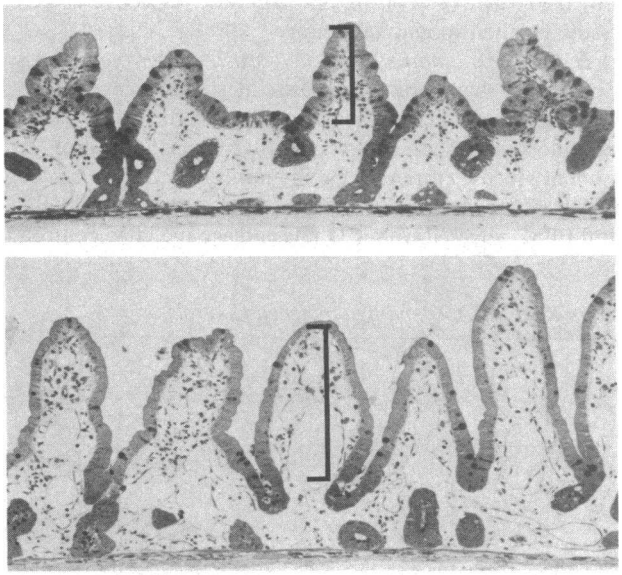

Figure 5. Light micrographs of chamber-mounted ileal mucosal sheets from control (*top*) and chronically diabetic (*bottom*) animals. The intestinal surface area of diabetic animals was expanded to a greater degree by villi (*brackets*) than control animals. Quantitative measurements revealed chronically diabetic animals had 1.5-fold greater amplification of ileal mucosal surface area than did control animals as a result of this morphological difference. The epithelium is well maintained in these stripped preparations (both ~ × 12).

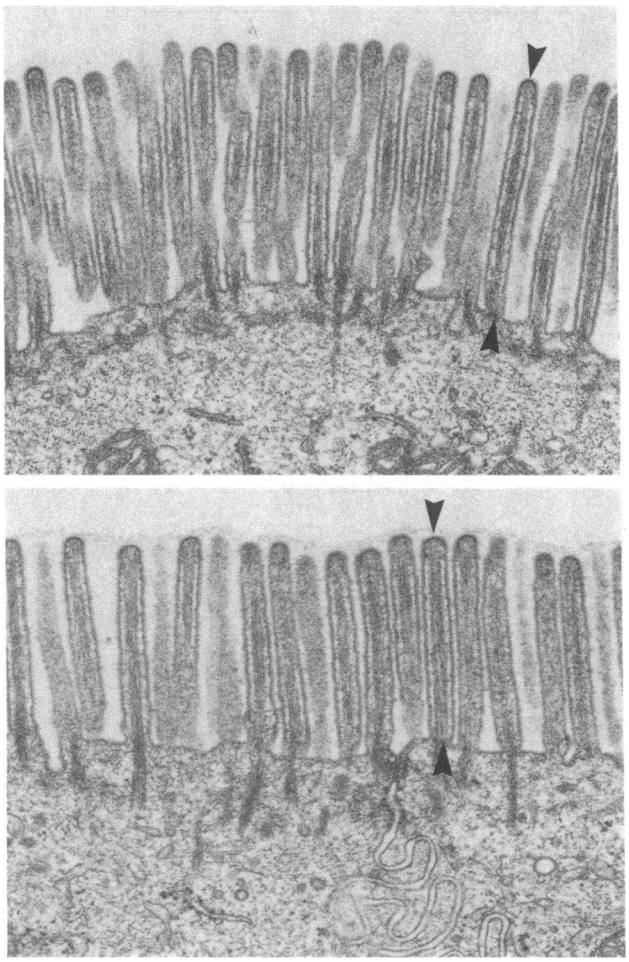

Figure 6. Electron micrographs of midvillus absorptive cell microvilli from ileum of control (*top*) and chronically diabetic (*bottom*) animals. Microvilli sectioned longitudinally and centrally (*arrowheads*) were utilized for measuring microvillus height, width, and density per unit absorptive cell width. Analysis of these data showed that midvillus absorptive cell apical membrane surface expansion by microvilli was similar in control and diabetic animals (both ~ × 22,500).

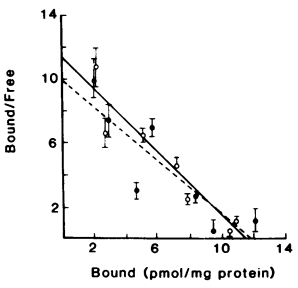

Figure 7. Determination by Scatchard analysis of specific [^{3}H]phlorizin binding sites in chronic diabetic (●) and control (○) ileal BBM. Lines are drawn by linear regression analysis. Chronic diabetes: $r = 0.940$; phlorizin binding sites, 11.4±0.9 pmol/mg protein; K_t = 1.0±0.2 μM. Control: $r = 0.850$; phlorizin-binding sites, 11.9±1.2 pmol/mg protein; K_t = 1.3±0.1 μM. Values are means±SEM of quadruplicate determinations in seven rats.

Table V. Comparison of [^{3}H]Phlorizin Binding to the Mucosal Surface with Net Transmural Fluxes of 3OMG in Ileum of Chronically Diabetic and Control Rats

(*n*)	[^{3}H]Phlorizin binding	Net 3OMG flux
	cpm × 10^4/*cm*^{2}	*μmol/cm^2/h*
Control (6)	1.5±0.5	0.26±0.15
Diabetes (6)	9.9±1.2*	1.80±0.20*

Values are means±SEM. Phlorizin binding and 3OMG fluxes were determined on paired tissues from the same animal. Unidirectional fluxes of 3OMG were as follows: diabetes, J_{ms} = 2.30±0.20 and J_{sm} = 0.50±0.06; and control, J_{ms} = 0.73±0.10 and J_{sm} = 0.47±0.02. See Methods for further details.
* Different from control, $P < 0.001$.

rats. Binding was 6.6-fold greater in CD than in control rat ileum (Table V). The relative effects of CD on phlorizin binding to intact ileum and on 3OMG transport were determined on paired tissues from the same animals and proved to be similar (Table V). These results suggest a "recruitment" of Na-dependent glucose carriers into the lower villus region of the ileal epithelium.

Discussion

These results describe the pattern of adaptation of Na-coupled nutrient absorption in rats made diabetic with streptozocin.

Because the diabetic state involves an inability to utilize circulating glucose at the cellular level and a loss of glucose in the urine, increased absorption of oral nutrients may represent a compensatory response. The simplest nonspecific means for such a response would be an increase in mucosal mass to uniformly increase absorption of all nutrients. In prior studies, however, intestinal glucose transport in rats was found to begin increasing within 4 h of induction of the diabetic state and to be significantly higher than control by 48 h, well before changes in mucosal morphology could be detected (32).

A simple increase in intestinal mass is unlikely, therefore, to explain enhanced glucose transport in experimentally induced diabetics. A more specific change of intestinal absorption appears likely. The results of the present study are consistent with this conclusion. During the first 10 d after streptozocin administration diabetic rats lost 20% of their body weight. Following development of hyperphagia, weight was rapidly regained to preinjection levels and then remained constant for at least the next 100 d. Normophagic age-matched controls injected with saline did not demonstrate the initial loss of body weight, and over the similar 120-d period doubled their body weight (Fig. 1). It is unlikely, however, that differences in body weight, growth, or food consumption are directly responsible for the observed enhancements of nutrient absorption. Increases in intestinal glucose absorption have been reported for normophagic diabetic rats fed a low-carbohydrate diet (33) and for diabetic rats pair-fed with nondiabetic controls (8). In addition, Crane (34) has shown an increase in intestinal glucose absorption in diabetes whether age- or weight-matched controls are used.

The present study confirms these prior observations in two respects: (*a*) Although enhanced glucose absorption was slow to develop, reaching a maximum between 45 and 60 d after injecting streptozocin, relative intestinal hypertrophy, as evidenced by an increase in dry weight per square centimeter and an amplification of villus height, was even slower to develop and was not significantly different from control at 90 d. Furthermore, the maximum increases in ileal dry weight and villus height were only 1.5-fold, which cannot account for the greater than five-fold increase in glucose transport. (*b*) The enhancement of active ileal absorption of solutes was also not uniform, the change being 5.1-fold for glucose and 2.2-fold for ALA. SO_4 transport, in contrast, was unaltered.

It is worth noting that 10 d of insulin therapy completely reversed the increase in glucose absorption that occurs in experimental diabetes. Thus the off-rate for this stimulation was faster than the on-rate. The reasons for this discrepancy are not at present clear.

The nature of the epithelial alteration(s) leading to increased nutrient absorption were explored mainly with sugar transport probes. For both 3OMG and ALA, the observed adaptation involves increases in m-to-s unidirectional fluxes but no significant changes developing in the s-to-m fluxes. Furthermore, the observed changes for both nutrients, reflected increases in V_{max} without any changes in carrier affinity, so there were either more carriers present per square centimeter surface area, a more rapid translocation through preexisting carriers, or less recycling though the carriers from cell to lumen, implying a greater electrochemical gradient for glucose and, to a lesser extent, alanine. The last of these possibilities seems very unlikely because the electrochemical gradient for Na and sugar or alanine is normally sufficiently steep to obviate a subtantial back flux through the carrier (35). Furthermore, ileal SO_4 transport, which also depends on the Na gradient (36), was unaltered in CD. Similarly, alanine transport in the jejunum was unaffected by CD, whereas 3OMG transport was affected, and it was altered far less than was 3OMG transport in the ileum. Thus the effect of CD on sugar transport appears to be sufficiently unique to rule out a general effect on the Na gradient.

The second of these possibilities is also unlikely because Brasitus and Dadeja (37), using the identical rat model, demonstrated that the intestinal microvillus membranes of CD rats have a lower lipid fluidity than do those of control rats. In consequence, an increase in carrier mobility in the diabetes-modified membrane seems improbable because lower lipid fluidity is generally associated with decreased, not increased, transport (38).

A change in [^{3}H]phlorizin binding to the mucosal surface of the intact epithelium parallels exactly the observed increase in 3OMG transport (see Table V) and suggests an overall increase in the number of sugar carriers as the explanation for the enhancement of sugar absorption. It is of interest that this overall increase was not reflected in an increase in the density of carriers near the villus tip, because there was no difference in isolated brush border membrane vesicles in specific [^{3}H]phlorizin binding. In preparation of membrane vesicles for the latter study, the villus tip region of the epithelium was employed due to light scraping of the mucosa before homogenization. This was confirmed by light microscopy of the tissue remaining after scraping. Thus, in the intact epithelium, a much larger fraction of mucosal surface area must have been recruited for sugar absorption. Radioautographic studies to test this hypothesis support this conclusion (Fedorak et al., manuscript in preparation).

The cellular signals responsible for these adaptive changes are presently unclear. Adrenergic denervation does not seem to play a role. We are currently exploring the roles of both insulin and glucagon in intestinal adaptation. Glucagon in particular

has been noted to increase protein synthesis and DNA synthesis in rat ileum (39).

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References

1. Vinnik, I. E., F. Kern, and K. E. Sussman. 1965. The effect of diabetes mellitus and insulin on glucose absorption by the small intestine in man. *J. Lab. Clin. Med.* 66:131–136.

2. Genel, M., D. London, P. G. Holtzapple, and S. Segal. 1971. Uptake of alpha-methylglucoside by normal and diabetic jejunal mucosa. *J. Lab. Clin. Med.* 77:743–750.

3. Madara, J. L., J. L. Wolf, and J. S. Trier. 1982. Structural features of the rat small intestinal microvillus membrane in acute experimental diabetes. *Dig. Dis. Sci.* 27:801–806.

4. Lal, D., and H. P. Schedl. 1974. Intestinal adaptation in diabetes: amino acid absorption. *Am. J. Physiol.* 227:827–831.

5. Flores, P., and H. P. Schedl. 1968. Intestinal transport of 3-*O*-methyl-*d*-glucose in the normal and alloxan-diabetic rat. *Am. J. Physiol.* 214:725–729.

6. Caspary, W. F. 1973. Increase of active transport of conjugated bile salts in streptozotocin-diabetic rat small intestine. *Gut.* 14:949–955.

7. Thomson, A. B. R., and R. Rajotte. 1983. Effect of dietary modification on the uptake of glucose, fatty acids and alcohols in diabetic rats. *Am. J. Clin. Nutr.* 38:394–403.

8. Olsen, W. A., and I. H. Rosenberg. 1970. Intestinal transport of sugars and amino acids in diabetic rats. *J. Clin. Invest.* 49:96–105.

9. Leese, H. J., and K. L. Mansford. 1971. The effect of insulin and insulin deficiency on the transport and metabolism of glucose by rat small intestine. *J. Physiol. (Lond.).* 212:829–838.

10. Miller, D. L., and H. P. Schedl. 1976. Effects of experimental diabetes on intestinal absorption in the rat. *Proc. Soc. Exp. Biol. Med.* 152:589–592.

11. Thomson, A. B. R. 1981. Uptake of glucose into the intestine of diabetic rats. Effects of variations in the effective resistance of the unstirred water layer. *Diabetes.* 30:246–255.

12. Schedl, H. P., and H. D. Wilson. 1971. Effects of diabetes on intestinal growth and hexose transport in the rat. *Am. J. Physiol.* 220: 1739–1745.

13. Miller, D. L., W. Hanson, H. P. Schedl, and J. W. Osborne. 1977. Proliferation rate and transit time of mucosal cells in small intestine of the diabetic rat. *Gastroenterology.* 73:1326–1332.

14. Schedl, H. P., and H. D. Wilson. 1971. Effects of diabetes on intestinal growth in the rat. *J. Exp. Zool.* 176:487–496.

15. Gabbay, K. H., J. M. Sosenko, A. Banuchig, M. J. Mininsohn, and R. Fluckiger. 1979. Glycosylated hemoglobins: increased glycosylation of hemoglobin A in diabetic patients. *Diabetes.* 28:337–340.

16. Chang, E. B., R. M. Bergenstal, and M. Field. 1985. Diarrhea in streptozocin-treated rats. *J. Clin. Invest.* 75:1666–1670.

17. Kostrzewa, R. M., and D. M. Jacobowitz. 1974. Pharmacological actions of 6-hydroxydopamine. *Pharmacol. Rev.* 26:199–259.

18. Tapper, E. J., J. S. Bloom, and D. L. Lewand. 1981. Endogenous norepinephrine release induced by tyramine modulates intestinal ion transport. *Am. J. Physiol.* 229:86–92.

19. Field, M., D. Fromm, and I. McColl. 1971. Ion transport in rabbit ileal mucosa. I. Na and Cl fluxes and short circuit current. *Am. J. Physiol.* 220:1388–1394.

20. Field, M., and I. McColl. 1973. Ion transport in rabbit ileal mucosa. III. Effects of catecholamines. *Am. J. Physiol.* 225:852–857.

21. Thompson, S. M., and D. C. Dawson. 1978. Sodium uptake across the apical border of the isolated turtle colon: conformation of the two barrier model. *J. Membr. Biol.* 42:357–374.

22. O'Grady, S. M., M. W. Musch, and M. Field. 1986. Stoichiometry and ion affinities of the Na-K-Cl cotransport system in the intestine of the winter flounder. *J. Membr. Biol.* 91(1):33–43.

23. Kessler, M., O. Acuto, C. Storelli, H. Murer, M. Muller, and G. Semenza. 1978. A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestine brush border membranes. *Biochim. Biophys. Acta.* 506:136–154.

24. Brasitus, T. A., D. Schachter, and T. G. Mamouneas. 1979. Functional interactions of lipids and proteins in rat intestine microvillus membranes. *Biochemistry.* 18:4136–4144.

25. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:256–275.

26. Toggenburger, G., M. Kessler, and G. Semenza. 1982. Phlorizin as a probe of the small intestine Na-glucose cotransporter. A model. *Biochim. Biophys. Acta.* 688:557–571.

27. Tannenbaum, C., G. Toggenburger, M. Kessler, A. Rothstein, and G. Semenza. 1977. High-affinity phlorizin binding to brush border membranes from small intestine: identity with a part of the glucose transport system, dependence on the Na-gradient, partial purification. *J. Supramol. Struct.* 6:519–533.

28. Smith, P. L., S. A. Orella, M. Field. 1981. Active sulfate absorption in rabbit ileum: dependence on Na and Cl and effects of agents that alter Cl transport. *J. Membr. Biol.* 63:199–206.

29. Chang, E. B., R. N. Fedorak, and M. Field. 1986. Experimental diabetic diarrhea in rats. Denervation hypersensitivity and treatment with clonidine. *Gastroenterology.* 91:564–569.

30. Semenza, G., M. Kessler, M. Hosang, T. Weber, and U. Schmidt. 1984. Biochemistry of the Na *d*-glucose cotransporter of the small-intestinal brush border membrane. *Biochem. Biophys. Acta.* 779:343–379.

31. Toggenburger, G., M. Kessler, A. Rothstein, G. Semenza, and C. Tannenbaum. 1978. Similarity in effects of Na gradients and membrane potentials on *d*-glucose transport by, and phlorizin binding to, vesicles derived from brush borders of rabbit intestinal mucosal cells. *J. Membr. Biol.* 40:269–290.

32. Csaky, T. Z., and E. Fisher. 1981. Intestinal sugar transport in experimental diabetes. *Diabetes.* 30:568–574.

33. Granneman, J. G., and E. M. Stricker. 1984. Food intake gastric emptying in rats with streptozocin-induced diabetes. *Am. J. Physiol.* 247:R1054–R1061.

34. Crane, R. K. 1961. An effect of alloxan-diabetes on the active transport of sugars by rat small intestine in vitro. *Biochem. Biophys. Res. Commun.* 4:436–440.

35. Kimmich, G. A., C. S. Christin, and J. Randles. 1977. Energetics of Na-dependent sugar transport by isolated intestine cells: evidence for a major role for membrane potentials. *Am. J. Physiol.* 233:E357–E362.

36. Lucke, H., G. Stange, and H. Murer. 1981. Sulfate-sodium cotransport by brush-border membrane vesicles isolated from rat ileum. *Gastroenterology.* 80:22–30.

37. Brasitus, T. A., and P. A. Dadeja. 1985. Correction of abnormal lipid fluidity and composition of rat ileal microvillus membranes in chronic streptozocin-induced diabetes by insulin therapy. *J. Biol. Chem.* 260:12405–12409.

38. Brasitus, T. A., and D. Schachter. 1982. Cholesterol biosynthesis and modulation of membrane cholesterol and lipid dynamics in rat intestinal microvillus membranes. *Biochemistry.* 21:2241–2246.

39. Miazza, B. M., M. Y. Mukhtar, M. Salmeron, M. A. Ghatei, M. Felce-Dachez, A. Filali, R. Villet, N. A. Wright, S. R. Bloom, and J. C. Crambaud. 1985. Hyperenteroglucagonemia and small intestinal mucosal growth after colonic perfusion of glucose in rats. *Gut.* 26:518–524.