NUTRIENT UPTAKE BY RAT ENTEROCYTES DURING DIABETES MELLITUS; EVIDENCE FOR AN INCREASED SODIUM ELECTROCHEMICAL GRADIENT

BY E. S. DEBNAM, W. H. KARASOV* AND C. S. THOMPSON

From the Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF and the *Department of Wildlife Ecology, University of Wisconsin, Madison, WI, U.S.A.

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SUMMARY

1. The effect of streptozotocin-induced diabetes (7 day duration) in rats on D-glucose uptake in vivo, the unidirectional uptake of D-glucose and L-proline in vitro, the passive uptake of L-glucose in vitro and the potential difference across the brush-border membrane has been studied.

2. Diabetes resulted in an increased carrier-mediated glucose uptake both in vivo and in vitro and a stimulation of L-proline uptake at a concentration of the amino acid (0.025 mm) at which uptake was largely Na⁺ dependent. Diabetes was without effect on uptake using a proline concentration of 50 mm at which transport was predominantly Na⁺ independent.

3. A marked hyperpolarization of the brush-border membrane and an enhanced passive glucose uptake were also evident during diabetes.

4. We conclude that the stimulation of glucose uptake in vivo in diabetic intestine involves events at the brush-border membrane. The mechanisms include an increased surface area for uptake and an enhanced transmembrane electrical gradient. The latter will have a major effect on the transport of other substrates when the uptake pathway is primarily Na⁺ dependent.

INTRODUCTION

It is well established that both acute and sustained hyperglycaemia stimulate active glucose transport across the intestinal enterocyte (Karasov & Diamond, 1983 a). As yet, the cellular locus of this response remains to be clarified, although the brush-border membrane (Hopfer, 1975), the basolateral membrane (Csaky & Fischer, 1981; Cheeseman & Maenz, 1985) or both these sites (Fischer & Lauterbach, 1984), have been implicated in the increased sugar uptake during hyperglycaemia.

The aim of this present work was to examine further the suggestion (Hopfer, 1975) that an alteration in the Na⁺ electrochemical gradient across the brush-border membrane might be responsible for the enhanced glucose uptake. In order to study this, we have used rats made diabetic by treatment with the B-cell cytotoxic agent streptozotocin, 7 days before experimentation. Using intestine from diabetic and
control rats, we have compared the effect of hyperglycaemia on the unidirectional uptake of L-proline and D-glucose using an in vitro method (Karasov & Diamond, 1983b; Karasov & Debnam, 1987). For comparison, the effect of diabetes on glucose uptake using electrical and chemical techniques in vivo (Debnam & Levin, 1975) has also been assessed. Finally, we have measured changes in the brush-border membrane potential ($V_m$) occurring in diabetes, since this is an important driving force for Na$^+$-dependent solute entry into the enterocyte. Preliminary accounts of part of this work have been published (Debnam, Karasov & Thompson, 1986a, b).

METHODS

Animals and anaesthesia

All experiments were carried out on male Sprague–Dawley rats (initial weight 250–300 g) which had been fed on a standard maintenance diet up to the time of experimentation. Diabetes was induced by a single injection of streptozotocin (80 mg/kg, dissolved in pH 4.5 citrate buffer) into the tail vein with the animal under light ether anaesthesia. Control rats were injected with buffer alone. Animals were used for absorption experiments 7 days after treatment. During this time period they were pair-fed and were allowed free access to water.

Anaesthesia was achieved using sodium pentobarbitone (90 mg/kg i.p.; Sagatal, May and Baker Limited). Blood samples for the determination of glucose concentration were obtained either after anaesthesia from the tail vein (experiments carried out in London) or prior to anaesthesia from the infraorbital sinus (experiments in Madison).

Measurement of the potential difference across the brush-border membrane

Full details of the methods used have been published (Debnam & Thompson, 1984). In brief, a 1 cm section of isolated jejunum was cut longitudinally and mounted as a flat sheet on a Perspex disc using cyanoacrylate adhesive applied to the muscle side. The disc formed the base of a tissue bath, a rubber gasket being used to prevent fluid leakage. The preparation was superfused with gassed (95% O$_2$–5% CO$_2$) bicarbonate saline at 35°C and a flow rate of 2 ml/min.

Microelectrodes were filled with filtered 3 m-KCl and had a resistance of 15–25 MΩ and a tip potential of less than 5 mV. An Ag–AgCl miniature half-cell connected the microelectrode to the input terminals of a high impedance preamplifier (Model KS 700, W.P. Instruments, New Haven, CT, U.S.A.). A 3 m-KCl–agar bridge in contact with the bathing solution was used as the reference electrode, the electrical potential of the bathing solution being taken as zero. The microelectrode was supported vertically and lowered gradually using a hydraulic drive micromanipulator (Trent Wells Inc., South Gate, CA, U.S.A.). The criteria for an acceptable impalement were (a) an immediate negative deflection, (b) maintenance of a stable potential difference for at least 15 s, (c) an abrupt return to baseline upon withdrawal of the microelectrode and (d) a similar electrode resistance (±10%) and tip potential (±2 mV) before impalement and after withdrawal of the electrode. A chart recorder (Model BD 41, Kipp & Zonen, F.T. Instruments, Tewkesbury, Gloucestershire) was used to obtain permanent traces of $V_m$.

In vivo glucose uptake and measurement of the transmural potential difference

A 25 cm length of upper jejunum of an anaesthetized rat was selected, washed through with warm NaCl (154 mm) and cannulated proximally with a perforated L-shaped cannula and distally with an L-shaped but unperforated cannula. The cannulae were connected to a fluid circuit through which gassed (95% O$_2$–5% CO$_2$) bicarbonate saline (Krebs & Henseleit, 1932), warmed to 37°C, was pumped (2 ml/min) using a peristaltic pump (Watson-Marlow, Falmouth, Cornwall). Rectal temperature was maintained at 37°C throughout the experiment using a heated blanket.

The potential difference (PD) across the intestine was monitored with salt bridges made of polyethylene tubing (o.d. 1 mm) containing 1 m-KCl in 3% agar. One salt bridge was inserted into the proximal cannula while the other made electrical contact with the peritoneal surface of the intestine. The salt bridges led to calomel cells connected to the input terminals of a high input impedance electrometer (Model 602, Keithley Instruments, Cleveland, OH, U.S.A.) the output of which was connected to a BD41 chart recorder (see above). The change in PD induced by the
presence of glucose (64 mm) was recorded and corrected for the osmotically induced PD using mannitol at a concentration of 64 mm (Debnam & Levin, 1975). Values for this correction in intestine from control and diabetic animals were 2.46 ± 0.27 mV (n = 5) and 2.65 ± 0.25 mV (n = 6) respectively.

Glucose uptake was determined by circulating the hexose (64 mm) dissolved in gassed (95% O₂-5% CO₂) bicarbonate saline (37 °C) through the intestinal segment for a period of 20 min. The sugar solution in the segment and circulation system was then washed out, deproteinized (0.3 n-BaOH₂-5% ZnSO₄) and glucose estimated using a colorimetric method (Nelson, 1944; Somogyi, 1945). Sugar uptake was calculated as luminal loss and calculated as nmol cm⁻¹ min⁻¹. In separate experiments the rate of glucose absorption was corrected for diffusive movement using phlorizin (2 mm) to block active sugar transport (Debnam & Levin, 1975). Subtraction of the amount of glucose absorbed in the presence of phlorizin from that absorbed in its absence gave values for uptake via the active pathway. In control animals, the kinetics of glucose uptake were obtained by circulating increasing concentrations of the sugar (4-64 mm) through the segment. All solutions were made to the same tonicity and active glucose uptake at each concentration was carried out using the techniques described above. Kinetic parameters of Vₘₐₓ (maximum transport capacity) and apparent Kₛₜ (the glucose concentration at 50% Vₘₐₓ) were determined by least-square analysis of the Lineweaver–Burk plots.

Glucose and proline uptake in vitro

Two independent groups of data were obtained. Those performed in London (Expt 1) measured L-proline uptake at 50 mm and those in Madison (Expt 2) measured L-proline uptake at 0.025 mm. Both studies measured D-glucose uptake at 64 mm and the passive uptake using L-glucose. Full details of the methods used are described elsewhere (Karavos & Diamond, 1983b; Karasov & Debnam, 1987). In brief, the preparation was an everted sleeve of small intestine, 1 cm in length, taken from the duodenum (i.e. adjacent to the pyloric sphincter), jejunum (25 cm from the pyloric sphincter) and ileum (8 cm from the ileal–caecal junction) and mounted on a grooved glass rod (5 mm diameter). Following pre-incubation for 5 min in buffer (Krebs & Henseleit, 1932) at 37 °C, the preparation was suspended in buffer at 37 °C over a stir-bar rotating at 1200 r.p.m. The method measured the unidirectional uptake of D-[1-³H]glucose, L-[5-³H]proline, and L-[1-¹⁴C]glucose into the tissue across the brush-border membrane (not transmural flux). At the end of incubation (1 min for D-glucose, 2 min for L-proline, 4 min for L-glucose) tissues incubated in D-glucose were rinsed with non-radioactive buffer (20 s, 2 °C) and blotted. Tissues were then removed from the rods and placed in pre-weighted scintillation vials. Further weighing allowed the determination of tissue wet weight. Tissue solubilization was achieved by overnight incubation of the sleeves at 55 °C in the presence of a methanol-based solubilizer (TS-1, Research Products International, Mount Prospect, IL, U.S.A.). Finally, scintillation fluid (Econofluor, New England Nuclear, Boston, MA, U.S.A.) was added and ³H or ¹⁴C activity determined by dual-channel liquid scintillation counting.

Uptake rates were normalized to either tissue wet weight or per centimetre length of intestine.

In studies of D-[1-³H]glucose uptake, L-[1-¹⁴C]glucose at tracer concentrations was used to correct simultaneously for D-glucose in adherent mucosal fluid and for D-glucose taken up passively, yielding stereospecific carrier-mediated uptake of D-glucose. In studies of L-[5-³H]proline uptake, [carboxyl-¹⁴C]inulin was used to correct for L-proline in adherent fluid. In studies of uptake of L-[1-¹⁴C]glucose, where uptake is passive, [³H]-inulin (mol. wt 5000-5500) at tracer concentrations was used as the adherent fluid marker.

Intestinal histology and morphology

A 1 cm section of jejunum was washed with NaCl (154 mm) and placed in 10% (v/v) formol saline. The tissue was later blocked in paraffin wax, sectioned longitudinally (5 µm) and stained with Haematoxylin and Eosin. Villus height and enterocyte column size (the number of enterocytes along one side of the villus) were determined using light microscopy. For mucosal weight measurements, a 5 cm section of jejunum was removed, blotted dry and cut longitudinally to form a flat sheet. The mucosal-submucosal layer was removed by scraping with the edge of a glass microscope slide. The separated layer was weighed after drying to a constant weight at 70 °C and the weight expressed as mg/cm.
Statistics

Results are expressed as mean ± s.e of the mean, with the number of experiments in parentheses. Analysis of variance was used to compare the uptakes of D-glucose and L-glucose in intestine from diabetic and control rats (Expts 1 and 2). The Student's t test was used to compare means of all other measurements. In both cases significance was taken as \( P < 0.05 \).

Chemicals

Radioactive isotopes were obtained from New England Nuclear, Boston, MA, U.S.A. Phlorhizin, L-glucose, L-proline, streptozotocin and L-valine were purchased from Sigma. All other chemicals were of Analar Grade from BBH Ltd.

RESULTS

Streptozotocin is known to induce a rapid elevation of blood glucose concentration, maximum values being obtained after about 48 h (Csaky & Fischer, 1981). In this present study plasma glucose levels in excess of 14 mm were found 7 days after treatment with streptozotocin. Control values were in the range 4·6-3·3 mm. Diabetic animals lost 16·3 ± 1·1% (\( P < 0·001 \)) whilst control animals gained 10·0 ± 1·0% of their initial body weight (\( P < 0·001 \)) over the course of the study. This weight loss was presumably a consequence of excessive levels of plasma glucose resulting in energy loss via the urine.

Jejunal histology and morphology

Tissue from diabetic rats showed significant increases in jejunal wet weight (control 88 ± 2 mg/cm (21); diabetic 109 ± 3 mg/cm (21) \( P < 0·001 \)), mucosal dry weight (control 10·5 ± 0·5 mg/cm (12); diabetic 11·9 ± 0·5 mg/cm (12) \( P < 0·005 \), enterocyte column size (control 91 ± 2 (24); diabetic 103 ± 2 (24) \( P < 0·001 \)) and villus height (control 427 ± 8 μm (54); diabetic 492 ± 5 μm (54) \( P < 0·001 \)).

Membrane potential difference

Hyperglycaemia for 7 day duration caused a significant increase in \( V_m \), the values being -55·7 ± 1·4 (20) and -44·7 ± 1·7 mV (72) for streptozotocin and diluent-injected animals respectively (\( P < 0·001 \)).

Kinetics of glucose uptake

A comparison of the concentration dependence of active glucose uptake in non-diabetic rats measured \textit{in vivo} and \textit{in vitro} is shown in Fig. 1. Active uptake displayed the expected hyperbolic relationship to glucose concentration, with saturation almost complete at a concentration of around 40 mm \textit{in vitro} and around 60 mm \textit{in vivo}. Values of apparent \( K_t \) and \( V_{max} \) were 400 ± 0·1 mm (6) and 414 ± 56 nmol cm\(^{-1}\) min\(^{-1}\) (6) respectively when measured \textit{in vitro} and 19·5 ± 3·7 mm (7) and 471·4 ± 41·2 nmol cm\(^{-1}\) min\(^{-1}\) respectively \textit{in vivo}. From these results a mucosal glucose concentration of 64 mm was chosen for subsequent experiments \textit{in vitro} and \textit{in vivo} since uptake at this concentration gives a good approximation of \( V_{max} \) in both preparations. Apparent \( K_t \) does not appear to differ in diabetic as compared to non-diabetic rats (Thomson, 1981).
Electrical and chemical determinants of glucose uptake in vivo

The administration of streptozotocin resulted in a significant increase of 25·3% in the magnitude of the glucose-induced increase in transmural PD when the hexose was present at a concentration of 64 mM (Table 1). Simultaneous measurements of

![Graph showing concentration dependence of active D-glucose uptake in the jejunum measured either in vitro (●) or in vivo (○). The data have been corrected for diffusion using L-glucose for in vitro measurements and phlorizin for in vivo measurements respectively (see Methods) and are shown as mean ± S.E.M. with number of experiments in parentheses. Curves were fitted by eye.](image)

**Table 1.** Effect of diabetes (7 days duration) on the glucose-induced change in transmural PD and on the active and passive components of glucose uptake measured in vivo. Glucose was present at an initial concentration of 64 mM. Results are given as mean ± S.E.M. with number of animals shown in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>PD (mV)</th>
<th>Glucose absorption (nmol cm⁻¹ min⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Active</td>
</tr>
<tr>
<td>Control</td>
<td>8.3 ± 0.6 (5)</td>
<td>421.2 ± 24.3 (6)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>10.4 ± 0.3 (6)</td>
<td>746.4 ± 46.9 (6)</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt; 0.05</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

active glucose uptake by the jejunum of diabetic rats revealed an 82·1% increase compared to that obtained using intestine of control animals. In marked contrast, the diffusive (phlorizin-insensitive) component of uptake was reduced by 31% during hyperglycaemia.

**Effect of diabetes on the uptake of D-glucose and L-glucose in vitro**

Carrier-mediated D-glucose uptake, when normalized to intestinal length, was significantly enhanced by diabetes in all regions of intestine (Table 2). When the data were normalized to mg/wet weight, the stimulatory effect of diabetes was evident only in the duodenum. The uptake of L-glucose was used to assess passive glucose
<table>
<thead>
<tr>
<th>Uptake (nmol cm(^{-1}) min(^{-1}))</th>
<th>Control</th>
<th>Diabetic</th>
<th>(P)</th>
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</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt 1</td>
<td>223 ± 26 (6)</td>
<td>484 ± 73 (7)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>D-Glucose uptake (64 mm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.19 ± 0.33 (6)</td>
<td>3.26 ± 0.33 (7)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>234 ± 29 (7)</td>
<td>422 ± 61 (7)</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Expt 1</td>
<td>2.56 ± 0.31 (7)</td>
<td>3.96 ± 0.77 (7)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Expt 2</td>
<td>4.55 ± 0.43 (5)</td>
<td>3.93 ± 0.28 (5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Jejunum</td>
<td>119 ± 28 (3)</td>
<td>158 ± 41 (5)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Expt 1</td>
<td>1.91 ± 0.55 (3)</td>
<td>1.92 ± 0.35 (4)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Expt 2</td>
<td>0.71 ± 0.20 (5)</td>
<td>1.44 ± 0.16 (5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ileum</td>
<td>56 ± 13 (5)</td>
<td>131 ± 19 (5)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1.61 ± 0.18 (7)</td>
<td>2.33 ± 0.32 (5)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Expt 1</td>
<td>0.019 ± 0.003 (7)</td>
<td>0.022 ± 0.003 (5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Expt 2</td>
<td>0.025 ± 0.002 (4)</td>
<td>0.020 ± 0.001 (5)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Table 3. Effect of diabetes (7 days duration) on the uptake of L-proline in vitro. Values have been normalized to either intestinal length or wet weight and are expressed as means ± s.e.m. with the number of animals used in parentheses. The \(P\) values are for comparisons between control and diabetic conditions and are based on analysis of variance of results of experiments 1 and 2; n.s. = not significantly different.

<table>
<thead>
<tr>
<th>Uptake (nmol cm(^{-1}) min(^{-1}))</th>
<th>Control</th>
<th>Diabetic</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt 2</td>
<td>0.69 ± 0.07 (5)</td>
<td>1.01 ± 0.02 (5)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1.07 ± 0.07 (5)</td>
<td>1.33 ± 0.09 (5)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Expt 2</td>
<td>0.0072 ± 0.0025 (5)</td>
<td>0.00087 ± 0.0012 (5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.57 ± 0.04 (5)</td>
<td>0.78 ± 0.07 (5)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Expt 2</td>
<td>0.0078 ± 0.0009 (5)</td>
<td>0.00099 ± 0.0009 (5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Jejunum</td>
<td>296 ± 48 (7)</td>
<td>323 ± 56 (4)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Expt 1</td>
<td>2.98 ± 0.63 (7)</td>
<td>2.43 ± 0.53 (4)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Jejunum</td>
<td>318 ± 45 (7)</td>
<td>388 ± 77 (5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Expt 1</td>
<td>3.51 ± 0.51 (6)</td>
<td>3.53 ± 0.66 (5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ileum</td>
<td>284 ± 50 (6)</td>
<td>283 ± 18 (6)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Expt 1</td>
<td>3.92 ± 0.45 (6)</td>
<td>3.55 ± 0.33 (6)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
uptake. Diabetes resulted in an increase in L-glucose uptake when expressed per centimetre but not when calculated per milligram (Table 2).

**Sodium dependence of L-proline uptake in vitro**

Following a 5 min pre-incubation in proline-free buffer, jejunal sleeves were incubated in solutions containing Na⁺ and L-proline (0.025 or 50 mM). Adjacent tissues were incubated in Na⁺-free buffer at the same proline concentrations.

At the lower concentration of proline, uptake in the presence of Na⁺ (7.75 ± 0.92 pmol mg⁻¹ min⁻¹ (5)) was significantly higher than that observed in the absence of Na⁺ (1.49 ± 0.04 pmol mg⁻¹ min⁻¹ (5)). Uptake at 50 mM-proline, however, yielded a Na⁺ dependence of 21.2 ± 4.6% (12), the values being 6.43 ± 0.32 nmol mg⁻¹ min⁻¹ (12) and 5.02 ± 0.26 nmol mg⁻¹ min⁻¹ (12) for Na⁺-containing and Na⁺-free buffer respectively (P < 0.005).

**Effect of diabetes on the uptake of L-proline**

At 0.025 mM-proline, uptake per centimetre was significantly enhanced by diabetes in all regions of intestine used (Table 3). In contrast, at 50 mM-proline, uptakes per centimetre were unaffected by diabetes. When normalized to tissue wet weight, diabetes was without effect on uptake using either concentration of the amino acid.

**DISCUSSION**

The aim of this study was to gain insight into the mechanistic basis for the enhanced glucose uptake which has been shown to occur in intestine from diabetic animals (Axelrad, Lawrence & Hazelwood, 1970; Leese & Mansford, 1971; Thomson, 1981). In particular, the use of electrophysiological techniques and the measurement of the uptake of nutrients with differing Na⁺ dependence have allowed us to investigate further the possibility that a change in the Na⁺ electrochemical gradient might be the basis for the functional adaptation.

The histological appearance of the mucosa 7 days after streptozotocin treatment showed clear evidence of hyperplasia. The regimen of feeding in pairs adopted implied that the structural and functional changes observed were unrelated to hyperphagia.

Although previous work has reported an enhanced glucose absorption in vivo following diabetes (Csaky & Fischer, 1981), no study to date has examined the effect of sustained hyperglycaemia on the active and diffusive components of glucose uptake in vivo. In this present work we have quantified the movement of the hexose via these separate pathways and the changes which occur during diabetes. The data confirm that diffusive movement accounts for a large proportion of total uptake (38% of total uptake in vivo in control rats).

Interestingly, whilst diabetes resulted in an enhanced active glucose absorption when measured in vivo both electrically and chemically, uptake via the phlorhizin-insensitive pathway was substantially reduced, presumably the consequence of a lowered concentration gradient for net glucose diffusion under conditions of an elevated plasma level of the sugar. Failure to separate and quantify the two components of glucose absorption may explain why previous studies in vivo (particularly those using high luminal glucose concentrations) have reported no
effect of diabetes on uptake (Lorenz-Meyer, Gottesburen, Menge, Bloch & Riecken, 1974; Costrini, Ganeshappa, Wu, Whalen & Soergel, 1977) while others have observed an inverse relationship between glucose uptake and blood glucose levels during diabetes (Lorenz-Meyer et al. 1974).

The in vitro preparation used in this present study has the advantage over others in giving information on unidirectional movement across the brush-border membrane. Using this method, our results provide an interesting insight into the mechanisms involved in the stimulation of the active component of glucose transport noted in vivo. When uptakes were expressed on a length basis, diabetes had marked effects on the uptakes of glucose and 0.025 mm-proline, i.e. where Na\textsuperscript{+}-dependent transport was the predominant process. When data were expressed per milligram wet weight, diabetes was found to be without effect on uptake, suggesting perhaps that changes in tissue weight were responsible for the increased transport. For several reasons, we believe this not to be a full explanation of the effects of diabetes. An alteration in intestinal weight is a poor index of surface area for uptake since much of the change in weight may occur in non-epithelial tissue. Interestingly, our study showed that diabetes increased whole tissue weight by 23.9% but increased the mucosal weight by only 13.3%. Even our observations of an increased villus height or enterocyte number in this condition may not reflect an increased surface area, since it is known that villus number is also decreased in diabetes with the result that surface area expressed per square centimetre serosa is unchanged (Lorenz-Meyer, Thiel, Gottesburen & Riecken, 1977). Additionally, since only a small proportion of villus enterocytes are involved in Na\textsuperscript{+}-dependent uptake (King, Sepulveda & Smith, 1981) it does not automatically follow that increased anatomical surface area is accompanied by an enlarged functional surface area.

What then is a more appropriate explanation for our diabetes-induced changes in uptake? Previous studies suggest two stages in the development of this response (Axelrad et al. 1970; Schedl & Wilson, 1971). In the first few days when intestinal weight is little changed, an increased absorptive capacity occurs. At a later stage, the intestine has undergone considerable hyperplasia (Miller, Hanson, Schedl & Osborne, 1977) which may account for some of the increased absorptive capacity still observed at this time. Our experiments were timed to fall in the transition period between stage 1 (no change in intestinal weight) and stage 2 (very large change). Whatever mechanism accounts for the changes observed in stage 1 may act in concert with tissue hyperplasia which occurs later. Thus, the changes observed in this present study probably reflect both mechanisms. This view is reinforced by two important findings. Firstly, in both Experiments 1 and 2 the increased uptake of L-glucose in jejunum from diabetic rats was proportionally smaller than that of solutes whose pathway is primarily Na\textsuperscript{+} dependent (0.025 mm-proline, 64 mm-d-glucose). The transport of these latter solutes is likely therefore to be due to both an increased surface area and an enhanced Na\textsuperscript{+} electrochemical gradient. Secondly, the hyperpolarization of $V_m$ noted in diabetic intestine implies an increased gradient for Na\textsuperscript{+} entry into the enterocyte (Schultz, 1977) and this may be responsible, at least in part, for the enhanced Na\textsuperscript{+}-dependent uptake both in vivo and in vitro. It is interesting to note that in two other experimental conditions, namely starvation and
hyperglucagonaemia, hyperpolarization of $V_m$ has been implicated in the enhanced intestinal Na$^+$-dependent solute accumulation (Thompson & Debnam, 1986a, b).

In summary, our results show that diabetes stimulates glucose uptake in vivo. An enhancement at the brush border is involved and the mechanisms include an increased surface area for uptake and an increased electrical gradient which has its greatest effect when the uptake mechanism is primarily Na$^+$ dependent. This conclusion is very much in accord with that of a previous study (Hopfer, 1975) which suggested that changes in the Na$^+$ conductance of the brush-border membrane may result in an increase in the electrochemical gradient of Na$^+$ across the brush-border membrane in diabetic rats.

The possibility of adaptation at the basolateral membrane cannot be excluded at the present time. Indeed, there is a growing body of evidence to suggest that following hyperglycaemia of 5–10 h duration, the induction of sugar carriers at this latter site may be responsible for the increased glucose uptake observed in vivo (Csaky & Fischer, 1981; Cheeseman & Maenz, 1985; Debnam et al. 1986a, b). It is unclear at the present time whether the stimulation of glucose uptake observed in this present study is due to lack of insulin per se, an altered rate of secretion of other hormones or an elevation of blood glucose concentration per se. Work is in progress to discriminate between these possibilities.

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