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DIABETES MELLITUS AND SUGAR TRANSPORT ACROSS THE BRUSH-BORDER AND BASOLATERAL MEMBRANES OF RAT JEJUNAL ENTEROCYTES

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SUMMARY

1. The effects of streptozotocin-induced diabetes mellitus on active jejunal glucose uptake *in vivo*, and on galactose movement across the brush-border (phlorhizin-sensitive) and basolateral (phlorhizin-insensitive) membranes of isolated upper and mid-villus enterocytes has been studied.

2. Chronic diabetes increased unidirectional phlorhizin-sensitive galactose uptake by mid-villus but not upper villus cells. In contrast, phlorhizin-insensitive uptake by both cell populations was enhanced by diabetes.

3. Diabetes increased glucose absorption *in vivo* by mechanisms which were unrelated to hyperphagia. Mucosal hyperplasia acting together with an epithelium containing a higher proportion of mature enterocytes is the most likely explanation for the response.

4. We conclude that, during diabetes, the mid-villus region is an important site of adaptation with functional changes occurring at both the brush-border and basolateral membranes. The increased hexose transport ability of the basolateral membrane is retained during cell transit along the villus.

INTRODUCTION

The stimulatory effect of experimental diabetes mellitus on intestinal sugar absorption, first reported in the rat nearly 50 years ago (Pauls & Drury, 1942), has since been confirmed in all animal species studied (see Karasov & Diamond, 1983 for discussion). Mucosal hyperplasia has long been implicated in the response (Schedl & Wilson, 1971; Lorenz-Meyer, Thiel, Menge, Gottesburen & Riecken, 1977) and it appears that in diabetic rats a prolonged enterocyte lifespan, together with a greater enterocyte number, results in a higher proportion of functionally mature cells on the villus (Debnam & Ebrahim, 1990). Sugar transport across the intestinal epithelial cell involves sequential events at the brush-border membrane and basolateral membrane, and the increased maturity of the villus cell population in diabetes is reflected in an enhanced electrochemical gradient for Na^+–sugar co-transport across the brush border (Debnam, Karasov & Thompson, 1988; Debnam & Ebrahim, 1989), and a greater density of hexose transporters at this site (Fedorak, Gershon & Field, 1989). There is, however, no information on the effect of diabetes on adaptation of

carrier-mediated transport at the basolateral aspect of the cell or on the specific villus regions involved in the adaptive response to diabetes.

For this present study, rats were made diabetic by treatment with streptozotocin, a B-cell cytotoxic agent, and were used after 7 days (acute diabetic) or 4–5 weeks (chronic diabetic). Initial experiments compared the effect of acute and chronic diabetes on active glucose uptake from the jejunal lumen *in vivo* and in further work the techniques of Weiser (1973) were used to harvest upper and mid-villus enterocytes from isolated jejunum. Since the inhibitor phlorhizin has a specific effect on Na^+-linked sugar uptake at the brush border, galactose uptake by enterocytes measured in the absence or presence of phlorhizin has allowed the effects of diabetes on both Na^+-linked sugar influx at the brush border and facilitated uptake at the basolateral membrane to be quantified separately. In parallel experiments, the effect of diabetes on the regional distribution of sucrase and alkaline phosphatase activity along the villus axis has been assessed. A preliminary account of this work has been published (Debnam, Ebrahim & Swaine, 1989).

METHODS

Animals and anaesthesia

Experiments utilized male Sprague–Dawley rats (initial weight 220–250 g). Diabetes was induced by a single injection of streptozotocin (50–60 mg kg^{-1}, dissolved in pH 4·5 citrate buffer) into the tail vein with the animal under light ether anaesthesia.

For studies on the acute effect of diabetes, animals were used after 7 days and during this time they were pair-fed with control (citrate buffer-injected) rats, i.e. the latter were given the same amount of food as consumed by paired diabetic rats on the previous day. For studies on the chronic effect of diabetes, animals were used after 30–40 days, and during this time they were allowed free access to a standard maintenance diet. Age-matched rats were used as controls. Both groups of diabetics were allowed free access to water.

Anaesthesia for *in vivo* experiments and prior to the removal of tissue for enterocyte isolation was achieved using sodium pentobarbitone (90 mg kg^{-1}, I.P., Sagatal, May & Baker Limited, Dagenham, Essex). Blood samples for the determination of plasma glucose concentration were obtained by cardiac puncture before removal of intestinal tissue and a glucose oxidase method used for the assay of the sugar.

Active glucose uptake in vivo

The methods used have been described in detail elsewhere (Debnam & Levin, 1975). In brief, 25 cm of upper jejunum of an anaesthetized rat, beginning 2 cm from the ligament of Treitz, was cleared of food residue by washing with warm NaCl (154 mmol l^{-1}) and cannulated at both ends. The cannulae were connected to a fluid circuit through which gassed (95% O_2:5% CO_2) bicarbonate saline at 37 °C was pumped (2 ml min^{-1}) using a peristaltic pump (Watson–Marlow Limited, Cornwall). Body temperature was maintained at 37 °C throughout the experiment using a heated blanket. The uptake of D-glucose was determined by circulating the sugar (64 mmol l^{-1}) through the segment for a period of 20 min duration. The fluid contained in the segment and circulation system was then washed out, proteins removed by using $Ba(OH)_2$ (0·15 M) and $ZnSO_4$ (5%) and estimated for glucose (Somogyi, 1945). The cannulated intestine was removed from the animal and its length measured without applying excess tension. The segment was then opened longitudinally to form a flat sheet and the mucosal/submucosal layer removed by scraping with the edge of a microscope slide and the separated layer was dried overnight at 70 °C and weighed. Glucose uptake was calculated as luminal loss of the sugar and expressed as either nmol cm^{-1} min^{-1} or nmol (mg dry weight)$^{-1}$ min^{-1}. Parallel experiments using phlorhizin in the

uptake buffer were carried out to enable values for active, Na^+-dependent glucose absorption to be derived (Debnam & Levin, 1975).

Enterocyte isolation

A modification of the technique of Weiser (1973) was used to isolate villus tip and mid-villus cells from the upper jejunum. In brief, a 25 cm length of small intestine beginning 10 cm from the Ligament of Treitz was removed and washed through with ice-cold NaCl (154 mmol l^{-1}) containing dithiothreitol (DTT, 0·5 mmol l^{-1}). One end was ligated and the segment filled with an oxygenated buffer (pH 7·3) containing (in mmol l^{-1}): NaCl, 96; KCl, 1·5; KH_2PO_4, 8; Na_2HPO_4, 5·6; sodium citrate, 27; β-hydroxybutyric acid, 0·5; and bovine serum albumin (BSA), 1 mg ml^{-1}. The other end was ligated and the sac was incubated for 15 min at 37 °C. The luminal solution was the discarded and the segment filled with a second oxygenated buffer containing (in mmol l^{-1}): NaCl, 137; KH_2PO_4, 11·5; Na_2HPO_4, KCl, 2·2; β-hydroxybutyrate, 0·5; EDTA, 1·5; DTT, 0·5; and BSA (1 mg ml^{-1}). The segment was incubated at 37 °C for 8 min (control) or 12 min (diabetic) and upper villus enterocytes were collected by manually dislodging the cells and washing through with ice-cold bicarbonate saline BSA (1 mg ml^{-1}). The sac was refilled with the EDTA buffer and incubated at 37 °C for a further 6 (control) or 8 min (diabetic) in order to harvest mid-villus cells. Cell fractions were washed twice in bicarbonate–BSA buffer and finally resuspended in this buffer to a protein concentration of 12–18 mg ml^{-1}. Cells were kept in plastic beakers on ice until their use 10–60 min later.

For the measurement of sugar uptake, 1 ml of cell suspension was added to 2 ml of gassed (95% O_2:5% CO_2) bicarbonate–BSA buffer and the cells pre-incubated for 10 min at 37 °C. Phlorhizin (1 mmol l^{-1}) or phloretin (1·34 mmol l^{-1}) were present in some experiments. The uptake process was initiated by adding 1 ml pre-warmed bicarbonate–BSA buffer containing D-galactose and [^{3}H]D-galactose (final concentration, 10 mmol l^{-1}, 0·28 Ci mol^{-1}) together with [^{14}C]inulin (0·23 μCi ml^{-1}) as an extracellular space marker. The mixture was shaken (70 oscillations min^{-1}) during the incubation to ensure adequate mixing of the cell suspension. The uptake process was terminated by adding 250 μl aliquots of the suspension to 500 μl ice-cold saline containing phlorhizin (1 mmol l^{-1}) and the cells separated by centrifugation (12000 *g*, 3 min) through a 250 μl layer of oil (di-*n*-butyl phthalate:dinonyl phthalate, 3:2 v/v). The cell pellets were lysed in 0·5% Triton X-100, 5% TCA was added to precipitate cellular protein and, after centrifugation, the supernatant subjected to dual-label scintillation counting. Galactose uptake was expressed as nmol (mg cell protein)$^{-1}$ with protein being estimated using the method of Bradford (1976). Sugar uptake measured in the presence of phlorhizin represented facilitated movement across the basolateral membrane whilst initial unidirectional entry across the brush border was estimated by subtracting data obtained in the presence of phlorhizin from obtained in its absence.

Brush-border enzyme activity

Upper and mid-villus enterocytes were harvested as described above. A lower villus cell population was also obtained using a third incubation with EDTA buffer (6 min, control and diabetic). The three cell populations were suspended in phosphate buffer (pH 7), homogenized (Polytron, half-maximal speed for 30 s), and stored at −70 °C. Alkaline phosphatase and sucrase activities were determined as described previously (Dahlqvist, 1964; Forstner, Sabesin & Isselbacher, 1968).

Protein:DNA ratio

Isolated enterocytes were suspended in 0·05 mol l^{-1} Na_2HPO_4:2·0 mol l^{-1} NaCl (pH 7·4), homogenized (Polytron, 30 s, full speed), and stored at −20 °C. Protein and DNA concentrations were determined on thawed, diluted samples using the methods of Bradford (1976) and Labarca & Paigen (1980) respectively.

Intestinal histology

In order to assess the extent of cell removal along the villus axis, 1 cm sections of jejunum were washed through with NaCl (154 mmol l^{-1}) and placed in 10% formol saline. The tissue was later blocked in paraffin wax, sectioned longitudinally (5 μm) and stained with Haemotoxylin and Eosin.

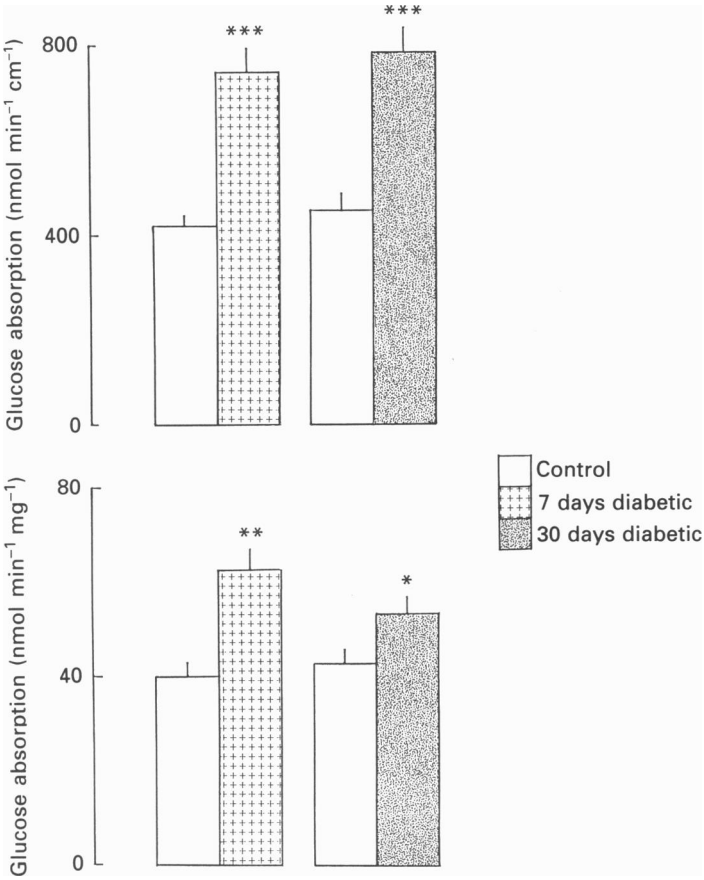

Fig. 1. Effects of acute and chronic diabetes on active glucose absorption measured *in vivo*. Uptake data have been normalized to mucosal length (upper) or mucosal weight (lower). Values are given as means ± S.E.M. of six or seven experiments per group. $*P < 0.01$; $**P < 0.005$; $***P < 0.001$.

Statistics

All values were assessed as means ± S.E.M. Differences between means were evaluated by Student's *t* test and considered significant at $P < 0.05$.

Chemicals

Radiochemicals were obtained from New England Nuclear (Dupont Limited, Stevenage, Herts). All other chemicals were of Analar Grade from either BDH Limited (Dorset) or Sigma Limited (Dorset).

RESULTS

Animals became glycosuric within 2 h of treatment with streptozotocin and elevated levels of plasma glucose were evident in both acute diabetes (control: 12.8 ± 0.7 mmol l^{-1} ($n = 9$); diabetic: 24.1 ± 2.2 mmol l^{-1} ($n = 10$), $P < 0.001$) and chronic diabetes (control: 10.9 ± 0.3 mmol l^{-1} ($n = 12$); diabetes: 36.4 ± 2.1 mmol l^{-1}

($n = 12$), $P < 0{\cdot}001$). Although chronically diabetic animals ingested approximately 38 g food day^{-1} (24 g day^{-1} in age-matched control rats), a slight reduction in body weight was noted (control: 228 ± 14 to 396 ± 35 g ($n = 18$), $P < 0{\cdot}001$; diabetic: 253 ± 11 to 238 ± 32 g ($n = 16$), $P > 0{\cdot}05$). Acute diabetic animals also lost weight over the 7 day feeding period (control: 234 ± 4 to 282 ± 5 g ($n = 8$) $P < 0{\cdot}001$; diabetic: 234 ± 3 to 196 ± 5 g ($n = 9$), $P < 0{\cdot}001$).

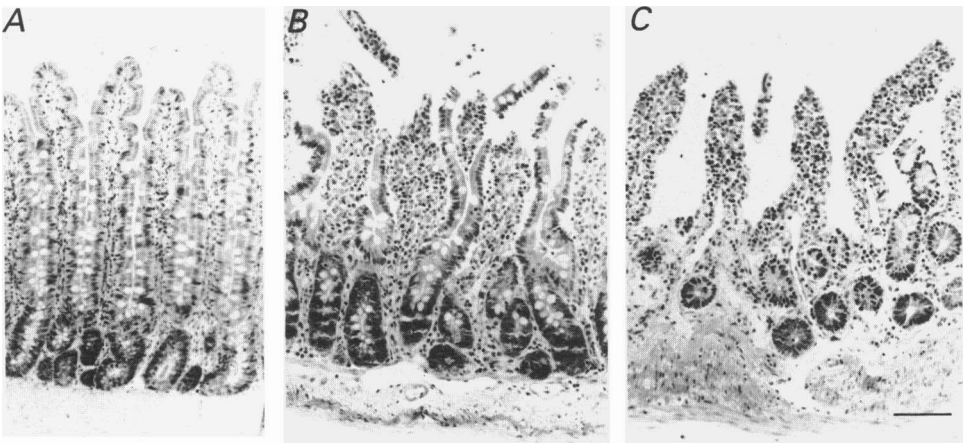

Fig. 2. Representative sections of jejunum from non-diabetic rats before cell isolation (*A*), or following removal of upper villus cells (*B*) and the entire villus epithelium (*C*). Details of the procedures used to remove cells are given in Methods. Scale bar: 100 μm, applies to all panels.

Diabetes and glucose absorption in vivo

When expressed per centimetre jejunal length, glucose absorption was increased by 78% ($P < 0{\cdot}001$) and 74% ($P < 0{\cdot}001$) respectively in acute and chronic conditions (Fig. 1). When uptake data were normalized to mucosal dry weight, the percentage increases were smaller but remained significant at 57% ($P < 0{\cdot}005$) and 24% ($P < 0{\cdot}01$) respectively for acute and chronic diabetes.

Isolated enterocytes – viability and origin

Routine examination of cell preparations showed that 85–90% of enterocytes were able to exclude Trypan Blue dye, non-viable cells usually being situated around the perimeter of cell sheets. The first incubation with Weiser B solution released cells at the upper region of the villus (Fig. 2*A* and *B*) and successive incubations removed enterocytes further down the villus axis until after three incubations only the crypt epithelium was intact (Fig. 2*C*). Functional viability of isolated cells was assessed by comparing galactose uptake under various conditions. Using upper villus cells from non-diabetic animals, sugar uptake was found to be saturable with time. The process was concentration and temperature dependent and inhibited by pre-incubation with phloretin or phlorhizin (Fig. 3). As described in Methods, the inhibition by phlorhizin was used in all subsequent experiments to separate and quantify movement of the sugar across the brush border from that across the basolateral membrane. The

inhibitor concentration used has been shown to block the increased transmural potential difference associated with active glucose uptake *in vitro* and *in vivo* (Barry, Dikstein, Matthews & Smyth, 1964).

Chronic diabetes and galactose uptake by isolated enterocytes

The magnitude of the phlorhizin-sensitive component of uptake was higher in upper villus compared to mid-villus cells (Fig. 4*A* and *B*). As an example, values

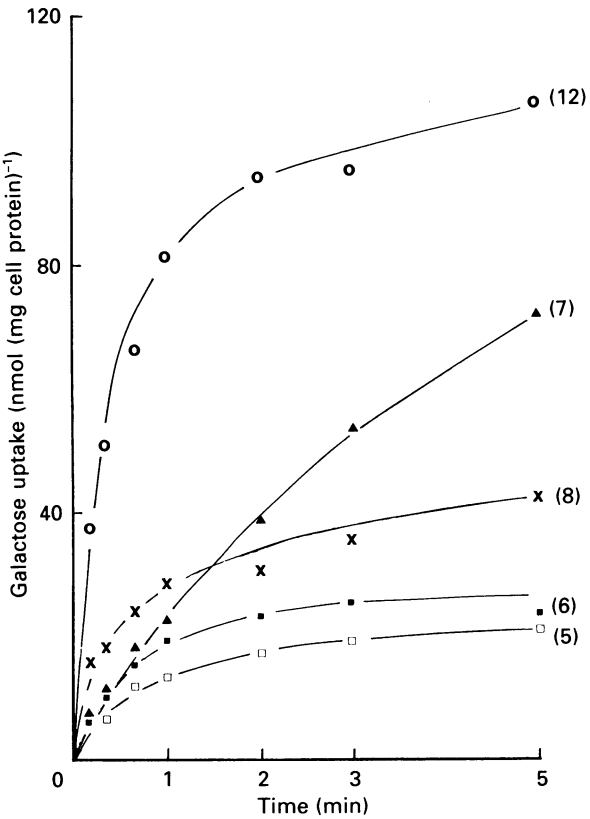

Fig. 3. General characteristics of galactose uptake by upper villus enterocytes. The sugar was present at a concentration of 10 mmol l^{-1} (○, ▲, ■, ×) or 1 mmol l^{-1} (□). Cells were pre-incubated for 10 min at 37 °C in the absence (○, □) or presence of phloretin (1·34 mmol l^{-1}, ▲) or phlorhizin (1 mmol l^{-1}, ×). ■, uptake, measured at 4 °C. Results are given as means with numbers of observations in parentheses.

obtained at 60 s in upper villus cells were increased by some 108% (control, $P < 0{\cdot}001$) and 38% (diabetic, $P < 0{\cdot}01$) compared to uptakes observed in mid-villus cells.

Diabetes increased phlorhizin-sensitive galactose uptake by mid-villus cells at all time points (Fig. 4*A* and *B*). However, there was a less obvious effect on uptake by upper villus cells, with significantly enhanced uptake only seen at 10 s. In contrast, phlorhizin-insensitive uptake of the sugar by both cell populations was enhanced by

diabetes (Fig. 4*C* and *D*) and was particularly evident in the first 60 s of uptake where the increases were 64 ($P < 0{\cdot}005$) and 66% ($P < 0{\cdot}005$) respectively for upper and mid-villus enterocytes.

Data obtained for maximal phlorhizin-insensitive galactose uptake were used to

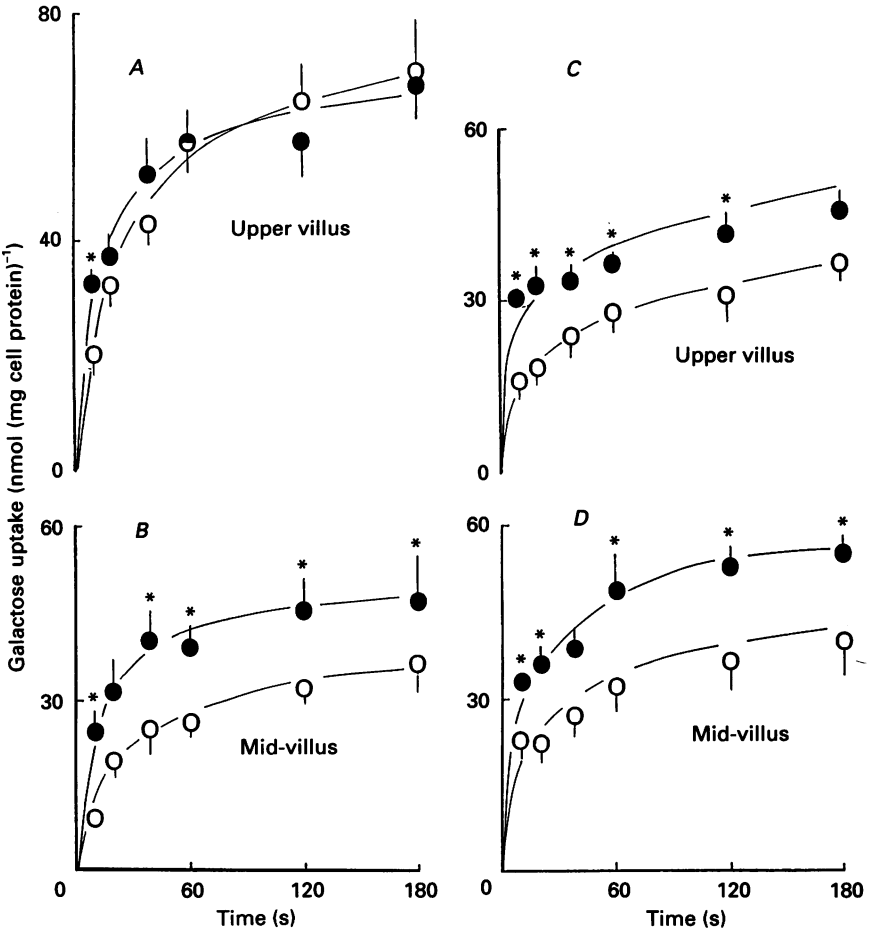

Fig. 4. Phlorhizin-sensitive (*A* and *B*) and phlorhizin-insensitive (*C* and *D*) galactose uptake by upper and mid-villus enterocytes prepared from control (○) and chronic diabetic (●) rats. Galactose was present in the incubation buffer at a concentration of 10 mmol l^{-1}. Values are given as means ± S.E.M. Each value is the mean of seven to thirteen observations. * $P < 0{\cdot}05$.

calculate intracellular volume and these values, together with data for maximal uptake in the absence of the inhibitor, allowed the determination of intracellular sugar concentration at equilibrium. Diabetes was without significant effect on both cell volume and intracellular galactose concentration in upper villus cells (Table 1). However, mid-villus cells from diabetic rats showed an enhanced ability (+33%, $P < 0{\cdot}01$) to concentrate galactose compared to non-diabetic cells, when exposed to the same extracellular level of the sugar.

Protein:DNA ratio

Protein:DNA ratios were 36% higher in upper compared to lower villus cells from control animals ($P < 0.02$, Table 1), but this villus gradient was not apparent in diabetes. Furthermore, diabetes was without effect on the relative amounts of protein and DNA at any defined locus on the villus axis.

TABLE 1. Effect of chronic diabetes on cell volume, intracellular galactose concentration and protein:DNA ratio of subpopulations of jejunal enterocytes. Galactose uptake experiments used the sugar at a concentration of 10 mmol l^{-1}

Cell origin	Cell volume (μl (mg protein)$^{-1}$)	Intracellular galactose concentration (mmol l^{-1})	Protein:DNA
Control			
Upper villus	4·9±0·5 (13)	25·4±1·7 (13)	12·4±0·7 (7)
Mid-villus	5·7±0·5 (14)	17·0±1·1 (14)**	10·8±0·6 (7)
Lower villus	—	—	9·2±0·5 (7)*
Diabetic			
Upper villus	5·6±0·5 (11)	23·1±2·6 (11)	11·7±1·1 (6)
Mid-villus	5·4±0·3 (10)	21·1±1·2 (10)†	11·4±1·0 (6)
Lower villus	—	—	10·7±0·6 (6)

Results are given as means±S.E.M. with numbers of observations in parentheses. *$P < 0.02$; **$P < 0.001$ compared to upper villus cells; †$P < 0.01$ compared to control.

Sucrase and alkaline phosphatase activity

As expected, activity gradients of both brush-border enzymes from villus tip to crypt–villus junction were noted and this was more obvious for alkaline phosphatase than for sucrase (Fig. 5).

Diabetes caused significant increases in sucrase activity at each level of the villus (upper villus: +200%, $P < 0.005$; mid-villus: +80%, $P < 0.005$; lower villus: +206%, $P < 0.005$). In marked contrast, alkaline phosphatase activity was lower in cells from diabetic rats compared to enterocytes from control animals (upper villus: −36%, $P > 0.05 < 0.1$; mid-villus: −55%, $P < 0.05$; lower villus: −33%, $P > 0.05 < 0.1$).

DISCUSSION

There is much information on the effects of experimental diabetes mellitus on the processes of digestion and absorption of nutrients. Early studies reported an enhanced sugar and amino acid absorption across the small intestine of the diabetic rat (Pauls & Drury, 1942; Axelrad, Lawrence & Hazlewood, 1970; Olsen & Rosenberg, 1970) and the involvement of mucosal hyperplasia in the adaptive response has been well documented (Schedl & Wilson, 1971; Lorenz-Meyer *et al.* 1977). More recent work has revealed that diabetes has a stimulatory effect, independent of changes in mucosal weight, on those substrates which utilize the Na^+ electrochemical gradient for movement across the brush border (Fedorak, Chang, Madara & Field, 1987; Debnam *et al.* 1988).

The process of differentiation and maturation of the intestinal epithelium results in a population of enterocytes with differing ages and cellular characteristics with only those cells situated at the upper region of the villus being functionally mature with respect to active nutrient uptake (Kinter & Wilson, 1965; Smith, 1985).

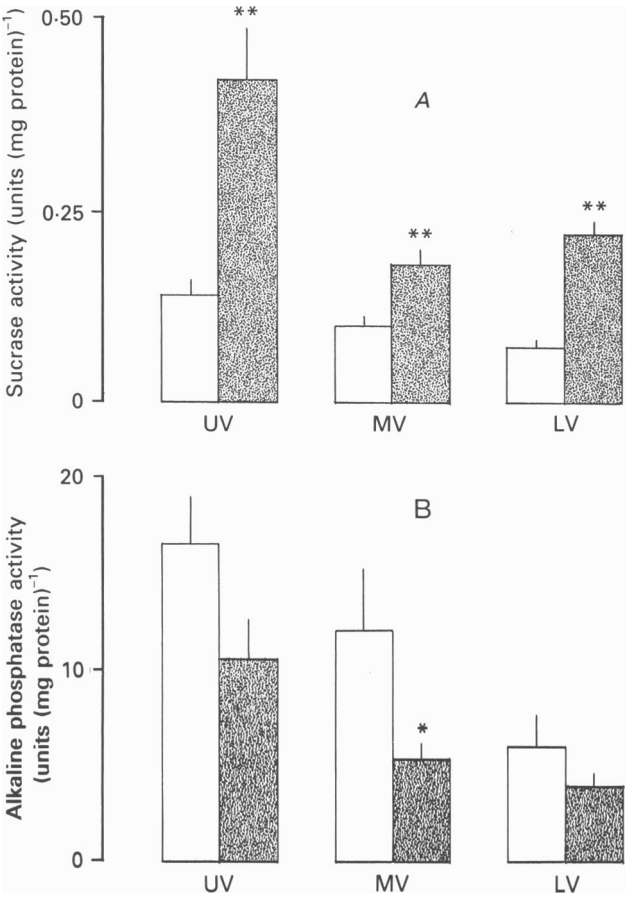

Fig. 5. Sucrase (*A*) and alkaline phosphatase (*B*) activity of upper villus (UV), mid-villus (MV) and lower villus (LV) jejunal enterocytes from control (open bars) and chronically diabetic (shaded bars) rats. Values are given as means ± S.E.M. of seven cell preparations per group. $*P < 0{\cdot}05$, $**P < 0{\cdot}005$.

However, the effect of diabetes on sugar uptake across the brush border of cells at specific loci on the villus is unknown. Moreover, there is no information on adaptation of facilitated transport at the basolateral membrane to diabetes. This present study provides important information on these areas.

Diabetes induced a highly significant enhancement of glucose absorption *in vivo*. A comparison of percentage increases obtained when uptake data were expressed per gram mucosal weight and per centimetre intestinal length (Fig. 1), showed that increased mucosal weight was responsible for approximately 21 and 50% of the adaptation to acute and chronic diabetes, respectively. Two other possible mechanisms could explain the remaining 57 and 24% enhanced uptake in acute and

chronic diabetes, respectively: (*a*) an increased uptake by mature upper villus enterocytes, and (*b*) a higher proportion of functionally mature cells on the villus. Uptake data from this present study using isolated enterocytes support the latter possibility.

More rigorous treatment was necessary for the isolation of enterocytes from diabetic intestine and we can offer no explanation for this observation at the present time. Structural integrity of the isolated cells from both control and diabetic rats was shown by their characteristic columnar appearance, together with the ability to exclude Trypan Blue. Enterocytes also displayed the expected response to pre-incubation with phloretin and phlorhizin (Randles & Kimmich, 1978). Whilst phloretin is recognized to inhibit sugar transport at both brush-border and basolateral membranes of isolated cells (Randles & Kimmich, 1978), the blocking effect of phlorhizin is more specific in its location – notably the active, electrogenic sugar transport process at the brush border (Kimmich & Randles, 1976; Hopfer, Sigrist-Nelson, Amman & Murer, 1977; Debnam & Thompson, 1984; Harig, Barry, Rajendran, Soergel & Ramaswamy, 1989). In the present study, this action of phlorhizin allowed unidirectional influx of galactose across the brush-border and basolateral membranes to be separated and quantified.

The loci of harvested cells with respect to villus structure were determined histologically and confirmed by measuring the activity distribution of two brush-border enzymes, alkaline phosphatase and sucrase. Previous studies have shown that alkaline phosphatase, in particular, shows a decreasing activity from villus tip to crypt–villus junction (Weiser, 1973; Freeman, Johnson & Quamme, 1987). The differential effect of diabetes on sucrase and alkaline phosphatase activity confirms and extends a previous study which showed that diabetes of 20 days duration had a proportionally greater effect on the activities of those brush-border enzymes involved in carbohydrate digestion. (Lorenz-Meyer *et al.* 1977).

The gradient of phlorhizin-sensitive galactose uptake from upper villus to mid-villus regions in cells from control animals was largely abolished by diabetes. Since the magnitude of Na^+-dependent nutrient uptake at the brush-border membrane reflects the degree of enterocyte development (Smith, 1985), our observation is a likely consequence of an increased cell age at each relative position on the villus in diabetes. In the present study, calculated values of cell volume and indirect indices (protein:DNA ratio) imply that the enhanced mid-villus sugar uptake in diabetes represents an enhanced ability of the cells to concentrate the sugar. Using autoradiographic methods, we have reported a similar change in the localization of valine accumulation in diabetes (Debnam & Ebrahim, 1990), and it appears therefore that Na^+–nutrient co-transport processes are affected by diabetes in the same general way. Possible explanations for the recruitment of normally immature cells for active nutrient transport across the brush border in diabetes include increased carrier density (Fedorak *et al.* 1989), and an increased electrochemical driving force for Na^+–sugar co-transport (Debnam & Ebrahim, 1989).

The basolateral membrane is now recognized to be an important site for the regulation of intestinal sugar absorption. Thus, I.V. glucose infusion of 4–10 h duration (Csaky & Fischer, 1981; Maenz & Cheeseman, 1986; Karasov & Debnam, 1987), ileal glucose perfusion (Karasov & Debnam, 1987), and an increased level of

dietary carbohydrate (Cheeseman, 1988) are associated with increased glucose movement across the basolateral but not brush-border membrane of jejunal enterocytes. This present study is the first to report alterations in basolateral hexose transport induced by diabetes mellitus.

Unlike events at the brush border, chronic diabetes stimulated galactose movement across the basolateral membrane of both upper and mid-villus enterocytes. It has been argued elsewhere (Karasov & Debnam, 1987) that sugar transport across the intestinal epithelium is normally limited by its efflux across the basolateral membrane. Thus, the membrane responses reported in the present work would facilitate transepithelial sugar transport, particularly in the context of an increased ability of mid-villus cells to concentrate luminal sugar. Our study, together with previous work (Csaky & Fischer, 1981; Maenz & Cheeseman, 1986; Karasov & Debnam, 1987), implies that, following the stimulus of hyperglycaemia, an enhanced basolateral sugar permeability is established in as little as 3 h duration and this is followed some days later by increased transport at the brush border. This sequence of events will minimize intracellular osmotic changes during transcellular sugar transport.

In conclusion, results from this study demonstrate a stimulatory effect of diabetes on sugar transport at both poles of jejunal mid-villus enterocytes. The increased basolateral transport is retained during subsequent migration of cells along the upper reaches of the villus. The recruitment of normally immature enterocytes acts in concert with an increased anatomical surface area (Lorenz-Meyer *et al.* 1977) to produce the enhanced absorption which is characteristic of this condition. The luminal and/or humoral signals which mediate the adaptive response are unknown, although the increased glucose uptake *in vivo* following pair-feeding of diabetic rats eliminates the involvement of hyperphagia. Finally, our data emphasize the importance of quantifying uptake by specific subpopulations of enterocytes in studies of the adaptation of intestinal nutrient absorption.

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