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EFFECT OF HYPERGLYCAEMIA ON SUGAR TRANSPORT IN THE ISOLATED MUCOSA OF GUINEA-PIG SMALL INTESTINE

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SUMMARY

1. The effect of hyperglycaemia on sugar transport was studied by comparing transepithelial permeation and tissue content of 3-*O*-methyl-D-glucose (3-*O*-MG), β-methyl-D-glucoside (β-MDG) and D-glucose in isolated mucosae of guinea-pig jejunum mounted in a flux chamber. Sugars were administered either to the luminal or the blood side of mucosae prepared either from normal animals or those maintained in a hyperglycaemic state by I.V. glucose infusion for 12 h.

2. In control animals, absorptive sugar fluxes increased in the order glucose > β-MDG > 3-*O*-MG. Only β-MDG was accumulated in the tissue beyond the medium concentration. Permeation of 3-*O*-MG and β-MDG in the direction blood-to-lumen was mainly paracellular as indicated by the strict correlation with the simultaneous permeation of polyethylene glycol (mol. wt. 900).

3. Luminal addition of 10^{-3} M-phlorhizin increased permeation and decreased tissue content of β-MDG and D-glucose when administered on the blood side, suggesting that these sugars are recaptured at the brush border even from vigorously mixed solutions.

4. For flux coefficient calculation the preparation was regarded as a three-compartment system. With all three sugars, the influx coefficient was higher at the luminal, but lower at the basolateral membrane than the corresponding efflux coefficient. 3-*O*-MG displayed the highest basolateral influx coefficient of all three sugars, being even higher than its luminal influx coefficient. The luminal influx coefficient of β-MDG was 22 times greater, and its basolateral efflux coefficient 2·5 times less than the corresponding values for 3-*O*-MG, resulting in cellular β-MDG accumulation. D-Glucose was suited best for transepithelial transport, having a luminal influx coefficient only 1·6 times less, and a basolateral efflux coefficient almost 10 times greater than those for β-MDG.

5. Prolonged hyperglycaemia increased the lumen-to-blood permeation of all three sugars 1·3–2-fold. No significant differences in tissue content to control values were observed after 45 min (3-*O*-MG, D-glucose) or 90 min (β-MDG) incubation. Therefore,

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flux coefficients increased by the same factors in luminal and basolateral membranes, i.e. 1·7, 1·3 and 1·7 for 3-*O*-MG, β-MDG and D-glucose, respectively.

6. These results indicate that changes in both the luminal and basolateral membranes play a role in the increase of sugar transport in hyperglycaemia and that a regulatory mechanism might exist between the transport systems located in both membranes. The time difference for approaching steady-state fluxes and tissue concentrations might be regarded as one indication of intracellular compartmentation of sugars.

INTRODUCTION

It has been pointed out that experimental diabetes produced by alloxan (Laszt & Vogel, 1946; Sols, Vidal & Larralde, 1948; Aulsebrook, 1961; Crane, 1961; Varma & Banerjee, 1963; Flores & Schedl, 1968; Axelrad, Lawrence & Hazelwood, 1970; Olsen & Rosenberg, 1970; Levinson & Englert, 1970; Schedl & Wilson, 1971) or streptozotocin (Olsen & Rosenberg, 1970; Rerup, 1970; Caspary, Rhein & Creutzfeldt, 1972; Caspary, 1973) causes significant changes in the function of the small intestine and markedly increases intestinal sugar absorption. It is also known that hyperglycaemia itself is able to enhance intestinal sugar transport (Csáky & Fischer, 1977, 1981).

Several theories have been published to explain these changes. However, their mechanism is still only poorly understood. Some data indicate that experimental diabetes increases the active transport of sugars, amino acids (Olsen & Rosenberg, 1970; Lal & Schedl, 1974) and bile salts (Caspary, 1973) as well as the activity of Na^{+}–K^{+}-dependent ATPase (Luppa, Hönicke, Reissig & Müller, 1978) in the small intestine. Sodium and water transport were also increased in diabetic rats (Aulsebrook, 1961); however, Levinson & Englert (1970) found no correlation between the changes of sugar and sodium absorption. Several experimental results support the hypothesis that changes in the carrier-mediated transport systems have an important role in the increased sugar transport produced by diabetes or hyperglycaemia (Vinnik, Kern & Sussman, 1965; Csáky & Fischer, 1981). Hopfer (1975) found an increased sugar uptake by vesicles isolated from the brush-border membranes of alloxan diabetic rats, whereas Csáky & Fischer (1981) claim that hyperglycaemia activates a substrate-specific adaptive transport system, presumably through synthesis of new carriers which are most probably located in the basolateral membrane.

The present study was designed to obtain new information about the mechanism of the increased intestinal sugar transport produced by hyperglycaemia, and especially about the relative importance of changes in the luminal and basolateral membranes. For this purpose experiments were performed with the isolated mucosa of guinea-pig jejunum mounted as a separating membrane in a flux chamber (Lauterbach, 1977). The preparation is almost free of additional tissues, thus permitting estimation of transepithelial substrate fluxes in both directions with equal accuracy. Regarding the experimental set-up as a three-compartment system, flux coefficients for influx and efflux across the luminal as well as the basolateral membranes of the mucosal cell can be calculated from transpithelial fluxes and the tissue content of sugar measured under steady-state conditions. Both metabolized (D-glucose) and non-metabolized (3-*O*-methyl-D-glucose, β-methyl-D-glucoside) sugars were used.

METHODS

Materials. Glucose was supplied by Merck AG, Darmstadt; β-methyl-D-glucoside by Fluka AG, Buchs SG; phlorhizin and phloretin by Carl Roth OHG, Karlsruhe; cytochalasin B by Sigma Chemical Company, Saint Louis, MO; 3-*O*-methyl-D-glucose was synthesized according to Freudenberg & Hixon (1923) and Freudenberg & Smeykal (1926). 3-*O*-[^{14}C]methyl-D-glucose (specific activity 12·2 GBq/mmol) and [^{3}H]polyethylene glycol (mol. wt. 800–1000, specific activity 159 kBq/mg) were obtained from New England Nuclear Corporation, Boston, MA, and β-methyl-D-[U-^{14}C]glucoside (specific activity 15·2 GBq/mmol) was purchased from The Radiochemical Centre, Amersham, England.

Animals. Guinea-pigs, preferably in a weight range of 350–500 g, were used (Fa. Rost, Witten-Annen). The animals were fed on a standard diet (Fa. Höveler, Langenfeld-Immigrath) for at least 2 weeks. In addition they received 0·5 ml multivitamin mixture (Multivitaminsaft Lappe, Lappe Arzneimittel, Niederlassung Bensberg der Bristol-Myers GmbH, Bergisch Gladbach) to which ascorbic acid was added to a final concentration of 20 mg/ml five times a week.

Preparation and incubation technique. Under light ether anaesthesia a polyethylene cannula (PE-50) was inserted into the carotid artery. The tubing was brought under the skin through a small incision in the back of the animal and fixed to a muscle. The animal was then placed in a cage, and a continuous flow of the solution to be administered was maintained through the polyethylene tubing by means of an infusion pump. During the infusion the animal could move freely and had free access to drinking water. Glucose solution (30 %, w/v) was infused at a rate of 3·75 ml/h in the first 2–3 h and then at 2·0 ml/h for 9–10 h. During the preparation a priming dose of glucose infusion solution (about 1·0 ml/100 g body wt.) was given enabling a high blood glucose level (15–30 mM) to be reached right at the start of infusion.

At the end of the glucose infusion the animal was laparotomized under ether anaesthesia, a segment of small intestine between 45 and 60 cm distal of the pylorus localized and flushed with a 'basic medium' (see below). The method of isolating and incubating the mucosa has been described before (Lauterbach, 1977). Briefly, small pieces of intestine were opened lengthwise, spread out and the mucosa scraped off by means of a sharp razor blade held at an angle of approximately 60 deg and quickly drawn over the tissue towards the preparator. The isolated mucosa was floated in oxygenated basic medium at 37 °C, captured on a little piece of nylon mesh and placed between two fenestrated polyvinyl chloride sheets where it occluded a window of 5 mm diameter, thus forming a separating membrane between two flux chambers. Both chambers were filled and emptied through drill holes in the chamber walls which also served as an inlet for moistened oxygen during the incubation. 0·2 ml of the respective incubation solutions were added per half-chamber when glucose and 3-*O*-methyl-D-glucose were used. When the fluxes of β-methyl-D-glucoside were investigated, in some cases the chambers were filled with 0·5 ml incubation solution, and 0·05 ml was taken out for analysis after 5, 10, 20 and 45 min incubation; however, the calculations in these cases were also made and related to the usual volume of 0·2 ml. Labelled sugars and polyethylene glycol were administered either to the luminal or the blood side of the isolated mucosa. Incubation was performed in a water-bath at 37 °C.

Incubation medium. The 'basic medium' had the following composition (mM): NaCl, 106; KCl, 7·7; $CaCl_2$, 3·3; $MgSO_4$, 1·1; Na phosphate buffer (pH 7·4), 1·0; Tris(hydroxymethyl)aminomethane buffer (pH 7·4), 32·4. The incubation medium was prepared by adding 1·0 ml 0·308 M-mannitol to 10 ml of the basic medium. Sugars were administered in a final concentration of 2·8 mM; in these solutions an isosmotic equivalent of mannitol was omitted. [^{3}H]polyethylene glycol was added to the sugar-containing incubation solutions for estimation of the extracellular space and extracellular shunt pathways.

Analytical methods. At the end of the experiments, the incubation solutions were withdrawn and the mucosa punched out by means of a little machine as described elsewhere (Lauterbach, 1977). The punched-out mucosa was weighed on a microbalance. For estimation of the substrate concentration of the tissue, 0·250 ml trichloroacetic acid (0·3 M) was added to the mucosa. The samples were homogenized, then soaked for 2 h and centrifuged. Of each sample of the incubation solution, 0·05 ml was mixed with 0·1 ml trichloroacetic acid (0·5 M) and centrifuged. An appropriate volume of the supernatants was used for determination of the substrate. Glucose was determined enzymatically using glucose oxidase (Boehringer Mannheim GmbH). ^{3}H- and ^{14}C-labelled substances were counted by means of a liquid scintillation spectrometer (model Packard Tricarb 3380) using

9 ml of Bray (1960) solution. Uniform quenching of all samples was checked by means of an external standard. Gross counts were corrected for measurements of ^{14}C in the ^{3}H channel and vice versa.

Calculations. Extracellular and intracellular space, intracellular amount and concentration, permeation of substrate and flux coefficients were calculated as described earlier (Lauterbach, 1977). Mucosal contents and permeation of the sugars are given in relative values. The amount of sugar in the mucosa is expressed in 'μl', which means the amount of sugar contained in 1 μl incubation solution at the beginning of the experiment. Intracellular concentrations of the sugars were calculated as intracellular amounts divided by the intracellular space and expressed as a percentage of the concentration administered. Permeation of sugar and polyethylene glycol are expressed as their concentrations in the counter-compartment at the end of the experiment as a percentage of the concentration originally administered.

For the calculation of flux coefficients, the experimental set-up was regarded as a three-compartment system consisting of the luminal solution, the intracellular water space and the blood-side solution. Though this view is clearly a simplification, these calculations should yield comparable parameters, though their absolute values have to be considered critically. Including the results on paracellular flux determinations and observations on trapping phenomena at the luminal membrane described later, the following definitions and assumptions have been made. (1) The steady-state lumen-to-blood fluxes can be derived from the differences of the permeation rates measured at 45 and 10 min. (2) The transcellular blood-to-lumen flux of 3-*O*-methyl-D-glucose is zero according to the ordinate intercept of the regression line. (3) The transcellular blood-to-lumen flux of glucose is zero because no glucose was detected in the intracellular compartment after blood-side administration. (4) The blood-to-lumen flux of β-methyl-D-glucoside can be obtained from the ordinate intercept of the regression line in the presence of phlorhizin. (5) Steady-state concentrations in cell water are represented by the tissue contents determined at 45 min. Since no significant differences have been found between the mucosal contents in control and hyperglycaemic animals, the averages of both groups were used. (After luminal administration of β-methyl-D-glucoside there was no significant difference between the *amounts* of sugar taken up by the mucosal tissue.) In the case of blood-side administration of sugars, the mucosal concentrations in the presence of phlorhizin on the luminal side were used to eliminate the trapping errors.

According to the method of calculation all flux coefficients are obtained as apparent permeabilities and expressed in cm/s. For calculations a pdp 8/e- computer (Digital Equipment Inc., Maynard, MA), with programs written in FOCAL was used. Tissue contents and permeation rates are given as means $\pm$ S.E. of mean. The significance of differences was tested by Student's t test.

RESULTS

Time course of sugar uptake and permeation

3-O-Methyl-D-glucose. The uptake and transepithelial permeation of 3-*O*-methyl-D-glucose (3-*O*-MG) were determined under four experimental conditions, namely administration to the luminal and the blood sides of mucosae isolated from normal and hyperglycaemic animals (Fig. 1). After luminal administration in the controls a linear increase in absorption was seen beyond a lag time of 5 min. The amount absorbed was relatively low, reaching about 2 % in the blood-side compartment after 45 min. Tissue concentration rose rapidly during the initial 5 min and increased slowly thereafter without reaching a plateau in 45 min. The cellular content of 3-*O*-MG determined at 45 min was less than 16 %. In hyperglycaemic animals the absorption of 3-*O*-MG was significantly increased and amounted to 3 % at 45 min. However, no significant changes in cellular content of 3-*O*-MG were found between normal and hyperglycaemic animals.

With blood-side administration the blood-to-lumen flux was likewise linear after 5 min. The permeation rate measured at 45 min was 1 % in control and 1·5 % in hyperglycaemic animals. Interpretation of this difference, however, has to take into

account corrections for paracellular shunt fluxes, as will be shown below. Tissue uptake of 3-*O*-MG was higher than with luminal administration. There was no significant difference between control and hyperglycaemic animals in the tissue content of 3-*O*-MG, except a small one in the values determined at 45 min.

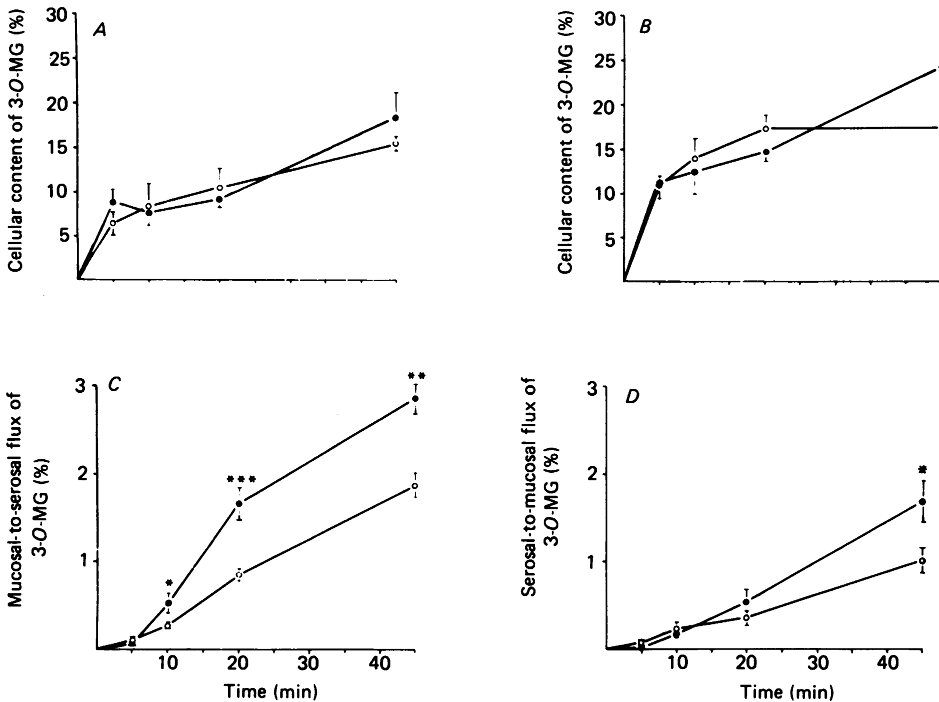

Fig. 1. Cellular content (*A* and *B*), lumen-to-blood (*C*) and blood-to-lumen flux (*D*) of 3-*O*-methyl-D-glucose (3-*O*-MG) in isolated mucosae from control (○—○) and hyperglycaemic guinea-pigs (●—●). Abscissa shows the incubation time. Initial concentration of 3-*O*-MG in the luminal (*A* and *C*) or blood-side compartments (*B* and *D*) was 2·8 mM. Data are expressed as a percentage of the initial concentration of 3-*O*-MG in the incubation solution. Significant differences between the values of control and hyperglycaemic animals: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

β-Methyl-D-glucoside. In the experiments with β-methyl-D-glucoside (β-MDG) the incubation time was prolonged to 90 min. From 10 to 90 min, a linear absorption of β-MDG was observed (Fig. 2). In normal animals the absorption rate of β-MDG was about twice as great as that of 3-*O*-MG. It is interesting to note that the mucosal content of β-MDG was extremely high, reaching 110% already after 5 min and increasing steadily up to 400%. In hyperglycaemic animals the absorption was significantly enhanced; however, the increase was less than that observed with 3-*O*-MG. The mucosal content of β-MDG measured between 10 and 45 min was elevated by hyperglycaemia whereas after 90 min incubation no significant change was found in this parameter.

The transepithelial permeation after blood-side administration of β-MDG was very low. The cellular content of β-MDG increased steadily, reaching about 60% (control)

and 90 % (hyperglycaemic) after 90 min incubation. It should be noted that these results have to be discussed in context with the phlorhizin experiments.

D-Glucose. Glucose was the sugar most rapidly absorbed. An absorption rate of 20 % was found after 45 min incubation in the controls (Fig. 3). In spite of the high absorption rate, no glucose accumulation was observed in the mucosa. The cellular

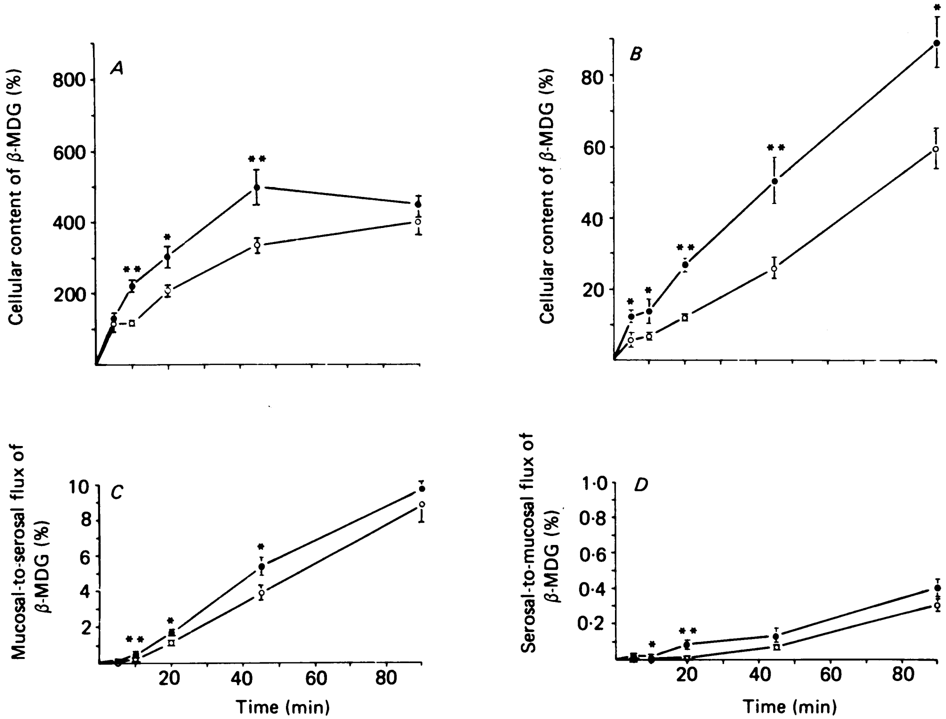

Fig. 2. Cellular content (*A* and *B*), lumen-to-blood (*C*) and blood-to-lumen flux (*D*) of *β*-methyl-D-glucoside (*β*-MDG) in isolated mucosae from control (○—○) and hyperglycaemic guinea-pigs (●—●). Abscissa shows the incubation time. Initial concentration of *β*-MDG in the luminal (*A* and *C*) or blood-side compartments (*B* and *D*) was 2·8 mM. Data are expressed as a percentage of the initial concentration of *β*-MDG in the incubation solution. (In some experiments with *β*-MDG the volume of incubation solution was 0·5 ml; however, the flux rates in these cases were also calculated and related to the volume of 0·2 ml, which was usually administered.) Significant differences between the values of control and hyperglycaemic animals: $*P < 0.05$, $**P < 0.01$.

content increased continuously to 74 % within 45 min. The absorption of glucose was significantly enhanced by hyperglycaemia. After 5 min it was 3 times, after 45 min twice as great as in the controls.

The cellular content of glucose was also increased in hyperglycaemic animals. In contrast to the values observed with 3-*O*-MG and *β*-MDG, the glucose content of the mucosa decreased during the incubation time and at 45 min no significant difference was found between the control and hyperglycaemic data.

As compared with the lumen-to-blood permeation, the blood-to-lumen flux of glucose was very low. The permeation rate in this direction was 0·14 % in controls,

but 1·8 % in hyperglycaemic animals. In controls no glucose could be detected in the mucosa after blood-side administration. In the hyperglycaemic animal the glucose content of the mucosa decreased from 90 to 40 % between 5 and 10 min; even after 10 min in this state no glucose was detectable.

The changes of glucose content and permeation observed in hyperglycaemic animals might be influenced by that amount of glucose which is taken up during the

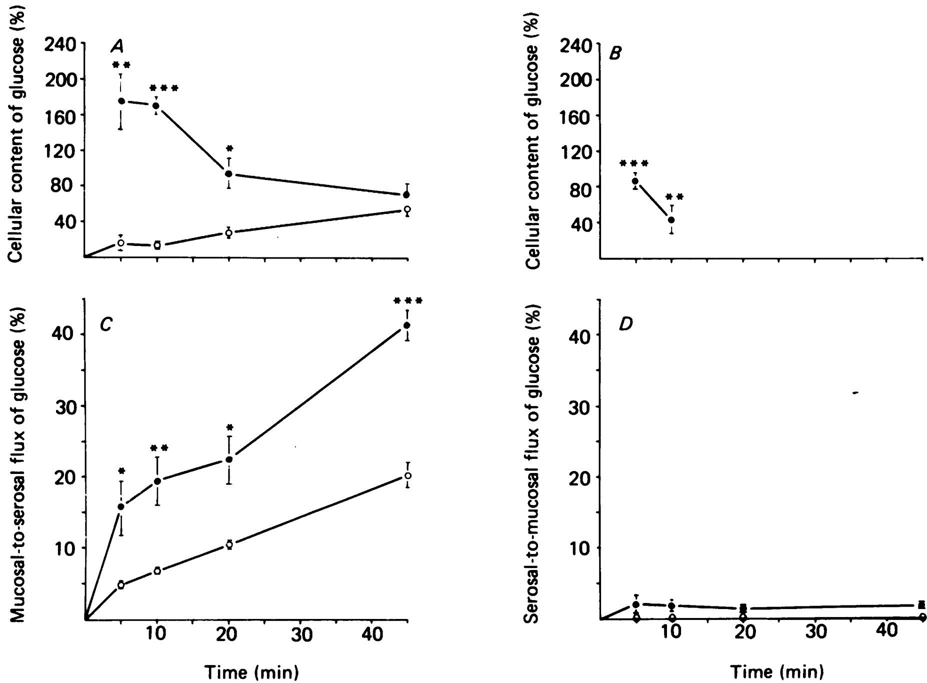

Fig. 3. Cellular content (*A* and *B*), lumen-to-blood (*C*) and blood-to-lumen flux (*D*) of D-glucose in control (○—○) and hyperglycaemic animals (●—●). Abscissa shows the incubation time. Initial concentration of glucose in the luminal (*A* and *C*) or blood-side compartment (*B* and *D*) was 2·8 mM. Data are expressed as a percentage of the initial concentration of glucose in the incubation solution. Significant differences between the values of control and hyperglycaemic animals: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

in vivo hyperglycaemic period and transferred to the *in vitro* system. Therefore, the possible magnitude of this effect has been estimated by a number of control determinations. Immediately after stripping off, the glucose content in a mucosal piece of approximately 10 mg (comparable in size to those used for incubations) was 46 nmol. (Glucose blood level in that case was 28·5 mM. Hence, tissue content was only one-sixth of, and intracellular concentration even less than, the extracellular concentration *in vivo*.) This amount was reduced by the short flotation procedure used for spreading the tissue to 24 nmol. By incubation in a glucose-free medium, sugar content was reduced further to 17 nmol within 5 min. After 10 min incubation between glucose-free media, no sugar was detectable in the tissue. Sugar was released almost exclusively to the blood side, where 24·2 nmol was found, in contrast to only

1·35 nmol on the luminal side. In experiments with the usual administration of 2·8 mM-glucose, these values would correspond to permeation rates of 4 and 0·2 %, respectively. Hence, the increase in glucose absorption from 20 to 40 % observed in hyperglycaemic animals cannot be attributed to a wash-out of glucose originating *in vivo*.

After 5 min incubation with 2·8 mM-glucose on the blood side, 19 nmol glucose per mucosa was found, almost the same value as with glucose-free incubation. Therefore, the initial difference in glucose content between normal and hyperglycaemic animals under blood-side sugar administration (see Fig. 3*B*) might be largely explained by glucose originating from the *in vivo* hyperglycaemic period. However, after luminal glucose administration, 38 nmol was detected in the mucosae of hyperglycaemic animals, which has to be compared with the 17 nmol still present after 5 min glucose-free incubation. Since sugar uptake of normal mucosae within the first 5 min was only 4–5 nmol, the origin of more than two-fifths of the amount in hyperglycaemic animals can neither be ascribed to pre-loaded sugar nor to the normal uptake rate. At present no ready explanation can be given for this initial 'overshoot' phenomenon of glucose uptake in hyperglycaemic mucosae.

Paracellular shunt fluxes: experiments with phlorhizin

For calculation of flux coefficients from steady-state experiments the transcellular fluxes in both directions have to be known. For this purpose the correlation between the permeation of sugar and that of polyethylene glycol (PEG) was calculated (note the permeation of PEG is limited to the paracellular pathways).

From previous experiments it was suspected that sugar crossing the paracellular pathways to the luminal side will be immediately taken up from the unstirred layer at the brush-border membrane and transported again into the cell ('trapping'). Therefore, after blood-side administration the paracellular sugar fluxes are underestimated, whereas the tissue content is over-estimated. In order to eliminate these errors, in a series of experiments phlorhizin (10^{-3} M) was used, which inhibited the uptake of sugars at the brush-border membrane. The use of phlorhizin also gave a chance to answer the question of whether the increased sugar transport in hyperglycaemic animals was phlorhizin sensitive or not.

3-O-Methyl-D-glucose. In the direction blood-to-lumen a close correlation can be seen between the permeation of 3-*O*-MG and that of PEG (Fig. 4). No significant difference exists between the regression lines of control and hyperglycaemic animals. However, as with the two other sugars the PEG flux measured in mucosae of hyperglycaemic animals was, on average, increased (Tables 1–3). Therefore, the hyperglycaemic values are shifted to the right on the common regression line. Thus, the increased permeation observed in hyperglycaemic animals after 45 min (Fig. 1) can be explained solely by an enhanced shunt permeability. The common regression line has a negative point of intersection on the ordinate. Obviously the blood-to-lumen flux is limited to the paracellular pathways.

The correlation between the lumen-to-blood permeation of 3-*O*-MG and that of PEG is considerably less; however, statistically it is still significant ($P < 0.01$). A positive point of intersection indicates in this case a transcellular transport. In hyperglycaemic animals no correlation was observed between the permeation of 3-*O*-MG and PEG.

Phlorhizin (10^{-3} M) administered on the luminal side inhibited the absorption of 3-*O*-MG completely both in control and hyperglycaemic animals. In this case the lumen-to-blood permeation of the sugar showed a close correlation with that of PEG. The values for lumen-to-blood and blood-to-lumen transepithelial fluxes of both control and hyperglycaemic animals are located around only one regression line

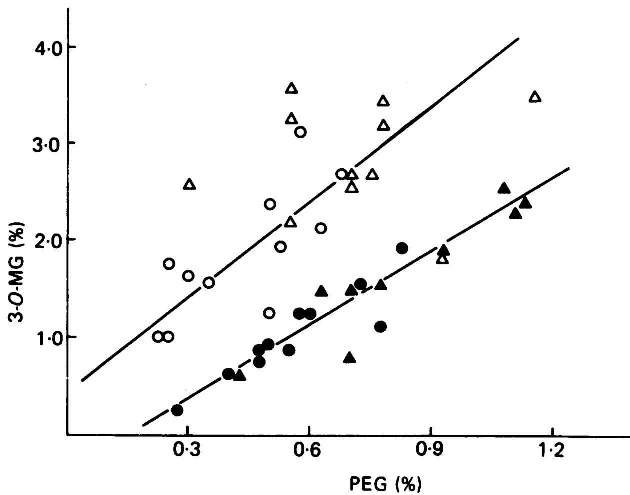

Fig. 4. Correlation between the permeation of polyethylene glycol (PEG) and 3-*O*-methyl-D-glucose (3-*O*-MG) in the direction lumen-to-blood (○, △) and blood-to-lumen (●, ▲). Values show the permeation rates determined after 45 min incubation of isolated mucosae from normal (○, ●) or hyperglycaemic guinea-pigs (△, ▲). 2·8 mM-3-*O*-MG added initially either to the luminal or the blood side. Equations for the regression lines are as follows. Normal, luminal sugar administration (○): $y = 3{\cdot}22x+0{\cdot}47$; $r = 0{\cdot}77$. Hyperglycaemic, luminal administration (△): no significant correlation. Normal, blood-side administration (●): $y = 2{\cdot}43x-0{\cdot}32$; $r = 0{\cdot}91$. Hyperglycaemic, blood-side administration (▲): $y = 2{\cdot}66x-0{\cdot}51$; $r = 0{\cdot}93$. Since no significant difference exists between the two latter regression lines, only the common line calculated from all experiments with blood-side administration ($y = 2{\cdot}53x-0{\cdot}39$; $r = 0{\cdot}94$) has been drawn.

(Fig. 5). The ordinate intercept of the regression line does not differ significantly from zero. The transepithelial sugar fluxes measured in the presence of phlorhizin are obviously limited to the paracellular pathways.

The blood-to-lumen flux of 3-*O*-MG was not influenced by phlorhizin administered on the luminal side. The regression lines calculated from the values of control and hyperglycaemic animals with and without phlorhizin treatment did not differ from each other (Fig. 6).

After luminal administration, the tissue content of 3-*O*-MG was reduced by phlorhizin in both control and hyperglycaemic animals by about 50 %, whereas after blood-side administration of 3-*O*-MG, phlorhizin had no effect in both groups (Table 1).

β-Methyl-D-glucoside. No correlation was found between the absorption of β-MDG and PEG permeation measured either in control or in hyperglycaemic animals in the absence of phlorhizin. However, in the presence of phlorhizin on the luminal side, a correlation existed between the permeation of β-MDG and PEG (Fig. 7). The values

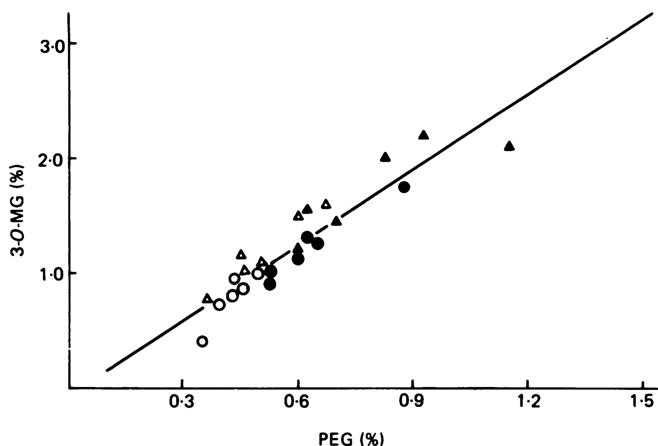

Fig. 5. Effect of phlorhizin on the absorption of 3-*O*-methyl-D-glucose (3-*O*-MG) in isolated mucosae from control and hyperglycaemic guinea-pigs. 2·8 mM-3-*O*-MG was added initially either to the luminal or to the blood side. Phlorhizin (10^{-3} M) was administered to the luminal side in all experiments. Correlation between the 45 min permeation rates of 3-*O*-MG and polyethylene glycol (PEG) in the direction lumen-to-blood and blood-to-lumen is shown. Symbols as in Fig. 4. Equation of the common regression line: $y = 2{\cdot}21x - 0{\cdot}08$; $r = 0{\cdot}94$.

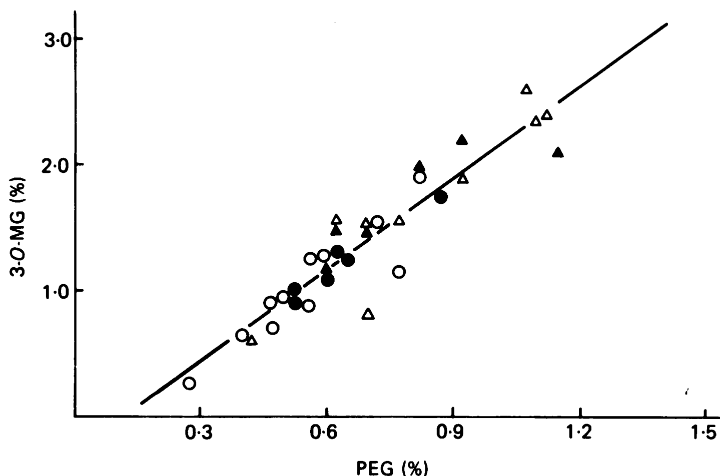

Fig. 6. Effect of phlorhizin on the blood-to-lumen flux of 3-*O*-methyl-D-glucose (3-*O*-MG) in isolated mucosae from control and hyperglycaemic guinea-pigs. Correlation between the 45 min permeation rates of 3-*O*-MG and polyethylene glycol (PEG) is shown. 2·8 mM-3-*O*-MG was added initially to the blood side. Phlorhizin was administered to the luminal side (10^{-3} M). Control animals without (○) and with phlorhizin (●); hyperglycaemic animals without (△) and with phlorhizin (▲). Equation of the common regression line: $y = 2{\cdot}43x - 0{\cdot}29$; $r = 0{\cdot}93$.

TABLE 1. 3-*O*-Methyl-D-glucose. Tissue content and permeation after luminal and blood-side administration

	Control animals		Hyperglycaemic animals	
	Without phlorhizin	With phlorhizin	Without phlorhizin	With phlorhizin
Luminal administration				
Wet weight (mg)	13·2±0·64	15·0±0·53	10·2±0·66	13·0±0·48
Extracellular space (%)	18·5±0·79	14·0±1·37	16·2±1·08	13·8±1·06
Intracellular amount (µl)	1·54±0·10	0·85±0·08	1·38±0·20	0·64±0·03
Intracellular concentration (%)	15·60±0·75	7·56±0·69	19·00±2·26	6·59±0·27
Sugar permeation (%)	1·86±0·17	0·79±0·08	2·87±0·17	1·17±0·13
PEG permeation (%)	0·43±0·02	0·43±0·02	0·70±0·07	0·51±0·04
n	11	6	11	6
Blood-side administration				
Wet weight (mg)	12·3±1·04	14·3±0·60	9·5±0·61	11·2±0·81
Extracellular space (%)	14·4±0·89	13·7±0·35	17·4±1·08	16·6±1·64
Intracellular amount (µl)	1·80±0·18	2·55±0·10	1·81±0·17	1·76±0·11
Intracellular concentration (%)	17·5±1·38	23·8±0·78	24·4±2·64	21·2±0·76
Sugar permeation (%)	1·04±0·14	1·22±0·12	1·69±0·23	1·24±0·17
PEG permeation (%)	0·56±0·05	0·63±0·05	0·83±0·08	0·80±0·09
n	11	6	9–11	6

The experiments were carried out on isolated mucosae of control and hyperglycaemic animals. Phlorhizin (10^{-3} M) was administered to the luminal side. Incubation time: 45 min. Values are expressed as means ± S.E. of mean; *n* = number of experiments.

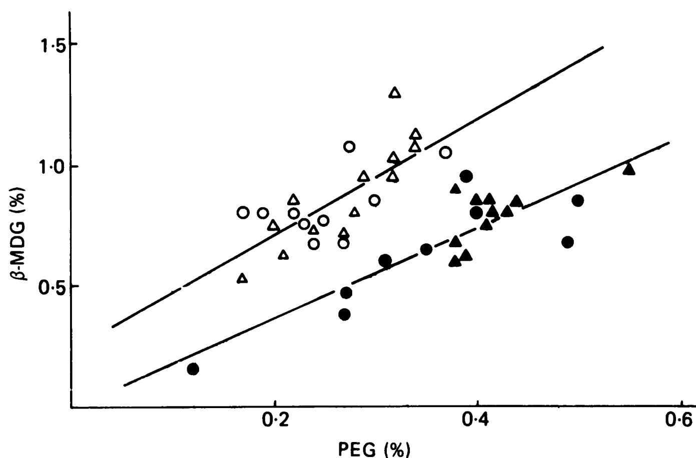

Fig. 7. Effect of phlorhizin on the absorption of β-methyl-D-glucoside (β-MDG) in isolated mucosae from control and hyperglycaemic guinea-pigs. 2·8 mM-β-MDG was added initially either to the luminal or the blood side. Phlorhizin (10^{-3} M) was administered to the luminal side in all experiments. Correlation between the 45 min permeation rates of β-MDG and polyethylene glycol (PEG) are shown. Symbols as in Fig. 4. Equations for the regression lines are as follows. Luminal sugar administration (○, △): $y = 2{\cdot}38x + 0{\cdot}23$; $r = 0{\cdot}73$. Blood-side administration (●, ▲): $y = 1{\cdot}85x - 0{\cdot}01$; $r = 0{\cdot}85$.

of both control and hyperglycaemic animals are scattered around one regression line. On the other hand, in contrast to the data on 3-*O*-MG, there are two separate regression lines for the lumen-to-blood and the blood-to-lumen fluxes of β-MDG.

The blood-to-lumen permeations of β-MDG and PEG measured without phlorhizin in control and hyperglycaemic animals are correlated and scatter around only one

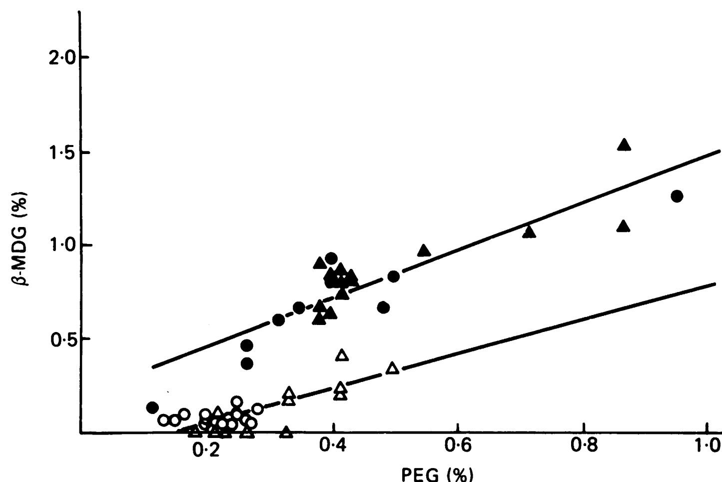

Fig. 8. Effect of phlorhizin on the blood-to-lumen flux of β-methyl-D-glucoside (β-MDG) in isolated mucosae from control and hyperglycaemic guinea-pigs. Correlations between 45 min permeation rates of β-MDG and polyethylene glycol (PEG) are shown. 2·8 mM-β-MDG was added initially to the blood side. Phlorhizin (10^{-3} M) was administered to the luminal side. Control animals without (○) and with phlorhizin (●); hyperglycaemic animals without (△) and with phlorhizin (▲). Equation for the common regression line of all experiments without phlorhizin (○, △): $y = 0·87x - 0·13$; $r = 0·76$. Equation for the common regression line of all experiments with phlorhizin (●, ▲): $y = 1·28x + 0·21$; $r = 0·89$.

regression line (Fig. 8). But, this regression line is conspicuously flat and has a negative point of intersection on the ordinate. The mean permeation rate of β-MDG is less than that of PEG (Table 2). In contrast to the effect observed with 3-*O*-MG, phlorhizin had a definite influence on the blood-to-lumen flux of β-MDG. The data obtained from control and hyperglycaemic animals in experiments with phlorhizin formed a new regression line, being steeper and shifted to higher sugar permeation values.

In the presence of phlorhizin the mucosal content of β-MDG was 9 % and 5 % in control and hyperglycaemic animals, respectively, of the values measured in its absence. These low values are still 4 times higher than those of 3-*O*-MG determined under phlorhizin inhibition. In accordance with the changes in permeation rates, phlorhizin used on the mucosal side decreased the tissue content of β-MDG when the sugar was administered on the blood side. Simultaneously, the difference between normal and hyperglycaemic animals seen in phlorhizin-free experiments was abolished (Table 2).

D-Glucose. No correlation was observed to exist between the permeation of glucose

and PEG in experiments with and without phlorhizin. The glucose absorption measured at 45 min was decreased by phlorhizin from 20 to 6 % in controls and from 41 to 14 % in hyperglycaemic animals (Table 3). The percentage inhibition was about the same (70 %) both in control and in hyperglycaemic animals.

The effects of luminal phlorhizin and of hyperglycaemia on blood-to-lumen permeation of glucose were noticeably more pronounced than those seen with 3-*O*-MG

TABLE 2. β-Methyl-D-glucoside. Tissue content and permeation after luminal and blood-side administration

	Control animals		Hyperglycaemic animals	
	Without phlorhizin	With phlorhizin	Without phlorhizin	With phlorhizin
Luminal administration				
Wet weight (mg)	16·9 ± 0·81	14·0 ± 1·08	13·4 ± 0·91	14·7 ± 0·37
Extracellular space (%)	18·5 ± 1·62	22·0 ± 0·62	16·1 ± 2·58	18·9 ± 1·72
Intracellular amount (μl)	43·3 ± 2·70	3·32 ± 0·43	48·5 ± 3·14	3·17 ± 0·35
Intracellular concentration (%)	337 ± 21·5	30·9 ± 3·02	500 ± 50·7	28·7 ± 3·10
Sugar permeation (%)	3·97 ± 0·39	0·82 ± 0·04	5·34 ± 0·59	0·88 ± 0·06
PEG permeation (%)	0·18 ± 0·02	0·29 ± 0·05	0·22 ± 0·02	0·27 ± 0·02
n	9 (20)	6 (11)	9 (15)	6 (13)
Blood-side administration				
Wet weight (mg)	16·3 ± 1·12	15·4 ± 1·16	14·2 ± 0·63	14·1 ± 0·38
Extracellular space (%)	14·1 ± 0·73	16·3 ± 1·27	14·4 ± 0·37	19·9 ± 0·67
Intracellular amount (μl)	3·10 ± 0·35	1·91 ± 0·45	5·34 ± 0·65	2·31 ± 0·11
Intracellular concentration (%)	25·9 ± 2·90	16·0 ± 2·66	50·6 ± 6·50	22·0 ± 1·13
Sugar permeation (%)	0·07 ± 0·01	0·61 ± 0·08	0·14 ± 0·04	0·88 ± 0·06
PEG permeation (%)	0·20 ± 0·01	0·34 ± 0·04	0·32 ± 0·03	0·50 ± 0·05
n	9 (16)	5 (9)	9 (12)	6 (14)

The experiments were carried out on isolated mucosae of control and hyperglycaemic animals. Phlorhizin (10^{-3} M) was administered to the luminal side. Incubation time: 45 min. Values are expressed as means ± S.E. of mean; *n* = number of experiments (*n* for permeation rates in parentheses).

and β-MDG. After blood-side administration, only 0·14 % glucose but 0·84 % PEG was found on the luminal side in control animals after 45 min. When phlorhizin was present on the luminal side, the blood-to-lumen flux of glucose was enhanced to 2·8 %.

Though the low glucose permeation in control animals in the phlorhizin-free state indicated a marked trapping of glucose reaching the brush border by the paracellular route, in hyperglycaemic mucosae a glucose permeation of 1·8 % was observed. This value was increased further to 4·8 % by luminal phlorhizin. However, glucose introduced into the luminal compartment from *in vivo* sources has, as just mentioned, been estimated to correspond to a permeation rate of only 0·2 %.

No cellular glucose was detectable in the mucosae of control and hyperglycaemic animals treated with phlorhizin irrespective of the side of sugar administration (Table 3).

TABLE 3. D-Glucose. Tissue content and permeation after luminal and blood-side administration

	Control animals		Hyperglycaemic animals	
	Without phlorhizin	With phlorhizin	Without phlorhizin	With phlorhizin
Luminal administration				
Wet weight (mg)	14·6 ± 0·66	9·9 ± 1·66	10·6 ± 0·36	9·4 ± 0·69
Extracellular space (%)	15·9 ± 1·25	21·0 ± 4·01	17·4 ± 1·56	19·0 ± 1·89
Intracellular amount (μl)	6·43 ± 0·91	0	5·83 ± 0·94	0
Intracellular concentration (%)	57·4 ± 11·3	0	73·1 ± 11·9	0
Sugar permeation (%)	20·2 ± 2·30	6·25 ± 0·80	41·5 ± 2·10	14·1 ± 1·12
PEG permeation (%)	0·32 ± 0·07	0·52 ± 0·08	0·70 ± 0·04	0·87 ± 0·12
n	3–8	5	13	6
Blood-side administration				
Wet weight (mg)	12·1 ± 0·96	10·5 ± 0·99	10·1 ± 0·52	10·4 ± 1·15
Extracellular space (%)	16·8 ± 1·37	17·9 ± 0·40	17·5 ± 0·92	19·0 ± 1·57
Intracellular amount (μl)	0	0	0	0
Intracellular concentration (%)	0	0	0	0
Sugar permeation (%)	0·14 ± 0·04	2·84 ± 0·60	1·80 ± 0·53	4·82 ± 0·27
PEG permeation (%)	0·84 ± 0·11	0·95 ± 0·11	0·81 ± 0·07	0·89 ± 0·07
n	12	5	14	6

The experiments were carried out on isolated mucosae of control and hyperglycaemic animals. Phlorhizin (10^{-3} M) was administered to the luminal side. Incubation time: 45 min. Values are expressed as means ± S.E. of mean; n = number of experiments. 0 = no glucose was detected.

TABLE 4. Effect of phloretin and cytochalasin B on the cellular content and lumen-to-blood flux of β-methyl-D-glucoside

	Intracellular concentration (%)	Permeation (%)
Control animals		
Control	399 ± 35·7	8·9 ± 1·09
+ 10^{-3} M-phloretin	435 ± 45·9	9·3 ± 1·11
+ 0·2 × 10^{-4} M-cytochalasin B	374 ± 31·0	11·3 ± 1·63
Hyperglycaemic animals		
Control	450 ± 21·9	9·6 ± 0·57
+ 10^{-3} M-phloretin	513 ± 11·1	12·8 ± 0·32
+ 0·2 × 10^{-4} M-cytochalasin B	342 ± 83·0	14·7 ± 0·54

Initial concentration of β-MDG in the mucosal compartment was 2·8 mM. Incubation time: 90 min. Phloretin and cytochalasin B were added to the incubation solution on the blood side. Values shown are means ± S.E. of mean of 4–11 experiments.

Effect of phloretin and cytochalasin B on sugar absorption in control and hyperglycaemic animals

It is known that phloretin and cytochalasin B inhibit the transport of sugars in basolateral membranes (Kimmich & Randles, 1975, 1976; Bihler, 1977; Murer & Hopfer, 1977; Randles & Kimmich, 1978). In our experiments phloretin and cytochalasin B had no inhibitory effect on the lumen-to-blood permeation and tissue uptake of β-MDG, either in control or in hyperglycaemic animals (Table 4). Thus, as compared with the data obtained by Kimmich and co-workers in chicken enterocytes, the guinea-pig jejunum behaved differently. However, no effect of

phloretin was observed in preparations of vascularly perfused intestine of the frog (Boyd & Parsons, 1979) and the rat (Bronk & Ingham, 1979).

DISCUSSION

Paracellular sugar permeation

The contribution of the paracellular route in the transepithelial sugar permeation may be determined by the correlation of sugar permeation with the simultaneous permeation of an extracellular marker (PEG), provided that this contribution is of quantitative importance. In this study, this has been the case for the permeation of 3-*O*-MG and β-MDG in the direction blood-to-lumen and for the permeation of 3-*O*-MG in the absorptive direction in normal animals. If the correlation between sugar and PEG permeation is obscured by the variation of high absorption rates, it may be unmasked by inhibiting the absorption with phlorhizin. This has been observed for the absorptive flux of 3-*O*-MG in hyperglycaemic and with β-MDG in both normal and hyperglycaemic mucosae.

10^{-3} M-phlorhizin inhibited the absorption of 3-*O*-MG (the poorest substrate for the luminal entrance system) completely, resulting in one common regression line for the permeation in the direction blood-to-lumen and vice versa. With β-MDG, two separate regression lines for the permeation lumen-to-blood and blood-to-lumen indicated an incomplete inhibition. The same conclusion has to be drawn for glucose, since even with phlorhizin no correlation between glucose and PEG permeation could be observed. Sugar absorption stimulated by hyperglycaemia is phlorhizin sensitive. Absorption values of 3-*O*-MG and β-MDG are suppressed by the inhibitor to the same regression line as are the controls, and the percentage inhibition of glucose absorption in normal and hyperglycaemic mucosae is identical.

The role of paracellular sugar permeation is very similar to that observed with substrates actively secreted by the intestine. The absorptive fluxes of cardiac glycosides like digoxin, organic acids like β-naphthol orange and others are strictly correlated with the simultaneous fluxes of extracellular markers. The secretory fluxes, however, display this correlation only in a state of secretory activity reduced by low temperatures or anaerobiosis (Lauterbach, 1979, 1981) or low affinity of the substrate to the transport system (Sund & Lauterbach, 1978).

Trapping of paracellular sugars at the brush border

Two observations indicate that sugars with a high affinity for the luminal entrance system which are administered to the blood side and reach the brush border via paracellular shunt pathways are recaptured before they can pass into the bulk solution. First, the slope of the regression line calculated from the experiments with 3-*O*-MG was about 2·5. According to the differences in molecular size it is reasonable to assume that the permeation of the monosaccharides through the paracellular pathways should have been more than twice as rapid as that of PEG. On the other hand, both the slopes of the regression line and a comparison of the mean permeation rates of β-MDG and PEG (Table 2) indicate that in the absence of phlorhizin, β-MDG appeared on the luminal side more slowly than PEG. Only in the presence of phlorhizin are the transepithelial blood-to-lumen flux of β-MDG and its relation to

the simultaneous permeation of PEG comparable to those of 3-*O*-MG. The blood-to-lumen permeation of glucose in normal animals is similarly increased by luminal phlorhizin from 0·14 to 2·8 %, raising the ratio of glucose/PEG permeation from 0·16 to 2·7.

Secondly, after blood-side administration of β-MDG its intracellular concentration is reduced by luminal phlorhizin both in normal and hyperglycaemic mucosae, suggesting that part of the tissue sugar in fact has been taken up across the brush border. The higher sugar uptake in hyperglycaemic mucosae where the permeation of PEG was increased supports this conclusion. Stimulation of glucose exsorption by luminal phlorhizin has been also observed *in vivo* (Axon & Creamer, 1975). Gomme (1981) discussed the protective role of glucose recycling in the unstirred layer surrounding marine polychaete worms.

TABLE 5. Flux coefficients for 3-*O*-methyl-D-glucose, β-methyl-D-glucoside and D-glucose. All values are expressed in 10^{-5} cm/s. For further details and explanations see text

	Control animals				Hyperglycaemic animals			
	Luminal		Basolateral		Luminal		Basolateral	
	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux
3-*O*-Methyl-D-glucose	0·43	0	0·57	1·60	0·74	0	0·97	2·80
β-Methyl-D-glucoside	9·70	0·85	0·44	0·64	11·0	0·85	0·50	0·84
D-Glucose	6·05	0	0	5·96	10·0	0	0	9·86

Hence, the trapping of high-affinity sugars at the luminal membrane in spite of continuously bubbling small chambers of only 4 × 6 mm^2 cross-sectional area seems obvious and necessitates the corrections listed in the section on calculations.

Comparison of flux coefficients of 3-O-MG, β-MDG and glucose in the normal state

The flux coefficients of the luminal and basolateral membranes calculated on the basis mentioned above are summarized in Table 5. Generally the ratio of influx/efflux coefficients deviates from 1 both at the luminal and the basolateral membranes. At the luminal membranes it is the influx coefficients, whereas at the basolateral membranes it is the efflux coefficients that are predominant. The results could be regarded as indicating not only the expected active uptake transport system in the luminal membrane but also an active extrusion system in the basolateral membrane. The magnitudes of the flux coefficients displayed remarkable differences between the three sugars.

3-*O*-MG had the least luminal (0·43 × 10^{-5} cm/s), but the highest basolateral influx coefficient (0·57 × 10^{-5} cm/s). According to the higher tissue content measured after blood-side administration of 3-*O*-MG, the influx coefficient was even greater at the basolateral than at the luminal membrane. For the basolateral membrane the efflux coefficient was almost 3 times as great (1·6 × 10^{-5} cm/s) as the influx coefficient.

The influx coefficient of β-MDG across the brush border is 22 times that of 3-*O*-MG and is even 1·6 times higher than that of D-glucose. However, the efflux coefficient across the basolateral membrane was only 0·4 times that of 3-*O*-MG and only 0·11

times that of D-glucose. Thus, β-MDG has a high affinity for the system for uptake and a low affinity for that for exit. Hence, the high accumulation of β-MDG in the tissue was not only due to the high uptake, but first of all to the low removal rate. From experiments with vascular perfusion in frog small intestine Boyd & Parsons (1979) concluded that α-methyl-D-glucoside had a higher uptake and lower removal permeability than 3-*O*-MG. Furthermore, they found a constant accumulation of α-methyl-D-glucoside but an accumulation of 3-*O*-MG only if the vascular perfusion was interrupted. Thus, in contrast to some other *in vitro* methods like the everted-sac technique, the isolated mucosa behaves similarly to a vascularly perfused preparation.

The tissue concentration of glucose was 6·5 times less than that of β-MDG, although the calculated luminal influx coefficient was only 1·6 times less. However, due to the high glucose absorption the basolateral efflux coefficient was almost 10 times as great (6×10^{-5} cm/s) as that of β-MDG. Rapid absorption of the physiological substrate glucose seems thus to be achieved not only by a high affinity to the entrance system in the brush border, but also to the exit system in the basolateral membranes.

After blood-side administration of glucose no sugar was found in the mucosa, therefore, the values of the basolateral influx and the luminal efflux coefficient were taken to be zero, which is probably an underestimated value. It is known that glucose is metabolized very rapidly in the epithelial cells, and the glucose concentration in these experiments was possibly too low to be detected by the method used.

Changes of flux coefficients in the hyperglycaemic state

The flux coefficients were increased by hyperglycaemia. As compared with the control values, all flux coefficients were equally enhanced when 3-*O*-MG (1·72-fold) or glucose (1·65-fold) was given. In hyperglycaemic animals receiving β-MDG, both influx coefficients increased 1·14-fold, and the basolateral efflux coefficient 1·32-fold, whereas the luminal efflux coefficient remained unchanged. This difference from the results with 3-*O*-MG and glucose has to be seen in the light of the difficulties in determining accurately the blood-to-lumen flux. Since the basolateral efflux coefficients for β-MDG were especially low, the result was influenced first of all by the blood-to-lumen flux and the luminal efflux coefficients calculated therefrom. If the blood-to-lumen flux were taken to be zero, then, in control animals, the luminal influx coefficient would decrease from $9{\cdot}7 \times 10^{-5}$ to $4{\cdot}2 \times 10^{-5}$ cm/s and the basolateral influx coefficient from $0{\cdot}44 \times 10^{-5}$ to $0{\cdot}19 \times 10^{-5}$ cm/s. In this case the increase of all coefficients would be 1·32-fold in hyperglycaemic animals as compared with controls.

Thus, artificial hyperglycaemia increases the activities of the luminal entrance *and* the basolateral exit system. Moreover, the relative changes in both systems are quite similar. Such a reaction seems to be biologically very suitable, because it results in an increase in transcellular substrate transport without enhancement of the intracellular substrate concentration. In this way loading of the osmotic and other regulatory mechanisms of the enterocytes can be avoided. Another interesting conclusion is that a relationship exists between the luminal and basolateral transport systems in the sense of a feed-back mechanism. A coupling of processes in both membranes has also been discussed by Boyd & Parsons (1978), because the removal

of 3-*O*-MG originating from a vascular loading could be stimulated by luminal administration of glucose. Esposito, Faelli, Tosco & Capraro (1981) emphasized that in everted sacs from hyperglycaemic rats glucose absorption was increased but tissue sugar content was unchanged. Tissue content of 3-*O*-MG was also unaltered in rat jejunal rings obtained after a 5 day glucose infusion (Olsen & Rosenberg, 1970). With these *in vitro* preparations however, sugar was concentrated in the tissue 2–4-fold. For the intestinal transport of sodium a coupling between the basal sodium pump and the passive luminal entry by the size of the sodium transport pool was supposed by Turnheim, Frizzell & Schultz (1977).

Compartmentation of intracellular sugar?

Though the conclusions drawn from a comparison of the flux coefficients are supported by other experiments with independent methods, the absolute values of the coefficients should be considered as only approximate data. It remains dubious whether the three-compartment model is suitable in every respect. Since the steady state of transepithelial fluxes was observed sooner than that of the mucosal content, the sugar in the mucosa might be distributed to more than one cellular compartment. Probably only one of these, rapidly filled, is directly involved in sugar transport. The different ratios between the β-MDG contents in control and hyperglycaemic mucosae at various incubation times could also be explained by differences in the dimensions of such compartments. The transepithelial glucose fluxes without a corresponding intracellular glucose content (permeation lumen-to-blood after addition of phlorhizin) or without a PEG permeation of expected magnitude (permeation blood-to-lumen in hyperglycaemic animals) might also be ascribed to the existence of a separate small transport compartment.

Various other findings also argue for an intracellular compartmentation. Efflux of 3-*O*-MG and β-MDG from pre-loaded mucosae proceeds in a multi-exponential fashion even after correcting for extracellular sugar (F. Lauterbach & E. Fischer, unpublished observations). Johnson & Bronk (1979) demonstrated a non-uniform distribution of labelled galactose in the enterocytes by electron microscope autoradiography. Differences in the metabolism of glucose (Hanson & Parsons, 1977) and amino acids (Gilles-Baillien, 1980) indicated that these substrates were distributed, obviously, to different compartments after luminal and blood-side administration. Finally, the transport pool for sodium represents only one part of cellular sodium (Turnheim, Frizzell & Schultz, 1978).

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