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TRANSPORT OF 3-*O*-METHYL D-GLUCOSE AND β-METHYL D-GLUCOSIDE BY RABBIT ILEUM

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SUMMARY

The intestinal transport of three actively transported sugars has been studied in order to determine mechanistic features that, (a) can be attributed to stereospecific affinity and (b) are common.

The apparent affinity constants at the brush-border indicate that sugars are selected in the order, β-methyl glucose > D-galactose > 3-*O*-methyl glucose, (the K_m values are 1.23, 5.0 and 18.1 mM, respectively.) At low substrate concentrations the K_i values for Na^+ activation of sugar entry across the brush-border are: 27, 25, and 140 mequiv. for β-methyl glucose, galactose and 3-*O*-methyl glucose, respectively. These kinetic parameters suggest that Na^+, water, sugar and membrane-binding groups are all factors which determine selective affinity.

In spite of these differences in operational affinity, all three sugars show a reciprocal change in brush-border entry and exit permeability as Ringer [Na] or [sugar] is increased. Estimates of the changes in convective velocity and in the diffusive velocity when the sugar concentration in the Ringer is raised reveal that with all three sugars, the fractional reduction in convective velocity is approximately equal to the (reduction of diffusive velocity)^2. This is consistent with the view that the sugars move via pores in the brush-border by convective diffusion.

Theophylline reduces the serosal border permeability to β-methyl glucose and to 3-*O*-methyl glucose relatively by the same extent and consequently, increases the intracellular accumulation of these sugars.

The permeability of the serosal border to β-methyl glucose entry is lower than permeability of the serosal border to β-methyl glucose exit, which suggests that β-methyl glucose may be convected out of the cell across the lateral serosal border.

INTRODUCTION

The Na^+-linked brush-border transport systems in small intestine and renal proximal tubules are closely similar. Three sugars which are transported via the Na^+-linked brush-border systems, but are not metabolized, have been extensively investigated in both tissues from a variety of species.

3-*O*-Methyl glucose first used by Csaky [1] is actively transported by the small intestine and concentrated to a maximum of 3–4-fold within the tissue fluid [2–4]. However, this sugar is not actively accumulated within the tissue fluid of rabbit renal cortex [5]. α and β-Methyl glucose have been shown to be actively transported by hamster intestinal epithelium without being metabolized [6], and Kleinzeller et al. [5] have demonstrated that α-methyl glucose is actively accumulated into rabbit renal cortex by a Na^+-linked process, to approximately twice the extent that galactose is accumulated [5]. Bihler [7] has compared the rate of uptake of several actively transported sugars into hamster intestine, he showed that the V for uptake is similar, whereas the affinities of the sugars are widely different, α-methyl glucose > galactose > 3-*O*-methyl glucose.

Although D-galactose is not metabolized appreciably by rabbit ileum [8], it is metabolized to a small extent by hamster jejunum [9] and Koopman and Schultz [10] have reported that galactose, unlike 3-*O*-methyl glucose or glucose causes an appreciable gain of Na^+ and loss of K^+ by rabbit ileum [10]. They suggested that a possible explanation for the observed effects of galactose is that it inhibits cell metabolism and thereby reduces Na^+ pump activity.

Since it is clear that the Na^+-linked transport mechanism accumulates α- and β-methyl glucose, galactose and 3-*O*-methyl glucose to different extents and since a method has been described whereby the unidirectional fluxes of sugars across the mucosal and serosal borders of sheets of intestine [8, 11] can be determined, it was considered worthwhile to characterize the differences in the handling of these sugars by the intestinal transport process in terms of their unidirectional fluxes and permeabilities. Furthermore, since some uncertainty exists as to whether galactose transport is "typical" [12], it was of interest to determine if the effects of variation in sugar and Na^+ concentration on the unidirectional fluxes and permeabilities of galactose observed previously [8, 11, 13, 14] are more generally applicable.

By analysis of transport data to obtain convective and diffusive velocities, an attempt has been made to establish that pores within the brush-border can have selective affinity for sugars.

MATERIALS AND METHODS

The method of estimating unidirectional fluxes across the mucosal and serosal borders of rabbit ileum stripped of its serosal muscle layers has been described previously [8, 11]. The Ringer solutions were the same as used previously. Na^+-free choline Ringer was made by substituting choline chloride for NaCl.

Materials. All chemicals used were Analar grade, except β-methyl D-glucose and 3-*O*-methyl glucose which were obtained from Koch-Light Ltd.

Radiochemicals. ^3H-labelled 3-*O*-methyl glucose and ^{14}C-labelled β-methyl glucose were purchased from New England Nuclear Ltd. ^{14}C-labelled 3-*O*-methyl glucose, (^3H, ^{14}C)-labelled D-galactose and [^3H]glucose were purchased from the Radiochemical Centre, Amersham. ^3H-labelled β-methyl glucose was synthesised according to the method described by Barnett et al. [15] for synthesis of β-propyl D-glucopyranoside except that methanol was used instead of propanol. [^3H]glucose was used as starting material. The product was purified by chromatography.

The formulae used to calculate the unidirectional fluxes and permeabilities

have been previously defined [8, 11, 13] where $P_{ij} = J_{ij}/C_i$. $J_{12} = J_{31} \cdot R + J_{13}$; $J_{21} = J_{31} \cdot (1+R)$; $J_{23} = J_{13}\ (1+1/R)$; $J_{32} = J_{31} + J_{13}/R$ where compartments 1, 2, 3 are the mucosal, tissue and serosal fluids respectively, and where

$$R = \frac{(\text{cpm})_2^T}{(\text{cpm})_2^C} \times \frac{(\text{cpm/ml})_3^C}{(\text{cpm/ml})_1^T}$$

$$V_i = \frac{J_i(1-e^{-Pe})}{C_1 - C_2 e^{-Pe}}$$

$$Pe = \ln \frac{P_{12}}{P_{21}}, \quad P_i = V_i/Pe$$

$$\bar{C} = \frac{C_1 - C_2 e^{-Pe}}{1-e^{-Pe}} - \frac{C_1 - C_2}{Pe}$$

V_i is the convective velocity of solute across the brush border, J_i is the net flux of sugar across the tissue. Pe is the Peclet number of the sugar across the brush border.

$$= V_i/P_i = \ln \frac{P_{12}}{P_{21}} \approx \ln \frac{P_{13}}{P_{31}}.$$

P_i = passive permeability of solute i across the brush-border which can be measured directly when $J_v = 0$. J_v is solvent velocity across brush border. $\bar{C}$ is the average brush border concentration of solute.

RESULTS

The effects of varying concentrations of Na^+ on transport and accumulation of 3-O-methyl glucose and β-methyl glucose

By substitution of choline chloride for NaCl in the Ringer's solution bathing both the mucosal and serosal surfaces of the rabbit ileum, it is possible to vary both the steady-state extracellular [Na_0^+] and intracellular [Na_i^+]. It was shown previously with D-galactose, that maximal asymmetry of sugar transport is obtained when the Ringer sugar concentration is low [11]. 1 mM β-methyl glucose and 2 mM 3-*O*-methyl glucose were chosen to give maximal transmural flux asymmetries of these sugars (see below) and were used in all the experiments in which the effects of variation of [Na^+] on sugar absorption and accumulation were determined.

Fig. 1a shows the effect of varying Ringer [Na^+] from 0 to 140 mequiv. on the bidirectional transmural fluxes of 3-*O*-methyl glucose and β-methyl glucose.

Since, at higher [Na^+] the asymmetry between mucosal-serosal flux J_{13} and serosal-mucosal flux, J_{31}, is large, the fluxes are plotted logarithmically. There is a 20-fold increase in mucosal-serosal flux, J_{13} of β-methyl glucose and a 4-fold increase in J_{13} of 3-*O*-methyl glucose on raising Ringer [Na^+] from 0 to 140 mequiv. Simultaneously, serosal-mucosal flux, J_{31} of β-methyl glucose decreases by 4-fold and of 3-*O*-methyl glucose by 2-fold. These results indicate that transepithelial fluxes of both sugars respond to increasing Ringer [Na^+] qualitatively in the same way as do galactose fluxes [11]. However, the relative changes in both J_{13} and J_{31} observed with β-methyl glucose are much greater than those observed with 3-*O*-methyl glucose. As well as

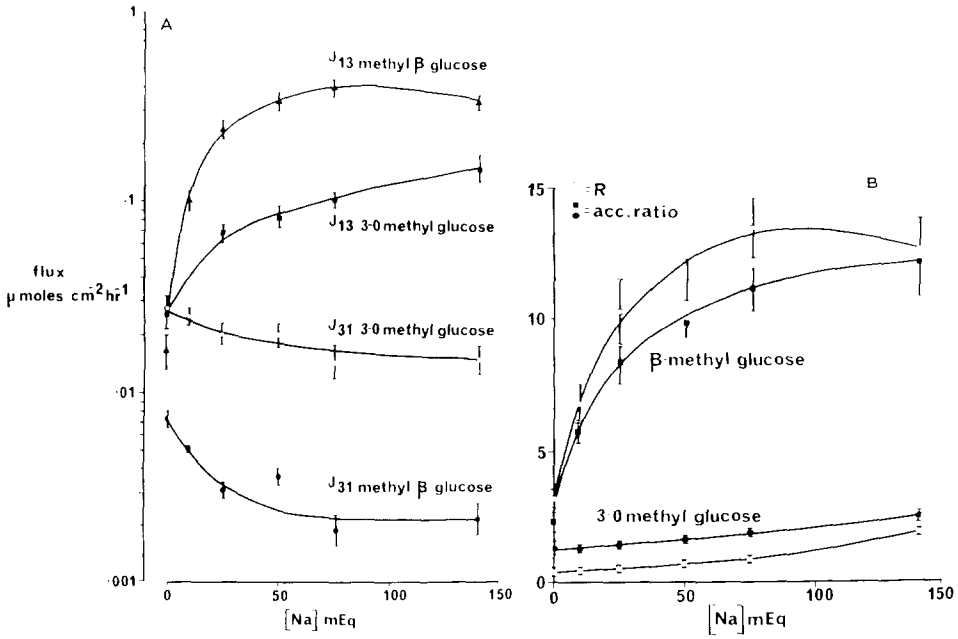

Fig. 1 (a) The effect of variation of Ringer $[Na^+]$ on the transmural fluxes J_{13} and J_{31} of β-methyl glucose (▲) and (●), respectively, and 3-*O*-methyl glucose (■) and (○), respectively. (b) The effect of variation of Ringer $[Na^+]$ on tissue accumulation ratio (closed symbols) and on the specific activity ratio, *R* (open symbols) of β-methyl glucose (squares) and of 3-*O*-methyl-D-glucose (circles). Ringer β-methyl glucose concentration is 1 mM. Ringer 3-*O*-methyl glucose concentration is 2 mM. Bars represent the S.E. of four experiments.

TABLE I

PARAMETERS OF 3-*O*-METHYL D-GLUCOSE AND β-METHYL D-GLUCOSE TRANSPORT ACROSS STRIPPED SHEETS OF RABBIT ILEUM AND ACROSS THE BRUSH-BORDER

The number of experiments is shown in parentheses.

Na^+ activation of flux.							
	1 mM β-methyl glucose				2 mM 3-*O*-methyl glucose		
	K_t (mequiv. Na^+)		V (μmol · cm^{-2} · h^{-1})			K_t (mequiv. Na^+)	V (μmol · cm^{-2} · h^{-1})
J_{13}	34.3 ± 5.0	(4)	0.57 ± 0.16	(4)	J_{13}	140 ± 7.5	0.33 ± 0.54
J_{12}	27.5 ± 6.8	(4)	0.55 ± 0.09	(4)	J_{12}	141 ± 22.0	0.33 ± 0.65
Ringer $[Na^+]$ = 140 mequiv.							
	β-Methyl glucose				3-*O*-Methyl glucose		
	K_m (mM)		V (μmol · cm^{-2} · h^{-1})			K_m (mM)	V (μmol · cm^{-2} · h^{-1})
J_{13}	1.36 ± 0.28	(4)	0.88 ± 0.59		J_{13}	13.6 ± 2.5 (6)	1.17 ± 0.32
J_{12}	1.23 ± 0.13	(4)	1.89 ± 0.33		J_{12}	18.17 ± 3.2 (6)	2.24 ± 0.32

differences in the maximal extent of the Na^+-dependent changes in transmural fluxes, the $[Na^+]$ giving half maximal changes in J_{13} for β-methyl glucose is significantly lower than for 3-*O*-methyl-glucose (see Table I) ($P < 0.01$).

Following the 90-min flux measurement period, the specific activity ratio R of ^3H- : ^{14}C-labelled sugar originating from the mucosal and serosal bathing solutions, respectively, and the total concentration of sugar within the tissue fluid (corrected for extracellular space) obtained from extracts of the tissues (see Materials and Methods) were measured. Fig. 1b shows both measured variables plotted against the Ringer $[Na^+]$. With both sugars the changes in specific activity ratio R and the accumulation ratio are closely correlated. The specific activity ratio of 3-*O*-methyl glucose rises from 0.3 in choline Ringer, to 2.0 in Na^+ Ringer; the accumulation ratio rises from approx. 1 to 3.0. Over the same range of Ringer $[Na^+]$, the change in both the specific activity ratio R and accumulation of β-methyl glucose is much more pronounced; R increases from 2.5 to 11.5 and accumulation from 3 to 14. (Repeated washing of the tissue in isotonic choline chloride to remove all traces of residual Na^+ prior to incubation reduces both the specific activity ratio and accumulation ratio of β-methyl glucose to unity. (Holman, G. D. and Naftalin, R. J., unpublished results). A difference between the effects of raising Ringer $[Na^+]$ on β-methyl glucose and 3-*O*-methyl glucose or galactose transport [11] can be noted in Fig. 1b, in that with the latter pair of sugars, the accumulation ratio generally exceeds the specific activity ratio, whereas with β-methyl glucose, the specific activity ratio is significantly greater than the accumulation ratio ($P < 0.01$) (see Discussion and Table II.)

TABLE II

	n	P_{23} (cm/h)	P_{32} (cm/h)	$\frac{P_{32}}{P_{23}}$	R/accumulation ratio
β-Methyl glucose	14	0.034 ± 0.0026	0.0308 ± 0.0029	0.89 ± 0.024*	1.14 ± 0.036*
3-*O*-Methyl glucose	16	0.047 ± 0.0038***	0.054 ± 0.004***	1.141 ± 0.033***	0.503 ± 0.062***
2 mM galactose	10	0.046 ± 0.0053	0.086 ± 0.0068***	1.87 ± 0.16***	0.342 ± 0.030**

Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ — calculated by Student's t-test (unpaired means solution) for significance of the difference between numbers in successive rows.

Effects of varying Ringer Na^+ on the unidirectional permeabilities of 3-O-methyl glucose and β-methyl glucose across the brush-border

A direct comparison of the effects of Na^+ on the mobility of 3-*O*-methyl glucose and β-methyl glucose within the brush-border can be made by comparing the entry and exit permeability of these sugars.

Unidirectional fluxes are calculated according to the formulae derived previously, utilizing the bidirectional transmural fluxes and the specific activity ratios. The unidirectional permeabilities $P_{ij} = J_{ij}/C_i$ require additionally the tissue concentrations of sugars.

Fig. 2a shows the effects of raising Ringer $[Na^+]$ from 0 to 140 mequiv. on the entry and exit permeabilities of 3-*O*-methyl glucose and β-methyl glucose across the

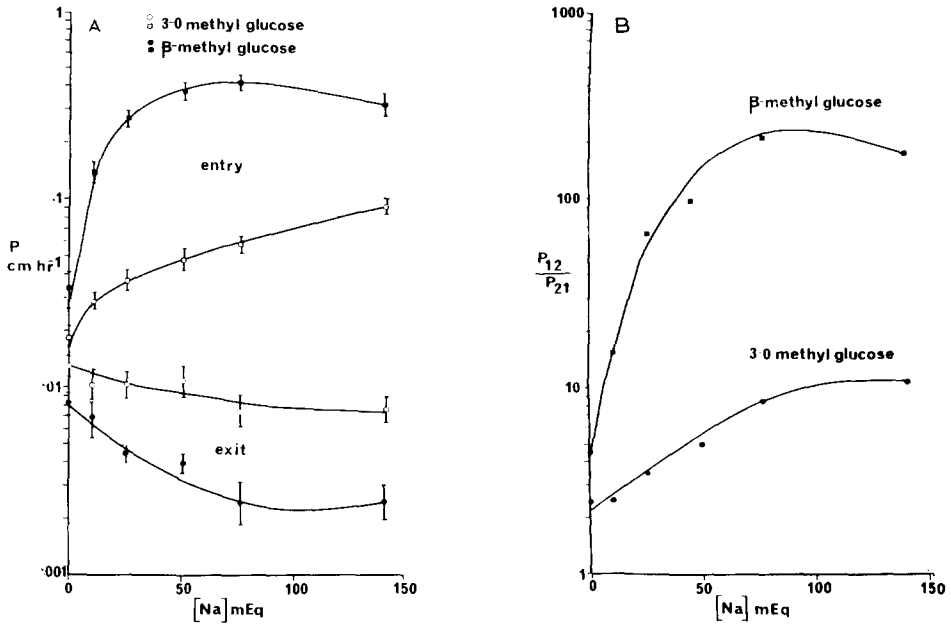

Fig. 2. (a) The effect of variation of Ringer $[Na^+]$ on the calculated brush-border entry permeabilities (squares) and exit permeabilities (circles) for β-methyl glucose (closed symbols) and for 3-*O*-methyl glucose (open symbols). (b) The effect of variation of Ringer Na^+ on the permeability ratio across the brush-border P_{12}/P_{21} for β-methyl glucose (■) and for 3-*O*-methyl glucose (●). Ringer β-methyl-glucose concentration is 1 mM, Ringer 3-*O*-methyl glucose concentration is 2 mM. Bars represent the S.E. of four experiments.

brush-border. As with the transmural fluxes, raising $[Na^+]$ increases the entry permeability of β-methyl glucose by 20-fold and of 3-*O*-methyl glucose by 6-fold. The K_m of Na^+ activation of β-methyl glucose entry is 27.5 mequiv., whereas the K_m for Na^+ activation of 3-*O*-methyl glucose entry is 140 mequiv. The K_m for Na^+ activation of 3-*O*-methyl glucose entry is similar to that found by Goldner et al. [3] for direct uptake of 5 mM 3-*O*-methyl glucose into rabbit ileum. Thus it can be inferred that transport of both D-galactose [11] and β-methyl glucose differ from 3-*O*-methyl glucose, in that at low concentrations of these sugars, the K_m for Na^+ activation of entry across the brush border is low.

It can also be seen that raising Ringer $[Na^+]$ (and consequently cell $[Na^+]$) reduces the exit permeability of β-methyl glucose by 5-fold and of 3-*O*-methyl glucose by 2-fold. By plotting the increment of exit resistance $= 1/P_{21}$ vs. Ringer $[Na^+]$ the K_m for Na^+-dependent activation of exit resistance to both sugars can be obtained; these do not differ significantly from the corresponding K_m values for entry.

These findings are qualitatively similar to those already reported for galactose [11], namely that on raising Ringer $[Na^+]$, there is a reciprocal increase and decrease in the entry and exit permeabilities, respectively, of the brush-border to actively transported sugars and are contrary to the predictions of the Na^+ gradient hypothesis [16, 17].

Fig. 2b shows the calculated ratios of brush-border entry: exit permeability of

β-methyl glucose and 3-*O*-methyl glucose, plotted as functions of Ringer [Na^+]. It can be seen that the ratio observed with β-methyl glucose exceeds that found with 3-*O*-methyl glucose by 25-fold. The Ringer [Na^+] giving half maximal activation of the permeability ratio is lower for β-methyl glucose: 40–50 mequiv., than for 3-*O*-methyl glucose, 90–100 mequiv.

The effects of variation in the concentration of 3-O-methyl glucose and β-methyl glucose in Ringer on the bidirectional fluxes of these sugars across rabbit ileum

Fig. 3(a and b) show the effects of variation in the concentration of β-methyl glucose and 3-*O*-methyl glucose, respectively, on the mucosal-serosal flux, J_{13}, and the serosal-mucosal flux, J_{31}, measured simultaneously.

The K_m for mucosal-serosal flux of β-methyl glucose is 1.36 mM and for 3-*O*-methyl glucose is 13.6 mM (see Table I). The V values for mucosal-serosal flux of both sugars are similar, as previously noted by Bihler [7]. (Over the range 0–10 mM serosal-mucosal flux increases linearly). The transmural permeability P_{31} of 3-*O*-methyl glucose is significantly greater than that of β-methyl glucose ($P < 0.001$). Above 10 mM, with both sugars, the relationship between serosal-mucosal sugar flux and sugar concentration becomes steeper. Similar findings have been previously described for galactose [8] and evidence was presented suggesting that this increase in serosal-mucosal sugar permeability at higher sugar concentrations results from dilation of the paracellular and submucosal spaces.

The effects of varying sugar concentration in Na^+ Ringer on the specific activity ratio R and accumulation of sugars within the tissue fluid

Both the accumulation ratio and specific activity ratio R of β-methyl glucose at low concentrations exceed those of 3-*O*-methyl glucose by approx. 4-fold. With both sugars it is found that on raising their concentration in the external Ringer, that there is a parallel fall in both the accumulation ratios and specific activity ratios (Fig. 3c). The concentrations of β-methyl glucose giving half maximal reductions in both the specific activity ratios and accumulation ratios are both approx. 4 mM, the corresponding values for 3-*O*-methyl glucose are 15 mM. At high concentrations of both sugars in the Ringer, both the accumulation ratio and the specific activity ratio R tend towards unity.

The effects of varying the concentrations of β-methyl glucose and of 3-O-methyl glucose on their entry and exit permeabilities across the brush-border

Fig. 4 shows the entry and exit permeabilities of both β-methyl glucose and 3-*O*-methyl glucose, as functions of the concentration of each of these sugars in the external Ringer. For clarity, both the permeabilities and the sugar concentrations are plotted logarithmically.

It can be seen that over the entire range of sugar concentrations the entry permeabilities of β-methyl glucose exceed those of 3-*O*-methyl glucose at the corresponding external sugar concentration ($P < 0.001$) and that the exit permeabilities of 3-*O*-methyl glucose over the same range of sugar concentrations exceed those of β-methyl glucose ($P < 0.001$). Thus here again there is a reciprocal relationship between the rise and fall of exit and entry permeability, indicating that the observed reciprocity between the entry and exit permeability of D-galactose observed previously,

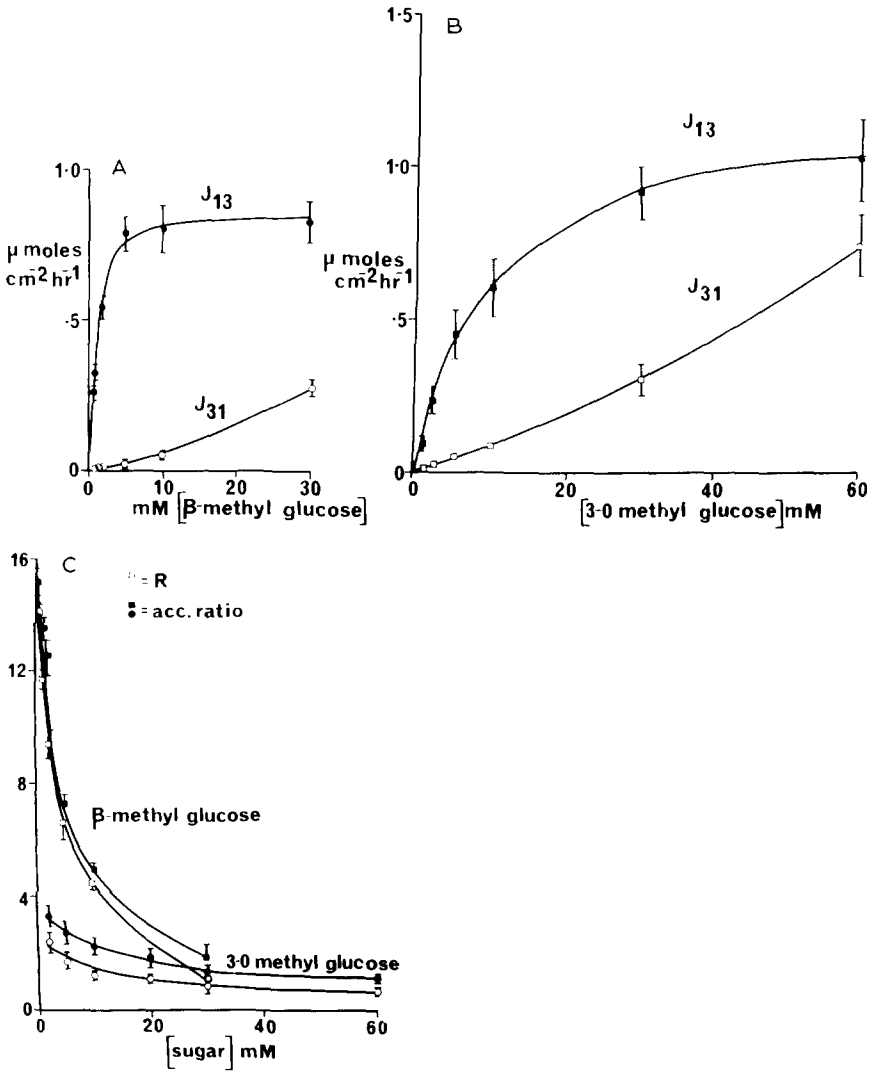

Fig. 3. The effect of variation of Ringer sugar concentration on the transmural fluxes J_{13} (closed symbols) and J_{31} (open symbols) for β-methyl glucose (a) and for 3-*O*-methyl glucose (b). Bars represent the S.E. of four experiments for β-methyl glucose and of six experiments for 3-*O*-methyl glucose. (c) The effect of variation of Ringer sugar concentration on the accumulation ratio (closed symbols) and the specific activity ratio R, (open symbols) for β-methyl glucose (squares) and for 3-*O*-methyl glucose (circles). Bars represent the S.E. of four experiments for β-methyl glucose and of six experiments for 3-*O*-methyl glucose.

[12] is not unique to galactose.

The K_m for the flux J_{12} for β-methyl glucose across the brush-border is 1.23 mM and for 3-*O*-methyl glucose 18.17 mM. This latter value is identical to that found by Goldner et al. [3] for direct uptake of 3-*O*-methyl glucose from Na^+ Ringer. However, the V obtained in this study is lower than that obtained by Goldner et al.

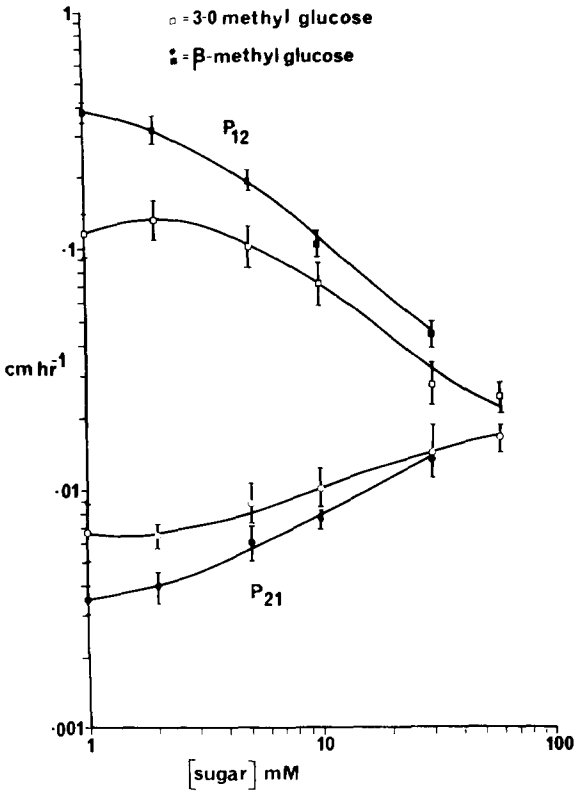

Fig. 4. The effect of variation of Ringer sugar concentration on the calculated brush-border entry permeabilities (P_{12}) and exit permeabilities (P_{21}) for 3-*O*-methyl glucose and *β*-methyl glucose. Bars represent the S.E. of four experiments for *β*-methyl glucose and of six experiments for 3-*O*-methyl glucose.

[3]. This difference may, in part, be due to the different techniques employed and in part, to the large between animal variation in V.

Comparison of the permeabilities of the serosal border to 3-O-methyl glucose, β-methyl glucose and galactose

Table II shows the calculated entry and exit permeabilities of 3-*O*-methyl glucose, *β* methyl glucose and galactose. Only a small reduction was noted in the entry and exit permeability of the serosal border to 3-*O*-methyl glucose on raising the sugar concentration from 1 to 60 mM in the bathing medium, no reduction in either the entry or exit permeability of *β*-methyl glucose was observed over the entire range of sugar concentrations tested. The calculated K_m for 3-*O*-methyl glucose across the serosal border is approx. 240 mM but this is probably an underestimate of sugar affinity for the membrane because of stagnant layer effects in the submucosal space [22, 23]. Since there are no significant concentration-dependent changes in serosal permeability to either 3-*O*-methyl glucose or *β*-methyl glucose, all the serosal entry and exit permeabilities measured in Na^+ Ringer for each sugar were averaged. The entry and exit permeabilities of galactose were obtained from experiments in which

galactose was present in Na^+ Ringer at 0.2 mM [13]. Both the entry and exit permeabilities of β-methyl glucose are significantly less than those of either 3-*O*-methyl glucose or galactose. There is no significant difference between the exit permeabilities of 3-*O*-methylglucose and galactose, however, the entry permeability of galactose exceeds that of 3-*O*-methyl glucose.

These results are consistent with previously obtained data [13] where it was shown that β-methyl glucose did not affect the entry of galactose across the serosal border and the K_i for 3-*O*-methyl glucose inhibition of galactose entry across the serosal border was found to be 80 mM. It can be seen that unlike either galactose or 3-*O*-methyl glucose the exit permeability of β-methyl glucose is significantly greater than the entry permeability (see Discussion). With 3-*O*-methyl glucose there is no difference between the entry and exit permeabilities and with galactose it has been previously shown that entry permeability significantly exceeds exit permeability [13].

The effects of theophylline on accumulation of β-methyl glucose and 3-O-methyl glucose

Previously [14] it has been shown that 5 mM theophylline increases D-galactose accumulation within the tissue fluid of rabbit ileum by up to 3-fold. This increase in sugar accumulation was shown to be due to a symmetrical reduction in entry and exit permeability of the serosal border to galactose. Since serosal permeability to galactose is slightly higher than to 3-*O*-methyl glucose and much higher than to β-methyl glucose, it is of interest to determine if the effect of theophylline on the serosal permeability and accumulation of 3-*O*-methyl glucose and β-methyl glucose is the same as for galactose.

Table III indicates that 5 mM theophylline causes an increase in both the specific activity ratio R and accumulation ratio of both 2 mM 3-*O*-methyl glucose and 1 mM β-methyl glucose. Additionally, theophylline reduces the bidirectional transepithelial fluxes of both sugars. It can be deduced that the major change in the calculated unidirectional permeabilities is the reduction by 30 % in both the serosal entry and exit permeabilities of β-methyl glucose and 3-*O*-methyl glucose.

TABLE III

THE EFFECT OF THEOPHYLLINE (5 mM) ON THE TRANSPORT OF 1 mM β-METHYL GLUCOSE AND OF 2 mM 3-*O*-METHYL GLUCOSE

The fluxes are expressed as $\mu mol \cdot cm^{-2} \cdot h^{-1}$ and the permeabilities as cm/h.

	n	J_{13}	J_{31}	J_{net}	Accumulation (mM)
		($\mu mol \cdot cm^{-2} \cdot h^{-1}$)			
1 mM β-methyl glucose	4	0.34 ± 0.048	0.0056 ± 0.0006	0.336 ± 0.048	11.35 ± 1.07
1 mM β-methyl glucose + 5 mM theophylline	4	0.21 ± 0.019*	0.0046 ± 0.0006	0.21 ± 0.019*	17.39 ± 0.69**
2 mM 3-*O*-methyl glucose	4	0.287 ± 0.015	0.011 ± 0.0012	0.275 ± 0.016	9.76 ± 0.43
2 mM 3-*O*-methyl glucose + 5 mM theophylline	4	0.207 ± 0.011**	0.005 ± 0.0013*	0.201 ± 0.011**	12.64 ± 0.51***

Significance levels were tested using Students' t-test (unpaired means solution): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

DISCUSSION

The results indicate that there are two reasons why β-methyl glucose is accumulated more than either galactose or 3-*O*-methyl glucose.

First, the maximal Na^+-linked permeability asymmetry of the brush-border found with β-methyl glucose is much greater than observed with either galactose or 3-*O*-methyl glucose (Fig. 2b) and secondly, the permeability of the serosal border towards β-methyl glucose is less than towards either galactose or 3-*O*-methyl glucose.

Since theophylline reduces the serosal permeability to all three sugars by the same fraction, it is clear that the lower serosal border permeability to β-methyl glucose results from stereospecific interaction between the sugar and the serosal membrane, rather than to any permeability change resulting from β-methyl glucose affecting cell metabolism.

As 3-*O*-methyl glucose is accumulated maximally only by 3–4-fold, the kinetic behaviour of this sugar is partially consistent with the Na^+ gradient hypothesis, i.e. the accumulation ratio of sugar is similar to the ratio of $[Na_0^+]/[Na_i^+]$ across the brush-border [16, 17]. However, the Na^+ gradient hypothesis cannot realistically be invoked to account for the 15–20-fold accumulation of β-methyl glucose or the 200–300-fold asymmetry of the entry: exit permeability of this sugar across the brush border (Fig. 2b). Nor can the Na^+ gradient hypothesis account for the previously observed fall in exit permeability to galactose [8, 13, 18] (now also found with β-methyl glucose and 3-*O*-methyl glucose) when Ringer $[Na^+]$ and the steady-state tissue Na^+ concentration are increased. Thus an alternative hypothesis is needed.

It was previously proposed that the asymmetry of sugar entry and exit permeability at the brush-border results from convective diffusion of sugars through aqueous channels in the brush-border. Sheetz and Chan [19] have shown that the hydraulic permeability of lecithin bilayers is critically dependent on the radius of curvature of the bilayers. At high curvatures the bilayer becomes highly permeant to water. Oschman et al. [20] have suggested by analogy, that since the radius of curvature

R	P_{12}	P_{21}	P_{23}	P_{32}
	(cm/h)			
15.13 ± 0.86	0.428 ± 0.047	0.008 ± 0.0004	0.032 ± 0.0048	0.028 ± 0.002
24.58 ± 2.33**	0.326 ± 0.012	0.007 ± 0.0013	0.0126 ± 0.0006**	0.013 ± 0.0008***
3.22 ± 0.12	0.162 ± 0.006	0.005 ± 0.0001	0.0388 ± 0.003	0.05 ± 0.0021
5.75 ± 0.24***	0.118 ± 0.006	0.003 ± 0.0007	0.019 ± 0.0015***	0.0207 ± 0.001***

at the bases and tips of the brush-border microvilli is very small, these regions are likely to be areas of high porosity.

Experimental evidence for this view comes from results presented in this and other papers, namely that reciprocal changes in sugar entry and exit permeability to the brush-border are seen (a) when the Na^+ pump is stimulated by raising intracellular $[Na^+]$ [11, 8], (b) when the Na^+ pump is inhibited by the action of ouabain [11, 21] or anaerobiasis [18]; (c) when the brush-border transport process is saturated by either high concentrations of a single sugar or competitive interactions with other sugars [13]. An explanation for the last-mentioned set of results is that saturation of entry flux occurs because the presence of sugar within the brush-border pores retards the flow both of sugar and solvent hence rectification of sugar exit caused by solvent drag will also be diminished when the brush-border sugar transport process is saturated.

The effect of variation of sugar concentrations on convective diffusion

Assuming that both sugar and solvent are transported via polar pores, as if in quasi-Poiseuille flow, then V_i, the solute convective velocity and P_i, the solute diffusive velocity, are related to the effective pore area, A, as follows:

$$V_i \propto A^2;\ P_i \propto A \quad [20, 24]. \tag{1}$$

This hypothesis predicts that the sugar concentration which reduces the diffusive velocity by 50 % should reduce the convective velocity by 75 %.

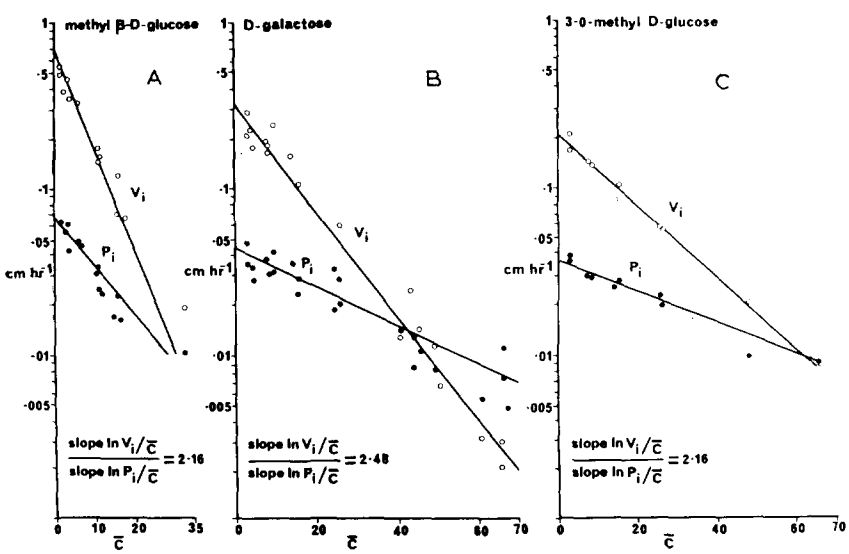

Fig. 5. The calculated convective velocities V_i and diffusive velocities P_i across the brush-border of rabbit ileum plotted as functions of the mean sugar concentration within the brush-border $\bar{C}$. V_i, P_i, $\bar{C}$ are obtained from the appropriate formulae as defined in Materials and Methods. The lines through the points are the least square linear regression lines through the points. The ratios of the slopes of the lines $\ln V_i/\bar{C}$ and $\ln P_i/\bar{C}$ for all three sugars; methyl glucose, galactose and 3-*O*-methyl glucose are all consistent with the hypothesis that $V_i \propto A^2$ and $P_i \propto A$. The data were obtained from experiments in which the brush-border [sugar] was varied by altering Ringer sugar concentration.

A primary purpose of this investigation is to test the hypothesis that sugar flow across the brush-border results from mass flow via aqueous channels which have selectivity for sugars.

3-*O*-Methyl glucose, *β*-methyl glucose and galactose have different affinities for the brush-border transport system, hence, if the relative change in convective velocity is approximately the same as the (relative change in diffusive velocity)^2 for each of these sugars on saturation of the brush border transport process, this is a fairly stringent test of the hypothesis. The convective and diffusive velocity components of brush-border sugar flux may be resolved according to the formulae defined previously [11], see Materials and Methods.

Figs. 5a–5c shows semilogarithmic plots of convective velocity V_i and diffusive velocity P_f of *β*-methyl glucose, galactose and 3-*O*-methyl glucose, respectively, versus the calculated brush-border concentrations of each set. The slopes of the log-linear plots of the decreases in convective velocities as brush-border sugar concentration is raised, are all approximately double the slopes of the corresponding falls in log diffusive velocities as predicted by Eqn. 1.

Thus, these findings are consistent with the hypothesis that actively transported sugars cross the brush-border by convective diffusion via aqueous channels. The brush-border pores must permit sugars with high affinity for the transport sites to pass more rapidly. This last point is illustrated in Fig 6 (a and b). The brush-border sugar concentrations giving half maximal convective and diffusive velocities of *β*-methyl glucose, galactose and 3-*O*-methyl glucose are approx. 5, 10 and 15 mM,

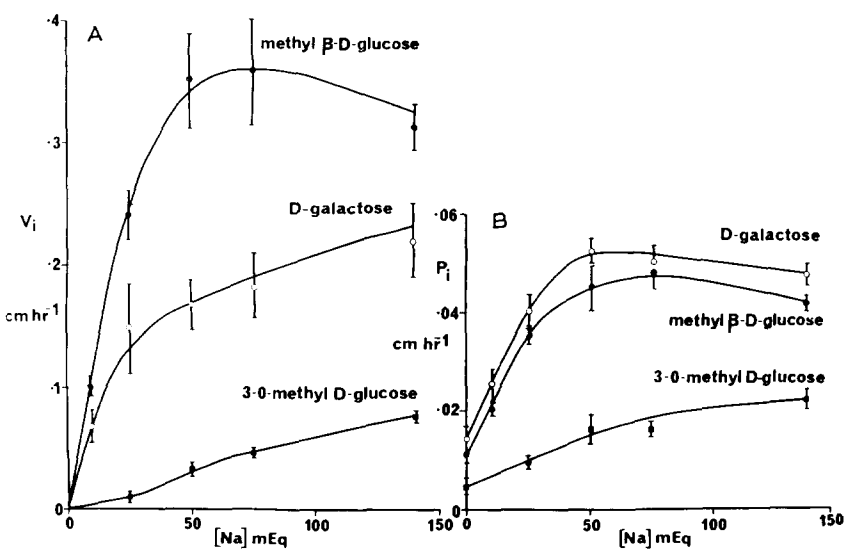

Fig. 6. (a) The effect of varying Ringer [Na^+] on the calculated convective velocities V_i of *β*-methyl glucose present in Ringer at 1 mM (four experiments), galactose at 2 mM (seven experiments) and 3-*O*-methyl glucose (four experiments). Vertical bars are the S.E. values of the data. The data for galactose were shown previously [14] and are inserted here for the purpose of comparison. (b) The effect of varying Ringer [Na^+] on the calculated diffusive velocities of *β*-methyl glucose and 3-*O*-methyl glucose. The data were obtained from the same experiments as were used to obtain those shown in (a).

respectively; the maximal convective velocities at infinite dilution (obtained by extrapolation) are approx. 0.7, 0.45 and 0.2 cm/h and the passive permeabilities are 0.07, 0.055 and 0.034 cm/h, respectively. Thus, there is a positive correlation between the operational sugar affinities for the brush-border transport "sites" and both convective and diffusive velocity components of sugar flux across the brush-border. A possible explanation for this correlation between the operational affinity of the sugar and its mobility within the pore, may be that the affinity is related to the distribution coefficient of the sugar between the external solution and the porous water.

The determinants of this distribution coefficient must be related to the ease with which the sugar interacts with its microenvironment.

The effect of variation of $[Na^+]$ *on convective diffusion of sugars*

Some insight into the role of Na^+ in brush-border sugar transport may be gained on examining the convective and diffusive velocities of all three sugars as functions of Ringer $[Na^+]$.

The sugars are all present at sufficiently low concentrations to ensure maximal asymmetry (Figs. 6a and 6b). On raising Ringer $[Na^+]$ from 0 to 140 mequiv., the convective velocity of all sugars increases from zero, β-methyl glucose responds most rapidly and to the greatest extent, closely followed by galactose, then 3-*O*-methyl glucose. The Ringer $[Na^+]$ giving half maximal velocity for both β-methyl glucose and galactose is approx. 25 mequiv. but for 3-*O*-methyl glucose, the $[Na^+]$ giving half maximal activation of convective velocity is 95 mequiv. Similarly, the Na^+ concentration giving half maximal increases in passive permeability of β-methyl glucose and galactose are also approx. 25 mequiv., whereas for 3-*O*-methyl glucose, the $[Na^+]$ giving half maximal increase in diffusive velocity is 100 mequiv. The passive permeability of the brush-border towards 3-*O*-methyl glucose is approximately half of that calculated for β-methyl glucose or galactose.

Previously [11] it was suggested that the low operational K_m for activation of galactose convective velocity was due to the activation of the Na^+ pump by cell $[Na^+]$. This simple hypothesis can no longer be sustained as it implies that the K_m for Na^+ activation of the convective velocity of all sugars should be similar. Since the K_m for Na^+-dependent activation of the convective velocity of 3-*O*-methyl glucose is approx. 3–4 times greater than for galactose or β-methyl glucose, it may be that the presence of sugars within the brush-border can alter the affinity of the "sites" for Na^+. Several kinetic models based on multivalent mobile carrier kinetics have already been propoposed which could adequately rationalize the part of the above data pertaining to influx [16], however, the conceptual basis of carrier kinetics, (i.e. sequential uptake and release of ligands at alternate sides of a membrane) is clearly inapplicable to a system in which there is evidence for simultaneous forward and backward flow with interaction between the opposing fluxes of both ligands (Simmons and Naftalin, in preparation) and solvent-solute coupling. The results are consistent with the view that the presence of Na^+ within the brush-border increases the mobility of sugars within the pores. At infinite dilution, the presence of sugar within the brush-border cannot affect solvent flow, hence since the convective velocity $V_i = (1-\sigma_i)J_v$, it follows that the convective velocity measured at infinite dilution is an index of the sugar reflexion coefficient. Thus the reflexion coefficient of 3-*O*-methyl glucose > galactose > β-methyl glucose. A possible explanation for the increase in convective velocity of

3-*O*-methyl glucose found on raising Ringer [Na^+] beyond the concentration at which maximal convective velocity of β-methyl glucose and galactose obtains is that Na^+ increases the effective pore area and thus reduces the reflexion coefficient of the sugars. Since the reflexion coefficient of 3-*O*-methyl glucose is largest, the mobility of this sugar will increase over a wider range of [Na^+].

Recent work with membrane vesicles obtained from brush-borders from small intestine [29] and renal cortex [30] ostensibly lends support to the Na^+ gradient hypothesis and certainly suggests, as we do here, that there is a stereospecific sugar transport mechanism within these membranes which is responsive to Na^+. However, the Na^+ gradient hypothesis provides a framework for linking unidirectional Na^+ and sugar fluxes which cannot be readily accommodated to the experimental findings from these systems without making additional assumptions, which are not normally required; e.g. Aronson and Sacktor [30] suggest that unidirectional glucose fluxes are strongly trans-inhibited by Na^+. An alternative explanation of these results is that sugar influx into vesicles is linked to net, rather than unidirectional Na^+ flux. Murer et al. [29] find that Na^+-dependent amino acid influx into brush-border vesicles is a function of the permeability of the major anion accompanying Na^+ and solute entry. This again suggests that the initial rate of solute entry into vesicles is dependent on net rather than unidirectional Na^+ flux. If this proves correct, then the observations with isolated membranes become wholly compatible with the observed Na^+-dependent acceleration of diffusive and convective sugar movements reported here.

Evidence for convective flow of β-methyl glucose across the serosal border

In previous papers it was demonstrated that the observed accumulation of D-galactose within the tissue fluid of rabbit ileum is consistent with the view that accumulation results from reflexion of sugar at the serosal border following convection through the brush-border. It was demonstrated that the high asymmetry of entry and exit permeability at the brush-border is quantitatively consistent with the observed level of accumulation when leaks at the mucosal and serosal borders are considered [13, 14]. Fig. 7a shows that accumulation of 3-*O*-methyl glucose is also consistent with this hypothesis. However, Fig. 7b indicates that the predicted accumulation ratio of β-methyl glucose is much higher than the observed accumulation, if it is assumed that accumulation results from total reflexion of sugar flow at the lateral-basal border. Since these assumptions are adequate for both 3-*O*-methyl glucose and galactose accumulation, the deviation in the case of β-methyl glucose might be explained by differences in the transport properties of this sugar across the serosal border, as this sugar's movement across the brush border is qualitatively similar to that of galactose and 3-*O*-methyl glucose. In contrast to the serosal transport of D-galactose where the ratio P_{32}/P_{23} is close to 2, indicating a weakly active transport uptake of galactose at this border [8, 13] : the results shown in Table III suggest that β-methyl glucose may be convected outwards across the lateral border of the epithelial cells, since the ratio P_{32}/P_{23} for β-methyl glucose is less than unity ($P < 0.05$). In vivo, it is likely that this serosal convection of sugars is much more pronounced, since it has been observed that the steady-state intracellular concentrations of 3-*O*-methyl glucose and glucose in rat small intestine are lower than in either the lumenal or capillary perfusion fluid [25, 26]. A possible explanation for the apparent difference between the in vitro behaviour of β-methyl glucose and galactose may be

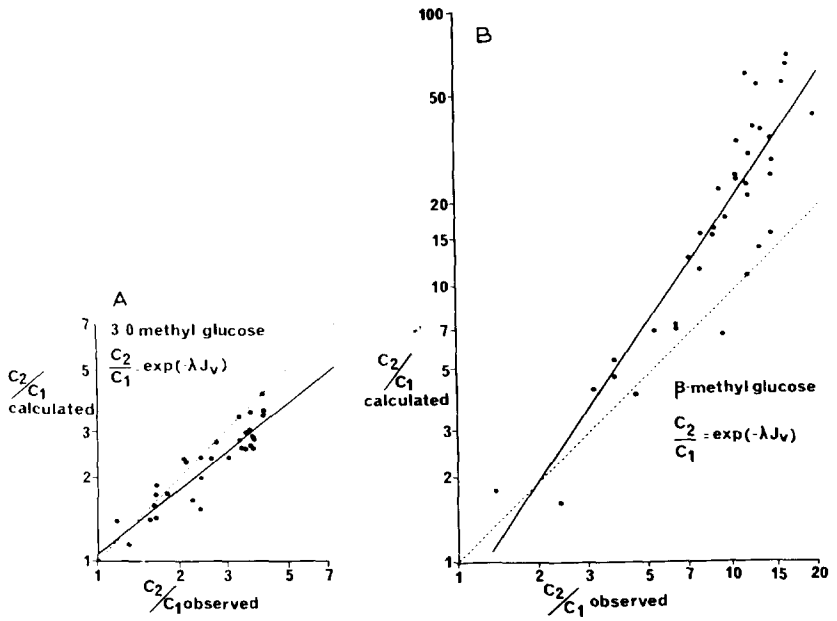

Fig. 7. A plot showing the calculated accumulation ratio versus the observed accumulation ratio. C_2/C_1 = accumulation ratio of sugar. The calculated accumulation ratio of sugar is obtained from the following relationship $C_1/C_2 = e^{-\lambda J_v}$. If it is assumed that the Peclet number = the ratio of convective: diffusive velocity of sugar within the membrane is approximately = ln P_{13}/P_{31} and the reflexion coefficient of sugar at the serosal border = 1 then it can be shown that $C_1/C_2 = e^{-\lambda J_v} = e(Pe/(1+Pe/R(1-e^{-Pe})))$ [14], where R is the specific activity ratio of $^3H^-$: ^{14}C-labelled sugar as described in Materials and Methods, $\lambda = (\sigma_s-\sigma_m)/(P_m+P_s)$. (a) Shows the least square linear regression line of ln C_2/C_1 (calculated) vs. ln C_2/C_1 (observed); slope = 0.79; $r = 0.93$ for 3-*O*-methyl glucose; dotted line is ideal line; slope = 1; ln C_2/C_1 (cal.) = ln C_2/C_1 (observed). (b) shows the least square linear regression line of ln C_2/C_1 (calculated) versus ln C_2/C_1 (observed); slope = 1.44; $r = 0.89$ for β-methyl glucose; dotted line is the line showing C_2/C_1 (observed) = C_2/C_1 (calculated).

that the low permeability of the basal lateral border to β-methyl glucose as was shown in the earlier study [13] and previously by Bihler and Cybulsky [27] prevents significant unstirred layer effects in the submucosal space, which may mask any in vitro convection of galactose at the lateral serosal border.

If it is assumed that the reflexion coefficient of β-methyl glucose at the lateral border is < 1, then the previous assumptions made for galactose must be modified to describe intracellular accumulation of β-methyl glucose.

Kedem and Katchalsky [28] showed that accumulation of solute within the intermembrane region of a double membrane series array may be described as follows:

$$\frac{C_2}{C_1} = \exp\left(\left(\frac{\sigma_s-\sigma_m}{P_m+P_s}\right) J_v\right) \tag{2}$$

Where C_1, C_2 are the extracellular and intracellular solute concentrations, respectively, σ_m and σ_s are the reflexion coefficients of solute across the mucosal and serosal

borders, respectively. P_m and P_s are the passive permeabilities of solute across the mucosal and serosal borders, respectively, and J_v is the net solvent velocity across the series membrane array.
Thus

$$\frac{C_2}{C_1} = \exp\left(\left(\frac{(1-\sigma_m)}{P_m+P_s} - \frac{(1-\sigma_s)}{P_m+P_s}\right) J_v\right) \tag{2}$$

since $V_j = (1-\sigma_j)J_v$ (where V is the solute convective velocity across membrane j).

$$\ln \frac{C_2}{C_1} = \frac{V_m}{P_m+P_s} - \frac{V_s}{P_m+P_s}$$

but $\exp(V_m/(P_m+P_s))$ is the predicted value of the accumulation ratio C_2/C_1 (cal) assuming that $\sigma_s = 1$ [14].
It follows therefore, that

$$\frac{V_s}{P_m+P_s} = \ln \frac{C_2}{C_1}\text{ (cal)} - \ln \frac{C_2}{C_1}\text{ (obs.)}.$$

The serosal convective velocity obtained in conditions giving optimal accumulation of β-methyl glucose can be calculated as follows

$$V_s = (P_m - P_s) \ln \frac{\frac{C_2}{C_1}\text{ (cal)}}{\frac{C_2}{C_1}\text{ (obs)}}$$

$$= (0.05+0.03)1.4 = 0.12 \text{ cm/h}$$

i.e. the convective velocity of β-methyl glucose across the brush-border is approximately four-times faster than across the serosal border (Figs. 5 and 6). The results of this paper are consistent with the view that sugar accumulation is caused by concentration polarization at the serosal boundary of the epithelial cells. Eqn. 2 indicates that there are five parameters which affect the level of steady-state accumulation. In Na^+ Ringer where the solvent velocity J_v can be assumed to be constant at low sugar concentration, the results indicate that the most important parameter controlling accumulation is the reflection coefficient of the sugar at the brush-border; the next most important factor is the passive permeability coefficients which control leak flux across the serosal boundary and to lesser extent across the brush-border. In the case of β-methyl glucose, where the serosal border passive permeability is low, sugar accumulation will be high, but a third factor, the reflexion coefficient of the sugar at the serosal border may be a factor reducing this accumulation.

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