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INTESTINAL SUGAR TRANSPORT: IONIC ACTIVATION AND CHEMICAL SPECIFICITY

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SUMMARY

The mode of absorption of several monosaccharides was studied *in vitro* with segments and everted sacs of hamster small intestine.

1. Na^+ reduced the apparent K_m of transport of the actively transported D-galactose, 3-methyl-D-glucose, α-methyl-D-glucoside, D-xylose and L-glucose. The 'nontransported' D-arabinose, L-arabinose, L-rhamnose, L-mannose and L-fucose showed Michaelis–Menten kinetics, but their K_m's were not significantly altered by Na^+. The v_{max} of all the sugars tested was identical and independent of Na^+. Inhibition of entry of nontransported sugars by D-galactose and by phlorizin was demonstrated.

2. By the criteria of Na^+ dependence, inhibition by phlorizin and ouabain and inhibition by transported sugars, D-mannose and D-fructose were also shown to interact with the joint sugar carrier. The transport of D-mannose appears to be active, but rapid metabolism of the two sugars precluded a quantitative determination of transport parameters.

3. The data suggest a dual specificity of a joint sugar carrier: In the absence of Na^+ its affinity for many diverse sugars is low; the presence of Na^+ increases the affinity for only some of these sugars. The potential for active transport depends on the extent of this activation by Na^+ and varies with different sugars from negligible to very great.

4. With low concentrations of sugar the asymmetry of transport increases towards a limiting value which depends on the ratio $K_{m(Na^+ \text{ free})}/K_{m(Na^+)}$. Active transport occurs with sugars where this ratio >3–5, provided their concentration is below a critical value which also parallels the K_m ratio.

INTRODUCTION

The intestinal absorption of glucose and several related compounds is carrier mediated and depends on the presence of Na^+ and the supply of metabolic energy (see ref. 1 for review). By the classical definition[2] this process is one of "active" transport which leads to the movement of sugar against a concentration gradient. Indeed, this was the experimental criterion first used to delineate the chemical specificity of active intestinal sugar transport[1]. In the absence of Na^+, sugars of the

glucose group enter the tissue only very slowly and are not accumulated against a concentration gradient. It is thought that this slow entry also occurs via the carrier for active transport, showing, in the hamster, the same maximal velocity but a drastically reduced affinity[3]. In contrast, the slow entry of other, so-called non-transported sugars appears to be independent of Na^+ and has often been assumed to occur by simple diffusion. Thus, Na^+ dependence of the rate of entry could be another indication of active transport. Another possible test could be the sensitivity of sugar entry to the specific inhibitor, phlorizin.

Several observations are at variance with this simple picture: The entry of D-xylose[4] and L-glucose[5] shows Na^+ dependence but uphill transport can be demonstrated only under special conditions; the nontransported sugar L-fucose[6] appreciably inhibits the transport of some actively transported sugars; also, in contrast to other compounds of similar molecular weight and hydrophilic character, which are restricted to the extracellular space, many so-called nontransported sugars definitely penetrate into the intracellular space. All this suggests that the intestinal sugar carrier may have some affinity for compounds which are not necessarily actively transported.

In this paper we examine the mechanism of entry of several nontransported sugars and discuss some of the implications of the finding that their entry appears to be mediated by the carrier for active sugar transport.

MATERIALS AND METHODS

Two preparations *in vitro* of hamster small intestine were used, everted jejunal sacs prepared according to WILSON AND WISEMAN[7] and transversely cut segments ("strips") prepared according to CRANE AND MANDELSTAM[8]. The tissue was incubated with gentle shaking at 37° in an atmosphere of O_2–CO_2 (95:5, v/v). For experiments in the presence of Na^+, bicarbonate buffer (pH 7.4) of KREBS–HENSELEIT[9] was used. For experiments with high sugar concentrations, the medium was modified by reducing the concentration of NaCl to 72 mM and adding mannitol in amounts appropriate to maintain the same osmolarity. For experiments in the absence of Na^+, the incubation medium consisted of 240 mM mannitol buffered with 24 mM Tris–HCl–bicarbonate (pH 7.4) and containing in addition the same concentration of K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-} and SO_4^{2-} as the Krebs–Henseleit buffer with which it was isoosmotic. For experiments with high concentrations of sugar, the mannitol concentration was reduced to maintain unchanged osmolarity. The media also contained a mixture of ^{14}C- or ^3H-labelled and unlabelled test sugars to give the desired initial concentration. Apparent extracellular space was determined with tracer amounts of mannitol[10] labelled with the isotope not added with the sugar. In experiments using strips the tissue was preincubated for 10 min in medium without sugar, rapidly transferred to fresh medium containing the additions indicated for each experiment, and incubated for 30 or 40 min. Samples of medium and tissue homogenates were deproteinized by the method of SOMOGYI[11] except in some experiments with D-galactose, D-fructose and D-mannose when 5% trichloroacetic acid was used. After centrifugation, the radioactivity of supernatants was determined by double-label liquid scintillation spectrometry as described previously[12].

Everted sacs were filled with the incubation medium and incubated for 40 min.

Analyses were usually performed on both the mucosal and serosal media, as well as on the tissue homogenate. L-Mannose was determined chemically by a method for reducing sugars[13] adapted to the Technicon Autoanalyzer. For thin-layer chromatography, samples of tissue extracts and medium were spotted on MN300 powdered cellulose and developed with isobutyric acid-NH_4OH-water (66:1:33, by vol.). After separation, 3-mm zones were scraped into counting vials and their radioactivity determined. The distribution of radioactivity was then related to the position of reference compounds in a guide strip sprayed with the benzidine reagent according to BACON AND EDELMAN[14].

The results are expressed in terms of percent filling, *i.e.* the concentration in the intracellular water as percent of the concentration in the medium. Calculations and statistical evaluation were done as described previously[12].

RESULTS AND DISCUSSION

The starting point of this study was the observation of CRANE *et al.*[3] that in hamster intestine, Na^+ appeared to act by increasing the affinity of the carrier for the actively transported sugar 6-deoxy-D-glucose, while leaving the maximal velocity unchanged. Their data indicated that entry occurred through the carrier even in the absence of Na^+. Fig. 1 shows LINEWEAVER–BURK[15] plots of the transport of 3-methyl-D-glucose, an actively transported sugar, and of L-arabinose a nontransported sugar. It shows that with 3-methylglucose also, Na^+ appeared to affect only the apparent affinity of the carrier but not its maximal velocity. In contrast, Na^+ did not significantly influence the apparent K_m of L-arabinose, but the intercept at the ordinate was significantly different from zero, suggesting that this sugar also enters the cell by a saturable process with a definite v_{max}. These data are in keeping with the current hypothesis[1] that all actively transported sugars enter the absorptive cell *via* the same carrier regardless of the presence or absence of Na^+. They indicate that the entry of some of the Na^+-independent sugars may also be carrier mediated and further suggest the possibility that the entry occurs *via* the carrier for active sugar transport.

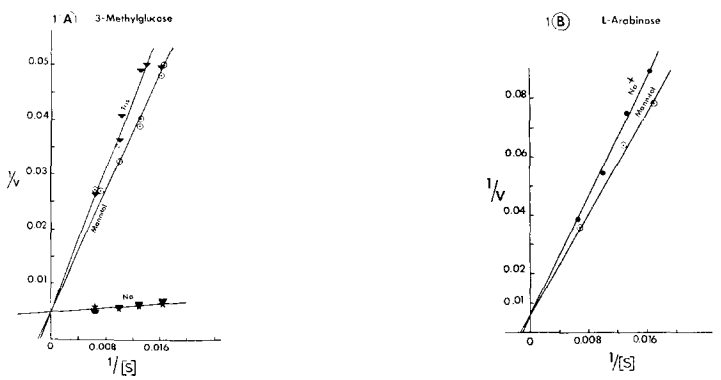

Fig. 1. Kinetics and Na^+ dependence of transport of 3-methyl-D-glucose (A) and L-arabinose (B). Intestinal strips were incubated for 30 min in medium containing 72 mM Na^+ (two separate experiments), in Na^+-free mannitol medium, (see MATERIALS AND METHODS) or in a Na^+-free Tris medium, pH 7.4. The difference in slope between the two Na^+-free lines in (A) and between the Na^+ and Na^+-free lines in (B) was not statistically significant. Each point represents the mean of 4 experiments. $[S]$ = mM, v = mmoles/l intracellular water per h.

To examine these possibilities, the penetration *in vitro* of a number of sugars into the intracellular water of hamster intestinal tissue was measured. To distinguish with certainty between diffusion and a saturable process with very low affinity, very high concentrations of substrate, up to 150 mM, were used in these experiments. This has the effect of "magnifying" the portion near the origin of the double-reciprocal plot and permits a more accurate determination of v_{max} and K_m. Fig. 2 compares

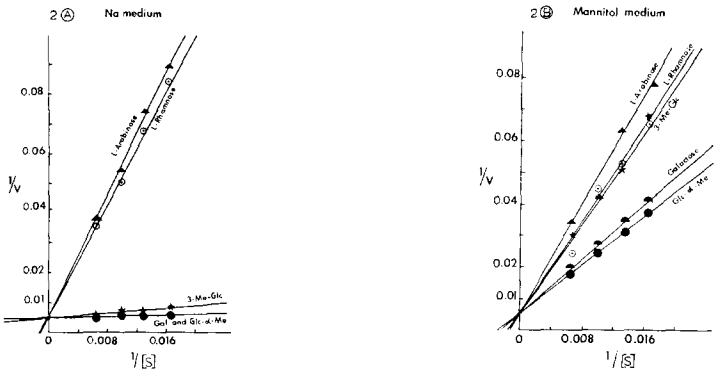

Fig. 2. Entry kinetics of Na^+-dependent and Na^+-independent sugars. Intestinal strips were incubated for 30 min. Other details as in Fig. 1. Glc-α-Me = α-methyl-D-glucoside.

the entry kinetics of two sugars which are not actively accumulated, L-rhamnose and L-arabinose and of three actively transported sugars, 3-methyl-D-glucose, D-galactose and α-methyl-D-glucoside. In the presence of Na^+, the low K_m of the latter three sugars is contrasted by the very high values for L-rhamnose and L-arabinose; their K_m, calculated by graphic extrapolation, was of the order of 300–500 mM, indicating extremely low affinity, but their v_{max} was the same as for the three actively transported sugars. In the absence of Na^+, in a Tris-buffered mannitol medium, the slopes for all five sugars were steep. Again there was one common v_{max} which, as shown in Fig. 1, was the same as in the presence of Na^+. The omission of Na^+ had no significant

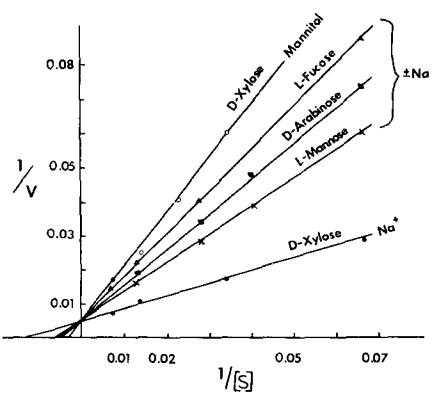

Fig. 3. Entry kinetics of four additional monosaccharides. Everted sacs were incubated for 40 min. Each point for D-xylose is the mean of 6 experiments; for the other sugars the effect of Na^+ was not statistically significant, and data with and without Na^+ were pooled; each point represents the mean of 12 experiments.

effect on the affinities of the nontransported sugars (see Fig. 1) but caused a sharp decrease of more than a 100-fold in the affinity of the three transported sugars. It may be noted that in the absence of Na^+ the affinity for α-methylglucoside and D-galactose was greater than for the other three sugars, although much less than in the presence of Na^+.

Fig. 3 shows the kinetic parameters of four additional sugars, L-fucose, D-arabinose, L-mannose and D-xylose. With all four sugars, with and without Na^+, the same v_{max} as that in Figs. 1 and 2 was reached, which, under the conditions of the present experiments, had a value of about 0.2 mole/l intracellular water per h. Na^+ had no significant effect on the affinity of the first three sugars. For D-xylose a definite effect of Na^+ was evident: Its K_m in the presence of Na^+ was about 6-fold lower than in the absence of the activating ion. This difference is highly significant but much less than for glucose or galactose, and parallels the much smaller extent of active transport exhibited by D-xylose.

The results of these experiments add four additional actively transported sugars and five 'nontransported' sugars to the list of compounds which exhibit a single and identical v_{max} regardless of the ion present, and in which the effect of Na^+ is limited to changing the affinity of the sugar for the carrier. The same v_{max} and the same type of dependence of affinity on Na^+ were also demonstrated recently in this laboratory for L-glucose[16].

Table I summarizes the effect of phlorizin on the uptake of these sugars. Four sugars whose K_m was not measurably affected by Na^+ were tested in a medium containing 145 mM Na^+: no inhibition was found with L-rhamnose and L-arabinose. With D-arabinose and L-fucose a small degree of inhibition was demonstrated which became significant at a greater ratio of inhibitor to test sugar. Three actively transported sugars, 3-methyl-D-glucose, α-methyl-D-glucoside and D-galactose were tested in a mannitol medium in which, as shown in Fig. 2, their affinity is low. There was

TABLE I

EFFECT OF 0.5 mM PHLORIZIN ON SUGAR UPTAKE

Everted sacs were incubated for 40 min. The percent inhibition is calculated from the mean percent filling in the presence and absence of inhibitor; $n = 6$. P is calculated by the one tailed t-test and refers to mean decrease in percent filling caused by the inhibitor. For other details see MATERIALS AND METHODS. n.s. = not significant.

Sugar	*Concn. (M)*	*Inhibition (%)*	*P*
Na^+ medium			
L-Rhamnose	$1.0 \cdot 10^{-3}$	0	
L-Arabinose	$1.0 \cdot 10^{-3}$	0	
D-Arabinose	$1.0 \cdot 10^{-3}$	5.0	n.s.
	$2.3 \cdot 10^{-6}$	14.5	<0.025
L-Fucose	$1.0 \cdot 10^{-3}$	9.0	n.s.
	$0.52 \cdot 10^{-6}$	53.3	<0.0025
Mannitol medium			
3-Methyl-D-glucose	$0.5 \cdot 10^{-3}$	4.0	n.s.
D-Galactose	$0.5 \cdot 10^{-3}$	15.5	<0.025
α-Methyl-D-glucoside	$0.5 \cdot 10^{-3}$	40.5	<0.0025

hardly any inhibition with 3-methyl-D-glucose, but the transport of α-methyl-D-glucoside and D-galactose was appreciably inhibited. Thus, the inhibition by phlorizin, shown in Table I, is only weak and increases in parallel with the affinities of the respective sugars shown in Figs. 2 and 3. The latter is the exact opposite of what would be expected if phlorizin were to act competitively. This anomalous effect occurs not only with Na^+-independent sugars (in the presence of Na^+) but also in the absence of Na^+ with sugars of the glucose group towards which phlorizin acts competitively when Na^+ is present. Whatever mechanisms are envisaged to explain the transport, with the same v_{max}, of both Na^+ dependent, actively transported and Na^+ independent, not actively transported sugars, it appears that the interaction of phlorizin with the low-affinity, Na^+-independent system is not of the competitive type and, indeed, cannot be readily explained at present. However, new insights into the mechanism of action of phlorizin may soon result from the recent discovery of phlorizin hydrolase[17,18].

TABLE II

EFFECTS OF D-GALACTOSE AND D-MANNITOL ON THE UPTAKE OF L-ARABINOSE AND L-RHAMNOSE

Intestinal strips were incubated in 145 mM Na^+ medium as described in MATERIALS AND METHODS. The data are calculated from the means of the percent filling in the presence and absence of the additions listed ($n = 3$). P refers to the mean difference in percent filling calculated by the one tailed t-test. The correction for osmotic shrinkage was calculated by taking the data with 30 mM mannitol as control values.

Addition	*Concn. (mM)*	L-*Arabinose (0.5 mM)*		L-*Rhamnose (0.5 mM)*	
		Change (%)	*P*	*Change (%)*	*P*
D-Mannitol	30	+15.2	<0.0125	+18.0	<0.10
D-Galactose	30	−17.5	<0.025	−28.3	<0.05
D-Galactose (corrected for osmotic shrinkage)	30	−28.8	<0.005	−39.5	<0.0025

The transport of sugars with very low affinity is also affected by actively transported sugars under appropriate conditions. Table II shows the effect of 30 mM D-galactose on the transport of 0.5 mM L-arabinose and L-rhamnose in the 145 mM Na^+ medium. Osmotic shrinkage of the cells may be expected to occur under these conditions, and an equivalent concentration of mannitol was used to measure the apparent increase in sugar penetration due to this effect. Assuming that galactose has the same osmotic effect, the data for galactose inhibition were then appropriately corrected. The corrected figures represent an upper limit; the true inhibition is probably somewhat smaller, since some D-galactose will have penetrated the cells. Considering the affinities of the respective sugars (see Fig. 2), the degree of inhibition is much less than would be expected for a simple competitive interaction between D-galactose and the two low-affinity sugars. This again points to the complexity of the relationship between sugars and the transport system.

Another expression of the affinity of these sugars for the active transport system is seen in Table III. It shows the effect of a very large excess (150 mM) of several so-called nontransported sugars on the transport of D-xylose. This sugar has been chosen because its affinity is only moderately increased by Na^+ (see Fig. 3).

The data show that the degree of inhibition, reflecting the relative affinities of the two sugars, depends on the presence of Na^+. The much stronger inhibition by D-mannose in the presence of Na^+ would suggest that the carrier's affinity for this sugar is increased by Na^+ more than it is for xylose; conversely the smaller inhibition caused by D-fructose and L-arabinose in the presence of Na^+ would mean that, if Na^+ increases the affinity for these sugars, it is to a lesser degree than for xylose. These conclusions are consistent with other data on these sugars. They also suggest that, contrary to earlier views, D-mannose and D-fructose may also interact with the carrier. Recently it was shown by CSÁKY AND HO[19] that D-mannose is actively transported in frog intestine but is not appreciably metabolized. It has also been pointed out [20] that the rapid absorption of fructose at higher concentrations would suggest a mechanism of entry different from diffusion. Thus it seemed worth reexamining the transport of these two sugars.

TABLE III

EFFECT OF A LARGE EXCESS (150 mM) OF SEVERAL SUGARS ON THE TRANSPORT OF 1 mM D-XYLOSE

Intestinal strips were incubated as described in MATERIALS AND METHODS in 72 mM Na^+ medium or in Na^+-free mannitol–Tris medium. The data refer to changes in the transport of D-xylose, when compared to transport in the control (addition of mannitol) and are calculated as in Table II ($n = 3$). The effect of L-mannose is not significant, that of all other sugars is highly significant ($P<0.01$). For D-mannose and D-fructose the difference between effects with and without Na^+ is also significant ($P<0.025$ and <0.05, respectively).

Addition (150 mM)	*Change (%)*	
	Na^+ (72 mM)	*Na^+ free*
Mannitol (control)	0	0
D-Fructose	−25.6	−35.9
L-Arabinose	−27.0	−33.9
L-Mannose	− 4.5	− 2.8
D-Mannose	−53.3	−37.2

Thin-layer chromatography of extracts of hamster intestine exposed to D-[^{14}C]fructose and D-[^{14}C]mannose showed that both these sugars undergo rapid metabolism in the tissue and that only a small part of the radioactivity in the tissue represents unchanged sugar. Therefore, in hamster intestine the appreciable accumulation of radioactivity observed with these two sugars cannot be taken as evidence for accumulation of the free sugar against the gradient. Attempts to inhibit metabolic transformation of these sugars with little damage to other metabolic activity were only partially successful, NaF proving most effective. This inhibitor was shown earlier to inhibit glucose metabolism strongly, but glucose transport only slightly[6]. Nevertheless, some qualitative conclusions can be drawn regarding interaction with the sugar carrier and with Na^+.

Table IV shows some such data for D-fructose. Two actively transported nonmetabolized sugars, phlorizin and the absence of Na^+ all strongly inhibited the uptake of radioactivity. When the uptake was about halved by NaF, the degree of inhibition with phlorizin or in the absence of Na^+ remained unchanged, which would be consistent with an effect on transport. Table V shows similar data for D-mannose,

TABLE IV

INHIBITION OF UPTAKE OF D-[^{14}C]FRUCTOSE

Intestinal strips were incubated as described in MATERIALS AND METHODS. Relative changes in the uptake of total radioactivity were calculated as described in Table II from the means of 6–12 experiments. When 20 mM NaF were present in the medium, NaCl was decreased proportionately and Ca^{2+} and Mg^{2+} were omitted.

Addition	*Change (%)*	
	No inhibitor	*NaF (20 mM)*
145 mM Na^+ medium		
None (control)	0	0*
0.5 mM phlorizin	−45.3	−41.5
15 mM 3-methyl-D-glucose	−40.5	−46.0
5 mM D-galactose	−26.0	−22.8
Na^+-free medium		
None	−73.3	−74.0
0.5 mM phlorizin	−79.8	−76.0

* −58.5% when compared to control without inhibitor.

TABLE V

UPTAKE OF D-[^{14}C]MANNOSE

To determine percent filling everted sacs were incubated as described in MATERIALS AND METHODS. Data are means of 4 experiments ± S.E. To measure inhibition of uptake intestinal strips were incubated for 30 min as described in MATERIALS AND METHODS. Relative changes in uptake of total radioactivity were calculated as described in Table II from the means of 4 experiments. For other details see Table IV.

Concn. (M)	*Filling (%)*	
	Na^+ (145 mM)	*Na^+ free*
$1.2 \cdot 10^{-5}$	370.0 ± 28.9	154.0 ± 8.9
$1 \cdot 10^{-3}$	107.1 ± 5.4	60.4 ± 4.7
$5 \cdot 10^{-3}$	74.5 ± 10.5	50.6 ± 8.0
$12 \cdot 10^{-3}$	56.2 ± 2.0	34.1 ± 1.0

Addition	*Change (%)*	
	No inhibitor	*NaF (20 mM)*
145 mM Na^+ medium		
None (control)	0	0
10^{-5} M ouabain	−32.0	−18.7
0.5 mM phlorizin	−78.4	−61.6
15 mM 3-methyl-D-glucose	−35.5	−35.3
1 mM D-glucose	−49.3	
5 mM D-glucose	−83.5	−74.4
Na^+-free medium		
None	−29.5	−28.0
0.5 mM phlorizin	−41.5	−48.0

also indicating Na^+ dependence and inhibition by actively transported sugars, phlorizin and ouabain.

In other experiments the uptake of D-mannose and D-fructose was found to obey Michaelis–Menten kinetics within a certain concentration range. Because of the rapid metabolism, the apparent K_m values cannot, of course, be compared with those for nonmetabolized, sugars. Yet it may be significant that they were increased in the absence of Na^+, more than 2-fold in the case of D-mannose. This shows that the entry of D-mannose and D-fructose occurs, at least in part, *via* the carrier for active sugar transport. The results in Table III also suggest that D-mannose is probably actively transported, and to a greater extent than D-xylose, but this could not be directly demonstrated owing to rapid metabolism. The demonstration of these interactions with the carrier does not, however, exclude additional or alternate pathways for the entry of these two sugars. Indeed, the rapid absorption of D-fructose in cases of glucose–galactose malabsorption[21] would suggest that, for fructose at least, an alternate pathway of entry is very likely.

The above data suggest that the actively transported sugars and several so-called nontransported sugars all share the same saturable entry process with a single joint v_{max}, both in the presence and absence of Na^+. It appears that the carrier for active transport is also instrumental in the entry of at least several sugars hitherto considered "nontransported", usually at the same low rate as shown by sugars of the D-glucose group in the absence of Na^+. However, while the affinity of the transport process towards them is only slightly affected by Na^+, the affinity for the actively transported sugars is greatly increased.

The simplest model consistent with this picture is a single site on a single carrier whose affinity for sugars is increased by Na^+ to a variable extent, up to several hundredfold for some compounds but little or not at all with most others. In other words, Na^+ is thought to change the carrier's specificity profile from that resembling a rather flat, wide plateau to a plateau with a number of peakes of varying height. The weak inhibition of entry of the low-affinity sugars by D-galactose and by phlorizin in the presence of Na^+ (Tables I and II) are, however, difficult to reconcile with this model.

An alternative concept is that of two separate carriers, one Na^+ dependent, with high affinity for actively transported sugars only, and one Na^+ independent, with low affinity for both actively transported and other sugars, both having the same v_{max}. This model agrees better with the inhibition data but fails because it requires that, as the Na^+-dependent carrier is activated by Na^+, the other carrier must be inhibited, otherwise the v_{max} would be doubled by Na^+. This is obviously not the case: the transport of the low-affinity sugars is not decreased in the presence of Na^+.

Thus, no detailed model can be proposed as yet for the interaction between the carrier and the two classes of sugars. Nevertheless, the broad outlines and functional relationships emerge clearly. The data presented here indicate (1) that the slowly penetrating sugars which are not actively transported, do, in fact, interact with the active transport system, and (2) that the affinities of the actively transported sugars are increased by Na^+ to varying degrees.

According to the model of CRANE *et al.* (see ref. 1 for review), Na^+-dependent active transport is a consequence of the asymmetry in affinity of the carrier exposed to high and low Na^+ concentrations outside and inside the cell, respectively. It is

therefore limited to sugars whose affinity is increased by Na^+. Its extent, as reflected in the degree of intracellular sugar accumulation at steady state, will depend on the degree of this increase in affinity. Since the Na^+-dependent increases in affinity range all the way from negligible to very large, there is no sharp limit to the 'specificity' of active transport.

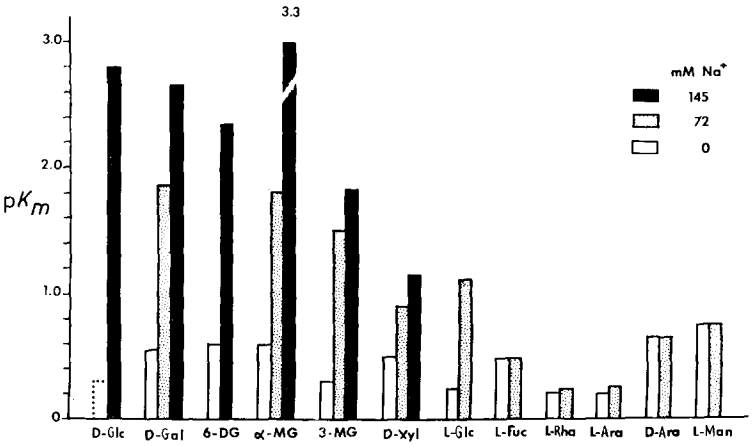

Fig. 4. Relative affinities for transport in hamster intestine. The affinities are expressed as pK_m and based, in part, on published data: D-glucose with Na^+ (ref. 6), without Na^+, estimated; L-glucose (ref. 16); D-galactose with 145 mM Na^+ (ref. 6); 6-deoxy-D-glucose (ref. 3); α-methyl-D-glucoside with 145 mM Na^+ (ref. 23); all other data from this study. Abbreviations: 6-DG, 6-deoxy-D-glucose; α-MG, α-methyl-D-glucoside; 3-MG, 3-methyl-D-glucose.

Fig. 4 illustrates these relationships for a number of sugars, using the negative logarithm of K_m, pK_m, as a measure of affinity. All data refer to transport *in vitro* by hamster intestine and are in part taken from the published literature and in part derived from the results of this study. As pointed out previously, a medium with 72 mM Na^+ was used to maintain isoosmotic conditions when higher sugar concentrations had to be used. In other cases, experiments with this intermediate concentration of Na^+ were added for comparison and showed the expected[3,10] smaller activating effect. The potential for active transport of the various sugars can be quantitatively estimated from the difference between the pK_m values with and without Na^+. Due to lack of reliable K_m values, the two rapidly metabolized sugars are not included in Fig. 4; D-mannose would probably show a pattern similar to D-xylose, and preliminary data with D-fructose suggest a relatively high affinity, with values in the presence and absence of Na^+ differing by about 20–30%. The main purpose of this figure is to emphasize the gradual nature of the transition between actively transported and nontransported sugars.

Intracellular sugar accumulation at the steady state provides an estimate of the relation of active transport to the affinities. Assuming that the sugar enters and leaves the cell only *via* the carrier and using the Michaelis–Menten equation in its simplest form, it can be shown that, at the steady state: $S_2/S_1 = K_2/K_1$ where 1 and 2 refer to the conditions inside and outside the cell, respectively. With sugars entering slowly steady state cannot be reached experimentally *in vitro*, and the relationship between accumulation and affinity is better described by the ratio of influx to efflux

at equilibrium (100% filling). Under these conditions, the flux ratio will be a direct expression of the extent of active transport. Again, from the Michaelis–Menten equation, this ratio at equilibrium is: $v_{1\to2}/v_{2\to1} = (S + K_2)/(S + K_1)$; and for $S\to0$: $v_{1\to2}/v_{2\to1} = K_2/K_1$; for $S\to\infty$: $v_{1\to2}/v_{2\to1} = 1$. This value can be calculated for any pair of apparent K_m values for influx and efflux and can be plotted as a function of the sugar concentration. Fig. 5 shows such a plot for several pairs of K_m

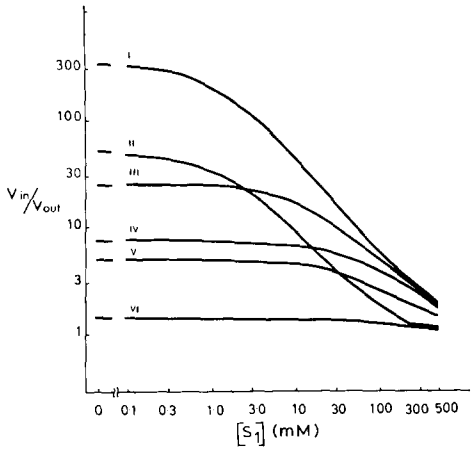

Fig. 5. Extent of active transport as a function of substrate concentration. The ratio $v_{\text{inward}}/v_{\text{outward}}$ (which equals the ratio of carrier saturation at the two faces of the membrane) was calculated for K_m values representing affinities at the outside (high Na^+) and the inside (low Na^+). Rounded off K_m's (in mM) of several sugars in 145 mM Na^+ and in mannitol medium were used The intra-and extracellular substrate concentrations were assumed to be equal.

Curve	$K_{m(Na^+)}$	$K_{m(Na^+\,free)}$	*Typical example*
I	1.5	500	D-Glucose
II	2	100	6-Deoxy-D-glucose
III	20	500	3-Methyl-D-glucose
IV	80	600	L-Glucose
V	70	350	D-Xylose
VI	350	500	L-Fucose, L-rhamnose

values chosen to represent typical situations. Note the logarithmic scale on both coordinates. Three phases can be distinguished in these calculated curves: (1) at very low sugar concentrations the flux ratio tends toward a limiting value which depends on the K_m ratio; (2) as the substrate concentration increases, the ratio drops; (3) at high concentrations the ratio levels off again at a value asymptotically approaching 1.

It has long been known that the percent filling of an actively transported sugar drops steeply as the sugar concentration in the medium is increased[8], and rather than levelling off at equilibrium, falls below 100% at a concentration characteristic for each sugar. Depending on the extent of the effect of Na^+ on the affinity for a given sugar, transport against the gradient will take place over a variable range of sugar concentrations. For example, the 'weakly transported' D-xylose is accumulated against the gradient only at concentrations below 10^{-4} M (refs. 4 and 16). The 'strongly transported' D-galactose is moved uphill up to approx. $7\cdot10^{-2}$ M (ref. 16).

This difference between the two sugars is qualitatively predicted by Fig. 4. There are, presumably, two causes for the existence of a critical concentration above which a sugar is not transported uphill: first, as the sugar concentration is increased, the net influx *via* the carrier will decrease because of the convergence of influx and efflux rates (see Fig. 1). Second, at the same time, sugar exit through portals other than the carrier will increase. Thus, Phases 2 and 3 have been demonstrated experimentally.

On the other hand, the predicted levelling off at low concentrations (Phase 1) has not been observed hitherto for intestinal sugar transport. Table VI provides such evidence: Sugar accumulation tends to level off at low substrate concentrations at values roughly parallel to the asymmetry of K_m values of the respective sugars. Thus, for each sugar there is a maximal flux ratio which cannot be improved by going to still lower concentrations.

TABLE VI

SUGAR TRANSPORT AT VERY LOW SUBSTRATE CONCENTRATIONS

Intestinal strips were incubated in Na^+ medium as described in MATERIALS AND METHODS. The results with the lowest practicable concentration of sugar (not diluted with nonradioactive sugar) are in the column 'undiluted'; the last column gives this concentration for each sugar. The figures are mean ± S.E. Values in parentheses indicate numbers of experiments.

Sugar	*Filling* (%)			*'Undiluted' concn. (M)*
	10^{-5} *M*	$3 \cdot 10^{-5}$ *M*	*'Undiluted'*	
D-Galactose		412.5 ± 17.7 (4)	415.5 ± 23.0 (4)	$1 \cdot 10^{-5}$
3-Methyl-D-glucose		271.5 ± 28.3 (5)	275.0 ± 11.8 (4)	$6 \cdot 10^{-6}$
D-Xylose	127.7 ± 7.3 (5)	130.0 ± 10.2 (4)*		
L-Glucose		144.8 ± 11.1 (4)	151.4 ± 18.4 (9)	$7 \cdot 10^{-6}$
D-Arabinose		34.9 ± 3.1 (3)	37.7 ± 3.5 (7)	$2 \cdot 10^{-6}$

* 'Undiluted' concentration.

While the general shape and mutual relationship of the curves in Fig. 4 is in qualitative agreement with experimental observations, quantitative agreement cannot be expected because of the many simplifications in the model. For example, the K_m values are only approximate and, being Na^+ dependent, will vary appreciably with the Na^+ concentrations in cell and lumen. Also, the ratios are computed for equal sugar concentrations inside and outside the cell, which leads to an underestimate if, as is often the case, the actual filling is less than 100%. In other words, downhill active transport will be faster and uphill active transport slower than predicted by Fig. 5. Most important, the contribution of exit pathways other than the carrier is not taken into account. This may perhaps explain why active transport becomes demonstrable by classical standards only when the flux ratio reaches values greater than 3 or 4. This discrepancy emphasizes the need for the intensive study of sugar transport from the epithelial absorptive cell inward, to the underlying tissue.

A discussion of how specificity of transport is correlated with chemical structure and conformation of the various sugars is beyond the scope of this paper. Indeed, current data are as yet insufficient to form a coherent picture. However, it seems likely that sugar–carrier interaction involves hydrogen bonding, perhaps to several hydroxyl groups of the sugar molecule[22]. In this context one may speculate that the

allosteric effect of Na^+ binding to the carrier could produce a conformational change which would greatly increase the contribution to affinity of a given bond, or bonds, leading to a preferential affinity for certain sugars.

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