Sodium and Sugar Fluxes across the Mucosal Border of Rabbit Ileum

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ABSTRACT Unidirectional influxes of sugars and Na from mucosal solution into the cells of rabbit ileum have been examined. The influxes of glucose, galactose, and 3-0-methyl glucose (3 MG) follow Michaelis-Menten type kinetics and are markedly dependent on the presence of Na in the mucosal solution. For 3 MG, reduction of Na concentration causes a decrease in maximal rate of influx and little change in the "apparent Michaelis constant." There appeared to be little mediated entry of 3 MG into the cells from Na-free solution. The influx of Na was increased by the presence of 3 MG in the mucosal solution and at all Na concentrations tested, there was a 1:1 ratio between sugar influx and the sugar-dependent Na influx. On the basis of these observations, a model has been developed for the sugar transport system involving a transport site that combines with both sugar and Na.

The mucosal cells of the small intestine are able to concentrate certain sugars to levels substantially above those in the extracellular medium (1, 2) and are able to bring about a net active transfer of these sugars from the mucosal to the serosal surface of the cell layer (3, 4). Both of these processes are dependent on the presence of Na ion in the bathing media (1, 5–8). In addition, active Na transport across the mucosa is stimulated by the presence of actively transported sugars in the mucosal solution (5, 9). Since this stimulation can be observed with nonmetabolized sugars that are actively transported, it cannot be explained simply in terms of an increase in energy available for Na transport as a result of metabolism of the sugar. On the basis of these and other observations, Crane and his coworkers (10, 11) have formulated an

hypothesis for sugar transport by intestine that has as its basis the concept of a "carrier" that combines with both sugars and sodium. According to this hypothesis, the driving force for "active" sugar transport is provided by the difference between the intracellular and extracellular Na concentrations.

While there is considerable evidence to support this concept, at least some of the experiments involved are subject to a degree of uncertainty. There seems little doubt that the primary site for sugar transport is at the brush border (12, 13). However, in most studies of sugar transport, transmural transfer or entry of sugars into rings or strips of intestine has been examined. These processes depend on events at both the serosal and mucosal sides of the cell so that interpretation in terms of a transport system at the mucosal border may be uncertain in the absence of knowledge of the contribution made by the serosal border. To overcome these difficulties, we have developed a method for direct measurement of unidirectional fluxes across the brush border from mucosal solution to cells (14). The present experiments were carried out to investigate certain aspects of sugar transport by intestine using this method to determine Na and sugar influxes across the mucosal border of rabbit ileum.

METHODS

The technique used to determine the influx[1] of sugars and Na across the mucosal border of the intestinal epithelial cells was similar to that described by Schultz et al. (14) for the measurement of amino acid and Na influxes. Briefly, the distal ileum of New Zealand white rabbits (sacrificed by intravenous injection of pentobarbital) was mounted in a Lucite chamber in which only the mucosal surface was bathed by Ringer solution. After a 30 min preincubation period in the appropriate Ringer solution free of sugar, the preincubation solution was removed from the chamber and test solution, containing sugar-^{14}C, inulin-^{3}H, and ^{22}Na, was rapidly injected. This solution remained in contact with the mucosa for 20–80 sec and was then withdrawn. The chamber was immediately rinsed with cold (4°C) isotonic mannitol solution and the tissue was punched out and extracted for at least 2 hr in 0.1 N HNO_3. Aliquots of the tissue extract and test solutions were assayed for ^{14}C, ^{3}H, and ^{22}Na in a three channel liquid scintillation spectrometer. The inulin-^{3}H in the test solution was used to correct for contamination of the tissue by adherent medium. Na and sugar influxes were calculated from the uptake of tracer by the tissue. Control experiments testing the validity of these methods have been reported previously (14).

The basic Ringer solution used contained 140 mM NaCl, 10 mM $KHCO_3$, 1.2 mM K_2HPO_4, 0.2 mM KH_2PO_4, 1.2 mM $CaCl_2$, and 1.2 mM $MgCl_2$. The pH was maintained at 7.2 by bubbling with 95% O_2–5% CO_2. Na concentration of the solution was altered by replacing NaCl with an equivalent concentration of KCl, LiCl, choline chloride, Tris chloride, guanidine chloride, or mannitol. The nonmetabolized sugar, 3-0-methyl D-glucose (3 MG), was used for most studies, but influxes of D-glucose and

[1]Throughout this paper, the term influx will be used to refer to a unidirectional flux across the brush border from mucosal solution to cell.

D-galactose were also determined. In a number of experiments, sugar concentrations as high as 40 mM were used. Consequently, sufficient mannitol was added to the test solutions to give a total nonelectrolyte (sugar plus mannitol) concentration of 40 mM. Sugars and mannitol were added from concentrated stock solutions. After the addition of appropriate volumes of isotope solutions, the test solution was approximately isosmotic with the normal Ringer solution but the ion concentrations were about 6% lower than those given above. In all experiments, eight influx measurements, were made on tissue from a single animal.

Transmural 3 MG fluxes were determined using the apparatus described by Schultz and Zalusky (15). The ileum was bathed on both sides with identical solutions (normal Ringer or Na-free choline Ringer) containing 20 mM 3 MG; ^{14}C-3 MG was added to one solution and, after allowing a 40 min equilibration period, its steady-state rate of appearance in the opposite solution determined. Mucosal-to-serosal flux was determined on one piece of tissue and serosal-to-mucosal flux on an adjacent piece from the same animal.

TABLE I

TRANSMURAL FLUXES OF 3 MG*

Bathing medium	Mucosa to serosa flux	Serosa to mucosa flux	Net flux
	μmole/hr cm²	μmole/hr cm²	μmole/hr cm²
Na Ringer	0.81±0.06 (15)	0.20±0.06 (20)	0.61±0.06
Choline Ringer	0.19±0.04 (12)	0.18±0.04 (12)	0.01±0.06

* 3 MG present in both bathing solutions at a concentration of 20 mM. Errors are given as ±1 SEM and the number of observations is given in parentheses.

RESULTS

Transmural 3 MG Fluxes

Previous experiments (1) with strips of mucosa from rabbit ileum have shown that the epithelial cells are able to concentrate 3 MG to levels two to three times those in the incubation medium. This ability is lost when the tissue is incubated in Na-free media or in Na medium containing ouabain. In order to demonstrate that this particular preparation is also capable of bringing about active transfer of sugars across the tissue, transmural fluxes of 3 MG were measured. The results are summarized in Table I. When both bathing solutions contain 20 mM sugar, there is a net flux from mucosa to serosa in the presence of Na. Replacement of all Na in the bathing media by choline completely abolishes net flux of 3 MG. The decrease in net flux is due entirely to a marked decrease in flux from mucosa to serosa; flux in the opposite direction is unaffected by Na removal. These observations of net transfer from mucosa to serosa accompanied by cellular accumulation are consistent with the concept that the primary step in active sugar transport is entry into the cells at the mucosal surface. Subsequent experiments were carried out to investigate this process directly.

Effects of Na on Sugar Influx

Preliminary experiments were carried out to examine the time course of the movement of 3 MG into the tissue from the mucosal solution. Previous work (14) had shown that sodium uptake remained linear for at least 60 sec and similar information was necessary for sugars in order to select appropriate time intervals for subsequent influx determinations. Fig. 1 shows the results of such an experiment; each point is the average of two determinations and all points were obtained using tissue from one animal. The uptake of 3 MG is linear over the entire 80 sec interval. As previously discussed (14), this observation indicates that there is no significant return of tracer from the tissue to the mucosal solution and that tissue uptake of ^{14}C sugar during this brief

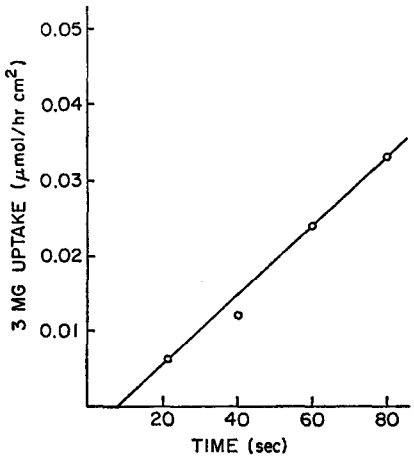

FIGURE 1. Tissue uptake of 3 MG as a function of time from a mucosal solution containing 140 mM Na and 5 mM 3 MG.

period provides an appropriate estimate of influx. All subsequent experiments, except those with glucose, involved exposure of the mucosal surface to test solution for 60 sec. To minimize the possibility of metabolism of glucose to $^{14}CO_2$ and subsequent loss of tracer from the tissue, an exposure of 20–30 sec was used for this sugar.

The initial set of experiments was designed to study effects on sugar influx of changes in Na concentration in the mucosal solution and in the epithelial cells. Cellular Na concentration was reduced by preincubation of the tissue for 30 min in Na-free choline medium, a procedure that has been shown to cause at least a 70% decrease in cellular Na concentration (14). Sugar influx was then measured from a test solution containing 130 mM Na or from Na-free choline medium. In the same series, some tissues were preincubated in 130 mM Na and influx was measured from the same solution.

The results of these studies are summarized in Table II. Glucose, galactose, and 3 MG influxes were markedly depressed by complete removal of

Na from the system; influxes observed when both preincubation and test solutions were Na-free were of the order of 10% of those found when both solutions contained 130 mM Na. The results obtained when cell Na was depleted by preincubation in Na-free medium and sugar influx measured from test solution containing 130 mM Na are less straightforward. Reduction in cell Na had no effect on glucose influx and caused a small but statistically insignificant decrease in galactose influx. However, in a more extensive series using 3 MG a somewhat larger reduction in influx was observed and the difference was significant if tissues from the same animal were treated as paired. Similar experiments were carried out on tissue preincubated in a

TABLE II

EFFECT OF Na ON INFLUX OF SUGARS

Sugar	Principal cation preincubation	test	Influx	n*
			μmole/hr cm²	
Glucose (10 mM)	Na	Na	3.00	3
	Choline	Na	3.03	3
	Choline	Choline	0.45	2
Galactose (20 mM)	Na	Na	2.30±0.50‡	9
	Choline	Na	2.08±0.35	9
	Choline	Choline	0.25±0.05	6
3 MG (20 mM)	Na	Na	2.53±0.23	24
	Choline	Na	2.04±0.15	24
	Choline	Choline	0.31±0.05	10

* Number of observations.

‡ Standard error of the mean.

solution in which all NaCl was replaced by Tris chloride rather than choline chloride. The influx of 3 MG was again depressed relative to control values observed for tissues from the same animals (control 1.62 ± 0.17 μmoles/hr cm², 130 mM Tris, 1.29 ± 0.14 μmoles/hr cm²) suggesting that the effect shown in Table II cannot be ascribed to a specific effect of choline in the preincubation solution. Thus, these experiments indicate that reduction in cellular Na concentration may have a small effect on the influx of actively transported sugars but further experiments are necessary to clarify this point. Clearly, the major Na requirement for sugar influx is a requirement for Na in the solution bathing the mucosal surface.

In order to examine the specificity of this Na requirement, experiments were carried out using other solutes to replace NaCl. In these studies, all tissues were preincubated in choline medium to minimize initial variations in cell Na concentration. The test solution contained 21 mM NaCl, 40 mM

3 MG, and 110 mM replacement solute (220 mM in the case of the nonelectrolyte, mannitol). The results are summarized in Table III A. The influx of 3 MG observed with 110 mM Tris chloride or 220 mM mannitol does not differ from that in the presence of 110 mM choline chloride, again suggesting that choline in the test solution does not have a specific inhibitory effect. The data also indicate that Li, K, and guanidine are ineffective in replacing Na in the sugar influx process. Li causes a small but statistically insignificant

TABLE III

EFFECTS OF Na REPLACEMENT ON 3 MG INFLUX*

	Test solution				
	NaCl	3 MG	Na replacement	Relative 3 MG influx ± SEM	
	mM	*mM*			
A	21	40	110 mM choline	1.00	
	21	40	220 mM mannitol	1.00±0.10	(6)‡
	21	40	110 mM Tris	0.91±0.10	(6)
	21	40	110 mM Li	0.82±0.10	(8)
	21	40	110 mM K	0.69±0.10	(12)
	21	40	110 mM guanidinium	0.51±0.05	(6)
	21	40	110 mM Na	2.80±0.30	(6)
B	0	20	130 mM choline	1.00	
	0	20	130 mM Li	1.29±0.21	(16)
	0	20	130 mM guanidinium	1.10±0.24	(8)
C	21	40	110 mM choline	1.00	
	21	40	122 mM K	0.71±0.07	(13)
	21	40	110 mM choline§	0.52±0.09	(4)
	21	40	122 mM K§	0.30±0.03	(8)

* Unless otherwise noted, all tissues were preincubated in Na-free solution containing 140 mM choline and 12 mM K.

‡ Number of observations.

§ Tissue preincubated in Na-free medium containing 142 mM K.

decrease in influx while K and guanidine cause decreases of 30 and 50%, respectively. For comparative purposes, the final line in Table III A illustrates the effect on 3 MG influx of adding 110 mM Na to the test solution. Thus, under the conditions of these experiments, an ion capable of replacing Na should cause an increase in influx rather than the decrease observed.

Two additional tests of the effects of cations other than Na on 3 MG influx have been carried out. Bihler and Adamic (16) have reported that Li causes an increase in sugar entry into rings of hamster intestine from Na-free solution. Because the possibility existed that the presence of 21 mM Na in the experiments reported in Table III A might obscure such an effect, we tested the effects of both Li and guanidine on 3 MG influx from Na-free solutions.

The results, shown in Table III B, indicate that in rabbit ileum neither of these ions stimulates 3 MG influx above the level observed in the presence of choline.

The influence of K on 3 MG influx was also examined in more detail with particular emphasis on the effect of preincubation of the tissue in high K media. In all experiments, the test solution for measurement of influx contained 21 mM Na and 40 mM 3 MG. The results are summarized in Table III C. When the tissue was preincubated in solution containing 142 mM K and influx measured from a test solution of the same composition, influx was depressed by 70% compared to control conditions (preincubation, Na-free choline medium; test, 21 mM Na, 110 mM choline, 12 mM K). The decrease is much greater than that observed for preincubation in choline medium and test using 122 mM K, and suggests an effect of preincubation in high K medium. This suggestion is confirmed by experiments in which the preincubation was carried out in 142 mM K but the test solution was the same as in control experiments (110 mM choline, 12 mM K). Influx was reduced 50% from control values. Thus, the inhibitory effect of K appears to involve two components, one due to prolonged exposure (30 min) of the tissue to high K and one due to a more direct effect that is apparent in short (60 sec) exposures. The two effects appear to be approximately additive in tissues preincubated and tested in high K.

Na influxes were also measured in these experiments involving the effects of different substitute solutes. The results obtained with mannitol, Tris, Li, and K were the same as those reported previously (14) for experiments in which amino acid influx was investigated. Tris and mannitol had no effect and Li (110 mM) caused a 50% decrease in Na influx. The percentage changes in Na influx caused by K did not differ significantly from those observed for 3 MG for the conditions shown in Table III C. This observation suggests that the inhibitory effects of K may be relatively nonspecific since, under the conditions of these experiments, approximately 70% of the Na influx is independent of the presence of sugar (see below). In the present series, guanidine was also investigated; it caused a 40% decrease in Na influx compared to the value observed in the presence of choline.

Kinetics of Sugar Influx

In order to determine the relation between sugar influx and concentration, experiments were carried out in which influxes were measured at four different sugar concentrations on tissue from the same animal. At a Na concentration of 130 mM, influxes of glucose, galactose, and 3 MG increased with increasing sugar concentration but showed a tendency toward saturation. A plot of sugar concentration divided by influx against the concentration gives straight lines with nonzero intercepts for the three sugars. Thus, influx can

be described in terms of Michaelis-Menten type kinetics and the relation between flux and concentration can be expressed as

$$J_s^i = \frac{J_s^{imax}[S]_m}{K_t + [S]_m} \quad (1)$$

or

$$\frac{[S]_m}{J_s^i} = \frac{K_t}{J_s^{imax}} + \frac{1}{J_s^{imax}}[S]_m \quad (2)$$

in which J_s^i is sugar influx, J_s^{imax} is maximal influx, $[S]_m$ is sugar concentration in the mucosal solution, and K_t is the "apparent Michaelis constant"

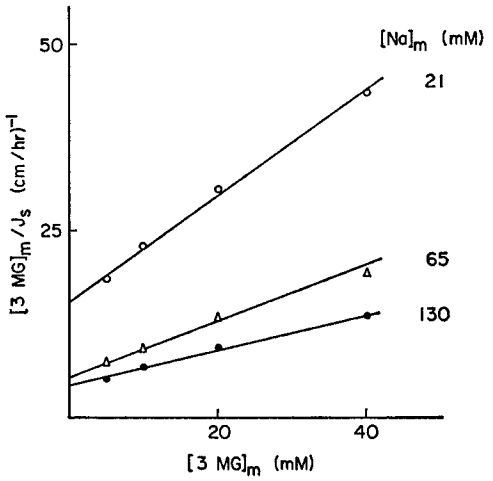

FIGURE 2. Relation between 3 MG influx and 3 MG concentration at different Na concentrations. Data plotted as indicated by equation 2. Each point is the average of at least 12 observations.

(the sugar concentration at which influx is half-maximal). All three sugars had approximately the same maximal velocity, 4.3 μmoles/hr cm^2. The values of K_t were 1.4 mM for glucose, 5.7 mM for galactose, and 18.2 mM for 3 MG.[2]

Similar experiments were carried out with 3 MG to examine the effects of varying Na concentration in the test solution. Because of the observed effect on 3 MG influx of preincubation in choline medium, two conditions were tested. In one series, tissues were preincubated in solution having the same composition as the test solution while in the other, all tissues were preincubated in Na-free choline medium. In these experiments, in which preincubations in choline medium and in Na medium were not carried out on tissue from the same animal, significant differences between the preincubation conditions were not apparent. Consequently the results of all experi-

[2]In all experiments of this type, the values of maximal rates have been determined by least squares analysis on plots of C/J_s vs. C. Subsequent analysis has shown that the values are very close to the ones obtained for the least squares fit to the original data expressed in the manner of equation 1.

ments were averaged to obtain the data illustrated in Fig. 2. The values obtained for maximal influx J_s^{imax} and K_t are summarized in Table IV. These data can also be used to examine the relation between 3 MG influx and Na concentration at constant 3 MG concentration. As shown in Fig. 3, these results can also be described in terms of Michaelis-Menten type kinetics so that

$$J_s^i = \frac{\mathcal{J}_s^{imax}[\mathrm{Na}]_m}{\mathcal{K}_t + [\mathrm{Na}]_m} \quad (3)$$

The values of maximal influx ($\mathcal{J}_s^{imax}$) and apparent Michaelis constant ($\mathcal{K}_t$) are given in Table IV.

TABLE IV

PARAMETERS OF 3 MG INFLUX

A. Constant Na, variable 3 MG		
$[Na]_m$	J_s^{imax}	K_t
mM	*μmole/hr cm²*	*mM*
130	4.3±0.3*	18.2±1.9
65	3.0±0.1	18.7±1.3
21	1.4±0.1	21.8±1.0
B. Constant 3 MG, variable Na		
$[S]_m$	$\mathcal{J}_s^{imax}$	$\mathcal{K}_t$
mM	*μmole/hr cm²*	*mM*
40	5.2±0.1	98.5±2.4
20	3.8±0.1	99.2±2.0
10	2.7±0.2	105.0±10.4
5	1.8±0.2	113.2±12.6

* Standard error of mean.

When the relation between 3 MG influx and concentration was investigated in Na-free media, no evidence for saturation could be obtained for sugar concentrations up to 40 mM; influx appeared to be a linear function of concentration. This observation suggests that 3 MG influx under these conditions may be due to simple diffusion. However, similar results would be obtained if the K_t for influx were extremely high so that a tendency toward saturation could not be detected without using much higher sugar concentrations. Such an approach seemed impractical since it would necessitate marked changes in either the osmolality or ionic composition of the solutions. Therefore other alternatives were used to examine this possibility.

Phlorizin is a potent inhibitor of intestinal sugar transport and, as shown in the upper portion of Table V, causes a marked reduction in 3 MG influx measured in the presence of 130 mM Na. Under similar conditions, addition of glucose to the test solution also causes a marked inhibition of 3 MG influx,

presumably as a result of competition for the transport system.[3] If 3 MG influx in the absence of Na is also due to a mediated process (i.e., a saturating process with a high K_t), these treatments might be expected to have similar effects. The results of experiments to test this possibility are given in Table V. Glucose has no effect on the influx of 3 MG in the absence of Na. However, this is not necessarily a definitive test since a minimal effect would be ex-

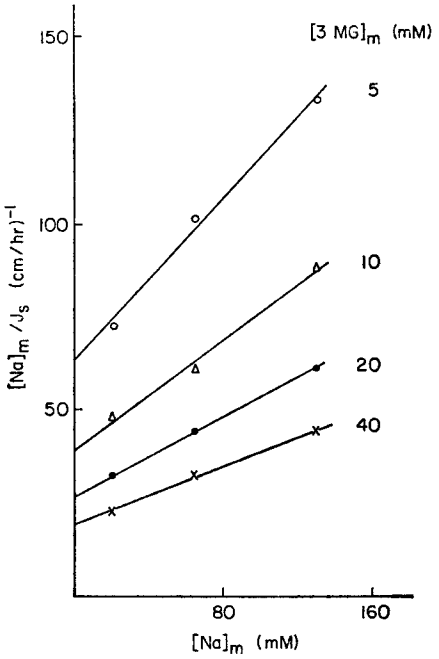

FIGURE 3. Relation between 3 MG influx and Na concentration at different concentrations of 3 MG.

pected if the K_t for glucose were also very high under these conditions. Phlorizin caused a small but statistically insignificant decrease in influx from Na-free solution suggesting that, at best, only a small fraction of influx under these conditions is the result of a mediated process. As a final test, we have compared the influxes of mannitol and 3 MG because these two substances have very similar diffusion characteristics but mannitol should not be subject to mediated transfer. The influxes did not differ significantly. All the results suggest that there is little mediated entry of 3 MG into the mucosal cells in the absence of Na.[4]

[3]The inhibition constant K_I for the effect of glucose on 3 MG influx calculated on the assumption of competitive inhibition is 1.1 mM, in good agreement with the value of 1.4 mM observed for the K_t of glucose influx.

[4]This result suggests that 3 MG influx should be corrected for a diffusion component before carrying out analyses of the type shown in Figs. 2 and 3. However, the correction appears to be small in most cases so that using it has little effect on the kinetic parameters and does not alter any of our conclusions. Consequently, uncorrected data have been used throughout because the exact magnitude of the correction is difficult to determine precisely.

A series of experiments were carried out to examine the effect of 3 MG in the mucosal cell on the influx of this sugar. Tissues were preincubated in normal Ringer solution containing 10 mM 3 MG which should lead to a sugar concentration in the cells of the order of 20 mM (1). Influx was then measured from a solution of the same composition. In 12 observations, influx into these preloaded tissues (0.74 ± 0.09 μmole/hr cm²) did not differ significantly from that into control tissues from the same animals preincubated in normal Ringer solution without added 3 MG (0.82 ± 0.10 μmole/hr cm²).

TABLE V

EFFECTS OF PHLORIZIN AND GLUCOSE ON 3 MG INFLUX

Principal cation test solution	Additions	3 MG	Influx	n*
		mM	μmole/hr cm²	
Na	—	40	3.29±0.59‡	4
Na	Phlorizin (10^{-4} M)	40	0.44±0.15	4
Na	—	20	1.83±0.08	8
Na	Glucose (20 mM)	20	0.18±0.05	8
Choline	—	20	0.25±0.04	6
Choline	Phlorizin (10^{-4} M)	20	0.15±0.03	8
Choline	—	40	0.52±0.07	12
Choline	Phlorizin (10^{-4} M)	40	0.42±0.06	12
Choline	—	20	0.25±0.04	6
Choline	Glucose (20 mM)	20	0.33±0.04	6

* Number of observations.
‡ Standard error of mean.

Relation between Sugar Influx and Na Influx

Since Na influx was also measured in most of the kinetic experiments discussed above, the effect of sugar on Na influx could be evaluated. Na influx was plotted against the simultaneously measured 3 MG influx for experiments at each Na concentration. Experiments involving the two different preincubation conditions were again combined because there appeared to be no difference between them. The results, shown in Fig. 4, indicate a linear relation between sugar and Na influxes. The slopes of the least squares lines through the points are essentially the same at all three Na concentrations and do not differ significantly from unity. Thus, the ratio of the sugar-dependent Na influx to 3 MG influx is approximately 1:1 at all Na concentrations tested.

DISCUSSION

Since the entry of sugars into the cell at the mucosal side of the intestine appears to be the primary step in the over-all process of active sugar transport, we felt it worthwhile to utilize our method for direct measurement of this influx in a further investigation of the transport process. This method has

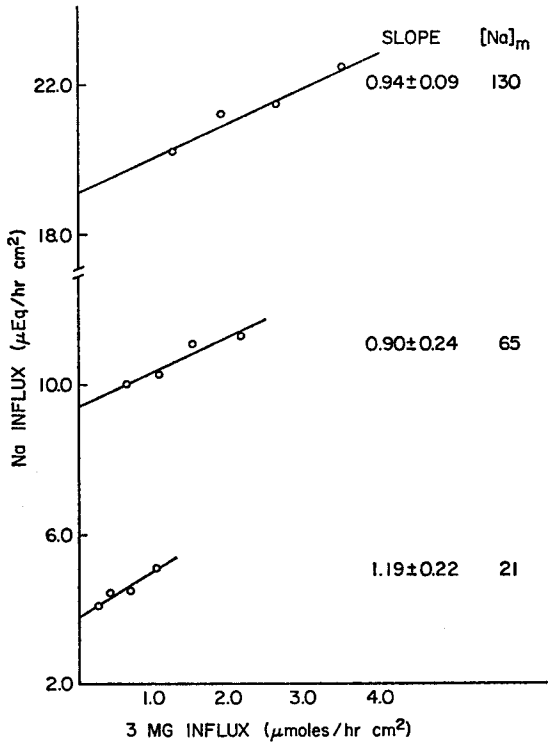

FIGURE 4. Relation between Na and 3 MG influxes. Each point represents the average of at least 12 observations in which both fluxes were measured simultaneously. The lines were determined by least squares analysis.

provided a substantial amount of information on amino acid influx in rabbit ileum (14, 17, 18) and since both sugar and amino acid transport are strongly Na-dependent, comparative data on these two nonelectrolytes in the same tissue obtained with the same method are important. In certain superficial aspects, the sugar influx system in rabbit ileum is similar to the system for neutral amino acid influx. Thus, both processes can be described by Michaelis-Menten type kinetics, both are dependent on Na concentration in the mucosal solution, and both involve simultaneous entry of Na and nonelectrolyte

into the mucosal cells. However, the details of the two processes differ in a number of aspects: (*a*) sugar influx appears to be appreciably more sensitive to Na removal than does amino acid influx; (*b*) in the absence of Na, amino acid influx is primarily via a mediated process while very little sugar influx appears to be mediated under these conditions; (*c*) reduction in Na concentration causes an increase in K_t for amino acids with little change in maximal influx while for sugars, maximal influx decreases with only a small increase in K_t; (*d*) the relation between nonelectrolyte-dependent Na influx and nonelectrolyte influx varies with Na concentration for amino acids but is constant for sugars. These factors must be taken into account in an effort to develop a model for sugar transport, and they indicate that the model discussed previously for amino acid transport (17) is inadequate for sugar transport.

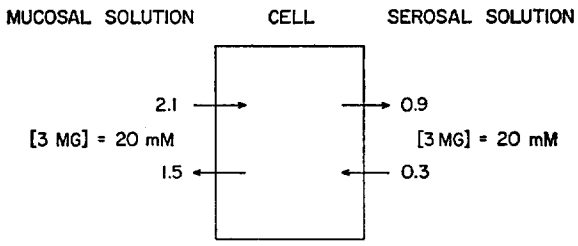

FIGURE 5. Unidirectional 3 MG fluxes across the mucosal and serosal membranes of rabbit ileum. The mucosal and serosal solutions contained 20 mM 3 MG and 130 mM Na. 3 MG fluxes are expressed in μmoles/hr cm^2.

Several aspects of the present results are somewhat different from those reported by others. Crane et al. (19) have found that sugar entry into rings of hamster intestine can be described by Michaelis-Menten kinetics and that reduction in Na concentration causes an increase in K_t with no change in maximal velocity, an effect opposite to that observed here. These differences could be due to species variation or to differences in method. In this respect, it is of interest to note that Kleinzeller et al. (20) have recently reported that sugar entry into rabbit kidney slices is Na-dependent and that Na removal decreases maximal velocity with little effect on K_t. The data of Schultz and Zalusky (5) on the effect of sugar on Na transport by rabbit ileum show a similar effect. The increased Na transport caused by sugar is a hyperbolic function of sugar concentration and reduction in Na concentration decreased the maximal effect with little change in "K_t." Thus, these observations on rabbit tissues appear to be consistent with the present findings.[5]

Crane et al. measured entry of sugar into rings of intestine over a 10 min

[5]In this respect it is of interest to note that Kolinska and Semenza (*Biochim. Biophys. Acta*, 1967, **146**: 181) have reported that the activity of intestinal sucrase from rat, hamster, and rabbit is stimulated by Na. Kinetic analysis has disclosed that Na decreases the K_m of rat and hamster sucrase with little or no change in the maximum velocity but that Na increases the maximum velocity of rabbit sucrase with little or no effect on the K_m.

period. Although the time interval seems relatively long for estimates of initial rates, they presented evidence indicating that the procedure was satisfactory. However, these measurements involve sugar entry into the tissue from both the mucosal and serosal sides. There is little direct information on the extent to which the serosal barrier is involved, but the present studies provide some initial insight. The observed transmural fluxes of 3 MG together with the influx across the brush border can be used to calculate fluxes across both faces of the mucosal cell as previously described (14). The results, at an external concentration of 20 mM, are shown in Fig. 5. Under these conditions, approximately 15% of the initial entry of 3 MG into a strip of rabbit mucosa would occur at the serosal side and the proportion could be appreciably higher under other conditions. For example, as Na concentration is lowered at constant sugar concentration, entry across the mucosal side decreases and that at the serosal side might remain nearly constant if this step is a Na-independent process. It is not clear, however, whether entry via the serosal side could account for the differences between our observations and those of Crane et al. As discussed below, these differences in kinetic detail do not necessarily affect the hypothesis that the sugar transport system is driven by a Na concentration difference between the cell and its environment since similar over-all behavior could occur in terms of both sets of observations.

There have been several investigations of the effects of sugar on electrical potential differences across the intestine (21–23) and attempts have been made to interpret these in terms of a coupled sugar-Na transport system. However, such interpretations are open to a number of serious questions and cannot be accepted in the absence of additional information. Some of the possible problems involved have been discussed by Schultz et al. (24). However, we should also note that changes in potential difference across the whole tissue need not bear any relation to Na entry into the cell, the process of particular interest. Further, in tortoise (25) and in hamster (26) intestine the change in potential difference caused by sugars occurs mainly across the serosal side of the cell. Thus, interpretation in terms of events at the mucosal border would seem open to considerable question. The advantage of the present approach is that it provides direct information on some of the fluxes of sugars and Na that are essential to understanding the transport system. Once sufficient information is available concerning these fluxes across the brush border, interpretation of some of the more complex experiments may be easier. Consequently we wish, at present, to concentrate primarily on analysis of the data on influx.

Model for Sugar Transport

In considering possible models for a transport system in the brush border membrane that could explain the interactions between Na and sugars, we

must take into account a variety of experimental observations. First, with respect to sugar transfer, the system must obey Michaelis-Menten type kinetics for sugar influx as a function of sugar concentration at constant Na and for sugar influx as a function of Na concentration at constant sugar. In both cases, the maximal influx varies with changes in concentration while the apparent Michaelis constant varies only slightly. Second, there appears to be very little mediated influx of sugar in the absence of Na. Third, sugar and Na influxes via the transfer mechanism are approximately equal at the Na concentrations tested. A model that will satisfy these requirements is shown in Fig. 6.

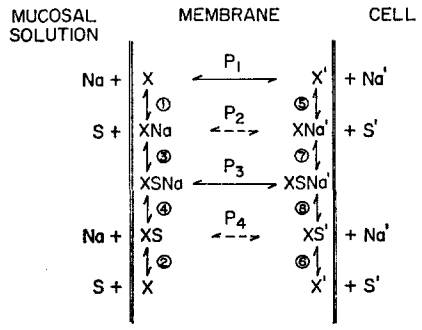

FIGURE 6. Model system for sugar and Na transport across the mucosal border of the intestinal epithelial cell.

We assume that there is a transport site confined to the membrane phase that can combine with both Na and sugar to form a ternary complex $XS\text{Na}$. This complex can be translocated across the membrane but the rates of translocation of the binary complexes represented by XS and $X\text{Na}$ are much slower than that of the ternary form. We also assume that the rates of all translocation steps are slow relative to the association-dissociation reactions at the membrane boundaries, that the rates of translocation of X and $XS\text{Na}$ are equal, and that effects of cellular Na are negligible. As described in more detail in the Appendix, the sugar influx J_s^i is then given by

$$J_s^i = \frac{2X_tP[S]_m[\text{Na}]_m}{2K_1K_3 + K_3[\text{Na}]_m + K_4[S]_m + 2[S]_m[\text{Na}]_m} \qquad (4)$$

in which X_t is total concentration of transport sites (assumed constant), P is the coefficient for translocation of X and $XS\text{Na}$, K_i is the dissociation constant for the i*th* reaction (Fig. 6), and $[S]_m$ and $[\text{Na}]_m$ are sugar and Na concentrations in the mucosal solution.

For experiments at constant $[\text{Na}]_m$ this expression indicates that

$$J_s^{i\max} = \frac{2X_tP[\text{Na}]_m}{K_4 + 2[\text{Na}]_m} \qquad K_t = \frac{K_3(2K_1 + [\text{Na}]_m)}{K_4 + 2[\text{Na}]_m}$$

For experiments at constant $[S]_m$ we find that

$$\mathcal{J}_s^{imax} = \frac{2X_tP[S]_m}{K_3 + 2[S]_m} \qquad \mathcal{K}_t = \frac{2K_1K_3 + K_4[S]_m}{K_3 + 2[S]_m}$$

These expressions predict hyperbolic relations between J_s^{imax} and $[Na]_m$ and between $\mathcal{J}_s^{imax}$ and $[S]_m$. As shown in Fig. 7, the experimental data conform to these predictions, and can be used to evaluate some of the parameters. The two lines give precisely the same values for the product X_tP (7.22 μmoles/hr cm^2) and the calculated values of K_3 and K_4 are 33 mM and 177

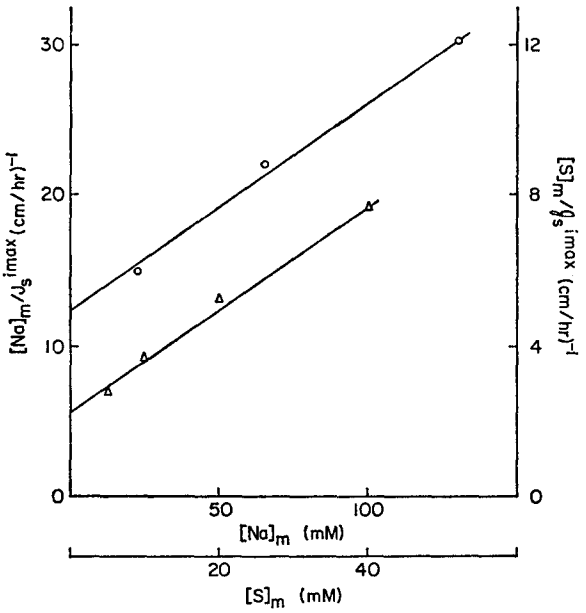

FIGURE 7. Maximal influx of 3 MG (J_s^{imax}) as a function of sodium concentration (0) and maximal influx of 3 MG ($\mathcal{J}_s^{imax}$) as a function of mucosal sugar concentration (Δ).

mM, respectively. With the use of these values we can obtain an estimate for K_1 from the expression for K_t and can then check the consistency of these values by comparing the calculated values of $\mathcal{K}_t$ with the observed. The results of these calculations, shown in Table VI, are satisfactory. The value of K_2 can be calculated from the requirement that $K_1K_3 = K_2K_4$. The resulting values are $K_1 = 60$ mM and $K_2 = 11$ mM. This model, with the indicated values of the dissociation constants, provides an adequate description of several aspects of the Na-sugar transport system. It also satisfies the requirement that sugar-dependent Na influx and sugar influx exhibit a 1:1 ratio (Fig. 4) since we have assumed that the rates of translocation of the forms XS and XNa are small relative to that of XSNa.

Although we have denoted the translocation steps by the symbol P, perhaps implying diffusion of a carrier, there is no compelling reason to postulate a mobile carrier for the transport system. We require only that the rate coefficients for translocation be relatively small and equal in the two directions. The assumption that the translocation rates for X and XSNa are equal is made for simplicity and does not appear to be required by any aspect of the present data. If the rates denoted by P_1 and P_3 differ, the coefficients K_3 and K_4 will be altered by a factor of $(1 + \alpha)/2$ where $\alpha = P_3/P_1$ but the values of K_1 and K_2 will be unaffected.

A number of other assumptions have been made in developing this model

TABLE VI
APPARENT MICHAELIS CONSTANTS

$[Na]_m$	K_t(obs)	K_t(calc)*
mM	mM	mM
130	18.2±1.9‡	18.6
65	18.7±1.3	19.6
21	21.8±1.0	21.0
$[S]_m$	$\mathcal{K}_t'$ (obs)	$\mathcal{K}_t'$(calc)*
40	98.5±2.4	97.7
20	99.2±2.0	102.7
10	105.0±10.4	108.1
5	113.2±12.6	112.6

* Calculated from equations in text using K_1 = 60 mM, K_2 = 11 mM, K_3 = 33 mM, and K_4 = 177 mM.
‡ Standard error of mean.

and some of these should be examined in more detail. There are several reasons for requiring that the translocations described by P_2 and P_4 be negligible. First, if they are not, the expressions for J_s^{max} and $\mathcal{J}_s^{max}$ do not have the required form. Second, sugar entry must be very small in the absence of Na; this will not occur if XS can be translocated at an appreciable rate. While this condition can be met by assuming that X is required to combine first with Na and then with sugar, other aspects of the data cannot be explained by such a model. Third, sugar flux and sugar-dependent Na flux must occur in a 1:1 ratio at several Na concentrations.

We have also neglected any effect due to changes in cellular Na concentration. This seems reasonable since in kinetic experiments with 3 MG at Na concentrations ranging from 21 to 130 mM, we could not detect a significant effect of reduction in cell Na concentration by preincubation of the tissue in Na-free choline medium. It seems possible that the small effect of preincubation in choline observed in paired experiments (Table II) may be a relatively nonspecific one not directly related to the transport system. As indicated by equation A-3, the expected effect of a reduction in cell Na

would be an increase in sugar influx rather than the decrease observed. At present, modifications of the model that would give rise to such a decrease in influx appear incompatible with other aspects of the data. Further investigations of the effects of changes in cellular Na, both increases and decreases, at different sugar concentrations are, however, clearly necessary. As indicated by equation A-3 neglect of effects of changes in cell Na means that the influence of the term $[Na']/K_5$ should be negligible. Calculations using the values of K_1, K_3, and K_4 given above indicate that if $[Na']/K_5 = 0.5$ under normal conditions, reduction of $[Na']$ to zero would alter sugar influx by 12% or less depending on sugar concentration. Such an effect would be quite difficult to detect with certainty by the present methods.

The full expression for sugar influx (equation A-3) also indicates that there should be a decrease in influx with an increase in sugar concentration within the cell, $[S']$. In experiments testing this effect, a small (10%) decrease in influx was actually observed although the effect was not statistically significant. These experiments were carried out with 130 mM Na and 10 mM sugar in the mucosal solution. If we assume that $[Na']/K_5 = 0.5$, that the K's on the cytoplasmic side are the same as at the outside, and that the cell sugar concentration is 20 mM, the model predicts a decrease in influx of only 14% for preloaded cells compared to cells containing no sugar. Again, the model seems reasonably consistent with the data but it also suggests further experiments. For example, it would be of interest to load the cells to a relatively high sugar concentration and measure influx from a low concentration; an appreciably larger effect should be observed.

Although the experimental results on which the model in Fig. 6 is based differ in several respects from those obtained by Crane and his coworkers with hamster intestine, the hypothesis that he has suggested for the role of Na in sugar transport remains feasible for rabbit intestine. Thus, in terms of the present model, sugar accumulation by the mucosal cells and subsequent transfer to the serosal solution could be due to the Na concentration difference between the cytoplasm and the mucosal solution and it may be unnecessary to postulate a direct link between sugar transport and metabolic energy. When the general expression for net sugar transfer (influx minus outflux) derived from the present model is used, it can be shown that if $[Na]_c < [Na]_m$ a net flux of sugar can occur from mucosal solution to cell even though $[S]_c > [S]_m$ where the subscript c denotes concentration in the cell. For example, if we assume that the K_i's are the same at both membrane boundaries, that $\alpha = 1$, that $[Na]_m = 130$ mM, and that $[Na]_c = 30$ mM, a net inward flux of 3 MG will occur if $[3\ MG]_m = 2$ mM and $[3\ MG]_c = 8$ mM.[6] The steady-state concentration ratio $[3\ MG]_c:[3\ MG]_m$ for rabbit ileum

[6]This conclusion is the more precise expression (equation A-3) based on the use of calculate fluxes. The maximum value of $[3\ MG]_c$ that can be achieved (zero net flux) is approximately 10 mM.

is 2.8 when $[3\ MG]_m = 2$ mM (1). Since the exit of sugar from the serosal side will reduce this ratio from the maximum value predicted by events at the mucosal side, these predictions of the model seem satisfactory. Although the present data would be consistent with the hypothesis that the Na gradient provides the energy for active sugar transport, they do not provide unequivocal evidence for such an hypothesis. None of the experiments rules out the possibility that there is a direct link to metabolic energy; for example, via ATP splitting, at some point in the over-all transfer process. A decision on this point must await further studies on sugar fluxes, in particular, careful examination of the effects of various metabolic inhibitors.

In terms of this model, the role of Na in sugar transport appears to be rather different than in amino acid transport. In the amino acid system previously proposed (12), Na combines with a site that is already complexed with amino acid and this leads to increased stabilization. The result is a shift of total carrier toward the forms combined with amino acid and hence enhanced influx. In such a system, removal of Na is equivalent to the addition of a competitive inhibitor. That is, the effect on influx of Na removal can be overcome by increasing the amino acid concentration. In the sugar system, removal of Na is approximately equivalent to adding a noncompetitive inhibitor; the effect cannot be overcome by increasing sugar concentration. The reason is apparent from Fig. 6. The role of Na is to produce a form suitable for translocation, *XS*Na; in the absence of Na, this complex cannot be formed and hence sugar transfer does not occur (or is extremely slow). It is clear from the values of the K_i's that combination of Na with X or XS does not provide increased stability of the system as it does in the amino acid system. This point is indicated by the finding that $K_3 > K_2$. We might also note that combination of X with sugar does not enhance the ability of the site to combine with Na since $K_4 > K_1$.

The factors that might be involved in the peculiar requirements regarding translocation of various complexes remain unclear. In order to fit the data available, we must require that *X*Na and XS are translocated very poorly compared to X and *XS*Na. The reasons cannot involve charge of the species because *X*Na and *XS*Na should have the same charge as should X and XS. One might suggest that a particular configuration is required for translocation and that it is achieved only when X is combined with both Na and S, although we are then hard pressed to explain why the completely uncombined form X can cross readily. In this respect, we should, however, keep in mind the point that the data can be fit reasonably well by assuming that *XS*Na can be translocated at a rate appreciably greater than X. It is perhaps of interest in this regard to note that the values of the dissociation constants indicate that *XS*Na is actually relatively less stable than are XS and *X*Na, and this property may be in some way associated with the relative translo-

cation rates of the three complexes. These considerations tend to suggest that in the sugar system, there is a distinction between the process of binding of sugar to the transport site and the actual process of translocation since binding alone (formation of XS) is not necessarily followed by translocation. However, further speculation along these lines seems unproductive; additional studies are clearly needed in order to obtain further information regarding the validity of this model before considering possible mechanisms that might give rise to the postulated properties of the system.

APPENDIX

We wish to examine the behavior of the model shown in Fig. 6, using the assumption that the translocation steps denoted by P_i are slow relative to the reactions occurring at the membrane boundaries. Under these conditions, the reactions may be considered at equilibrium so that the concentrations are determined by a series of dissociation constants of the form

$$K_1 = \frac{[\mathrm{Na}][X]}{[X\mathrm{Na}]} \qquad K_2 = \frac{[S][X]}{[XS]}$$

$$K_3 = \frac{[S][X\mathrm{Na}]}{[XS\mathrm{Na}]} \qquad K_4 = \frac{[\mathrm{Na}][XS]}{[XS\mathrm{Na}]}$$

and a similar set for the ($'$) side of the membrane. It is apparent from the above equations that

$$K_1K_3 = K_2K_4$$

so that of the eight expressions for the K_i's, six are independent. We also assume that the total concentration of "transport site" or carrier is constant so that

$$X_t = \frac{[X] + [X']}{2} + \frac{[XS] + [XS']}{2} + \frac{[X\mathrm{Na}] + [X\mathrm{Na}']}{2} + \frac{[XS\mathrm{Na}] + [XS\mathrm{Na}']}{2}$$

Finally, if the system is in a steady state,

$$P_1([X'] - [X]) = P_2([X\mathrm{Na}] - [X\mathrm{Na}']) + P_3([XS\mathrm{Na}] - [XS\mathrm{Na}']) + P_4([XS] - [XS'])$$

There are thus eight equations to be solved for the eight unknown concentrations. The influxes of sugar (J_s^i) and Na (J_{Na}^i) are then obtained from the following expressions:

$$J_s^i = P_4[XS] + P_3[XS\mathrm{Na}] \qquad \text{(A-1)}$$

$$J_{\mathrm{Na}}^i = P_2[X\mathrm{Na}] + P_3[XS\mathrm{Na}] \qquad \text{(A-2)}$$

We have observed that $J_{\mathrm{Na}}^{\mathrm{total}} = J_{\mathrm{Na}}^i + J_{\mathrm{Na}}^{i'}$ where J_{Na}^i is sugar-dependent Na influx and $J_{\mathrm{Na}}^{i'}$ is sugar-independent influx assumed to occur via a path or paths in parallel to

that shown in Fig. 6. Also, according to Fig. 4, $J_s^i = J_{Na}^i$. Consequently, the terms represented by $P_4(XS)$ and $P_2(X\text{Na})$ in equations A-1 and A-2 must be small. We shall therefore assume that P_2 and P_4 are much smaller than P_1 and P_3.

Under these conditions, the general expression for J_s^i is

$$J_s^i = \frac{2X_tP_3[\text{Na}][S]}{K_1K_3 + K_4[S] + K_3[\text{Na}] + [\text{Na}][S] + K_1K_3\left\{1 + \alpha\frac{[S][\text{Na}]}{K_1K_3}\right\}} \cdot\left\{1 + \frac{[S']}{K_6} + \frac{[\text{Na}']}{K_5} + \frac{[S'][\text{Na}']}{K_5K_7}\right\}\Big/\left\{1 + \alpha\frac{[S'][\text{Na}']}{K_5K_7}\right\} \quad \text{(A-3)}$$

where $\alpha = P_3/P_1$. In most experiments, $[S'] = 0$ and since we have observed minimal effects of variation in $[\text{Na}']$ we can assume that the term $[\text{Na}']/K_5$ can be neglected. Equation A-3 then becomes

$$J_s^i = \frac{2X_tP_3[\text{Na}][S]}{2K_1K_3 + K_4[S] + K_3[\text{Na}] + (1 + \alpha)[\text{Na}][S]} \quad \text{(A-4)}$$

The application of this expression to the experimental data is discussed in the text. In most cases, we have also assumed that $\alpha = 1$. Expressions for outflux of sugar can be obtained by interchanging $[S]$ and $[S']$, $[\text{Na}]$ and $[\text{Na}']$, K_1 and K_5, K_2 and K_6, K_3 and K_7, and K_4 and K_8.

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