

# Ion Transport in Isolated Rabbit Ileum

## II. *The interaction between active sodium and active sugar transport*

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**ABSTRACT** The addition of actively transported sugars to the solution bathing the mucosal surface of an *in vitro* preparation of distal rabbit ileum results in a rapid increase in the transmural potential difference, the short-circuit current, and the rate of active Na transport from mucosa to serosa. These effects are dependent upon the active transport of the sugar *per se* and are independent of the metabolic fate of the transported sugar. Furthermore, they are inhibited both by low concentrations of phlorizin in the mucosal solution and by low concentrations of ouabain in the serosal solution. The increase in the short-circuit current,  $\Delta I_{sc}$ , requires the presence of Na in the perfusion medium and its magnitude is a linear function of the Na concentration. On the other hand,  $\Delta I_{sc}$  is a saturable function of the mucosal sugar concentration which is consistent with Michaelis-Menten kinetics suggesting that the increase in active Na transport is stoichiometrically related to the rate of active sugar transport. An interpretation of these findings in terms of a hypothetical model for intestinal Na and sugar transport is presented.

Previous studies (1) have indicated that when isolated segments of distal rabbit ileum are perfused with a physiological buffer, the serosal surface is electrically positive with respect to the mucosal surface and the initial potential difference (PD) in the presence of glucose averages approximately 9 mv. We have further demonstrated that the transmural PD and the short-circuit current ( $I_{sc}$ ) result from the active transport of Na from mucosa to serosa, that this process is dependent upon intact aerobic metabolic pathways, and that it is inhibited by low concentrations of ouabain in the serosal solution. The unidirectional serosa-to-mucosa Na flux,  $\Phi_{s,m}^{Na}$ , on the other hand, may be attributed to passive diffusion with no evidence for the presence of exchange diffusion or the influence of solvent-drag.

In the course of these studies, it was observed that the addition of actively

transported sugars, and/or amino acids, to the mucosal solution resulted in a rapid increase in both the  $I_{sc}$  and net active Na transport. These effects are independent of the metabolic fate of the added solute. Preliminary reports of these findings have been published (2, 3). The present communication is concerned with a more detailed investigation of the interaction between active intestinal Na and sugar transport.

#### METHODS

Segments of distal ileum from New Zealand white rabbits (2.5 to 4 kg) were utilized in these studies. The surgical techniques, perfusion apparatus, and bathing media, as well as the methods employed for the determination of transmural PDs, the  $I_{sc}$ , and

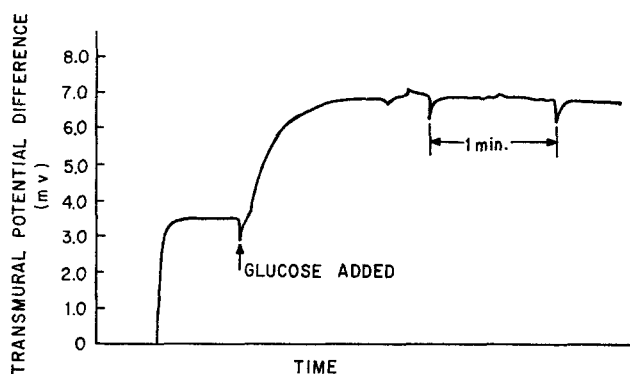


FIGURE 1. Time course of the increase in the transmural PD following the addition of glucose (final concentration = 10 mM) to the mucosal and serosal reservoirs previously containing a glucose-free medium. The three downward spikes are timing artifacts.

unidirectional Na fluxes, have been previously described in detail (1). All studies were carried out at 38.5°C, and the pH of the bathing media varied from 7.2 initially to 6.7 after a period of 45 to 60 minutes.

All chemicals used were reagent grade, and the 3-O-methylglucose and  $\alpha$ -D-methylglucose were demonstrated to be glucose-free by means of paper chromatography (4, 5).

#### RESULTS

##### *Effect of Sugars on the $I_{sc}$ and Active Na Transport*

As reported previously (1), the initial transmural PD and  $I_{sc}$  in the absence of glucose averaged  $5.4 \pm 0.1$  mv and  $83 \pm 1 \mu a$  (114 experiments)<sup>1</sup> respectively. Both values are significantly different from those of  $9.1 \pm 0.4$  mv and  $136 \pm 6 \mu a$  (36 experiments) observed in the presence of glucose (11 mM). When glucose was added to the mucosal and serosal solutions which were

<sup>1</sup> All errors will be expressed as standard errors of the mean unless otherwise designated.

initially glucose-free, a rapid and marked increase in both the PD and  $I_{sc}$  was observed. The effect of the addition of glucose on the transmural PD, as shown in Fig. 1, commenced within 5 seconds and the maximum PD was usually achieved within 1 minute. The increase in the  $I_{sc}$  followed a similar time course (Fig. 2).

Although the direction of the response was not unexpected, the rapidity of the glucose effect suggested a role for external glucose in Na transport in addition to supplying a source of metabolic energy. Indeed, since the glucose effect commenced within the mixing time of the perfusion system, a surface effect was suggested. In Table I we have summarized the results of a series of experiments, utilizing a variety of sugars and sugar analogs, designed to

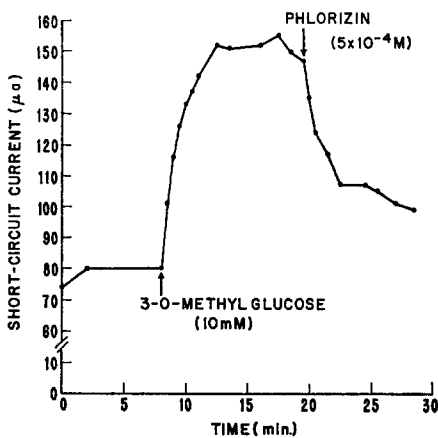


FIGURE 2. The time course of the increase in the  $I_{sc}$  following the addition of 3-0-methylglucose to a sugar-free perfusion medium. The effect of phlorizin ( $5 \times 10^{-4}$  M), added to the mucosal solution alone, is also shown.

determine whether the rapid effect on the PD and  $I_{sc}$  is the result of either the entrance of the sugar into metabolic pathways, with the subsequent production of ATP, or the active transport of the sugar *per se*. It should be noted that the only requirement for the sugar-induced increase in the  $I_{sc}$  is that the added sugar is itself actively transported by the intestine and that the increase in the  $I_{sc}$  is not dependent upon the subsequent metabolic fate of the transported sugar. Thus, fructose, which is readily metabolized by the intestine but is not actively transported (6, 7), had no effect on the  $I_{sc}$ . On the other hand, 3-0-methylglucose, which is not metabolized but is actively transported (6, 7), produced a rapid rise in the  $I_{sc}$  as shown in Fig. 2. Furthermore, it is known that fructose after passively entering the intestinal cell is converted to glucose which may then enter the metabolic cycle (6) and that not only is 3-0-methylglucose not utilized by the intestine, but it also can be recovered quantitatively in these systems (8). Therefore, the effect on the  $I_{sc}$  cannot be linked to an early step in sugar metabolism but must be attributed to the transport process itself.

Further evidence implicating the active intestinal sugar transport mechanism in the stimulation of the  $I_{sc}$  is derived from the observation that this effect was obtained only when the actively transported sugar was added to the mucosal solution; addition to the serosal solution alone was ineffective. Kinter (9) and McDougal *et al.* (10) have presented convincing evidence that the mechanism mediating the active uptake of glucose by the intestinal cell is located on or near the mucosal or brush border of the cell.

TABLE I  
EFFECT OF SUGARS ON THE SHORT-CIRCUIT  
CURRENT IN ISOLATED RABBIT ILEUM\*

Sugar	Active transport (6,7)	Metabolized (6,7)	Effect on $I_{sc}†$
Glucose	Yes	Yes	+
D-Galactose	Yes	Yes	+
3-O-Methyl-D-glucose	Yes	No	+
$\alpha$ -D-Methylglucose	Yes	—	+
Fructose	No	Yes	0
D-Mannose	No	Yes	0
D-Ribose	No	Yes	0
6-Deoxy-L-galactose	No	No	0
2-Deoxy-D-glucose	No	—	0

\* The sugars (final concentration 10 mM) were added simultaneously to the reservoirs containing the mucosal and serosal perfusing solutions.

† A positive effect (+) on the  $I_{sc}$  indicates a rapid increase to 50 per cent or more over the base line value commencing within 10 seconds after the addition of the sugar to the perfusing solutions; (0) indicates no change in the  $I_{sc}$  following the addition of the sugar.

We have previously demonstrated that there is good agreement between the rate of net active intestinal Na transport and the  $I_{sc}$ , expressed in units of flux of a monovalent cation, both in the presence and in the absence of glucose. These data are presented in Table II, together with the average values of the unidirectional ( $\Phi_{ms}^{Na}$  and  $\Phi_{sm}^{Na}$ ) and net Na fluxes ( $\Phi_{net}^{Na}$ ) and the  $I_{sc}$  determined in the presence of 20 mM 3-O-methylglucose using  $Na^{24}$  (1). It is clear from the data in Table II, and further evidence which will be presented below, that the increase in  $I_{sc}$  and PD observed after the addition of an actively transported sugar to the mucosal solution may be attributed to an increase in the rate of active Na transport from mucosa to serosa.

Fig. 3 shows the relationship between the time which has elapsed after the onset of perfusion and the increase in the  $I_{sc}$  which follows the addition of an actively transported sugar. Glucose was used in the experiment shown; however, similar results have been obtained using 3-O-methylglucose. The two segments of distal ileum were obtained from the same animal and studied

TABLE II  
COMPARISON OF NET Na FLUXES AND SHORT-CIRCUIT  
CURRENT IN ISOLATED RABBIT ILEUM\*

$\Phi_{ms}^{Na}$	$\Phi_{sm}^{Na}$	$\Phi_{net}^{Na}$	$I_{sc}$
$\mu\text{mol}/\text{cm}^2 \text{ hr.}$			
<i>No Glucose</i>			
9.5 $\pm$ 0.2 (42)	6.7 $\pm$ 0.3 (33)	2.8 $\pm$ 0.4	2.6 $\pm$ 0.1 (75)
<i>10 mM Glucose</i>			
9.4 $\pm$ 0.2 (60)	5.7 $\pm$ 0.2 (54)	3.7 $\pm$ 0.3	3.5 $\pm$ 0.1 (114)
<i>20 mM 3-O-methylglucose</i>			
10.5 $\pm$ 0.2 (28)	6.6 $\pm$ 0.2 (28)	3.9 $\pm$ 0.3	3.7 $\pm$ 0.1 (56)

\* The number of observations is indicated in parentheses. The subscripts *m* and *s* refer to the mucosal and serosal solutions respectively.  $\Phi_{ms}^{Na}$  is the unidirectional Na flux from mucosa to serosa.

simultaneously as described previously (1). In the absence of glucose the  $I_{sc}$  fluctuated about a relatively constant value for periods which often exceeded 2 hours. Following stimulation, the  $I_{sc}$  achieved a maximal value within 1 minute and thereafter declined at approximately 1  $\mu\text{a}/\text{min}$ . If the control tissue (*i.e.*, glucose absent) was stimulated at some later time, the increase in the  $I_{sc}$  was considerably reduced and the maximal level reached closely approximated the value to which the previously stimulated tissue had de-

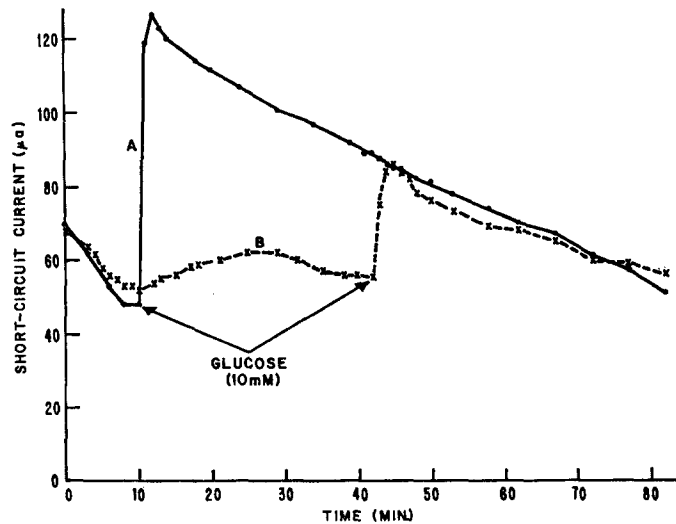


FIGURE 3. The effect of the age of the *in vitro* preparation on the ability of the tissue to respond to the addition of an actively transported sugar. Tissues A and B were adjacent segments of distal ileum obtained from the same animal (1). The arrows indicate the times at which glucose was introduced.

clined. Thereafter, the  $I_{sc}$  of both tissues continued to decline at approximately equal rates until the prestimulation level was reached. These results indicate that the ability of the tissue to respond to the addition of an actively transported sugar with an increase in the  $I_{sc}$  diminishes with time, and that this accounts for the gradual decline in the  $I_{sc}$  observed in the presence of glucose but not in its absence (1). Several factors in the perfusion medium have been examined in an attempt to explain this time-dependent decline in the ability of the tissue to respond to the addition of actively transported sugars, without success. The increase in the  $I_{sc}$  is not affected by the omission of  $PO_4$  from the bathing medium; thus, the possibility that this ion becomes

TABLE III  
TIME COURSE OF NET Na TRANSPORT AND  
SHORT-CIRCUIT CURRENT IN THE PRESENCE AND  
IN THE ABSENCE OF SUGAR\*

Period	Sugar absent			3-o-Methylglucose (20 mM)		
	$\Phi_{net}^{Na}$	$I_{sc}$	$\Phi_{net}^{Na}/I_{sc}$	$\Phi_{net}^{Na}$	$I_{sc}$	$\Phi_{net}^{Na}/I_{sc}$
	$\mu\text{mol}/\text{cm}^2 \text{ hr.}$			$\mu\text{mol}/\text{cm}^2 \text{ hr.}$		
1	3.1	3.1	1.00	3.8	3.9	0.97
2	3.0	3.2	0.94	4.7	3.8	1.24
3	2.9	3.3	0.89	3.8	3.6	1.05
4	3.0	3.1	0.97	3.3	3.4	0.97
5	2.6	2.8	0.93	3.3	3.2	1.03
6	2.5	2.8	0.89	2.8	3.1	0.90
7	2.9	2.8	1.04	2.5	2.9	0.86
Average	$2.8 \pm 0.1$	$3.0 \pm 0.2$	$0.93 \pm 0.2$	$3.5 \pm 0.3$	$3.4 \pm 0.1$	$1.03 \pm 0.5$

\* Sampling commenced 20 minutes after the addition of the  $Na^{24}$  and the sugar to the perfusion medium, after which time a steady-state Na flux is achieved (1).

rate-limiting during the course of the experiment is unlikely. Furthermore, the increase in the  $I_{sc}$  is essentially independent of the pH of the bathing medium over the range 7.4 to 6.2. At present, we cannot exclude the possibilities that morphological alterations of the microvilli constituting the brush border of the cell, or that limiting concentrations of either essential intermediates or hormonal factors are responsible for this effect. It is of interest to note that Fisher and Parsons (11) have reported that the rate of glucose transport by *in vitro* segments of rat small intestine decreases rapidly with time.

The data presented in Table II are average values obtained during the course of experiments of 55 minute duration. Because of the spontaneous decline in the  $I_{sc}$  in the presence of sugars, the agreement between the av-

average values of  $\Phi_{\text{net}}^{\text{Na}}$  and  $I_{sc}$  in the presence of actively transported sugars does not establish that net Na transport and the  $I_{sc}$  follow the same time course. In Table III the net Na fluxes and the  $I_{sc}$  are given for 5 minute sampling periods during two representative experiments, one in the presence and one in the absence of sugar. The agreement between the net Na flux and the average  $I_{sc}$  during each 5 minute period, and their decline in the presence of 3-0-methylglucose should be noted.

#### *The Effects of Phlorizin and Ouabain*

It is well established that phlorizin, in low concentrations, inhibits glucose transport in a wide variety of cells (6, 7). Newey *et al.* have recently demonstrated that, in the rat small intestine, low concentrations of phlorizin prevent the entry of glucose into the epithelial cells from the mucosal side and have no effect on the uptake of glucose from the serosal solution (12). These authors have further reported that, in higher concentrations, phlorizin inhibits endogenous metabolism. The effect of the addition of phlorizin ( $5 \times 10^{-4}$  M) to the mucosal solution after the  $I_{sc}$  had been stimulated by the addition of 3-0-methylglucose is shown in Fig. 2. In all instances the addition of phlorizin resulted in an immediate decline in the  $I_{sc}$  which rapidly reached values approximating those observed in the absence of actively transported sugars. Furthermore, the presence of phlorizin in the mucosal solution, while having no effect on the  $I_{sc}$  in the absence of glucose, completely prevented the increase in the  $I_{sc}$  following the addition of actively transported sugars. This inhibitory action was observed only when the glycoside was present in the mucosal solution; addition of phlorizin to the serosal solution alone was ineffective. The possibility that the action of phlorizin is secondary to metabolic inhibition can be ruled out in two ways. First, phlorizin has no effect on the  $I_{sc}$  in the absence of actively transported sugars whereas metabolic inhibitors markedly decrease the  $I_{sc}$  under these conditions (1). Second, phlorizin does not reverse or prevent the increase in the  $I_{sc}$  which follows the addition of actively transported amino acids to the mucosal solution (3, 13).

These data indicate that the increases in the  $I_{sc}$  and net Na transport are dependent upon the actual active transport of sugars *per se*, and not simply upon the *presence* of these sugars in the mucosal solution.

We have previously reported that the addition of ouabain ( $5 \times 10^{-4}$  M) to the serosal solution markedly inhibits the  $I_{sc}$  and net Na transport (1). This concentration of ouabain, in the serosal solution, also prevents or reverses the increase in the  $I_{sc}$  observed after the addition of actively transported sugars. If ouabain is added to the mucosal solution alone, this inhibitory action is either absent or much reduced. These observations indicate that either a single, ouabain-sensitive mechanism is responsible for active Na trans-

port both in the presence and in the absence of sugars, or, if two mechanisms are present, both are ouabain-sensitive.

#### *The Effect of Temperature on the Na-Sugar Interaction*

The effect of temperature on the response of the  $I_{sc}$  to the addition of actively transported sugars was studied in four experiments, as described previously (1). The response of the  $I_{sc}$  to the addition of glucose is highly temperature-sensitive and averaged  $7 \mu a$  at  $22.5^\circ C$  as compared with  $61 \mu a$  at  $38.5^\circ C$ . The calculated apparent activation energy of  $3.0 \times 10^4$  cal/mole ( $Q_{10} =$

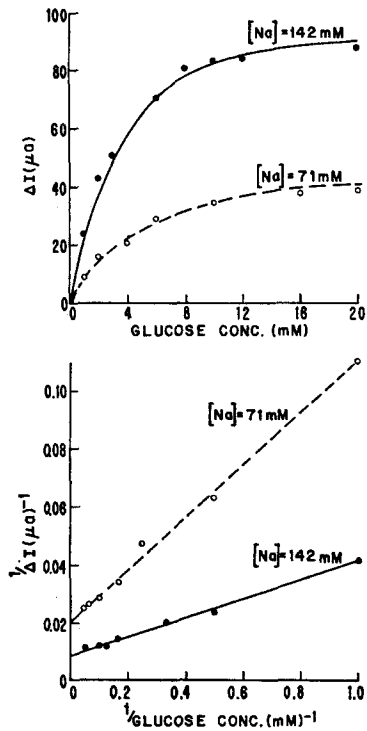


FIGURE 4. Upper, the increase in the  $I_{sc}$  ( $\Delta I_{sc}$ ) as a function of the concentration of glucose in the presence of 71 and 142 mM Na. Lower, reciprocal plots of the above data. The lines were obtained by the method of least squares.

4.9) is quite high compared with those reported for intestinal sugar transport (14, 15) and other active transport systems (16). This may be attributable to the fact that the increase in the  $I_{sc}$  is the result of the interaction between two carrier-mediated transport systems.

#### *The Effect of Sugar Concentration on the Na-Sugar Interaction*

In order to determine the effect of the concentration of the added sugar on the subsequent increase<sup>2</sup> in the  $I_{sc}$  ( $\Delta I_{sc}$ ), the following series of experiments

<sup>2</sup>  $\Delta I_{sc}$  is the difference between the maximal  $I_{sc}$  achieved following stimulation and the  $I_{sc}$  immediately prior to the addition of the sugar to the reservoirs.



was undertaken: the ileal segments were perfused with the glucose-free medium (Na concentration = 142 mM), and, after a 10 minute period, small aliquots of a glucose-containing medium (0.2 – 0.4 M) were added to the known volume of medium in the mucosal and serosal reservoirs. The maximum level achieved by the  $I_{sc}$  was recorded, and then another aliquot of the glucose medium was introduced. Since the  $I_{sc}$  usually achieved its maximum within 1 minute, additions were made at approximately 2 minute intervals, and the experiment was terminated after three such additions. During this brief period the spontaneous decline in the  $I_{sc}$  is negligible compared with the  $\Delta I_{sc}$  and thus the time effect described above can be ignored.

TABLE IV  
THE KINETICS OF THE Na-SUGAR INTERACTION

Sugar	$K_I^*$	$\Delta I_{max}^*$	$\Delta \Phi_{max}^{Na}$	$K_s \ddagger$
	mM	$\mu$	$\mu\text{mol}/\text{cm}^2 \text{ hr.}$	mM
Glucose	4 ± 1	111 ± 26	3.7 ± 0.9	2.5 (20), 7 (19), 9 (11)
Galactose	10 ± 2	153 ± 32	5.1 ± 1.0	12 (20), 35 (18)
3-0-Methylglucose	17 ± 3	141 ± 30	4.8 ± 1.0	10 (20)

\* Calculated from least squares fit of Lineweaver-Burk plots.

‡ Data obtained from the literature. References are given in parentheses.

The results of 6 experiments, using glucose, are shown in Fig. 4. Each point represents the average of at least 2 determinations. The upper portion of this figure suggests that the relationship between  $\Delta I_{sc}$  and the added glucose concentration is consistent with Michaelis-Menten kinetics for saturable enzyme (or carrier) systems. This impression is confirmed by a reciprocal plot of the data, after the method of Lineweaver and Burk (17), shown in the lower half of Fig. 4. A similar relationship has been found for 3-0-methylglucose and galactose. The concentrations of glucose, 3-0-methylglucose, and galactose which produce half-maximal stimulations of the  $I_{sc}$ ,  $K_I$ , and the calculated maximum  $\Delta I_{sc}$ ,  $\Delta I_{max}$ , expressed both in electrical terms and in units of net Na flux,  $\Delta \Phi_{max}^{Na}$ , are given in Table IV.

Finally, if an actively transported sugar was added after the  $I_{sc}$  had been maximally stimulated by the previous addition of another actively transported sugar, no further increase in the  $I_{sc}$  was observed. On the other hand, if the  $I_{sc}$  was stimulated by the addition of an actively transported amino acid, the further addition of an actively transported sugar produced a second increase in the  $I_{sc}$ .

It is well established that (a) the rate of active sugar transport by small intestine is a saturable function of the mucosal sugar concentration, and (b) all actively transported sugars exhibit mutual inhibition and appear to share

a common carrier mechanism (6, 7, 11, 18, 21, 22). In Table IV we have listed the values, obtained from the literature, for the sugar concentrations,  $K_s$ , which result in half-maximal rates of active sugar transport by the mammalian small intestine. Although  $K_s$  varies considerably depending upon the animal studied and the methods employed, the similarity between  $K_I$  and  $K_s$  and the evidence that a single, saturable mechanism is involved in the Na-sugar interaction further support the contention that the mechanism by which Na transport is increased is intimately linked with the mechanism for active intestinal sugar transport.

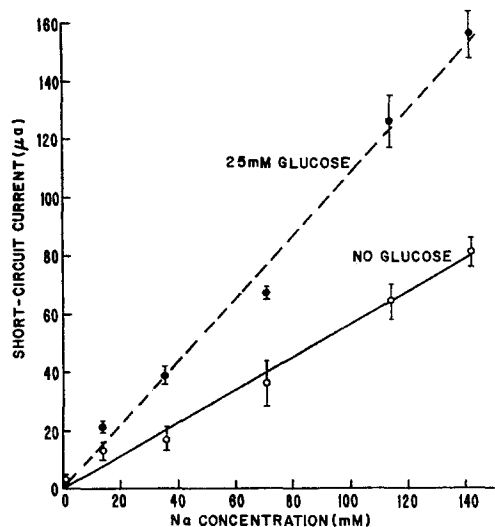


FIGURE 5. The  $I_{sc}$  as a function of the Na concentration of the perfusion medium before and after the addition of 25 mM glucose.

#### *The Effect of Na Concentration on the Na-Sugar Interaction*

Fig. 5 shows the results of a series of experiments in which 25 mM glucose was added, after a 10 minute stabilizing period, to tissue bathed by a glucose-free medium in which the Na concentration was varied by substitution with K. The  $I_{sc}$ , both in the presence and in the absence of glucose, and the  $\Delta I_{sc}$  are linear functions of the Na concentration of the medium. Furthermore, the  $I_{sc}$  and the  $\Delta I_{sc}$  do not differ significantly from zero in the absence of Na. These data support the conclusions drawn from the Na flux data, presented above, that the  $I_{sc}$  in both the presence and absence of glucose (and, thus, the  $\Delta I_{sc}$ ) may be attributed to net Na transport. Since the Cl concentration remained constant in these experiments, these results also indicate that a significant independent Cl current does not exist.

The data presented in Fig. 5 suggest that  $\Delta I_{max}$  is a linear function of the Na concentration, providing 25 mM glucose is sufficient to elicit a nearly maximum  $\Delta I_{sc}$  at all Na concentrations. To test this point, experiments were carried out in which the  $\Delta I_{sc}$  was determined as a function of the added glu-

cose concentration in the presence of 71, 106, and 142 mM Na. The data for 8 experiments in the presence of 71 mM Na are shown in Fig. 4 (dashed line). The calculated values of  $K_T$  in the presence of 71, 106, and 142 mM Na were  $4.0 \pm 1.3$  mM,  $5.0 \pm 2.5$  mM, and  $3.6 \pm 1.3$  mM, respectively; these values do not differ significantly. The calculated  $\Delta I_{\max}$  is plotted as a function of the Na concentration in Fig. 6. This plot confirms the above impression that the  $\Delta I_{\max}$  is a linear function of the Na concentration in the bathing medium.<sup>3</sup>

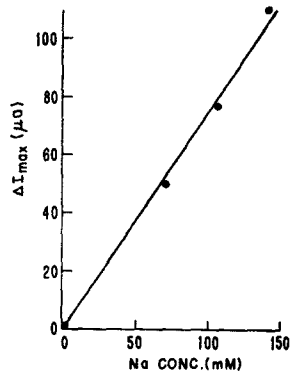


FIGURE 6.  $\Delta I_{\max}$  as a function of the Na concentration of the perfusion medium.

#### DISCUSSION

The dependence of active transport systems on chemical energy derived from cellular metabolism is so widely accepted that it has become common practice to attribute the effects of the presence or absence of glucose on active transport systems to the function of glucose as a source of metabolic energy. The results of the present experiments underline the danger of assigning glucose to the role of a "nutrient" in the absence of further evidence.

In the instance of the small intestine numerous observations have been reported concerning the effect of glucose on bioelectric phenomena, ion transport, and water movement. Clarkson *et al.* (23) have reported that the transmural PD across midjejunal segments of rat intestine rapidly undergoes a 60 per cent decrease when the tissue is transferred to a glucose-free medium. On returning the tissue to a medium containing glucose the original PD is restored within 5 seconds. Curran (24) has reported that net transport of Na and Cl across *in vitro* rat small intestine is diminished in the absence of glucose. Smyth and Taylor (25) and Fisher (26) have reported that intestinal water absorption is dependent upon the presence of glucose in the mucosal solution, and, on the basis of this and other observations, have suggested that water absorption is an active process. Parsons, Smyth, and Taylor (27) have shown

<sup>3</sup> It follows that if  $\Delta I_{\max}$  is a linear function of the Na concentration, and  $K_T$  is independent of the Na and sugar concentrations, then  $\Delta I_{sc}$  is also a linear function of the Na concentration.

that phlorizin inhibits intestinal absorption of both glucose and water, and have concluded that water transport is dependent on cellular glucose metabolism with the effect of phlorizin attributable to its inhibition of glucose uptake by the cell. Finally, Barry *et al.* (28) have recently reported that water transport by rat small intestine, *in vitro*, can be divided into glucose-dependent and glucose-independent fractions. The glucose-dependent water absorption is most prominent in the upper intestine, whereas, in the ileum approximately 60 per cent of the total water absorption is glucose-independent.

In each of the reports cited above, the effect of glucose was interpreted in terms of its function as a source of metabolic energy. Although it is difficult at present to rule out the possibility that this was a contributory factor in these experiments, all the above observations may be attributed solely to the interaction between active sugar and active Na transport which is independent of the metabolic fate of the transported sugar. Recently, Barry *et al.* (29) and Sawada and Asano (30) have independently concluded that actively transported sugars affect both the transmural PD and water movement in the small intestine, and that these effects are unrelated to the subsequent metabolism of these sugars. These conclusions are supported by the evidence of Newey *et al.* (12) that the isolated small intestine displays significant endogenous metabolic activity in the absence of external glucose, and the observation of Fisher and Parsons (11) that glucose is accumulated in the wall of *in vitro* rat intestine during glucose absorption. Curran and Solomon (31) have presented convincing evidence that water absorption by rat intestine is a passive process and is dependent upon net solute flux. The role of glucose in water absorption may be in part attributed to the fact that in its presence not only is this solute transported but there is a concomitant increase in active Na transport. The effect of low concentrations of phlorizin on water transport may be attributed to its inhibition of both active sugar transport and the increased Na transport.

#### *The Mechanism of the Na-Sugar Interaction*

The present experiments indicate that not only is active sugar transport accompanied by an increase in net Na transport, but, as suggested by the kinetic studies, the magnitude of this increase is related to the rate of sugar transport. In the absence of studies on the simultaneous rates of sugar and Na transport we cannot define the stoichiometry of this interaction. However, Fisher and Parsons (11, 18) have studied the kinetics of glucose and galactose transport by *in vitro* rat intestine and have reported that the maximum rate of transport is approximately  $4 \mu\text{mol}/\text{cm}^2 \text{ hr.}$  for glucose and approximately  $6 \mu\text{mol}/\text{cm}^2 \text{ hr.}$  for galactose. The similarity between these data and the values of  $\Delta\Phi_{\text{max}}^{\text{Na}}$  for glucose and galactose given in Table IV suggests that if similar

fluxes obtain in rabbit ileum the relationship between sugar transport and increased Na transport may be 1 for 1.

Two groups of mechanisms must be considered in attempting to define the Na-sugar interaction; *i.e.*, direct and indirect interactions. For example, if the rate-limiting step for active Na transport is the rate at which Na enters the cell across the mucosal membrane, then our observations could be explained by postulating that glucose transport is associated with a concomitant increase in the passive permeability of the mucosal membrane to Na. A similar mechanism has been proposed by Curran *et al.* (32) to explain the effects of Ca and antidiuretic hormone on Na transport in frog skin. At present, we are unable to exclude this possibility. A second indirect interaction which must be considered as possibly providing the force responsible for the increased Na transport is solvent-drag secondary to the increased water movement which accompanies glucose absorption. Several observations make this possibility unlikely. Ussing has pointed out that in the presence of solvent-drag the solute movement in the direction of the net solvent movement is accelerated and the solute flux in the opposite direction is reduced (33); this is a necessary consequence of the concept of solvent-drag as arising from the frictional interaction between water and solute movements in common channels. We have demonstrated, however, that the passive unidirectional serosa-to-mucosa Na flux is not influenced by the presence or absence of actively transported sugars, and that this flux behaves as if it were uninfluenced by solvent-drag forces (1). Furthermore, Curran (24) and Green *et al.* (34) have reported that solvent-drag does not play a significant role in Na and Cl transport in rat small intestine.

In recent years considerable evidence has been presented which suggests that the Na-sugar interaction which we have described is the result of a direct interaction between the sugar transport mechanism and Na, which results in an obligatory transfer of Na from the mucosal solution into the cell during the process of sugar absorption. In 1958, Riklis and Quastel (19) reported that glucose absorption by guinea pig intestine is dependent upon the presence of Na in the mucosal medium. Crane and coworkers (35-37) have extended these observations and have shown: (a) that the rate of active sugar accumulation by hamster small intestine is a function of the Na concentration of the medium [see Fig. 1, reference (37)] and (b) that the entrance of sugars into the intestinal cell, under anaerobic conditions where active transport is abolished, is Na-dependent. Csáky (38-41) has demonstrated that, in both the frog and rat small intestine, Na is required not only for active sugar transport, but for the active transport of amino acids and pyrimidines as well. He has further shown that the cardiac glycosides, potent inhibitors of active Na transport in a wide variety of systems, inhibit these Na-dependent active transport mechanisms (42, 43).

Recently it has been reported that active sugar transport by rabbit kidney cortex (44) and active amino acid transport by both Ehrlich ascites tumor cells (45) and a marine pseudomonad (46) are Na-dependent processes.

On the basis of these observations and our findings, it is possible to construct a hypothetical model for the interaction between active intestinal Na and sugar transport. This model, schematically depicted in Fig. 7, is similar to that which has been proposed by Crane (35) to explain the Na requirement for active intestinal sugar transport. The essential features of this model are the presence of (a) an energy-dependent, ouabain-sensitive Na carrier mechanism on or near the serosal membrane whose rate of transport is a function of the intracellular Na concentration,  $[Na]_i$ ; and (b) a Na-coupled, phlorizin-sensitive sugar transport mechanism on or near the brush border of the cell

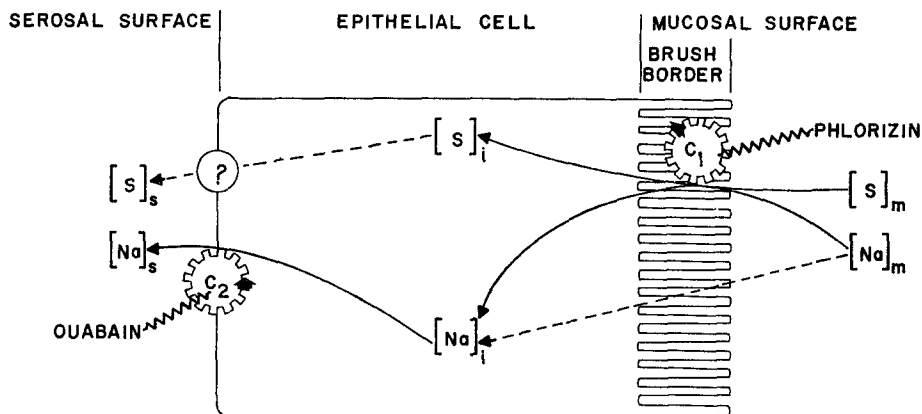


FIGURE 7. A hypothetical model of the Na-sugar interaction in distal rabbit ileum.

whose rate of transport is a function of both the Na and the sugar,  $[S]$ , concentrations. Considerable evidence supporting the locations of these two transport mechanisms has been documented both for the intestine (1, 9, 10) and for other epithelial tissues (14, 32, 47, 48). Furthermore, Frazier *et al.* (47) and Curran *et al.* (32) have presented evidence that the rate of active Na transport in toad bladder and frog skin is a function of the intracellular Na concentration.

As shown in Fig. 7, in the absence of sugar mucosal Na enters the cell by means of some "downhill" transport process, either simple diffusion or carrier-mediated transport (32). The steady-state  $[Na]_i$  is maintained at a level lower than that in the surrounding medium by means of the active Na transport mechanism,  $C_2$ . In the presence of an actively transported sugar,  $[Na]_m$  and  $[S]_m$  combine with either the same carrier to form a ternary complex, or with two coupled carriers (system  $C_1$ ). Within the cell,  $C_1$  equilibrates with  $[S]_i$  and  $[Na]_i$  with the forward (dissociation) reaction favored by the low

$[\text{Na}]_i$ . The rate of active Na transport is increased as a result of the increase in the intracellular Na concentration which in turn is the result of an increased rate of Na entry through the mucosal membrane. Sugar accumulation within the cell is, according to this model, a consequence of the Na carrier system  $C_2$  and the low  $[\text{Na}]_i$ . If the kinetics of the carrier system  $C_1$  resemble the relationships between  $I_{sc}$  and  $[S]_m$  shown in Figs. 4 to 6, sugar could be accumulated within the cell, reaching high concentrations, simply because the unidirectional fluxes of sugar on carrier(s)  $C_1$  (*i.e.*,  $m$  to  $i$  and  $i$  to  $m$ ) saturate at relatively low sugar concentrations, but are linearly related to the Na concentration. Ouabain, by inhibiting  $C_2$ , abolishes the gradient between intracellular and extracellular Na concentrations and thereby inhibits net sugar accumulation by the cell (*i.e.*, the forward and backward reactions of  $C_1$  are equal when  $[\text{Na}]_i = [\text{Na}]_m$  and  $[S]_i = [S]_m$ ).

Bihler and Crane (36) have demonstrated that Na is required for the entrance of sugars into the cell, and have suggested that sugar and Na combine with a single carrier to form a ternary complex. Csáky, on the other hand, has reported that sugar can be transported in the absence of Na if the mucosal concentration is sufficiently high so that transport is in the direction of the concentration gradient. He concludes that Na is linked to sugar and amino acid transport *via* the reaction(s) which provides the energy for non-electrolyte active transport (40). The carrier system  $C_1$ , described above, does not exclude either of these alternatives. If, as Crane suggests, Na and sugar combine to form a ternary complex, a possible role for Na in this process is suggested. Since the cell interior is, in all likelihood, electrically negative with respect to the extracellular medium, the binding of Na by the sugar-carrier complex could provide for or contribute to the force necessary for the translocation of the active site(s) of  $C_1$  from the outward-facing to the inward-facing aspects of the mucosal membrane. The recycling of the carrier would follow dissociation of the complex within the cell.

The views expressed herein are those of the authors and do not necessarily reflect the views of the United States Air Force or the Department of Defense.

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