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### Research Article

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# The Role of Intraluminal Sodium in Glucose Absorption In Vivo

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**ABSTRACT** Active glucose absorption is thought to depend on a gradient of sodium ion concentration across the brush border membrane of intestinal epithelial cells. This concept is generally accepted, although its validity has never been adequately evaluated in the human small intestine in vivo. According to this hypothesis, the rate of glucose absorption should decrease markedly if the luminal sodium concentration is markedly reduced, and glucose absorption against a concentration gradient should cease entirely if luminal sodium is lower than intracellular sodium concentration. In the present series of experiments we were not able to show an important role of intraluminal sodium concentration in the active absorption of glucose from the human, rat, and dog ileum in vivo. Specifically, glucose absorption was minimally reduced or not reduced at all when intraluminal sodium concentration was reduced from 140 to as low as 2.5 mEq/liter. The discrepancy between our results and those of previous workers whose data suggest that removal of intraluminal sodium should markedly inhibit active glucose absorption is not entirely clear, but there are a number of differences in experimental design between most previous studies and our own. Although our data show that active glucose absorption proceeds at a near normal rate even when lumen sodium concentration is reduced below 3 mEq/liter, our results do not disprove the sodium gradient theory because of the theoretic possibility that the microclimate adjacent to the brush border has a high concentration of sodium even when luminal sodium concentration is markedly reduced. The validity of the sodium gradient hypothesis would appear to be critically dependent on such a microclimate.

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## INTRODUCTION

Recent studies have suggested that glucose absorption cannot proceed unless sodium is present in the gut lumen (1), and that inhibition of the sodium pump (e.g. with cardiac glycosides) results in inhibition of glucose transport (2). Based mainly on these observations, the sodium gradient hypothesis for active glucose absorption was proposed by Crane (3, 4). Crane's experiments have been carried out primarily in the hamster intestine, and there are two main features of his model: first, the affinity of a mobile carrier for glucose, located in the brush border membrane of the epithelial cells, is directly proportional to sodium concentration; second, the sodium concentration within the absorbing cells is maintained at a low level by an outwardly directed sodium pump. Because the sodium concentration in the gut lumen is higher than that inside the cell, the affinity of the carrier for glucose at the luminal surface of the brush border membrane is higher than that at the intracellular surface. This differential affinity results in net movement of glucose from gut lumen into the cell. Once inside the cell, glucose diffuses passively across the serosal membrane to complete the absorptive process.

Extensive studies in the rabbit ileum have provided strong support for this hypothesis, although differences in kinetic detail between the rabbit and hamster have been observed, and the original hypothesis has been modified slightly (5). In the revised (rabbit) model, sodium and sugar combine with a mucosal transport site, which may or may not be a mobile carrier; in the absence of sodium in the mucosal solution, this ternary complex cannot be formed, and sugar transfer does not occur (5). In both models active sugar transport is driven by a sodium concentration difference across the brush border membrane, and this is the essence of the sodium gradient hypothesis.

According to the sodium gradient hypothesis (3-7), a lower concentration of sodium inside the cell than in the lumen is essential for the active transport of glucose. If the sodium pump mechanism were poisoned by cardiac glycosides, the intracellular sodium concentration would increase and active glucose absorption would cease. Likewise, according to this hypothesis, the rate of glucose absorption should decrease markedly or cease entirely if the luminal sodium concentration is markedly reduced.

This concept is now widely accepted (4) and is also applied to the absorption of other substrates, such as amino acids (8, 9), and to transport in many tissues other than the intestine (4, 10). However, the hypothesis has not, in our opinion, been adequately evaluated in the human small intestine or in any other *in vivo* system. In the present experiments, we have examined the effects of luminal sodium concentration on glucose absorption in the intact perfused ileum of humans, rats, and dogs.

## METHODS

*Human studies.* Studies were performed in normal adults of both sexes, ages 21-35 yr. Constant perfusion, non-absorbable marker techniques (11, 12) were utilized with the infusion site of the tube positioned at 200 cm from the teeth under fluoroscopy. Test solutions contained polyethylene glycol (PEG)<sup>1</sup> as the volume marker, and were perfused through the infusion tube and collected through one or two distally located aspiration sites. The distance from the infusion to the proximal aspirating site was always 10 cm; unless stated otherwise, the distance from proximal to distal aspirating site was 30 cm. The details of our perfusion methods have been previously published (11-13). Each specimen was collected over ice and 3 drops of toluene were added to prevent bacterial degradation of sugar.

*Kinetics of glucose absorption.* A triple-lumen tube was used, and glucose concentration in the test solutions was varied from 5 to 150 mM; sodium (as NaCl) concentration was 50 mEq/liter, and mannitol was added to achieve isotonicity with plasma. Test solutions were infused at a rate of 16 ml/min. The study segment was either 10 or 30 cm, the shorter test segment being used for solutions having low glucose concentrations. Absorption rates from 25 mM glucose solutions were measured six times with a 10 cm study segment and six times with a 30 cm study segment; expressed per centimeter of segment length, the results were virtually the same.

*Glucose absorption from sodium-free and 140 mEq/liter sodium test solutions as studied by the two-lumen perfusion method.* The two-lumen tube method (11, 12) was used with a 10 cm distance from the infusion to the collecting site. Perfused solutions contained 1.4 or 4.6 mM glucose and either 280 mM mannitol or 140 mEq/liter of sodium (as NaCl). Perfusion rate was 16 ml/min. After a 30 min equilibration period, fluid was collected continuously at 30-min intervals. From three to six 30 min samples were collected, and then the test solution was changed. The

<sup>1</sup> Abbreviations used in this paper:  $K_M$ , Michaelis constant; PEG, polyethylene glycol.

order of testing between high and low sodium solutions was randomized. With the 4.6 mM glucose solution, six normal subjects were studied with 24 30 min study periods with the high sodium and 32 30 min study periods with the sodium-free test solutions. With the 1.4 mM glucose solutions, three normal subjects were studied: 17 30 min periods with the high sodium solutions and 18 30 min periods with the sodium-free solutions. In addition, two diabetic patients with marked hyperglycemia were studied by a similar technique, the details of which are given in the Results section.

This two-lumen method gives an accurate measure of sugar absorption but results cannot be expressed on a per centimeter basis, since the test segment length is not known (12). The method is not valid for measuring net sodium absorption (12), but for present purposes only knowledge of the sodium concentration in the aspirated fluid was needed. In all of these studies fluid was also aspirated via a third lumen, which opening was 10 cm distal to the proximal aspiration site; however, with the low concentration of sugar infused, many samples obtained from the distal site contained no glucose (i.e. all glucose had been absorbed) so that glucose absorption by the three-lumen method could not be calculated.

*Effect of sodium concentration and sodium absorption or secretion rate on glucose absorption as measured by the triple-lumen perfusion technique.* The triple-lumen tube method (11-13) was used, the distance between the proximal and distal collecting sites was 20 cm, and test solutions were infused at a rate of 10 ml/min. Each test solution contained 4 mM glucose and 75 mM galactose. This large amount of galactose reduced the rate of glucose absorption to a level that could be accurately measured by the three-lumen tube method. In addition to glucose and galactose, test solutions contained either 10, 20, or 105 mEq/liter sodium (NaCl) and sufficient mannitol to maintain osmolality at 290 mOsm/kg.

*Rat studies.* Male Sprague-Dawley rats weighing between 280 and 390 g were fasted for 24 hr before study and were anesthetized with 50 mg/kg of sodium pentobarbital *i.p.* A loop of distal ileum 25 cm in length was isolated and cannulated at both ends. Solutions contained either: (a) 4 mM glucose, 75 mEq/liter sodium (as NaCl) and 140 mM mannitol, (b) 4 mM glucose and 280 mM mannitol, or (c) 4 mM glucose, 75 mEq/liter potassium (as KCl)<sup>2</sup> and 140 mM mannitol. All solutions contained PEG. These were recirculated sequentially through the isolated loop at a rate of 2 ml/min from an initial reservoir of 12 ml. A 3 ml sample was removed after 10 min (equilibration period) and all unabsorbed fluid was removed at the end of 50 min of recirculation. A drop of toluene was added to each specimen immediately after its collection. Glucose absorption was determined by comparing the 10- and 50-min samples. Before each perfusion the ileal loop was rinsed with the solution to be used next, so that remnants of the preceding test solution were removed. Blood was obtained at the end of each experiment for glucose determination.

*Dog studies.* Conditioned mongrel dogs were used for these studies. The animals were fasted for 24-36 hr before the study. After sodium thiopental induction, they were anesthetized with a 3% chloralose-6% urethane-10% etha-

<sup>2</sup> Preliminary studies revealed that perfusion of solutions containing 140 mEq/liter of potassium (as KCl) caused a high mortality in rats, hence the choice of 75 mEq/liter of potassium.

TABLE I  
Effect of Incubating a Glucose Solution with Stool Suspensions

Time	0.1 ml 50 mM glucose +0.9 ml thin suspension	0.1 ml 50 mM glucose +0.9 ml thick suspension	0.1 ml 50 mM glucose +0.9 ml saline
	mM glucose		
0	5.03	4.77	4.89
0 + 1 drop toluene	5.05	4.77	
1 hr + 1 drop toluene	4.85	4.02	
2 hr + 1 drop toluene	4.98	2.52	

nol solution. A distal ileal loop of 20 cm was isolated, cannulated at both ends and perfused with solutions containing PEG, glucose 5 mM, and either mannitol 280 mM or sodium 140 mEq/liter (as NaCl); these were recirculated sequentially through the isolated loop at 2 ml/min from a 30 ml reservoir. These experiments were otherwise performed as in the rat studies.

**Bacteriological studies.** To determine the effect of bacteria in lowering glucose concentration in aspirated samples, one thick and one thin stool suspension (from a normal individual) in saline were prepared. Known amounts of glucose were added, and the suspension incubated at 37°C. At zero time, and at 1 and 2 hr, bacterial counts were made on blood agar medium, after which 1 drop of toluene was added to stop bacterial metabolism, and glucose recovery determined. The results are shown in Tables I and II. These studies reveal that > 600,000 colonies/ml and 1 hr or greater incubation are necessary for a significant lowering of the glucose content.

During nine human studies on the effect of sodium concentration on glucose absorption, bacterial counts on aspirated fluid were measured and shown to contain from 0 to 150,000 colonies/ml. These relatively low colony counts, plus the fact that toluene was always added as soon as samples were removed from the intestine, rule out bacterial contamination as a factitious cause of glucose "absorption."

**Analytical methods.** Samples were analyzed for PEG and electrolytes by methods described previously (13), for glucose by glucose oxidase (Glucostat, Worthington Biochemical Corp., Freehold, N. J.) and for galactose by the Somogyi method. Absorption rates were calculated from the perfusion rate, the change in concentration of the nonabsorbable marker, and the change in concentration of

TABLE II  
Colony Count on Stool Suspensions from 0 to 2 Hr after Addition of Glucose

Time	No. bacterial colonies per ml	
	Thin suspension	Thick suspension
hr		
0	110,000	>600,000
1	114,000	>600,000
2	125,000	>600,000

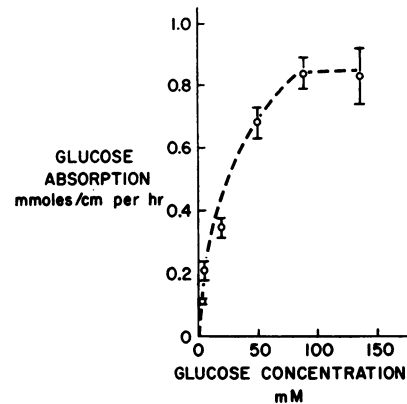


FIGURE 1 Rate of human ileal glucose absorption as a function of glucose concentration at the proximal aspiration site of a three-lumen perfusion tube. From 7 to 23 studies were performed at each point.

the test substance (11-13). Results are expressed as the mean  $\pm$  1 SE.

## RESULTS

### Human studies

**Kinetics of glucose absorption.** As shown in Fig. 1, glucose absorption increased rapidly with slight increases in luminal glucose concentrations between 0 and 85 mM/liter. Above a concentration of 85 mM, the rate of glucose absorption did not increase. The maximum rate of glucose absorption was approximately 0.84 mmoles/cm per hr. One-half maximal transport was achieved at a concentration of approximately 15 mM. Blood from these subjects had a glucose concentration of  $4.5 \pm 0.3$  mM. Since glucose was absorbed from luminal contents having concentrations as low as  $1.3 \pm 0.2$  mM, absorption against a concentration gradient has been demonstrated.

**Glucose absorption from sodium-free and 140 mEq/liter sodium test solutions.** Data comparing the effect

TABLE III  
Influence of Sodium Concentration on 4.6 mM Glucose Absorption in the Human Ileum

Solution perfused	Concentration at aspiration site		Absorption of glucose mmoles/hr
	Sodium mEq/liter	Glucose mM	
Glucose 4.60 $\pm$ 0.16 mM NaCl 140 mM No. = 24	139 $\pm$ 1.1	1.64 $\pm$ 0.08	2.83 $\pm$ 0.10
Glucose 4.58 $\pm$ 0.23 mM NaCl 0 Mannitol 280 mM No. = 32	10.4 $\pm$ 1.2	2.02 $\pm$ 0.10	2.44 $\pm$ 0.13

Average blood glucose in all studies =  $4.8 \pm 0.3$  mM.

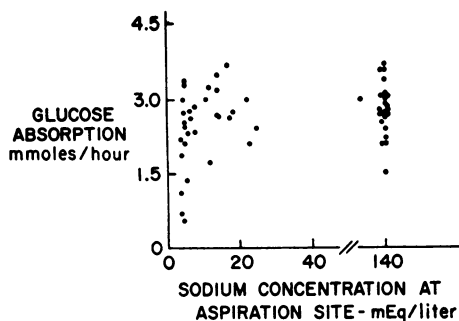


FIGURE 2 Rate of human ileal glucose absorption from a 4.6 mM glucose test solution at low and high intraluminal sodium concentration. The sodium concentration in aspirated ileal fluid is shown.

of luminal sodium concentration on glucose absorption from a 4.6 mM glucose solution are shown in Table III and Fig. 2. The average glucose concentration at the aspiration site for these studies was  $1.6 \pm 0.1$  mM for the solution containing 140 mM NaCl and  $2.0 \pm 0.1$  mM for the solution with no NaCl, while the mean serum glucose concentration was  $4.8 \pm 0.3$  mM. Thus, sugar absorption from these solutions was occurring against a concentration gradient. Mean values for glucose absorption were 2.8 mmoles/hr with the high sodium solution and 2.4 mmoles/hr with a no-sodium solution. As shown in Fig. 2, glucose absorption continued to occur even when the luminal sodium concentration was as low as 3 mEq/liter.

An additional 18 study periods in three subjects were done with test solutions containing only 1.4 mM glucose, and the mean results are shown in Table IV. Glucose absorption rate when sodium was 141 mEq/liter was 0.72 mmoles/hr, compared to an absorption rate of 0.58 mmoles/hr when the solution containing no sodium was perfused. As shown in Fig. 3, glucose absorption occurred even when the luminal sodium concentration was as low as 3.5 mEq/liter.

In order to study glucose absorption against a lumen to plasma concentration gradient when lumen glucose concentration was higher than normal plasma concentration of 4.5 mM, experiments in two insulin-dependent diabetic subjects were done. These patients had plasma sugar concentrations of 20–30 mM, and their lower ileum was perfused with 15 mM glucose solutions, with and without the addition of 135 mEq/liter NaCl. As shown in Fig. 4, glucose absorption in these subjects was against a steep lumen-to-plasma concentration gradient, and the rate of glucose absorption was 10-fold higher than when 1.4 mM glucose was perfused in normal subjects. Nevertheless, the rate of glucose absorption was not significantly reduced when sodium was omitted from the test solution and when lumen sodium fell from 135 to as low as 2.5 mEq/liter.

TABLE IV  
Influence of Sodium Concentration on 1.4 mM  
Glucose Absorption in the Human Ileum

Solution perfused	Concentration at aspiration site		Absorption of glucose mmoles/hr
	Sodium mEq/liter	Glucose mM	
Glucose 1.40 $\pm 0.01$ mM NaCl 140 mM No. = 17	141 $\pm 0.6$	0.625 $\pm 0.06$	0.719 $\pm 0.06$
Glucose 1.38 $\pm 0.01$ mM NaCl 0 Mannitol 280 mM No. = 18	10.1 $\pm 1.8$	0.732 $\pm 0.06$	0.583 $\pm 0.07$

Average blood glucose in all studies =  $4.5 \pm 0.2$  mM.

*Effect of sodium concentration and sodium absorption or secretion rate on glucose absorption as measured by the triple-lumen perfusion technique.* In order to measure net sodium absorption or secretion at varying luminal sodium concentrations, and to assess the effect of sodium absorption or secretion (as well as sodium concentration) on sugar absorption, the triple-lumen method was employed, and galactose was added in relatively high concentrations so as to reduce the rate of glucose absorption to a level that could be measured by this technique. The results in Table V demonstrate that glucose and galactose absorption were the same whether sodium was secreted (with the 10 mEq/liter Na solution), absorbed at a slow rate (with the 20 mEq/liter Na solution) or absorbed rapidly (with the 105 mEq/liter Na solution). Water was absorbed rapidly with each test solution; this was mainly secondary to galactose absorption with the low Na solutions, and due to both galactose and sodium absorption with the 105 mEq/liter Na solution.

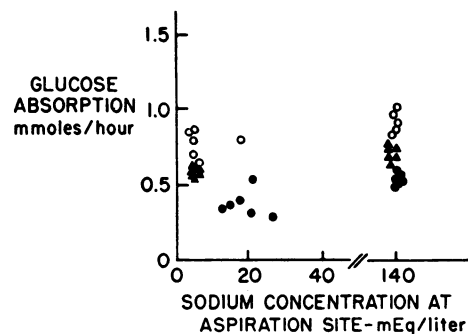


FIGURE 3 Rate of human ileal glucose absorption from a 1.4 mM glucose test solution at low and high intraluminal sodium concentration. The sodium concentration in aspirated ileal fluid is shown. Results for three individual subjects with high and low sodium concentrations are indicated by the different symbols.

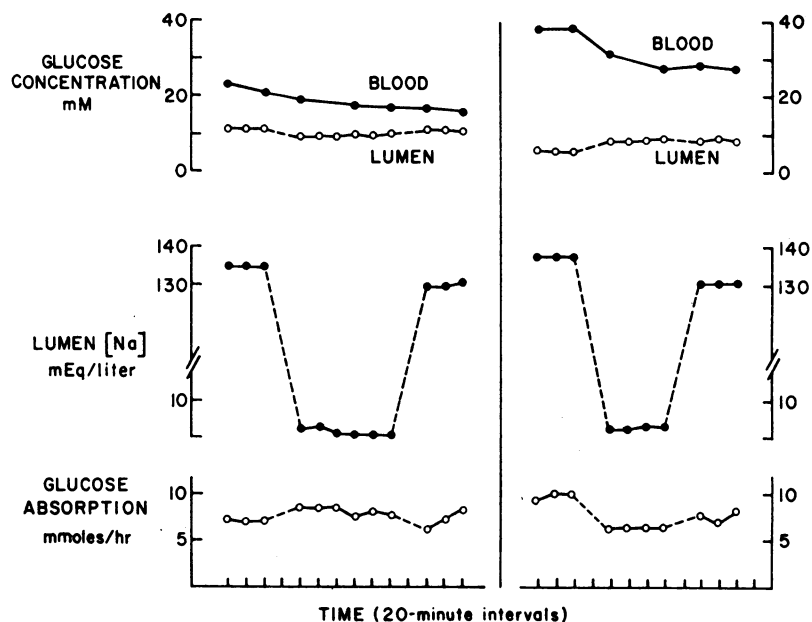


FIGURE 4 Effect of luminal sodium concentration on ileal glucose absorption rate in two insulin-dependent diabetic patients. Each subject had marked hyperglycemia. The ileum was perfused with 15 mM glucose solution. Glucose absorption, against steep lumen to plasma concentration gradients, was not affected when lumen sodium concentration (substituted by mannitol) was markedly reduced.

#### Rat studies

Fig. 5 depicts the relationship of intraluminal sodium concentration and glucose absorption in the rat. The average glucose absorption rate from the solution containing 75 mEq/liter sodium was  $0.85 \pm 0.03$   $\mu$ moles/cm per 40 min as compared with  $0.88 \pm 0.01$   $\mu$ moles/cm per 40 min when the perfusing solution contained mannitol and no NaCl. The mean sodium concentration achieved in the lumen during perfusion of this latter

solution was  $10.3 \pm 0.5$  mEq/liter. With a solution containing 75 mEq/liter potassium, glucose absorption was  $0.85 \pm 0.05$   $\mu$ moles/cm per 40 min, with a mean intraluminal sodium concentration of  $15.5 \pm 1.1$  mEq/liter. Mean blood glucose concentration was 5.1 mM.

#### Dog studies

As shown in Table VI, glucose absorption rate was 2.2  $\mu$ moles/cm per 40 min when intraluminal sodium

TABLE V  
Effect of Sodium Concentration and Sodium Absorption Rate on Glucose and Galactose Absorption in the Human Ileum\*

Test solution†	Mean concentration in test segment			Absorption or secretion			
	Na	Glucose	Galactose	H <sub>2</sub> O	Na	Glucose	Galactose
	mEq/liter	mM	mM	$\mu$ liter/hr/cm	$\mu$ Eq/hr/cm	$\mu$ moles/hr/cm	$\mu$ moles/hr/cm
[Na] = 10	15.0	2.46	49.7	-2676	+ 48	-56	-550
[Na] = 20	24.2	2.75	54.4	-2506	- 19	-47	-530
[Na] = 105	106.8	3.52	59.5	-4646	-420	-46	-500

\* Five studies were done with each test solution; the order of perfusion was randomized. -, absorption; +, secretion.

† Each test solution had 75 mM galactose, 4 mM glucose, and a chloride concentration equal to that for sodium. Mannitol was added in amounts necessary to make each solution have an osmolality of 290 mOsm/kg.

concentration was 137 mEq/liter, and 1.8  $\mu$ moles/cm per 40 min when intraluminal sodium concentration was reduced to 10 mEq/liter.

## DISCUSSION

The present studies were designed to determine the role of intraluminal sodium concentration on glucose absorption rate in humans, rats, and dogs in vivo. The ileum was chosen for study because, in contrast to the jejunum, its passive permeability to NaCl is negligible, and it can therefore maintain steep concentration gradients of sodium between blood plasma and intestinal lumen.

Before testing the effect of luminal sodium concentration on active glucose absorption, it was necessary to determine the Michaelis constant ( $K_m$ ) for glucose, since previous in vitro studies have suggested an important role of intraluminal sodium when sugar concentration is near or below the  $K_m$ , but not when the sugar concentration greatly exceeds the  $K_m$  (4). As shown in Fig. 1, glucose was clearly absorbed against a concentration gradient, indicating an active process. In addition, the transport process exhibited saturation kinetics with a  $V_{max}$  of approximately 0.84 mmoles/cm per hr, and a  $K_m$  of approximately 15 mM.

In the present human studies the effect of luminal sodium concentration on glucose absorption was studied by perfusing either 4.6 or 1.4 mM glucose solutions, with and without the addition of NaCl, into the ileum. These experiments revealed that lowering the sodium concentration (substituting with mannitol) of ileal fluid from approximately 140 to as low as 3 mEq/liter had only a minimal effect (<20% inhibition) on the rate of glucose absorption, even though the glucose concentration was far below the glucose  $K_m$ . Similar

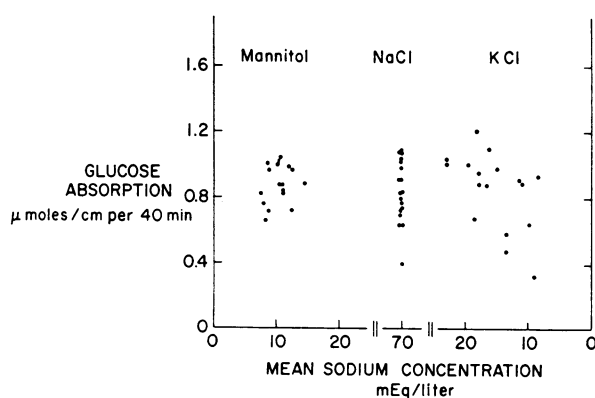


FIGURE 5 Rate of glucose absorption from 4 mM glucose solutions in the rat ileum at varying luminal concentrations of sodium. The average of initial and final sodium concentration is plotted. Either mannitol or KCl was substituted for NaCl.

TABLE VI  
Influence of Sodium Concentration on Glucose Absorption in the Dog Ileum\*

Solution perfused	Mean concentration $\ddagger$		Absorption of glucose $\mu$ moles/cm per 40 min
	Sodium mEq/liter	Glucose mM	
Glucose 5 mM NaCl 140 mM	137 $\pm$ 0.3	4.4 $\pm$ 0.1	2.2 $\pm$ 0.3
Glucose 5 mM NaCl 0 Mannitol 280 mM	10 $\pm$ 2.4	4.4 $\pm$ 0.1	1.8 $\pm$ 0.3

\* No. = 7. Average blood glucose in all studies = 5.2  $\pm$  0.7 mM.  
 $\ddagger$  Arithmetic average of initial and final concentrations.

results were noted in the dog ileum. In the rat, lowering luminal sodium concentrations from 75 to as low as 7 mEq/liter had no inhibitory effect at all on glucose absorption.

Although these experiments were done with glucose concentrations lower than that in plasma, it cannot be stated with certainty that glucose was being absorbed against a concentration gradient since it is theoretically possible that glucose absorbed from such low concentrations might be metabolized by the gut mucosal cells rather than be delivered into the bloodstream. In other words, if the rate of glucose utilization approached the rate of glucose absorption, glucose absorption might not be against a concentration gradient even though lumen glucose concentration is lower than that in plasma. In order to examine this possibility, studies were performed in insulin-dependent diabetic subjects in whom blood glucose was much higher than normal and in whom ileal absorption of glucose against concentration gradients could be studied when lumen glucose was higher than 4.5 mM. As shown in Fig. 4, perfusion of 15 mM glucose solution resulted in a 10-fold higher rate of glucose absorption than with 1.4 mM glucose solutions (from about 0.6 to about 7 mmoles/hr). At these high rates of glucose absorption the relative effect of tissue metabolism would be minimized, and it is reasonable to conclude that glucose absorption under these conditions is active, i.e., against a concentration gradient. Nevertheless, the rate of glucose absorption in these diabetic subjects was not significantly reduced when luminal sodium concentration was lowered from 135 to as low as 2.5 mEq/liter.

These results are surprising in view of the large body of evidence (14) which suggests that removal of sodium from the lumen markedly reduces or completely inhibits active sugar and amino acid absorption. To illustrate the discrepancy, the present results

can be compared with those of Crane, Forstner, and Eichholz (15), who noted an eightfold reduction in *in vitro* 6-deoxyglucose transport when the sodium concentration was reduced from 145 to 24 mEq/liter (estimated from their Fig. 1, using a sugar concentration of 3.3 mM). We, on the other hand, noted no reduction in *in vivo* sugar absorption when luminal sodium concentration was reduced from 140 to 15 mEq/liter (Table IV), and even when lumen sodium concentration was reduced to as low as 2–3 mEq/liter, active glucose absorption continued to occur at greater than 80% of the control rate (Figs. 2–4). The present results as well as previous studies must be carefully examined in an attempt to explain this discrepancy.

Previous *in vivo* studies on the effect of sodium removal on glucose absorption are few in number. Cšaky and Zollicoffer (16) perfused an isolated loop of rat upper jejunum with solutions containing various cations and a glucose concentration of 2.8 mM, so that absorption of glucose was against a concentration gradient. They noted marked inhibition of glucose transport in the solutions containing ionic substitutes as compared to sodium solutions. In three rats, they showed 91% inhibition of glucose uptake if the animals were perfused with a lithium solution, 86% inhibition with potassium, and a 75% inhibition with magnesium. In a later paper, these investigators found only 40% inhibition of glucose absorption with mannitol substituting for sodium (17). Annegers (18) measured glucose absorption from 14 mM glucose solutions in the presence of different ionic substances in the ileum and jejunum of the dog. Although he noted a 60% inhibition of glucose absorption in the ileum perfused with KCl as compared with NaCl chloride, there was only a 16% inhibition with substitution of ammonium for sodium. Thus, the absence of sodium was not consistently associated with a marked diminution in the rate of glucose absorption. Olsen and Ingelfinger (19), perfusing the ileum and jejunum of human subjects, noted a slight inhibition of glucose absorption when sodium was omitted from their test solutions (replaced by mannitol or Tris) and when glucose concentration was 3.4 mM or less. Absence of sodium from their test solutions had no effect when sugar concentration was 6 mM or greater. Although sodium-free solutions were infused, the concentration of sodium in the lumen rose to as high as 25 mEq/liter in the ileum and as high as 55 mEq/liter in the jejunum. Therefore, very low levels of intraluminal sodium concentration were not consistently achieved, and the authors were not able to decide whether or not their studies indicated a difference between *in vivo* and *in vitro* dependence of glucose absorption on luminal sodium concentration. Unfortunately, glucose absorption as a

function of intraluminal sodium concentration for individual studies was not reported.

These previous *in vivo* studies suggest that removal of sodium inhibits glucose absorption, but that the degree of inhibition depends to a large extent on the nature of the osmotic substitute. Substitution with lithium and potassium caused a marked inhibition of glucose absorption, while substitution with ammonium, mannitol, and Tris resulted in much less inhibition. The only serious discrepancy between our results and previous *in vivo* data concerns the rat studies. Cšaky found a marked inhibition of glucose absorption in the rat when sodium was replaced by potassium and a 42% inhibition when sodium was replaced by mannitol, whereas we found no reduction in glucose absorption when sodium was replaced by these two solutes. However, Cšaky's studies were carried out in the jejunum, while ours were in the ileum.

*In vitro* studies favoring an important role for intraluminal sodium concentration on sugar absorption are extensive, but there are a number of differences in the experimental design of our experiments and previous *in vitro* experiments. First, in most *in vitro* studies sodium was replaced on both the mucosal and serosal surfaces, whereas we replaced sodium only on the mucosal surface. Removal of sodium from both surfaces may have a profound effect on all cellular metabolic processes, and such studies cannot, in our opinion, be used as evidence for the sodium gradient theory. Second, in most of the *in vitro* studies analogues of glucose, such as 3-methyl glucose, galactose, or 6-deoxyglucose, rather than glucose, were studied. It is possible that sodium removal may reduce absorption of such sugars to a greater degree than it reduces glucose transport. Third, we used mannitol as an osmotic substitute for sodium (except in our rats, where potassium was also used), while others have used mainly ionic substitutes, especially potassium and lithium. Finally, the stirring and mixing rate in *in vitro* experiments has probably been greater in most instances than in our *in vivo* perfusions.

Differences in the degree of mixing might theoretically explain the discrepancy, since poor mixing *in vivo* might allow the concentration of sodium immediately adjacent to the brush border membrane to be much higher than in the bulk luminal fluid due to an "unstirred layer" effect. Since in perfusion experiments with zero sodium in the initial test solution some sodium diffuses into the lumen, the presence of an unstirred layer of fluid adjacent to the mucosal cells might impede sodium diffusion away from the membrane, and result in local concentrations higher than in the core luminal fluid, which we sampled. Poor stirring would emphasize the importance of the unstirred layer. How-



ever, as shown in the Appendix, calculations based on the rate of sodium entry into the perfused solutions, the diffusion constants for NaCl and glucose in water, and the glucose transport data, suggest that the unstirred layer would cause local concentration gradients of sodium between brush border membrane and luminal fluid of a *maximum* of 3–4 mEq/liter. Such small gradients could not account for our failure to find an important role for luminal sodium concentration on glucose transport. In addition to these calculations, the data in Table V place a limit, by direct experiments, on the extent to which an unstirred layer and poor mixing in vivo could cause a discrepancy between measured intraluminal sodium concentration and the sodium concentrations immediately adjacent to the brush border membrane. As shown in Table V, reducing luminal sodium concentration from 105 to 24 mEq/liter had no effect on glucose absorption. Since sodium was absorbed from the 24 mEq/liter sodium solution, the concentration of sodium at the mucosal surface must have been less than 24 mEq/liter, if the unstirred layer impedes sodium diffusion. By extrapolation of the experiments with 10 and 20 mEq/liter sodium concentrations shown in Table V, net sodium movement was zero at approximately 20 mEq/liter luminal sodium concentration, and under conditions of zero net movement, the sodium concentration in the lumen must equal that at the mucosal border, regardless of the unstirred layer.

Although we consider these observations to militate against inadequate mixing as an explanation for the discrepancy between our in vivo results and the data of others in vitro, there is a second possible mechanism whereby sodium concentrations adjacent to the brush border might be high in spite of a very low luminal sodium concentration. It has been suggested that the brush border or fuzzy coat might have a high density of fixed negative charges and that these maintain a high concentration of hydrogen ions in the microclimate of the luminal surface of the brush border (20). It is theoretically possible that such a mechanism might maintain high local concentrations of sodium, even when lumen sodium concentration is very low. However, it is not clear whether or not sodium ions maintained in the vicinity of fixed negative charges would be available for reaction with the glucose carrier, or, if available, would have the necessary electrochemical potential to maintain the near normal rates of glucose transport we observed when lumen sodium concentration approaches zero. Nevertheless, if membrane-bound negative charges did maintain a high sodium concentration in the microclimate, this might explain the discrepancy between our data and some of the previous work in vitro, since we used mainly mannitol and others used mainly ionic substitutes as sodium is re-

moved. Ionic substitutes might react with the fixed negative charges, and thus limit their ability to maintain a microclimate high in sodium, whereas mannitol would not have this property.

It is also possible that some of the discrepancy is due to the fact that our experiments were carried out in vivo while most of the data which support the hypothesis are from in vitro studies. However, to our knowledge, no in vitro studies have been done where mannitol was the replacement solute, where glucose was the test sugar, and where sodium was removed from only the mucosal surface of the epithelial cells; therefore, direct comparison of our in vivo studies with similar in vitro experiments is not possible.

Our interpretation of the data presented in this paper is that luminal sodium can be lowered drastically with only a trivial reduction of active glucose transport, and that glucose absorption is the same when sodium is being secreted into the ileal lumen as when sodium is being absorbed.

With regard to the sodium gradient hypothesis, our data show that the first evidence which suggested this concept (i.e. that removal of sodium ions from the lumen markedly inhibited active glucose absorption) does not apply to the in vivo absorption of glucose. However, it is theoretically possible that the luminal sodium concentration immediately adjacent to the brush border could be high, even when lumen sodium is zero, if the brush border membrane or fuzzy coat is electrically charged in such a way as to trap sodium ions on the luminal surface. Although there is no convincing evidence to suggest that the membrane does, in fact, trap sodium ions, the possibility exists, and for this reason the present data do not disprove the sodium gradient theory. It is our opinion, however, that the validity of this hypothesis is now critically dependent on the existence of some mechanism for maintaining a microclimate of high sodium concentration adjacent to the brush border membrane.

## APPENDIX

*Role of the unstirred layer.* When sodium-free solutions were perfused into the ileum, fluid collected 10 cm distally had a sodium concentration of from 2.5 to 25 mEq/liter. This sodium has two possible origins: first, the infused test solution may be mixed with a variable amount of endogenous fluids which come from above the infusion site and which are rich in sodium; second, sodium may diffuse across the ileal mucosa, even though previous studies have shown the ileum to be relatively impermeable to sodium diffusion (compared to the upper small intestine) (13, 21). The contribution of this second source, which is important in interpreting the present results (see below), was estimated by the use of a third collection, situated 10 cm beyond the first collecting tube. Any increase in sodium concentration between the proximal and distal collecting sites must be due to sodium diffusion across the ileal mu-

cosa. In the experiments shown in Figs. 2 and 3, the rise in sodium concentration over this 10 cm ileal segment averaged  $4.1 \pm 0.6$  mEq/liter.

It is theoretically possible that the diffusion of this amount of sodium might create a locally high sodium concentration at the outer border of the brush border membrane, provided a poorly mixed, unstirred layer of fluid coating the membrane is postulated. Thus, while the aspirated luminal contents might have a sodium concentration as low as 2.5 mEq/liter, the sodium concentration adjacent to the mucosal membrane might be much higher. The degree to which such a sodium gradient between the outer border of the microvillous membrane and the core solution could be maintained is dependent on the thickness of the unstirred layer. Fortunately, the effect of this unstirred layer on the local concentration of sodium can be estimated from the glucose absorption data.

If the unstirred layer constitutes a diffusion barrier for sodium entering the luminal fluid from plasma, and this causes high local concentrations of sodium which could influence glucose transport, the location of the barrier must necessarily be such that it would also constitute a barrier to diffusion of glucose from lumen to mucosal transport sites.

In steady-state perfusion experiments, the following assumptions are made: (a) diffusion of sodium (NaCl) across unstirred layer must equal diffusion of sodium (NaCl) from plasma to lumen; (b) diffusion of glucose across unstirred layer must equal rate of glucose absorption.

The following equations may then be written:

$$T_G = J_G = \frac{AD_G\Delta C_G}{\Delta X}, \quad (1)$$

where  $T_G$  is the glucose transport rate ( $\mu\text{moles cm}^{-1} \text{sec}^{-1}$ ),  $J_G$  the rate of movement across the unstirred layer,  $A$  the surface area per centimeter length of perfused intestine,  $\Delta X$  the thickness of the unstirred layer,  $\Delta C_G$  the glucose concentration gradient across the unstirred layer, and  $D_G$  is the diffusion coefficient of glucose in free solution. The value for  $D_G$  is  $6.7 \times 10^{-6} \text{ cm}^2 \text{sec}^{-1}$  (International Critical Tables).

$$J_{\text{NaCl}} = \frac{AD_{\text{NaCl}}\Delta C_{\text{NaCl}}}{\Delta X}, \quad (2)$$

where  $J_{\text{NaCl}}$  is equal to diffusion of NaCl across the unstirred layer (and also the diffusion of NaCl from plasma to lumen),  $\Delta C_{\text{NaCl}}$  the NaCl concentration gradient across the unstirred layer and  $D_{\text{NaCl}}$  is  $1.84 \times 10^{-6} \text{ cm}^2 \text{sec}^{-1}$  (International Critical Tables).

Although the absolute values for  $A$  and  $\Delta X$  in the perfused intestinal segments are not known they are the same in equations 1 and 2. It is possible, therefore, by simultaneous solution of these two equations to obtain an estimate of the sodium concentration gradient across the unstirred layer.

$$\Delta C_{\text{NaCl}} = \frac{J_{\text{NaCl}}}{T_G} \cdot \frac{D_G}{D_{\text{NaCl}}} \cdot \Delta C_G. \quad (3)$$

From Fig. 1, the rate of glucose absorption when the luminal concentration was 5 mM was  $0.21 \text{ mmoles cm}^{-1} \text{hr}^{-1}$  or  $58 \times 10^{-3} \mu\text{moles cm}^{-1} \text{sec}^{-1}$ . At this concentration, the glucose concentration gradient across the unstirred layer could be only 5 mM as a maximal limiting value. Since

absorption of glucose was still taking place, the glucose concentration at the transport site must have been greater than zero, and the concentration gradient across the unstirred layer must have been less than this maximal value of 5 mM. However, for purposes of present calculations, 5 mM is used for the value of  $\Delta C_G$  in equation 3.

In the steady state,  $J_{\text{NaCl}}$  must equal the inward diffusion of NaCl from plasma to lumen. Since the sodium concentration rose an average of 4 mEq/liter over a 10 cm length of ileum at a perfusion rate of 16 ml/min, the net influx was  $6.4 \mu\text{Eq/min}$  per cm length of ileum, or  $107 \times 10^{-3} \mu\text{Eq cm}^{-1} \text{sec}^{-1}$ .

Substituting these values into equation 3 gives:

$$\begin{aligned} \Delta C_{\text{NaCl}} &= \frac{107 \times 10^{-3} \mu\text{Eq cm}^{-1} \text{sec}^{-1}}{58 \times 10^{-3} \mu\text{moles cm}^{-1} \text{sec}^{-1}} \\ &\times \frac{6.7 \times 10^{-6} \text{ cm}^2 \text{sec}^{-1}}{18.4 \times 10^{-6} \text{ cm}^2 \text{sec}^{-1}} \times 5 \mu\text{moles ml}^{-1}, \end{aligned}$$

$$\Delta C_{\text{NaCl}} = 3.4 \mu\text{Eq ml}^{-1} \quad \text{or} \quad 3.4 \text{ mEq/liter.}$$

Thus, the concentration of sodium at the glucose transport site would be 3.4 mEq/liter higher than the concentration of sodium in the luminal fluid. This is a maximum value, since the calculation was made on the basis of a maximum value for  $\Delta C$  for glucose.

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