

Augmentation of Neutral Sodium Chloride Absorption by Increased Flow Rate in Rat Ileum In Vivo

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Abstract

Studies in intact animals have shown that intestinal solute absorption is enhanced with increasing flow rates; the mechanism of this phenomenon has not been explored in detail. We used single pass perfusions of rat ileum to study the effect of higher flow rate on electrolyte absorption. Augmenting perfusion rate from 0.5 to 5.0 ml/min resulted in increased rates of sodium (11.0 ± 0.9 vs. 23.5 ± 2.7 $\mu\text{eq}/\text{min} \cdot \text{g}$) and chloride (12.1 ± 0.8 vs. 25.0 ± 2.2 $\mu\text{eq}/\text{min} \cdot \text{g}$) absorption, reduction in the estimated unstirred layer thickness (668 ± 31 vs. 433 ± 28 μm), minimal changes in intraluminal pressure and transmural potential difference, and a small, though significant, increase in intraluminal volume ($19.4 \pm 8.4\%$). Removal of sodium from the perfusion medium abolished the effect of increased flow rate on chloride absorption as did removal of chloride on sodium absorption; addition of furosemide or acetazolamide to Ringer's solution also inhibited this effect. In separate experiments, stepwise increases in intraluminal volume were induced by elevating the outflow tubing; no effect on electrolyte transport was observed.

These studies demonstrate that neutral sodium chloride absorption is enhanced in rat ileum at higher flow rates, perhaps as a result of a decrease in the thickness of unstirred layers.

Introduction

The influence of motor function on absorption in the normal and diseased intestine is poorly understood (1, 2). Cholera enterotoxin, for example, elicits both electrolyte secretion (3) and abnormal, perhaps propulsive motor activity, which results in shortened transit time and mucosal contact (4). Opiates, long recognized as antidiarrheal agents, increase both transit time (5) and electrolyte transport (6). The effects, if any, of changes in contractile activity on intraluminal pressure, changes in intraluminal pressure on flow rate, and changes in flow rate on mucosal contact and intraluminal mixing are uncertain and need clarification.

Previous perfusion studies have demonstrated that higher luminal flow rates are associated with enhanced rates of absorp-

tion of various solutes (7–10). To explain this phenomenon, it has been suggested that faster flow rates result in thinning of unstirred layers, thus increasing solute concentration at mucosal transport sites (11, 12). The effects of unstirred layers on intestinal solute transport have been demonstrated in vitro by exposing isolated pieces of rat jejunum to increased rates of mechanical stirring (11). Increased rates of stirring resulted in enhanced rates of solute transport and a reduction in apparent Michaelis constant (K_m). The unstirred layer phenomenon, however, is distinctly different in an isolated mucosal sheet and in the intact perfused intestine. Isolating the intestine from its blood supply results in mucosal edema, villus engorgement, and subsequent obliteration of the intervillus space (13). Hence, diffusion through the intervillus space may no longer be a factor in solute absorption. Furthermore, in the perfused intestine, under conditions of laminar flow (14), a concentration gradient develops for a rapidly absorbed solute between the center of the fluid column and the brush border. Under these conditions the "unstirred layer" becomes essentially equal to the radius of the intestine (15–17). Therefore, the unstirred layer in a perfused intestinal segment should be of much greater thickness than that over a mucosal sheet. Discrepancies between in vitro and in vivo transport data have been attributed, in part, to the differences in unstirred layer thickness (18).

Changes in intraluminal volume or pressure may also affect intestinal transport. Prior studies have suggested that increased intraluminal volume may result in enhanced intestinal absorptive surface area, or that increased intraluminal pressure may result in increased intestinal pore size and permeability (7, 8, 19–21). Under perfusion conditions, the effects of increasing flow rate, volume, and pressure can be difficult to discern.

We performed single pass perfusions in order to determine the effect of flow rate on electrolyte transport in the rat ileum. In addition, we examined the relationship between intraluminal volume, pressure, and electrolyte absorption in this organ. These studies demonstrate that ileal Na and Cl transport is enhanced with increasing flow rates; this enhancement appears to be independent of changes in both intraluminal volume and pressure and may be related to thinning of unstirred layers.

Methods

Male Sprague-Dawley rats, 190–300 g (Charles River Breeding Laboratories, Inc., Wilmington, MA), were used in all experiments. Single pass perfusions of the distal ileum (20 cm) were performed in anesthetized animals (Inactin; 100 mg/kg body wt) at flow rates of 0.5, 2.5, and 5.0 ml/min in random sequence. These flow rates were chosen to avoid significant changes (>5%) in Na concentration along the longitudinal axis of the study segment as the perfusion speed was varied. The composition of the perfusion solution was (in millimolars): Na, 140; K, 5; Cl, 120; HCO_3 , 25; and mannitol, 30 (pH 7.4 by gassing with 95% O_2 /5% CO_2). In the experiments in which Na concentration was varied between 40 and 140 meq/liter, sodium was replaced by choline. In other

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experiments, Cl was replaced with sulfate. [³H]Polyethylene glycol (PEG)¹ plus unlabeled PEG 4000 (2 g/liter) was used as a nonabsorbable marker of water movement. Measurements of unidirectional Na fluxes were performed by the addition of ²²Na (8 μCi/liter) to the perfusion medium, using equations derived by Curran and Solomon (22). In some studies, 1 mM furosemide (Hoechst-Roussel Pharmaceuticals, Somerville, NJ) or 1 mM acetazolamide (Sigma Chemical Co., St. Louis, MO) was added to the perfusion medium. Effluent was collected for four 10–15-min intervals after equilibration periods of 40, 15, and 10 min at 0.5, 2.5, and 5.0 ml/min, respectively. Transmural potential difference (PD) was measured by placing 4% agar bridges into both the proximal infusion catheter and peritoneum. Junctional potentials were minimized by constituting the bridges with the same solution as used during the perfusion. A semi-rigid cannula was placed into the proximal part of the study segment and connected to a column manometer for measurement of intraluminal pressure.

Intraluminal volume measurements. A technique involving continuous marker infusion was developed that permitted repetitive measurements of intraluminal volume. After irrigating the study segment with saline and allowing the residual fluid to drain, the test solution (containing [³H]PEG) was perfused until equilibrium was reached. The effluent and infused volumes were determined to an accuracy of 0.01 ml (assuming a specific gravity of 1.0) by weighing the reservoir and collection vessels. After determination of the [³H]PEG activity in the test solution and effluent fluid, intraluminal volume was calculated (see Appendix). Measurements could be repeated by rinsing the study segment with 75 ml of warmed saline. By expressing the results as “relative” volume in each animal (ratio, Table I, or percent change, Fig. 4), scattering of the data, which arises from wide variations of absolute volumes in animals of comparative body weight, was circumvented.

This method was validated by repetitive measurements of intraluminal volume in 12 animals at a flow rate of 0.5 ml/min. There was no significant variation in volume over four consecutive study periods (Table I). Measurements of Na absorption made concurrently (after equilibration was reached) indicated that Na absorption did not vary during the time when repetitive volume determinations were made.

Unstirred layer measurements. We used the technique described by Diamond (23) and adapted by Read et al. (24) for measurement of unstirred layer thickness *in vivo*. This technique relates the time course (*t*_{1/2}) of a change in diffusion potential after a rapid change in Na concentration to unstirred layer thickness. In these experiments, the luminal solution (containing 25 mM Na) was rapidly replaced with a solution containing 140 mM Na, and the resulting transmural PD was recorded over time. By the equation derived by Diamond,

$$\delta = \left(\frac{D_{Na} t_{1/2}}{0.38} \right)^{1/2}, \quad (1)$$

where *D*_{Na} is the diffusion coefficient of Na in aqueous solution at 37°C (2.0 × 10⁻⁵ cm²/s) (13), the effective thickness (*δ*) of an unstirred layer of water could be calculated. Diffusion potential was measured by positioning an agar bridge in a nonconducting polyethylene catheter within 1 cm of the entrance to the study segment, and the site for changing the solutions was placed 2 cm proximal to the tip of the bridge. The purpose of taking measurements of the electrical transient from a proximal position in the segment was to record this change instantaneously as the new solution appeared at the entrance of the study segment, i.e., before any mixing of the two solutions more distally in the perfused segment. The appearance of the first observed change in PD matched that predicted from the dead space of the tube and the flow rate. Furthermore, shortening of the study segment from 20 to 10, 7, and 3 cm had no effect on the time course of the change in diffusion potential from this position in the study segment (Table II). Technical aspects of the surgical preparation precluded further shortening of the segment.

1. **Abbreviations used in this paper:** PD, potential difference; PEG, polyethylene glycol.

Table I. Repetitive Measurements of Intraluminal Volume and Na Absorption*

	Period				<i>P</i> ‡
	1	2	3	4	
Intraluminal volume	(1.00)	1.18±0.14	0.99±0.14	0.95±0.09	NS
Na absorption	12.0±1.1	11.0±0.9	10.9±0.8	12.2±1.0	NS

* At flow rate of 0.5 ml/min in four study periods (*n* = 12). Volume expressed as that relative to period 1 in each animal. Na absorption is expressed as microequivalents per minute per gram of dry weight. Results are mean±SE.

‡ *P* > 0.10 for each period by paired *t* test.

An agar bridge was also placed in the distal outflow catheter for making simultaneous recordings of the transient as the perfusion solution was changed. Placing the bridge in the distal portion of the study segment significantly lengthened the time course of the PD change; shortening the study segment (thereby reducing the available mixing space proximal to the recording site) reduced this change (Table II). The onset of the PD change was also delayed at the distal recording site. The apparent maintenance of a PD in free solution between proximal and distal ends of the intestinal segment is explained by treating the intestine as a poorly insulated conducting cable of finite length (25). Since the electrical conductivity of the bowel wall approximates the conductivity of the fluid-filled lumen, a large PD may be dissipated through the bowel wall over a short distance (25). Although the electrical transient recorded at the proximal bowel was dissipated before its arrival at the distal recording site, insulating the proximal electrical bridge in the polyethylene inflow catheter assured reliable detection of PD immediately adjacent to the inflow catheter. Dissipation of PD changes over short distances also assured that artifactual changes in PD due to mixing in distal parts of the study segment would not be conducted back to the proximal intestine. These findings suggested that changes in PD in the most proximal intestine were transmitted reliably to the proximal recording site and were not influenced by luminal mixing.

Analytical methods. Na and K were determined by flame photometry and Cl by electrometric titration with a silver nitrate solution. For isotope determination, 1 ml of perfusion solution or sample was mixed with 5 ml of scintillation cocktail (Ready Solv HP, Beckman Instruments, Inc., Fullerton, CA). Samples were counted in a liquid scintillation counter (L3300, Beckman Instruments, Inc.). In some experiments, quench cor-

Table II. Proximal vs. Distal Recording of *t*_{1/2} (s)

	Segment length			
	20	10	7	3
	cm	cm	cm	cm
1 Proximal	60±15	61±14	75±20	51±18
2 Distal	149±26*	125±22*	100±10‡	71±22‡

Measurements of the time course (*t*_{1/2}±SE, *n* = 7) of the change in diffusion potential (in seconds) after rapid change in Na concentration (25–140 mM) from agar bridges positioned at the entrance (proximal) and exit (distal) of the intestinal segment, and the effect of changes in study segment length. Shortening the study segment did not affect *t*_{1/2} measured at the proximal recording site (row 1). In contrast, *t*_{1/2} measured at the distal site (row 2) decreased as the segment was shortened (‡ *P* < 0.05 compared with 20 cm). Distal measurements were greater than proximal (* *P* < 0.05 compared with proximal) at 20 and 10 cm; these differences were minimized as the intestinal segment was shortened.

rection was made by external standardization. Since quenching remained constant between collected samples, quench correction, when implemented, did not alter measurements of absorption. Radioactivity in samples containing ^3H and ^{22}Na was determined by standard methods for doubly labeled samples. Counts per minute were converted into disintegrations per minute for each isotope with a computer program that corrected for quenching and spillover of ^{22}Na into the ^3H -channel. Spillover of ^3H into ^{22}Na was $<1\%$ in all cases.

Calculations and statistical analysis. Net water and electrolyte movements were calculated by standard formulas from the changes in PEG and solute concentrations (26). The results are expressed as mean \pm SE. Net water movement is expressed as microliter per minute per gram dry tissue weight, and electrolyte absorption as microequivalent per minute per gram dry tissue weight. Tissue dry weight was determined by incubating the excised intestinal study segment overnight at 110°C . Differences in absorption were evaluated by paired or unpaired t test analysis.

Results

Net fluid and electrolyte movement. Fig. 1 A demonstrates the rate of net Na absorption at luminal flow rates of 0.5, 2.5, and

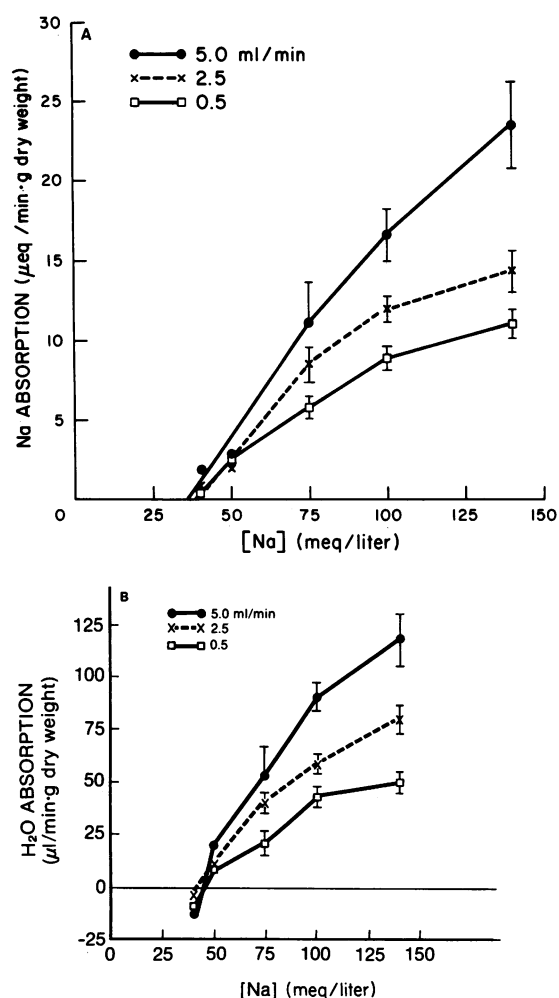


Figure 1. Effect of increasing flow rate (0.5, 2.5, and 5.0 ml/min on A) Na absorption ($\mu\text{eq}/\text{min} \cdot \text{g}$ dry weight), and (B) H_2O absorption ($\mu\text{l}/\text{min} \cdot \text{g}$ dry weight) at Na concentrations of 40, 50, 75, 100, and 140 meq/liter. Values are expressed as mean \pm SE ($n = 6, 10, 10, 11,$ and $12,$ respectively). Na and H_2O absorption at the two higher flow rates were significantly greater than at 0.5 ml/min ($P < 0.05$) at 75, 100, and 140 meq/liter Na concentration.

5.0 ml/min. There were progressive increases in net Na absorption with increasing flow rates. The effect of flow rate on Na absorption was directly dependent on the Na concentration; no effect was observed at lower Na concentrations. At the 140 mM Na concentration, a fivefold increase in flow rate (0.5–2.5 ml/min) resulted in a 31% increase in Na absorption. A further increase in flow rate (2.5–5.0 ml/min) enhanced Na absorption by more than twice this amount (83%). Fig. 1 B demonstrates the net increase in water absorption that was observed under these conditions. Increases in water absorption roughly paralleled the increases noted with Na absorption.

Lumen-positive PDs were recorded at low Na concentrations that were in agreement with earlier studies (Fig. 2) (22). A slightly negative PD was noted at the 140 mM Na concentration, which was not statistically significant from zero. Increasing the perfusion rate from 0.5 to 5.0 ml/min resulted in a small though statistically significant downward shift (i.e. more negative) in the PD during perfusion with 75 and 100 mM Na, but no significant change in transmural PD occurred with changes in perfusion rate at 40, 50, and 140 mM Na concentrations. Thus, large increments of net Na absorption induced by faster flow rates were not paralleled by substantial changes in transmural PD.

Unidirectional Na fluxes. Measurement of net and unidirectional Na fluxes were made in a separate group of eight rats perfused with isotonic (140 mM Na) Ringer's solution. Increasing the flow rate from 0.5 to 5.0 ml/min caused an increase in unidirectional lumen-to-plasma Na flux from 45.6 ± 4.4 to 54.6 ± 5.1 $\mu\text{eq}/\text{min} \cdot \text{g}$ (16% increase), whereas there was no significant change in unidirectional plasma to lumen Na flux (35.8 ± 3.1 to 32.7 ± 4.1 $\mu\text{eq}/\text{min} \cdot \text{g}$). The change in net Na absorption (lumen-to-plasma minus plasma-to-lumen Na flux) was comparable with earlier experiments (9.8 ± 0.7 to 21.9 ± 1.4 $\mu\text{eq}/\text{min} \cdot \text{g}$) and again represented a greater than twofold increase in absorption.

Changes in pressure, volume, and unstirred layers. Three mechanisms were considered whereby increased flow rate may have increased Na absorption: (a) Increased intraluminal hydrostatic pressure could act as a driving force for fluid and electrolyte absorption (27), although higher pressures could also obstruct venous outflow (21, 28) or stimulate secretory processes by inducing cholinergic reflexes (29); (b) Increased intraluminal volume (surface area) could result in recruitment of epithelial transport sites (7, 8); and (c) Decreased unstirred layers could facilitate access of electrolytes to epithelial transport sites (11, 12).

Fig. 3 demonstrates the relationship between flow rate and intraluminal pressure observed in these studies. No significant increase in pressure was noted over a 100-fold range of flow rate, and at no point did intraluminal pressures approach that reported to affect either mucosal permeability (27, 28) or blood flow (30).

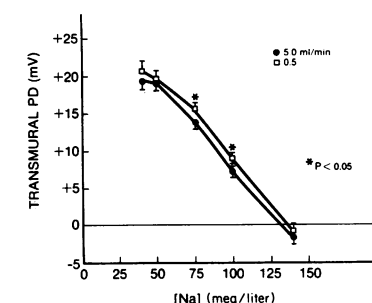


Figure 2. Effect of increasing flow rate on transmural PD, in millivolts, at Na concentrations between 40 and 140 meq/liter. Values represent PD of the luminal relative to the peritoneal potential. Results are expressed as mean \pm SE ($n = 8$). * $P < 0.05$, 5.0 ml/min compared with 0.5 ml/min.

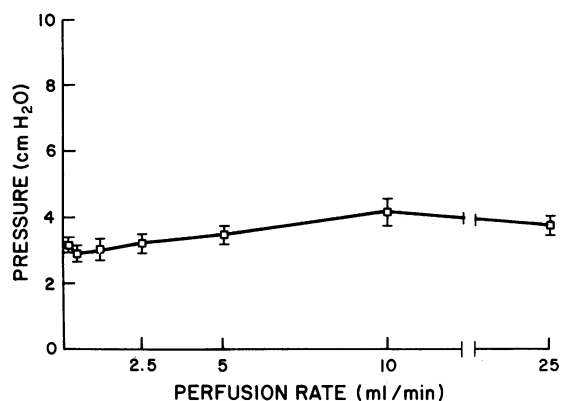


Figure 3. Effect of increasing perfusion rate (milliliters per minute) on intraluminal pressure (centimeters H₂O) measured from a proximally placed manometric catheter. Results are expressed as mean \pm SE ($n = 11$). No statistical differences in mean pressure were observed between perfusion rates.

Next, measurements of ileal intraluminal volume were performed at flow rates of 0.5, 2.5, and 5.0, using isotonic (140 mM Na) Ringer's solution. There was only a small (19.4 \pm 8.4%) increase in measured loop volume between flow rates of 0.5 and 5.0 ml/min, whereas no significant alteration of intraluminal volume occurred between 0.5 and 2.5 ml/min (5.2 \pm 7.4%). Therefore, the initial enhancement (31%) of Na absorption between 0.5 and 2.5 ml/min was not the result of a change in intraluminal volume. The relationship between increasing intraluminal volume and Na absorption was further examined in separate experiments (Fig. 4). Perfusions were performed with isotonic Ringer's solution at 0.5 ml/min using preset elevations of the outflow tubing to effect desired increases in intraluminal volume from baseline volumes. The intraluminal pressure in the absence of any outflow tubing elevation was 2.8 \pm 0.1 cm H₂O. Outflow tubing elevations of 1, 3, and 5 cm resulted in mean increases of 1.3 \pm 0.5, 2.3 \pm 0.4, and 5.8 \pm 0.6 cm H₂O, re-

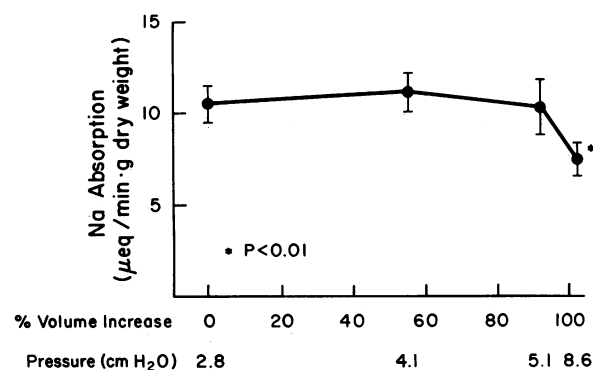


Figure 4. Relationship between increasing intraluminal volume and Na absorption measured at preset elevations of outflow tubing (1, 3, and 5 cm) above baseline at 0.5 ml/min. Volume is expressed below as percent increase above baseline (defined as zero elevation of outflow tubing). Na absorption is expressed as $\mu\text{eq}/\text{min} \cdot \text{g}$ dry tissue weight. Pressures measured at these respective elevations of outflow tubing are recorded in the lower line (baseline pressure 2.8 cm H₂O). Values for Na absorption are expressed as mean \pm SE ($n = 8$). * $P < 0.01$ compared with baseline.

spectively, above the initial value (no statistically significant difference between observed and predicted increases in pressure). The absolute pressure obtained by this manipulation (see lower line "Pressure," Fig. 4) were below those previously described to affect electrolyte transport in the rat small intestine (21). Thereafter, simultaneous measurements of sodium absorption were made. Despite a >100% increase in intraluminal volume, there was no enhancement of sodium absorption (at the highest volume, a decrease in sodium absorption was observed). Thus, the more marked enhancement of sodium absorption at 5.0 ml/min could not be related to increases in luminal volume.

Lastly, experiments were performed to test the hypothesis that faster flow rates resulted in diminution of unstirred layers (Table III). The $t_{1/2}$ of the electrical transient was significantly reduced at 2.5 and 5.0 ml/min compared with 0.5 ml/min. Using the diffusion coefficient of Na in aqueous solution to solve Eq. 1, the equivalent unstirred layer thickness was reduced by 20 and 35%, respectively.

Characterization of enhanced Na transport. Tables IV and V demonstrate the results of studies to determine the nature of the Na transport process augmented by flow rate. The left panel in Table IV represents the net rate of Na and Cl absorption observed in Ringer's solution at 140 mM Na concentration. The increase in the rate of Cl absorption between 0.5 and 5.0 ml/min paralleled the increase in Na absorption. Because transmural PD was little affected by increased flow rate at this Na concentration (Fig. 2), despite large changes in absorption (Fig. 1 A), further studies were performed to determine if the increase in Na and Cl absorption at 5.0 ml/min was due to induction of neutral NaCl absorption (which may represent either coupled NaCl co-transport or Na-H and Cl-HCO₃ exchanges) (31).

Replacement of Na in the perfusion medium by choline abolished the effect of luminal flow rate (represented as Δ) on Cl absorption (Table IV). Similarly, substitution of sulfate for Cl also prevented the increase in Na absorption produced by increases in flow rate. In an additional set of experiments (Table V), furosemide (1 mM) or acetazolamide (1 mM), which inhibit neutral NaCl absorption in the ileum (32, 33), was added to Ringer's solution. Acetazolamide abolished Na and Cl absorption and eliminated the increases noted at 5.0 ml/min. Furosemide reduced Na and Cl absorption at 0.5 ml/min from 11.0 \pm 0.9 to 3.5 \pm 0.3 and from 12.1 \pm 0.8 to 5.1 \pm 2.7 $\mu\text{eq}/\text{min} \cdot \text{g}$ dry tissue weight, respectively; enhancement in net Na absorption observed by increasing flow to 5.0 ml/min was reduced by 75% and the increase in Cl absorption was inhibited completely. Neither ac-

Table III. Measurement of Unstirred Layer Thickness

Flow rate	$t_{1/2}$	δ
ml/min	s	μm
0.5	84.8 \pm 8.0	668 \pm 31
2.5	53.4 \pm 5.4*	530 \pm 26*
5.0	35.6 \pm 4.8*	433 \pm 28*

The $t_{1/2}$ of the electrical transient (in seconds) after a change in Na concentration in the perfused solution from 25 to 140 mM, and the equivalent thickness (δ) of an unstirred layer of water, as derived from Eq. 1 (see text), at flow rates of 0.5, 2.5, and 5.0 ml/min. Recordings of $t_{1/2}$ were made at the proximal infusion site. Results are expressed as mean \pm SE ($n = 11$).

* $P < 0.05$ compared with 0.5 ml/min.

Table IV. Effect of Ion Substitution on the Increase in Electrolyte Absorption Induced by Increasing Flow Rate

	Ringer's solution (12)		Na free (8)		Cl free (6)	
	Na	Cl	Na	Cl	Na	Cl
0.5	11.0±0.9	12.1±0.8	-7.8±0.9	1.0±1.0	1.0±1.0	-0.8±1.0
5.0	23.5±2.7*	25.0±2.2*	-10.9±2.2	-1.3±3.0	-0.7±2.1	-2.8±0.9
Δ	12.5±2.0	12.9±1.4	-3.1±2.0‡	-2.3±7.1‡	-1.7±2.0‡	-2.0±1.4‡

Na and Cl absorption expressed as microequivalents per minute per gram of dry weight at 0.5 and 5.0 ml/min. Na-free experiments represent replacement of Na by choline and Cl-free experiments replacement of Cl by sulfate. Differences (Δ) in absorption were calculated as 5.0 ml/min minus 0.5 ml/min. Positive values represent net absorption and negative values net secretion at the two respective flow rates. Positive values for Δ represent an increase in absorption at 5.0 ml/min compared with 0.5 ml/min, and negative values represent a decrease. Results are expressed as mean±SE. Numbers in parentheses are the number of experiments. * $P < 0.01$ compared with 0.5 ml/min. ‡ $\Delta P < 0.01$ compared with Ringer's solution.

etazolamide nor furosemide caused a significant change in transmural PD at either flow rate (data not shown).

Discussion

These studies demonstrate that faster flow rates stimulate Na, Cl, and water absorption in the rat ileum. The increase in Na absorption (a) is electrically neutral, Cl-dependent, and inhibited by both furosemide and acetazolamide, consistent with augmentation of neutral NaCl absorption; (b) can be demonstrated in the absence of a significant change in intraluminal volume (at 0.5–2.5 ml/min) and pressure; and (c) is associated with significant reductions in the measured unstirred layer thickness (Table III).

It has been stated that expansion of intraluminal volume at higher flow rates may result in increases in the functional absorptive surface of the intestine (7, 8, 19, 20). In previous studies of the rat intestine, the expected enhancement of solute absorption, as flow rate was increased, was inhibited (but not abolished) by imposing constant distention at all flow rates (7, 8). Because intraluminal volumes were never measured directly in these experiments, the assumption that volume had been increasing in parallel with perfusion rate in controls (in the absence of distention) may not have been correct. In one study in which volumes were measured, determinations were made by expressing luminal contents into a glass cylinder, a method of uncertain accuracy (19). Adherent fluid in the segment could have intro-

duced large errors into these measurements in view of the small volumes being measured (1–3 ml) (34). Also, manipulation of the intestine, by inducing secretion, could have made these tissues unsuitable for studying transport (35).

Most studies dealing with the effect of intraluminal volume on solute transport have dealt with the absorption of nonelectrolytes. Matuchansky et al. (36) reported that metoclopramide, a dopamine antagonist, reduced electrolyte and fluid absorption in human intestine by reducing intraluminal volume. However, it is known that dopaminergic agents may influence electrolyte transport directly (37).

A continuous marker infusion technique was developed in our experiments in order to more accurately assess intraluminal volume in a small animal preparation. The method yielded acceptable levels of error, and repetitive measurements yielded constant transport values (Table I).

We determined that beyond a perfusion rate of 0.5 ml/min there is little change in intraluminal volume. These results are consistent with similar findings in the human intestine at comparatively fast rates of perfusion (38). We also found that there was no significant change in net Na absorption even when intraluminal volume increased nearly twofold (at 5.8 cm H₂O pressure). At greater degrees of distention, there was a loss of intestinal compliance (a steeper rise in pressure for the increase in volume effected) and a decrease in net Na absorption. The failure of distention to augment electrolyte absorption is in agreement with more recent studies by Swabb et al. (21) and

Table V. Effect of Furosemide and Acetazolamide on the Increase in Electrolyte Absorption Induced by Increasing Flow Rate

	Ringer's solution (12)		Ringer's solution + 1 mM furosemide (8)		Ringer's solution + 1 mM acetazolamide (12)	
	Na	Cl	Na	Cl	Na	Cl
0.5	11.0±0.9	12.1±0.8	3.5±0.3	5.1±2.7	1.1±0.8	0.5±0.7
5.0	23.5±2.7*	25.0±2.2*	6.2±3.2*	3.3±3.7	-0.1±3.2	0.7±0.8
Δ	12.5±2.0	12.9±1.4	2.7±1.0‡	-1.8±0.8‡	-1.0±3.3‡	0.2±1.0‡

Na and Cl absorption expressed as microequivalents per minute per gram of dry weight at 0.5 and 5.0 ml/min. Difference (Δ) calculated as 5.0 ml/min minus 0.5 ml/min. Positive values represent net absorption and negative values represent net secretion at the two respective flow rates. Positive values for Δ represent an increase in absorption at 5.0 ml/min compared with 0.5 ml/min; negative values represent a decrease. Results are expressed as mean±SE. Numbers in parentheses are the number of experiments. * $P < 0.01$ compared with 0.5 ml/min. ‡ $\Delta P < 0.01$ compared with Ringer's solution.

suggests that the relationship assumed between intraluminal volume and intestinal surface area should be reexamined. The discrepancy with earlier studies suggesting a positive effect of intraluminal volume on absorption could be reconciled by hypothesizing that the absorptive rates of electrolytes and non-electrolytes are not affected equally by distention. Increased pressure at greater degrees of distention has been demonstrated not to adversely affect nutrient absorption (28), but has been shown to induce electrolyte secretion (21). An electrolyte secretory process induced at higher intraluminal pressures may have negated any visible effect of increased surface area on net electrolyte absorption in our experiments.

There exists little experimental data supporting a direct relationship between intraluminal volume and absorptive surface area. In vitro histologic studies have not demonstrated any change in surface area when the bowel is passively distended (39, 40). In vivo, the problems in quantitating surface area has been the subject of extensive discussion (41–43). Functional chemical markers of absorptive surface area (urea, $^3\text{H}_2\text{O}$, xylose) (7, 44) have been used in many perfusion studies, but problems with these markers question their reliability. Urea and $^3\text{H}_2\text{O}$ absorption are both affected by bulk water transport (45), and urea absorption may be sensitive to the effects of the unstirred layer (8). Xylose may be absorbed by a facilitated mechanism (46). The limitations of available methods to assess absorptive surface area in vivo did not permit us to make definitive statements about changes in absorptive surface area in our studies.

We sought to examine the effect of faster flow rate independent of changes in intraluminal volume on electrolyte absorption. This line of inquiry was supported by earlier reports relating changes in electrolyte concentration in the unstirred layer to changes in luminal flow (47, 48). Although the rapidity of electrolyte diffusion through aqueous solutions would tend to minimize electrolyte concentration gradients across the unstirred layer, we reasoned that even small changes in electrolyte concentration could have considerable effects on net electrolyte flux. Net electrolyte transport in rat ileum represents the algebraic sum of oppositely directed absorptive and secretory processes (31). Although net transport may be minimal, unidirectional fluxes are normally of considerable magnitude (22). In our studies, at a faster flow rate, small increases (16%) in lumen-to-plasma Na flux led to large changes in net Na flux (114%). This finding provided a mechanism by which the effect of even small changes in Na concentration in the unstirred layer could be magnified. Further analysis of this problem also pointed to the specific mechanism of electrolyte transport present in this tissue. In the rat ileum, neutral NaCl absorption (either coupled NaCl co-transport or Na-H and Cl-HCO₃ exchanges) is the predominant sodium transport mechanism (32). To our knowledge, studies have not been conducted specifically to measure Na and Cl concentrations at the mucosal surface, but the existence of a zone of high hydrogen ion concentration has been documented in the rat intestine (49). The effect of flow rate could therefore have been to dissipate either an inwardly directed gradient of Na and Cl or an outwardly directed H-ion gradient; either effect could result in apparent stimulation of neutral NaCl absorption.

In contrast to the rather minimal changes in pressure and volume, there was significant reduction in the effective unstirred layer thickness measured at 2.5 and 5.0 ml/min. The derived values for the unstirred layer thickness are in the range reported for the intact rat small intestine (50–53) and confirm the inverse relationship postulated previously between flow rate and un-

stirred layer thickness (7, 8). It should be stressed that strict quantitative comparisons of the unstirred layer thickness at different flow rates is somewhat unrealistic, and that these changes be considered only as supportive evidence for possible mechanisms by which flow rate stimulates electrolyte transport for the following reasons. First, the Diamond technique was derived mathematically for diffusion to a flat epithelium and takes on additional complexity in the intact small intestine (23). Second, the diffusion of Na, Cl, and H ions is inhibited considerably by the mucus gel layer (54, 55). By using aqueous diffusion coefficients in Eq. 1, the dimension of the unstirred layer may be misrepresented. Therefore, changes in unstirred layer thickness at higher flow rates as represented in Table III are, in essence, changes in an equivalent unstirred layer of water. Third, measurements of unstirred layers performed in this study, due to the limitations noted, reflect only electrical transients appearing in the most proximal portion of the perfused segment. The technique for measurement of unstirred layers used in this study may have underestimated the change in mean unstirred layer thickness, since the unstirred layer could grow along the length of a perfused segment (15–17). Lastly, changes in the "surface area of the unstirred layer" (see reference 11) cannot be quantitated for compounds absorbed by active transport processes. In these studies, at 5.0 ml/min, a 35% decrease in measured unstirred layer thickness resulted in a 16% increase in lumen-to-plasma Na flux. Although difficulties in quantifying unstirred layers preclude their absolute predictive value, these proportionate changes in unstirred layer thickness and unidirectional Na flux appear reasonable.

These studies, designed to elucidate the observed increase in net Na absorption at higher flow rates, indicate that resulting changes in pressure and volume are not responsible, but permit the speculation that unstirred layers might influence electrolyte absorption and secretory processes. Other explanations for this phenomenon have not been eliminated and are important considerations for further study. For example, faster perfusion speeds abolish migrating motor complex cycling in the dog ileum (Kruis, W., and S. F. Phillips, personal communication), and it is likely that an enteric reflex stimulated at higher flow rates could induce augmented rates of Na absorption. α -2 adrenergic agonists, for example, stimulate Na and Cl absorption in several animal models (56). Reflex arcs involving cholinergic neurons, conversely, may mediate secretion induced by luminal obstruction (29). In our study, motor activity was not monitored, but significant distention of the bowel was not observed at faster flow rates because there was little change in pressure and volume. Hence, a sympathetic reflex mediated by mechanical receptors (perhaps deformation of villi) may have been unmasked by faster flow rates under these conditions, and would be an alternative explanation for the observed effects.

For reasons noted previously in the Methods section, these studies were carried out at perfusion speeds resulting in no more than a 5% change in solute concentration along the longitudinal axis of the loop. Although 0.5–5.0 ml/min probably exceeds the mean flow rates ever present in the rat ileum, it is possible that rates of flow over short distances, the result of rapid bursts of muscle activity, might be as high. Oppositely directed flow is often observed in the absence of any net flow, the foremost example being the relative "retardation" of ongoing flow by segmental bowel contractions, which may account for the majority of the motor activity present in the proximal colon or after opiate administration (57). Hence, the mean flow rate in this situation

would insufficiently describe overall intraluminal movement. Although techniques are available for making measurements of mean flow rate *in situ*, the present methodology excludes proper assessment of instantaneous fluid movement. Therefore, despite the hazards inherent in relating experimental observations to physiological circumstances, we speculate that flow rates of this magnitude are achievable in ileum under normal circumstances over short distances, and may have important implications for net fluid and electrolyte absorption.

Appendix

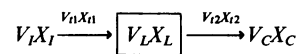
Continuous marker perfusion technique

Symbols:

V_I ,	volume of solution perfused from reservoir
V_{I1} ,	volume of infusion tubing
V_{I2} ,	volume in effluent tubing
V_L ,	final volume of the loop at equilibrium
V_C ,	volume of effluent fluid
X_I ,	concentration of [^3H]PEG in reservoir fluid
X_{I1} ,	concentration of [^3H]PEG in infusion tubing at equilibrium
X_{I2} ,	concentration of [^3H]PEG in effluent tubing at equilibrium
X_L ,	mean concentration of [^3H]PEG in the intestinal loop at equilibrium
X_C ,	concentration of [^3H]PEG in effluent fluid at equilibrium

$J_{ABS}\text{H}_2\text{O}$, rate of water absorption

$J_{ABS}S$, rate of solute absorption



Under equilibrium conditions:

$$V_I X_I = V_{I1} X_{I1} + V_L X_L + V_{I2} X_{I2} + V_C X_C.$$

Assuming $X_{I1} = X_I$, the equation may be rewritten as:

$$V_I X_I = V_{I1} X_I + V_L X_L + V_{I2} X_{I2} + V_C X_C.$$

Under these conditions, X_{I2} is the concentration of the nonabsorbable marker after perfusion through the study segment (also denoted as X_O), and X_C represents further dilution of the initial effluent solution by the residual fluid in the loop at the onset of the perfusion.

By the equation derived by Soergel (58):

$$X_L = X_I - (X_I - X_O) 0.683.$$

It therefore follows that:

$$V_I X_I = V_{I1} X_I + V_{I2} X_O + V_L X_L + V_C X_C,$$

or

$$V_L = \frac{(V_I - V_{I1})X_I - V_C X_C - V_{I2} X_O}{X_I - (X_I - X_O) 0.683}. \quad (2)$$

At equilibrium:

$$J_{ABS}\text{H}_2\text{O} = V_{II} \left(1 - \frac{X_I}{X_O}\right) \quad (3)$$

$$J_{ABS}S = V_{II} \left(S_I - S_O \frac{X_I}{X_O}\right).$$

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