

The Mechanism of Decreased Intestinal Sodium and Water Absorption after Acute Volume Expansion in the Rat

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ABSTRACT Studies were performed in rat small intestine *in vivo* to determine the effect of saline infusion on intestinal transport of Na^+ and H_2O . Saline infusion decreased net Na^+ flux (J_{Na^+}) from 12.7 ± 0.8 to 6.4 ± 1.5 $\mu\text{Eq/hr per cm}$ in the jejunum when the intestinal perfusate contained both Na^+ and glucose. A similar fall in J_{Na^+} occurred in ileum. When mannitol was substituted for glucose in the perfusate, control absorption decreased 29% in jejunum and 18% in ileum, but saline infusion still caused a decrease in J_{Na^+} quantitatively similar to that seen when glucose was present. When choline was substituted for Na^+ in the perfusate, there was net movement of Na^+ from blood to lumen during control and this net secretion was increased further after saline infusion. These observations suggest that saline infusion has a similar effect to decrease intestinal J_{Na^+} under three widely different conditions of basal sodium transport. Permeability of intestinal mucosa to inulin was very low under basal conditions but increased fivefold after saline infusion, and the unidirectional flux of Na^+ from blood to lumen doubled. This increase in unidirectional flux of Na^+ was greater than the observed decrease in J_{Na^+} .

Thus, saline infusion decreased net absorption of Na^+ and H_2O from small intestine through mechanisms which did not appear to be dependent upon the rate of Na^+ flux from lumen to blood, and in association with an increased flux of inulin and Na^+ into the intestinal lumen. The data suggest that the effect of saline infusion to decrease net absorption from the intestine could be due either to an increase in passive permeability of the epithelium which could disrupt solute gradients within the membrane or to an increase in flow of solution into the intestinal lumen.

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INTRODUCTION

Abundant evidence has been accumulated in recent years consistent with the view that saline infusion increases the renal excretion of Na^+ through mechanisms dependent, at least in part, on a depression of net tubular reabsorption of Na^+ in the proximal tubule (1-3). This effect of saline infusion to decrease tubular reabsorption may result from changes in intrarenal hemodynamics and physical factors (4, 5) or from changes in some humoral substance which regulates tubular Na^+ transport (6). Little information exists on the mechanism by which factors initiated by volume expansion regulate the tubular transport of Na^+ . If changes in a circulating humoral substance are responsible for the decrease in Na^+ reabsorption after volume expansion, then it seems reasonable that the decreased reabsorption could be due to inhibition of the active component of tubular Na^+ transport. On the other hand, evidence indicates that hemodynamic and physical factors influence net proximal tubular transport through strictly intrarenal mechanisms as a result of a primary change in peritubular capillary absorption (7-10). Although such intrarenal effects of capillary absorption on net tubular transport could result also from an effect on the active component of tubular transport, it is possible that these physical influences occur at other steps determining net transtubular transport.

The mucosa of the mammalian small intestine resembles renal tubular epithelium in several functional and morphological aspects (11-17). Furthermore, it has been demonstrated recently that saline infusion depresses the net absorption of Na^+ and water from small intestine in rat (18), dog (19), and cat (20), a response resembling that of the renal proximal tubule. Therefore, it seems likely that information on the mechanism whereby saline infusion influences Na^+ and H_2O absorption by the small intestine would provide information applicable

to the mechanisms whereby acute volume expansion decreases net Na⁺ transport by the renal tubule.

Results of the present study indicate that saline infusion depresses net absorption of H₂O and Na⁺ in both jejunum and ileum by a mechanism not dependent on the presence of luminal glucose or Na⁺, and in some instances, this depression of intestinal absorption could be partly reversed by the infusion of hyperoncotic albumin. Also, saline infusion increased the movement of Na⁺ and inulin into the intestinal lumen suggesting that decreased net absorption related to either increased passive diffusion or increased flow of solution into the intestinal lumen, neither of which requires that active Na⁺ transport be depressed during saline infusion.

METHODS

Studies were performed in 54 male Sprague-Dawley rats weighing 190–420 g which were allowed free access to food and water until the time of the experiment. The animals were anesthetized with intraperitoneal Inactin, 120 mg/kg body weight, and placed on a heated operating table which maintained rectal temperature constant at 37 ± 1°C. A tracheostomy was performed and polyethylene catheters were placed in a jugular vein for infusion of fluids and in a femoral or a carotid artery for obtaining blood samples and monitoring arterial blood pressure with a transducer and direct-writing recorder (Hewlett-Packard Co., Palo Alto, Calif.). The abdomen was opened in the midline and approximately 10-cm lengths of jejunum (just distal to the ligament of Treitz) and ileum (proximal to the ileocecal junction) were located. Small incisions were made in the antimesenteric borders at each end of these segments and luminal contents were gently expressed by hand. Each segment was irrigated with 5–10 ml of a Ringer's solution. Flexible Tygon catheters (o.d. 0.125 inch) were inserted through the incisions and tied in place, care being taken not to interfere with the blood supply of the perfused segment. The catheters from the proximal end of each segment were coiled one to two times before leading out of the abdominal cavity and the wound was closed with metal clips. A sustaining intravenous infusion of a Ringer's solution was delivered throughout all experiments at rates of 33–42 μl/min and in 29 experiments, this solution contained sufficient inulin to achieve plasma concentrations of approximately 100 mg/100 ml. In four of these rats carboxy-inulin-¹⁴C (New England Nuclear Corp., Boston, Mass.) was infused to achieve a radioactivity in plasma of approximately 1 × 10⁶ cpm/ml. All but three animals received an intramuscular injection of 0.25 mg desoxycorticosterone acetate during the surgical preparation.

Intestinal segments were perfused in the proximal to distal direction at a rate of approximately 200 μl/min by a Harvard constant infusion pump (Harvard Apparatus Co. Inc., Millis, Mass.). The exact rate was calibrated for each segment at the end of the experiment. Composition of the perfusion fluids was as follows: group I perfusate was a modified Tyrode's solution containing NaCl 137, NaHCO₃ 11.9, NaH₂PO₄ 0.4, KCl 3.4, CaCl₂ 1.4, MgCl₂ 0.1, and glucose 5 mM/liter, respectively; the osmolality of this solution was 290 mOsm/kg. Group II perfusate was identical in composition except that 5 mM mannitol was substituted for the glucose. Group III perfusate was identical

to that in group I except that all Na⁺ salts were replaced with the appropriate choline salts. In one experiment in this group, equiosmolar mannitol was used to replace all Na⁺ salts.

At the completion of surgery, perfusion was started and 30–60 min later, control collections of the effluent were made for three or four 15-min periods. Isotonic Ringer's solution (Na⁺ 140, K⁺ 4.0, Cl⁻ 124, HCO₃⁻ 20 mEq/liter, respectively) was then infused intravenously at a rate of 1 ml/min until the volume infused equaled 10% of the animal's body weight, after which the infusion rate was slowed to 382 μl/min. 15–20 min after completing the rapid infusion of saline, three to four additional 15-min collections were made. 22 rats were then infused with 1.5–2.5 ml of a 30 g/100 ml solution of bovine albumin in Ringer's solution over a 2 min period, after which the infusion of Ringer's solution was discontinued. Effluent was collected for an additional two to four 10-min periods. Arterial blood samples were obtained in heparinized capillary tubes at the midpoint of alternate collection periods.

In nine rats, the unidirectional flux of ²²Na⁺ was measured from blood to intestinal lumen. 20 μCi of ²²NaCl (Amersham/Searle Corp., Des Plaines, Ill.) in 0.1 ml was injected intravenously as a loading dose, and sufficient isotope added to the sustaining infusion to maintain plasma levels in the range of 0.5–1 × 10⁶ cpm/ml. In order to maintain the concentration of isotope in the collected effluent below 2% of that in plasma, shorter intestinal segments (approximately 6 cm) were perfused and the rate of perfusion was increased to 1.9 ml/min. 1 hr after infusing the isotope, five to seven consecutive 3-min collections of intestinal effluent were obtained and arterial blood samples were collected during alternate periods. These measurements were repeated after the infusion of the Ringer's solution. In five of these rats, measurements were made after infusion of 30 mg/100 ml albumin. In four animals, net Na⁺ absorption before and after the rapid perfusion rate was not statistically different (*P* > 0.50) either before or after volume expansion.

In five animals, two segments of ileum or jejunum of different lengths were perfused simultaneously in order to relate the total movement of inulin into the intestinal lumen to the length of segment perfused. Three of these animals received albumin-¹²⁵I (RISA) 15 min before beginning collections to achieve a level of radioactivity in plasma of 2–4 × 10⁴ cpm/ml. The purpose of these studies was to determine if the entry of inulin into the intestinal lumen was related to segment length and if inulin was accompanied by a proportional entry of albumin, as would be expected if random trauma to the perfused segments were permitting the entry of plasma or blood.

Phenolsulfonphthalein (PSP)¹ was added to all intestinal perfusion fluid to achieve a concentration of approximately 1 mg/100 ml and served as an index of volume change (21). The dye was measured in alkaline solution at a wavelength of 563 mμ. Effluent from intestinal segments perfused with the modified Tyrode's solution without PSP produced no absorption at this wavelength. Recovery of PSP from three jejunal segments averaged 95.2% and from seven ileal segments averaged 100.2%. Sodium, chloride, osmolality, total protein, and inulin were measured by techniques described previously (22). ¹⁴C-radioactivity (inulin) was measured in a Nuclear-Chicago liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.). ²²Na⁺

¹ Abbreviation used in this paper: PSP, phenolsulfonphthalein.

and ^{125}I -radioactivity were measured in a Packard Tri-carb gamma spectrophotometer (Packard Instrument Co., Downers Grove, Ill.).

Calculations. Net water flux ($J_{\text{H}_2\text{O}}$) was calculated from the following equation:

$$J_{\text{H}_2\text{O}} = V_i \left(1 - \frac{\text{PSP}_i}{\text{PSP}_0} \right) \cdot \frac{60}{L}$$

where V_i = perfusion rate in $\mu\text{l}/\text{min}$, PSP_i and PSP_0 are the initial and final concentrations of PSP respectively, and L is the intestinal segment length in centimeters. The net flux of any solute (J_{Na^s}) is given by the equation:

$$J_{\text{Na}^s} = V_i \left([\text{S}_i] - \frac{\text{PSP}_i}{\text{PSP}_0} [\text{S}_0] \right) \cdot \frac{60}{L}$$

where $[\text{S}_i]$ and $[\text{S}_0]$ are initial and final concentrations of the solute in μM or $\mu\text{Eq}/\text{ml}$. Permeability to inulin (cm^2/hr) was calculated as:

$$V_0 \cdot \left(\frac{E}{P} \right)_{\text{In}} \cdot \frac{60}{L}$$

where V_0 is the collected volume of effluent in $\mu\text{l}/\text{min}$ and $(E/P)_{\text{In}}$ is the ratio of inulin concentration in effluent to that in plasma. The unidirectional flux of Na^+ from blood into intestinal lumen (J_{Na^+}) in microequivalents/hour per centimeter was calculated as:

$$J_{\text{Na}^+} = V_0 \left(\frac{E}{P} \right)_{22\text{Na}^+} \cdot \frac{60 P_{\text{Na}^+}}{L}$$

where $(E/P)_{22\text{Na}^+}$ is the ratio of counts/minute per milliliter of $^{22}\text{Na}^+$ in the effluent to that in plasma, and P_{Na^+} is the average concentration of sodium in plasma determined in 24 rats at the end of experiments. Statistical significance of changes was calculated using Student's t test.

RESULTS

Effects of saline infusion. Data on intestinal absorption of Na^+ and H_2O in 17 jejunal and 14 ileal segments perfused with the modified Tyrode's solution are presented as group I in Table I. Net Na^+ flux before volume expansion was 12.7 ± 0.8 (SEM) and 8.9 ± 1.0 $\mu\text{Eq}/\text{hr}$ per cm in jejunum and ileum, respectively. Simultaneous net H_2O flux was 86.3 ± 5.3 and 52.8 ± 5.8 $\mu\text{l}/\text{hr}$ per cm, respectively. The infusion of a volume of Ringer's solution equal to 10% of body weight lowered the hematocrit and plasma total protein concentration, and arterial pressure increased an average of 5 mm Hg (group I, Table I). Net Na^+ flux after volume expansion fell in 16 of 17 jejunal and 13 of 14 ileal segments and simultaneous net water flux decreased proportionately. These changes were highly significant ($P < 0.005$ for all changes). In 12 jejunal and 10 ileal segments of group II animals (glucose-free perfusate) net Na^+ flux before volume expansion was decreased 29% ($P < 0.01$) and 18% ($P > 0.10$), respectively, from the control values observed in group I experiments (glucose-containing perfusate). Volume expansion in this group of rats was associated with changes in hematocrit, plasma protein concentra-

tion, and blood pressure similar to those in group I animals (Table I). The infusion of the Ringer's solution resulted in a further decrease in net Na^+ flux in both jejunum and ileum (Table I) and these changes after volume expansion in the studies with glucose-free perfusate were not significantly different from the changes observed after volume expansion in group I animals, ($P > 0.50$ and > 0.40 , respectively).

In seven rats, all Na^+ in the perfusate was replaced with choline (group III, Table I). Before volume expansion, net H_2O flux in these seven jejunal and ileal segments was close to zero, and net Na^+ flux was -9.9 ± 0.4 and -3.4 ± 0.7 $\mu\text{Eq}/\text{hr}$ per cm^2 in jejunum and ileum, respectively (Table I). Volume expansion in this group of rats produced changes in hematocrit, total protein, and arterial pressure similar to those in groups I and II (Table I). After volume expansion, the mean changes in net Na^+ flux were -3.3 ± 1.0 and -2.1 ± 1.0 $\mu\text{Eq}/\text{hr}$ per cm in the jejunal and ileal segments. This change was statistically significant in jejunum ($P < 0.05$), but overall did not reach statistical significance in ileum ($P < 0.10$, > 0.05). However, the mean change in net Na^+ flux after volume expansion in group III animals (Na^+ -free perfusate) was not significantly different from the decreases after volume expansion in group I and group II animals ($P > 0.05$ for jejunum and $P > 0.10$ for ileum).

Effect of infusing hyperoncotic albumin after volume expansion. A 30 g/100 ml solution of bovine albumin was infused after volume expansion in 22 animals of the three experimental groups. The results of these experiments are summarized in Table II. The infusion of hyperoncotic albumin resulted in an increase in plasma protein concentration averaging 1.37 g/100 ml. The effects of the albumin infusion on net H_2O and solute fluxes are shown in Table II. On the average, net Na^+ flux, depressed by previous infusion of Ringer's solution, increased in group I and II in jejunum, and in all three groups in ileum. Because of variable responses among the animals these changes did not achieve statistical significance in some of the groups studied (Table II). However, it seems likely that the increased net Na^+ and H_2O flux which occurred in most animals after infusing concentrated albumin was due to the infusion and not to spontaneously occurring changes in intestinal absorption. In seven animals, measurements were continued after infusion of Ringer's solution for a period of time exceeding that in 17 of the 22 experiments in which albumin was infused. Intestinal absorption in these animals remained depressed and showed no tendency to increase spontaneously with time.

^aThe negative sign indicates that the net movement of Na^+ was in the direction of blood to lumen, in contrast to net absorption of Na^+ in groups I and II.

TABLE I
Effects of Volume Expansion with Isotonic Ringer's Solution on Intestinal Absorption in the Rat*

	J_{H_2O}		J_{Na^+}		J_{Cl^-}		J_{Osm}		Hematocrit		Arterial protein		Arterial pressure	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E
	$\mu l/hr \cdot cm$													
I. Normal perfusate	$\mu Eq/hr \cdot cm$													
Jejunum	86.3	37.8	12.7	6.4	7.4	4.5	20.8	9.1	47.8	41.5	5.48	3.91	122	127
n = 17	± 5.3	± 9.7	± 0.8	± 1.5	± 1.8	± 2.1	± 1.3	± 2.9	± 1.2	± 1.2	± 0.17	± 0.18	± 3.9	± 5.0
P [†]	<0.001		<0.001		<0.10		<0.001							
Ileum	52.8	25.4	8.9	5.2	11.0	9.0	13.6	7.0						
n = 17	± 5.8	± 9.6	± 1.0	± 1.3	± 2.9	± 3.4	± 1.7	± 2.5						
P	<0.005		<0.005		NS		<0.005							
II. Glucose-free perfusate	$\mu Osm/hr \cdot cm$													
Jejunum	52.0	1.1	9.0	1.8	7.6	1.5	14.3	-1.5	48.8	39.0	6.13	4.16	130	133
n = 12	± 5.0	± 9.6	± 1.0	± 1.7	± 1.8	± 2.5	± 1.3	± 2.9	± 1.0	± 1.8	± 0.18	± 0.19	± 4.4	± 6.6
P	<0.001		<0.001		<0.02		<0.001							
Ileum	36.3	-1.0	7.3	2.3	14.2	8.2	12.4	2.2						
n = 10	± 7.1	± 10.7	± 1.1	± 1.8	± 1.0	± 2.1	± 1.5	± 2.9						
P	<0.005		<0.01		<0.01		<0.01							
III. Sodium-free perfusate	$\mu Eq/hr \cdot cm$													
Jejunum	-8.7	-36.1	-9.9	-13.2	-5.1	-8.2	-11.1	-17.3	47.9	39.7	5.49	4.01	124	134
n = 7	± 5.4	± 9.3	± 0.4	± 1.1	± 1.0	± 1.5	± 2.3	± 2.2	± 1.9	± 1.8	± 0.39	± 0.19	± 10.9	± 11.0
P	<0.01		<0.02		<0.10		<0.05							
Ileum	11.7	-6.6	-3.4	-5.5	8.9	6.0	-3.0	-6.4						
n = 7	± 10.2	± 13.2	± 0.7	± 1.1	± 1.7	± 1.7	± 3.6	± 2.7						
P	<0.10		<0.10		NS		NS							

* Values are means \pm SEM of three to four consecutive collections in each animal during control (C) and after volume expansion (E) with isotonic Ringer's solution.

J_{H_2O} , J_{Na^+} , J_{Cl^-} , J_{Osm} : net flux of water, sodium, chloride, and solute, respectively.

[†] J_{H_2O} was determined in 10 jejunal and 6 ileal segments in group I and 8 jejunal and 6 ileal segments in group II.

[‡] Student *t* test for paired data; significance of difference between C and E.

TABLE II
Effects of Intravenous Infusion of 30 g per 100 ml Bovine Albumin on Intestinal Absorption in the Volume-Expanded Rat*

	J_{H_2O}		J_{Na}		J_{Osm}		Hematocrit		Arterial protein		Arterial pressure	
	C	E	C	E	C	E	C	E	C	E	C	E
	$\mu/hr \cdot cm$		$\mu Eq/hr \cdot cm$		$\mu Osm/hr \cdot cm$		%		g/100 ml		mm Hg	
I. Normal perfusate												
Jejunum	31.6	69.6	4.8	9.9	8.1	16.5	42.8	32.1	4.06	5.32	131	128
n = 9	± 15.7	± 13.7	± 2.5	± 2.1	± 5.0	± 3.6	± 1.8	± 1.3	± 0.35	± 0.43	± 5.4	± 7.0
P	<0.01		<0.05		<0.05							
Ileum	21.4	45.9	4.3	7.3	7.5	13.7						
n = 8	± 13.3	± 12.1	± 2.0	± 1.7	± 3.8	± 3.8						
P	<0.001		<0.02		<0.005							
II. Glucose-free perfusate												
Jejunum	5.4	13.6	3.0	3.5	0.5	0.6	35.5	24.6	4.02	5.51	126	117
n = 7	± 10.7	± 12.9	± 1.7	± 2.5	± 2.4	± 4.2	± 1.9	± 2.7	± 0.29	± 0.25	± 8.3	± 7.0
P	NS		NS		NS							
Ileum	-1.8	35.1	2.7	7.7	1.9	12.5						
n = 6	± 17.2	± 25.2	± 2.7	± 2.3	± 4.7	± 8.0						
P	<0.10		<0.05		NS							
III. Sodium-free perfusate												
Jejunum	-36.8	-51.2	-13.6	-17.0	-17.0	-23.3	39.2	32.3	3.97	5.35	136	136
n = 6	± 11.0	± 14.5	± 1.3	± 1.9	± 3.8	± 3.2	± 2.0	± 2.0	± 0.18	± 0.25	± 13.9	± 16.7
P	NS		<0.05		NS							
Ileum	-16.3	8.3	-5.9	-4.7	-6.3	-0.4						
n = 6	± 10.7	± 10.0	± 1.3	± 0.8	± 3.2	± 3.5						
P	<0.05		NS		<0.10							

* Presentation of data the same as in Table I.

Effects of volume expansion on permeability to inulin. In 29 animals, inulin was measured in the intestinal effluent before and after volume expansion. The calculated permeability to inulin (see Methods) of jejunum and ileum, although quite low, clearly increased after volume expansion in every experiment ($P < 0.001$). These results are shown for all experiments in Fig. 1.

There was a positive correlation between the change in the rate of movement of inulin into the intestinal lumen and the change in net H_2O absorption after both volume expansion and the infusion of hyperoncotic albumin (Fig. 2). When the ratio of effluent/plasma inulin concentration after saline infusion was plotted against the length of the perfused intestinal segment over a range

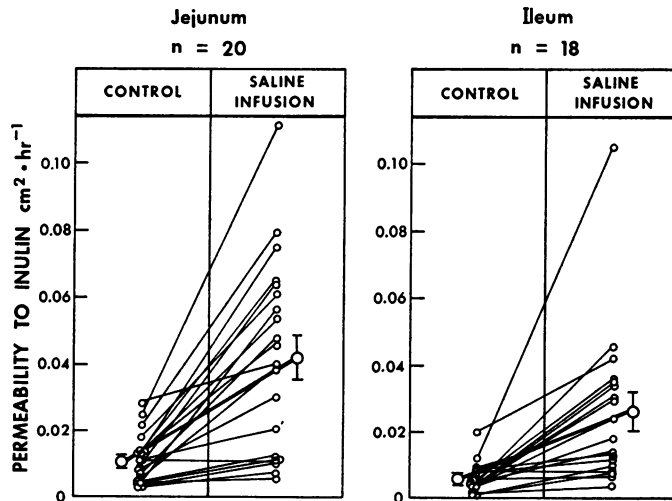


FIGURE 1 Permeability of the rat small intestine to inulin before and after saline infusion. Lines connect points from the same segment. Heavier lines connect mean ± 1 SEM.

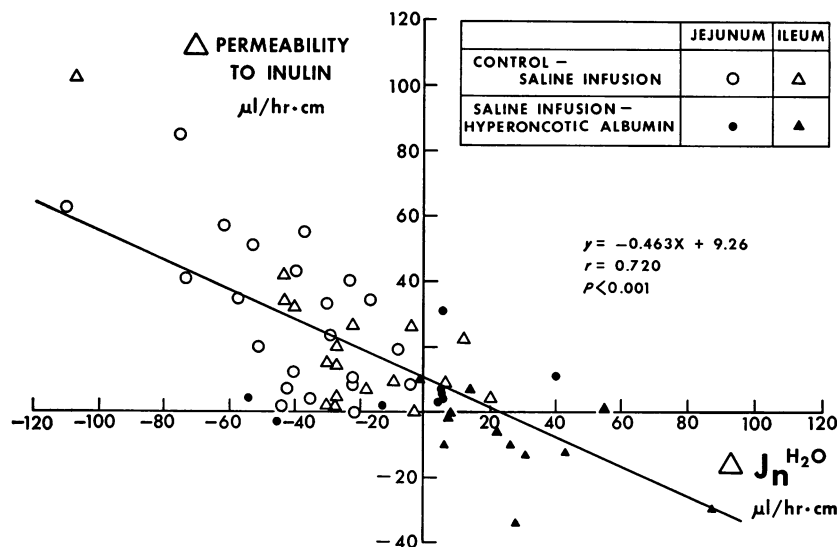


FIGURE 2 Relationship between the changes in net water flux ($J_n^{H_2O}$) and the simultaneous change in permeability to inulin, expressed in $\mu\text{l/hr}\cdot\text{cm}$, after saline infusion (open points) and after infusion of hyperoncotic albumin (closed points). Circles denote data obtained from jejunal segments; triangles from ileal segments.

of 1.5–28 cm in jejunum and 3.6–31 cm in ileum, significant positive slopes were obtained by least squares analysis for each segment. This linear regression of effluent/plasma inulin concentration against segment length took the form $y = 0.0037x - 0.0023$ ($r = 0.61$, $P < 0.001$) for jejunum, and $y = 0.001x + 0.007$ ($r = 0.49$, $P < 0.05$) for ileum.

Three rats received an injection of albumin- ^{131}I and the mean effluent/plasma ^{131}I was 0.0016 before and 0.0029 after saline infusion, which on the average was only 14% of the simultaneously measured effluent/plasma inulin concentration. Moreover, only 30% of the ^{131}I appearing in the effluent was precipitable with trichloroacetic acid, indicating that most of the radioactivity present in gut effluent was not protein bound.

Effects of volume expansion on unidirectional Na^+ flux. The movement of $^{22}\text{Na}^+$ from blood to lumen was used to calculate unidirectional fluxes of Na^+ in nine jejunal and ileal segments before and after saline infusion. These results are presented in Table III. If the movement of $^{22}\text{Na}^+$ from blood to lumen is regarded as a reliable means of measuring unidirectional passive flux of Na^+ then the calculated unidirectional flux of Na^+ from lumen to blood (sum of passive and net fluxes) was 1.6 times as great as the flux from blood to lumen before volume expansion. Volume expansion increased the rate of accumulation of $^{22}\text{Na}^+$ in the effluent, and therefore the calculated unidirectional flux of Na^+ from blood to lumen, in all experiments in jejunum and in eight of nine ex-

periments in ileum. The mean change for each group was highly significant (Table III). In both jejunum and ileum the mean increases in Na^+ flux from blood to lumen after volume expansion were greater than the mean decreases in net Na^+ flux determined from all experiments (Table I). In groups I and II (Na^+ -containing perfusate) the calculated flux ratio (lumen to blood/blood to lumen) decreased to an average of 1.2 after volume expansion. In four animals net and unidirectional fluxes of Na^+ were measured sequentially in the same intestinal segment, both before and after volume expansion (Table III). The decreased net Na^+ flux in each jejunal segment after volume expansion was less than the increase in flux of Na^+ into the lumen. Overall, similar results were observed in the ileum. These findings indicate that volume expansion with Ringer's solution decreases net Na^+ absorption in association with an increase in the unidirectional flux of Na^+ from blood to lumen.

In five experiments, the unidirectional flux of Na^+ from blood to lumen was measured in volume-expanded rats before and after infusing the 30 g/100 ml solution of albumin (Table III). In jejunum calculated flux of Na^+ from blood to lumen decreased in two experiments and increased in three after infusion of hyperoncotic albumin, and the mean change from 32.9 to 35.4 $\mu\text{Eq/hr}$ per cm was not of statistical significance ($P > 0.60$). However, in ileum calculated, flux of Na^+ from blood to lumen decreased in all experiments after the infusion of

TABLE III
Effects of Infusion of Ringer's Solution and 30% Albumin on Net and Unidirectional Flux of Sodium from Blood to Lumen in Rat Small Intestine†*

Experiment	J_a^{Na}		J_i^{Na}			Hct		
	C	E	C	E	A	C	E	A
	$\mu\text{l/hr}\cdot\text{cm}$		$\mu\text{l/hr}\cdot\text{cm}$			%		
Jejunum								
1	11.1	0.5	24.8	40.4		53.0	46.4	
2	14.5	12.7	29.2	36.1		44.0	38.4	
3	8.8	2.8	26.1	49.1		51.9	39.3	
4	7.5	1.0	16.3	29.1		51.3	43.0	
5			14.9	33.1	28.1	51.0	37.0	33.7
6			11.2	43.7	50.4	48.0	40.2	29.2
7			32.0	49.1	43.9	49.8	43.4	31.5
8			18.3	24.2	37.7	52.2	41.9	30.3
9			10.0	14.5	16.7	44.7	29.5	20.5
Means	10.5	4.3	20.3	35.5	35.3	49.5	39.9	29.0
	± 1.5	± 2.9	± 2.7	± 3.9	± 2.7	± 1.1	± 1.6	± 2.3
<i>P</i>	<0.001 NS							
Ileum								
1	8.9	2.1	19.4	17.6				
2	17.1	8.9	18.2	28.7				
3	5.2	2.2	22.7	39.8				
4	3.4	4.3	12.4	14.0				
5			11.6	17.4	16.7			
6			10.0	18.5	17.4			
7			10.3	21.2	14.6			
8			9.4	15.8	12.2			
9			8.4	13.6	11.5			
Means	8.7	4.4	13.6	20.7	14.5			
	± 3.0	± 1.6	± 1.7	± 2.8	± 1.2			
<i>P</i>	<0.005 <0.05							

* In experiments 1-4, J_a^{Na} was measured in the same intestinal segments immediately before measurement of unidirectional flux of sodium (J_i^{Na}) before and after volume expansion.

† Data are means of multiple consecutive collection periods during control (C); after volume expansion with isotonic Ringer's solution (E); and after administration of 30% albumin (A).

albumin ($P < 0.05$) in keeping with the more consistent effect of albumin to increase net Na^+ absorption in this intestinal segment (Table II).

DISCUSSION

The present studies demonstrate that volume expansion with an isotonic electrolyte solution decreases net absorption of Na^+ from rat small intestine, confirming recent observations of Richet and Hornyh in the rat (18), Higgins in the dog (19), and Gutman and Benzakein in the cat (20). Moreover, this effect of saline infusion to depress net Na^+ absorption occurred under experimental

circumstances expected to decrease the transport of Na^+ from lumen to blood. Maximal net absorption from both jejunum and ileum was observed before volume expansion when both Na^+ and glucose were present in the luminal fluid, and the rates observed agree well with values reported by others under similar experimental conditions (18, 23). Replacement of glucose in the perfusion fluid by mannitol was associated with a reduction in net Na^+ absorption of approximately 29% in jejunum and 18% in ileum, in agreement with the previously demonstrated partial dependence of intestinal Na^+ transport on glucose absorption (24, 25). In a different group of animals, Na^+ was omitted entirely from the perfusion

fluid, a maneuver which must have minimized or nearly eliminated the movement of Na^+ from intestinal lumen to blood. These experimental designs permitted evaluation of the effects of saline infusion on intestinal Na^+ transport under conditions which presumably resulted in markedly different rates of outward (lumen-to-blood) movement of Na^+ , the direction of active transport. Active outward transport of Na^+ should have been maximal when both Na^+ and glucose were present in perfusion fluid (group I), intermediate when glucose was omitted from perfusion fluid (group II), and minimal when Na^+ was eliminated from the perfusion fluid (group III). Despite these divergent conditions of outward Na^+ movement, volume expansion produced quantitatively similar effects to decrease net transport from lumen to blood in each experimental group. In view of these results it seems unlikely that saline infusion decreases net intestinal Na^+ absorption by decreasing the unidirectional movement of Na^+ from lumen to blood, a component of which includes active Na^+ transport. It follows then that the effect of volume expansion to decrease net Na^+ absorption in these experiments probably resulted in some way from an increase in the movement of Na^+ into the intestinal lumen.

Assuming that the unidirectional flux of Na^+ from blood to lumen measured in the present studies represents the bidirectional rate of passive movement of Na^+ , the total flux of Na^+ from lumen to blood before saline infusion and in the presence of glucose was approximately 60% greater than the flux into the lumen. In other words, the rate of net active transport of Na^+ from intestinal lumen to blood was approximately 60% of the unidirectional passive flux. This ratio of active to passive transport of Na^+ is greater than has been reported for the renal proximal tubule of the rat (26) and is similar to findings of others for jejunal and ileal epithelium (23, 27, 28). After the infusion of Ringer's solution, the unidirectional flux of Na^+ from blood to lumen increased by an average of 75% in jejunum and 65% in ileum as net absorption of Na^+ decreased 50 and 42%. Accordingly, net active transport decreased to approximately 20% of the unidirectional passive flux. If this increment in Na^+ movement into the intestinal lumen represented a unidirectional flow of solution then the change was more than adequate to account for the decrease in net absorption of Na^+ and H_2O . On the other hand, the observed increased movement of Na^+ into the intestinal lumen after volume expansion could represent an increase in bidirectional passive flux of Na^+ due, perhaps, to an increase in permeability of the mucosa. Furthermore, a decrease in the rate of active Na^+ efflux should permit a more rapid accumulation of $^{22}\text{Na}^+$ in the intestinal lumen. The latter seems unlikely as a cause for the apparent increase in unidirectional flux of Na^+ since

the increased influx was greater than the decrease in net efflux.

The present studies also indicate that acute volume expansion increases the permeability of the intestine to inulin. Others have reported leakage of inulin from serosa to mucosa in dog ileum *in vitro* in response to increased hydrostatic pressure (29). In the present studies, leakage of inulin from blood to lumen was barely detectable before volume expansion but increased markedly after the infusion of Ringer's solution. The possibility was considered that the movement of inulin into the intestinal perfusate represented leakage of plasma or interstitial fluid from sites of trauma rather than a physiologic permeability of intestinal epithelium. For several reasons this did not seem likely. (a) No protein was demonstrable by boiling effluent samples containing a concentration of inulin that should have represented an easily detected concentration of protein if the inulin had entered as a leak of plasma or blood. Furthermore, saline infusion resulted in the movement of only trace amounts of ^{125}I into the intestine of rats which had received intravenous injections of albumin ^{125}I , and the relative amount of inulin in these intestinal segments was invariably many times greater than the amount of ^{125}I . (b) The rate of inulin movement into the lumen always increased after infusion of Ringer's solution. Although this increase could have been due to increased leakage across areas of trauma as a result of volume expansion, the movement of inulin into the lumen decreased in experiments in which net absorption increased after infusing hyperoncotic albumin, despite further expansion of the vascular compartment (Fig. 2). In addition, the total amount of inulin entering the intestinal segment was proportional to the length of segment perfused. (c) After volume expansion, the entry of inulin into jejunum was much greater than that into ileum, and this correlated with both a greater permeability of jejunum to $^{22}\text{Na}^+$ and a greater effect of volume expansion to decrease net absorption in jejunum. Leakiness of the epithelium to inulin-containing plasma should have been independent of segment length, accompanied by a similar amount of plasma protein, and unrelated to changes in net absorption and unidirectional flux of Na^+ . For these reasons we believe the entry of inulin into the intestinal lumen in these experiments represents a physiologic permeability to inulin and not an artefact produced by manipulation of the intestine. It should be emphasized that the permeability of both jejunum and ileum to inulin was very low and the actual concentrations in the effluents ranged from a low of 0.05% of the plasma concentration during control to a high of 9.5% after saline infusion.

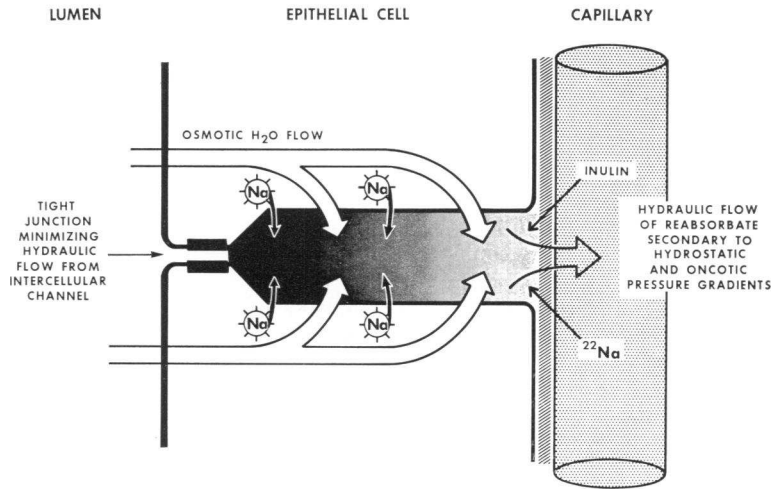
If the movement of inulin into the intestinal lumen was associated with the inward (blood to lumen) move-

ment of an equivalent volume of plasma H₂O and electrolyte, such an increase in flow from blood to lumen could have accounted for a large percentage of the decrease in net H₂O absorption observed during volume expansion. Implicit in this interpretation is the conclusion that volume expansion could decrease intestinal net absorption of Na⁺ and H₂O as a consequence of an increase in unidirectional flow of Na⁺ and H₂O from blood to lumen without any primary effect on the unidirectional movement from lumen to blood. Exactly how such a bulk flow of solution from blood to lumen could occur is unknown. Inulin is not known to cross cell membranes, and consequently any route of flow of inulin-containing fluid from blood to lumen would occur presumably via extracellular channels. Anatomically, the only such extracellular channels known to exist in the intestinal mucosa are the lateral intercellular spaces (30), which are thought to be the major sites of net isotonic transport in the direction of lumen to blood (31, 32). Furthermore, these spaces are bound at the apical end by what appears to be a tight junction (33) which presumably would represent a barrier to the flow of H₂O and solute. Fordtran, Rector, and Carter (34) have suggested that transport in human jejunum may be characterized by a fluid circuit system in which bulk flow of H₂O and solute occurs in the direction of lumen to serosa, but H₂O movement into the lumen across the cell membrane is presumably diffusional and would not account for the entry of inulin into the intestinal lumen observed in the present studies.

Curran and MacIntosh (35) and later Diamond (36) have proposed a three-compartment model to account for isotonic epithelial transport. In this model, solute is transported actively into an extracellular compartment located between the cells (the lateral intercellular space) but within the total membrane structure. The accumulation of solute in this intercellular compartment would create a gradient for the passive flow of H₂O which in turn would generate a sufficient hydrostatic pressure to drive the reabsorbate out of the channel in the direction of greatest hydraulic conductivity. Since the channel is tightly closed at the luminal surface of the cell, the column of reabsorbate would move in the serosal direction. Continued inward diffusion of H₂O would result in delivery of an isotonic reabsorbate across the basement membrane of the epithelium. Such intercellular spaces have been demonstrated in epithelial structures that perform isotonic absorption, including the gall bladder (31, 32), the intestinal mucosa (30), and the renal tubule (37). In view of the large body of evidence indicating that the rate of capillary uptake of reabsorbate may be a determinant of net reabsorption in the renal proximal tubule, we would propose a modification of the model of Curran and MacIntosh (35) and Diamond (36)

which includes transcapillary hydrostatic and oncotic pressures as additional factors determining the rate of transepithelial reabsorption. This modified model is represented schematically in Fig. 3. The rate at which the column of absorbate moves across the epithelial basement membrane would depend not only on the hydrostatic pressure generated within the intercellular channel by the osmotic inflow of H₂O but also on the hydrostatic and oncotic pressures within the capillary. Capillary hydrostatic pressure would tend to oppose flow out of the intercellular channel whereas plasma oncotic pressure would facilitate flow out of the channel. Increases in capillary hydrostatic pressure and decreases in plasma oncotic pressure, as might occur during volume expansion with colloid-free solution, should retard outflow from the intercellular channels. Continued active transport of solute into the channels and the osmotic inflow of H₂O should result in a rise in hydrostatic pressure within the intercellular space. Such an increased pressure could result in enlargement of the channel and the resultant stretching of the cell membrane could result in greater passive permeability to Na⁺ and other solute within the intercellular channel. As a consequence, increased flux of Na⁺ from the hypertonic intercellular space into the cell (and ultimately the lumen) could decrease net transport. In other words, as the membrane became more leaky to sodium the active transport system would become less efficient as part of the transported Na⁺ diffused into the lumen, reducing the degree of intercellular hypertonicity and the force for absorption of water. The present observations of increased permeability of the epithelium to Na⁺ and inulin during volume expansion are compatible with such a proposal. Alternatively, increased hydrostatic pressure within the intercellular channel and enlargement of this compartment as a consequence of decreased capillary uptake could increase hydraulic conductivity of the intercellular reabsorbate in the direction of the epithelial lumen. Although this (apical) end of the intercellular channel is thought to be tightly closed (31-33), it seems possible that increased pressure and stretching within the intercellular channel could force the flow of a part of the reabsorbate back in the lumen (Fig. 3). Since basement membranes in general must be permeable to inulin it seems likely that inulin would be present inside the intercellular compartments. Therefore, the present observation of increased movement of inulin into the intestinal lumen during volume expansion (when net absorption was decreased) is consistent with an increased flow of solution from intercellular spaces into the lumen. To us, this possibility seems more likely than the alternative one which requires the movement of inulin across cell membranes. This proposal that volume expansion decreased net absorption as a consequence of in-

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SALINE INFUSION

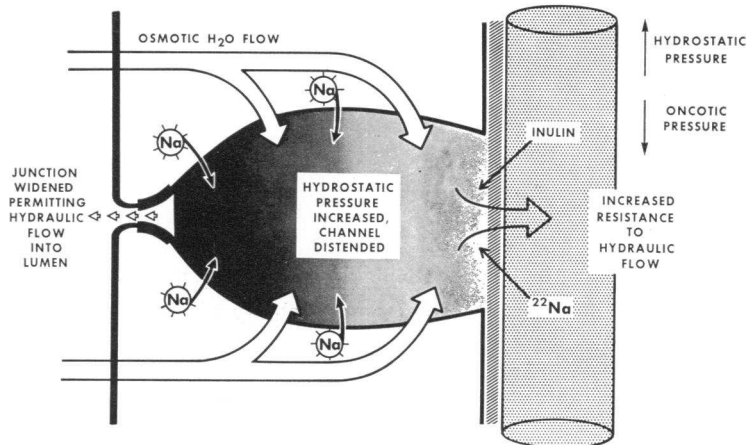


FIGURE 3 Schematic representation of transepithelial transport. During hydropenia, active transport of sodium into the intercellular channel creates a gradient for osmotic water flow. Uptake of this fluid from the intercellular channel into the adjacent capillary is governed by the effect of Starling forces acting across the capillary wall. After saline infusion, capillary Starling forces are altered to decrease uptake of fluid into the circulation. Hydrostatic pressure within the intercellular channel may then increase due to the continued transport of sodium and passive influx of water into the intercellular channel, leading to widening of the apical tight junction and thereby permitting bulk flow of fluid back into the intestinal lumen.

creased hydraulic conductivity across the apical end of the lateral intercellular channels is compatible with continued net absorption, since hydraulic flow of absorbate could occur across both ends of the channel, and net absorption would depend on the relative rates of flow at the basal and apical ends of the channel. In fact, such a mechanism could be associated both with increases in the absolute rate of net absorption (if active Na^+ trans-

port is accelerated, as could be the case in renal proximal tubule with increased filtered load after saline infusion) and with decreases in the fraction of actively reabsorbed Na^+ returned to the circulation if a part of the reabsorbate was forced back into the lumen.

Either increased diffusional permeability or flow of solution into the intestinal lumen could account for the smaller decrement in net absorption after infusion of

Ringer's solution observed in these studies in rats in which Na^+ was omitted from the intestinal perfusate. In the absence of luminal Na^+ , the concentration of Na^+ achieved by active transport into the intercellular space should be reduced. Any increase in cell membrane permeability or flow of solution back into the lumen resulting from volume expansion under this condition would be associated with a reduced amount of Na^+ moving into the lumen.

Bank, Yarger, and Aynedjian (38) recently reported an increased movement of sucrose into the renal proximal tubular lumen when net tubular reabsorption was decreased by renal venous constriction. In their studies, the increased movement of sucrose into tubular lumen was not accompanied by an equivalent movement of inulin, and therefore suggested an increase in selective permeability rather than an increased flow of solution. However, if sucrose entered the tubular lumen from intercellular channels the entry of inulin into the tubular lumen may not have been detectible if its concentration in the intercellular fluid was much lower than that of sucrose. It seems reasonable that the relative concentrations of species in the intercellular channels may not be the same as their relative concentrations in plasma. It is of interest that MacCallum (39) in 1904 and Fisher and Moore (40) in 1907 reported that dextrose as well as sucrose entered the intestinal lumen from blood in rabbits after intravenous injection of saline.

In support of the present proposal that the change in net absorption during volume expansion could be due to increased hydrostatic pressure within intercellular channels are some morphologic observations. Studies of small intestine by electron microscopy reveal that the lateral intercellular spaces of the intestinal mucosa are collapsed during conditions of minimal net transport but become dilated when net transport is increased by feeding (30), the addition of glucose (41), or when an osmotic gradient favors the movement of H_2O from mucosa to serosa (42, 43). Also, the intercellular spaces of the rat proximal tubule become dilated after the infusion of saline (37). Thus, the size of the intercellular compartment can be altered in response to maneuvers which change the rate of net epithelial transport.

The present observations that the infusion of concentrated albumin increased intestinal net Na^+ absorption in some animals, supports the view that the decreased net absorption after the infusion of Ringer's solution could be due partially to a decrease in plasma oncotic pressure as has been demonstrated for the renal tubule (4, 9, 10). Although the effects of concentrated albumin on intestinal Na^+ transport were inconsistent, it should be pointed out that the effect of concentrated albumin to increase proximal tubular reabsorption also is variable in that the effect may be transient and dependent on the

preexisting level of tubular reabsorption. Infusion of concentrated albumin in the dog in the absence of prior salt loading decreases proximal tubular reabsorption (44), presumably within several minutes. Even when infused in the presence of salt loading the effect of concentrated albumin to increase tubular Na^+ reabsorption disappears after several minutes (7, 45). This is not surprising since in addition to increasing plasma oncotic pressure the infusion of albumin produces further intravascular volume expansion and could activate factors favoring decreased tubular reabsorption which outweigh the effect of increased plasma oncotic pressure. These additional factors could include further changes in any natriuretic humoral substance released in response to volume expansion (6), vasodilatation (4), increased capillary hydrostatic pressure (7), and decreased hematocrit (46). For these reasons we believe that the observation of increased intestinal absorption after infusion of albumin may be important and cannot be dismissed simply because it was not observed in all animals. It is important also to emphasize that in addition to increasing net absorption of Na^+ the infusion of concentrated albumin also decreased the unidirectional flux of Na^+ from blood to lumen in several animals in which this unidirectional flux had been increased by prior infusion of Ringer's solution. We would conclude, then, that the present data support the view that net intestinal absorption of Na^+ may be determined, in part at least, by plasma oncotic pressure and presumably, therefore, by other factors determining capillary absorption.

In summary, the present studies demonstrate a decrease in net intestinal Na^+ and H_2O absorption after infusion of a Ringer's solution under circumstances which should minimize the importance of a decrease in the movement of sodium from lumen to blood. The depression of net absorption was accompanied by increased permeability of the intestinal mucosa to inulin and an increase in the unidirectional flux of Na^+ from blood to lumen. These findings suggest that saline infusion increases the passive permeability of intestinal epithelium, and this increased permeability may be responsible for decreases in net absorption of Na^+ . A model is proposed whereby volume expansion, by decreasing capillary absorption, may result in increased flow of reabsorbate directly out of intercellular channels into intestinal lumen, as a consequence of increased hydrostatic pressure within the intercellular channels.

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