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Sodium Chloride Transport of Normal and Dietary Enlarged Rat Cecum in vitro

Key Words
Cecum, rat
Diet adaptation
Polyethylene glycol
Ion transport
Histology

Abstract
Sodium chloride transport across isolated cecum mucosa was investigated in normal rats and rats with adaptive cecum growth induced by dietary polyethylene glycol (PEG). The normal cecum absorbed Cl in excess of Na with a small short-circuit current (I_{SC}). Dietary adaptation led to large equivalent increments of Na and Cl net absorption without adequate I_{SC} change. Inhibitor studies (mucosal amiloride 10^{-3} and 10^{-4} *M;* mucosal 4,4-diisothiocyanatostilbene-2,2-disulfonic acid 5×10^{-5} *M;* serosal furosemide 10^{-3} *M;* serosal ouabain 10^{-3} *M*) suggested that normal cecal NaCl absorption involves electroneutral Na/H and Cl/HCO_3 exchange at the apical and Na-K-ATPase-mediated exit across the basolateral cell membrane. Dietary adaptation stimulates the loosely coupled antiports and possibly activates a small serosally located NaCl cotransport. Comparative histology showed flattening of all tissue layers and widening of crypts in PEG animals. Crypt widening may facilitate ion access to underutilized transport sites and, at least in part, explain the increased absorption of the enlarged cecum.

Introduction

Within animal species, the cecum is small in carnivores and large in monogastric herbivores in whom it is an important site of nutrient fermentation [1]. In the omnivorous rat, cecal size varies depending on the luminal content. The organ grows when rats are fed certain large molecules or antibiotics, after small intestinal resection and in the germ-free state [1]. Under some of these circumstances, an increase in ion and water absorption has also been documented [2–4]. These morphological and functional changes are part of the spectrum of intestinal adaptation [1].

A simple means to stimulate cecal growth in the rat is to add the poorly absorbable polymer polyethylene glycol (PEG 4000) to the drinking water. This dietary manoeuver increases the cecal macrosurface about threefold and tissue dry weight twofold within 2 months [4]. Mucosal growth occurs by hyperplasia [5]. Sodium chloride (NaCl) and water net absorption in vivo approximately doubles per unit tissue mass, significantly at day 2 and fully developed at days 10–14 after the start of feeding [6]. Following the same time course, Na-K-ATPase specific activity doubles by incorporation of additional enzyme molecules into the basolateral cell membrane [7]. The larger Na transport was not mediated by adrenal and pituitary hormones and not reversed by extracellular volume expansion [8].

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These in vivo studies could not establish the detailed mechanism responsible for the stimulation of NaCl transport. In the present investigation we therefore compared the normal and adapted stripped cecum mucosa in vitro. Na and Cl unidirectional fluxes, net transport rates, and electrical parameters were measured without and with transport inhibitors. In addition, we used conventional histology to study the cecal mucosa under both conditions.

Methods

Animals

Cecum adaptation was induced in female 170–220 g Wistar rats by adding 160 g/l PEG 4000 (Serva, Heidelberg, Germany) to the drinking water for 10–14 days [6]. Control animals received tap water without PEG. Both groups had free access to standard rat chow (ssniff®, Spezialdiäten GmbH, Soest, Germany) up to the time of the experiment.

Tissue Preparation

Under ether anesthesia, the cecum was removed and rinsed free of feces with ice-cold bathing solution. It was opened along its small curvature and placed on a plexiglass plate, mucosal side up. With a glass microscope slide the mucosa was incised transversely at the distal cecum end and gently pushed away from the underlying muscularis mucosae, submucosa, muscularis propria and serosa [9]. The mucosal preparation, consisting of mucosa and inner circular muscle layer of muscularis mucosae, was mounted as a flat sheet between two halves of a modified Ussing chamber and sealed to it with silicone grease (Silikon, Hochvakuumfett, schwer, Merck, Darmstadt, Germany). The exposed surface area was 1 cm^2. It was bathed on each side with 3 ml solution gassed continuously with 5% CO_2 in O_2 and maintained at 37°C. Only one or two tissue pieces were taken from each animal.

Electrical and Ion Flux Measurements

Electrical measurements were made by an automatic voltage-clamp device (Dr. G. Haas, Darmstadt, Germany) and corrected for offset potential and solution resistance. Tissues were first incubated under open-circuit conditions for 15–20 min and then short-circuited. The electrical potential difference (PD) and the short-circuit current (I_{SC}) were recorded every 5 min. The tissue resistance (R) was calculated from PD and I_{SC}. 20–30 min after mounting, isotopes (^{22}Na and ^{36}Cl) were added to the bath on one side of the tissue. Following an equilibration period of 20 min, fluxes were measured over 2 successive 30-min periods before and 15 min after the addition of a transport inhibitor to the mucosal or serosal side of the tissue. At each time point two 0.25-ml samples, replaced by an equal volume of unlabeled solution, were taken from the initially unlabeled side. Isotope activities were measured by standard liquid scintillation spectrometry and gamma counting. Unidirectional Na and Cl fluxes and net residual ion flux were calculated using standard equations. Electrical parameters and ion transport were stable (in experiments without inhibitors) over at least 3 h. In the study using amiloride (10^{-3} and 10^{-4} *M*), control periods were evaluated as one group.

Solutions

The bathing solution contained (m*M*): NaCl 107; KCl 4.5; $NaHCO_3$ 25; Na_2HPO_4 1.8; NaH_2PO_4 0.2; $CaCl_2$ 1.25; $MgSO_4$ 1.0, and glucose 12 (pH 7.4). The solutions of amiloride, furosemide, 4,4-diisothiocyanatostilbene-2,2-disulfonic acid (DIDS) and ouabain (G-strophanthin) were prepared with the bathing solution. Chemicals were obtained from Sigma (Munich, Germany), and radioisotopes from New England Nuclear (Dreieich, Germany).

Histological Studies

The cecum (with content) was left in situ and ligated at the ileocecal and ceco-colonic junction. It was excised completely and freeze-fixed instantaneously with liquid nitrogen. The snap-frozen cecum was then separated into small segments, placed on cork plates and stored at –20°C. The segments were cut into longitudinal 8- to 10-µm-thick sections on a microtome (Cryostat HR, Slee, Mainz, Germany) and stained with hematoxylin and eosin. With the aid of an ocular micrometer the thicknesses of the mucosa, submucosa and muscularis propria were measured under a light microscope (Leitz, Wetzlar, Germany). The crypt depth, crypt width and intercryptal distance were also determined.

Statistics

Results are given as arithmetic means ± SEM. Significances of difference were tested using Student's t test for unpaired data. $p < 0.05$ was considered significant.

Results

Basal Ion Transport

As shown in table 1, the normal rat cecum absorbed both Na and Cl with a small I_{SC} and PD. The tissue had a resistance of about 110 $\Omega \cdot cm^2$. Cl was absorbed at a much higher rate than Na and there was a high calculated residual ion net transport which probably represents bicarbonate secretion.

In the adapted cecum Na net absorption increased by 10.3 $\mu Eq/cm^2 \cdot h$ without an adequate change in I_{SC} (+0.6 $\mu Eq/cm^2 \cdot h$). Cl net transport increased by 11.9 $\mu Eq/cm^2 \cdot h$. The higher Na and Cl net transport was due to higher mucosal to serosal fluxes. Resistance was unchanged (table 1).

Effects of Mucosal Amiloride 10^{-4} and 10^{-3} M

Mucosal amiloride (10^{-4} *M*), known to inhibit electrogenic Na absorption in many epithelia, had no effect on Na transport and I_{SC} in the control group (table 1). In the enlarged cecum 10^{-4} *M* amiloride inhibited both J^{Na}_{ms} and J^{Na}_{net} but did not alter I_{SC}.

High concentrations (10^{-3} *M*) of amiloride, which inhibit electro-neutral Cl-dependent Na/H exchange, reduced J^{Cl}_{net} and J^{R}_{net} in the normal mucosa (table 1). A decrease in J^{Na}_{net} to almost zero was not significant because

Table 1. Effects of mucosal amiloride 10^{-4} and 10^{-3} *M* on ion fluxes and electrical parameters in normal and adapted rat cecum mucosa

	Na fluxes, μEq/cm²·h			Cl fluxes, μEq/cm²·h			I_{SC} μEq/cm²·h	PD mV	R Ω·cm²	J^R_{net}
	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}				
Controls										
Control period	12.7±0.8	10.8±0.6	1.9±0.9	47.4±2.5	29.2±1.2	18.2±2.5	1.3±0.1	3.5±0.3	103.6±4.3	17.6±2.4
Amiloride 10^{-4} *M*	11.7±1.3	10.1±0.8	1.6±1.3	42.9±2.6	29.0±2.1	13.9±3.1	1.4±0.2	3.9±0.4	104.8±4.8	13.8±3.1
Amiloride 10^{-3} *M*	8.0±1.1*	7.7±0.5*	0.4±1.3	35.5±4.1*	30.2±1.8	5.3±4.6*	1.8±0.2*	5.5±0.7*	116.0±5.9	6.8±3.8*
PEG										
Control period	21.6±0.8	9.5±0.6	12.2±1.0	65.6±0.8	35.5±2.1	30.1±4.3	1.9±0.2	5.6±0.4	113.5±3.4	19.8±3.7
Amiloride 10^{-4} *M*	14.8±0.7*	8.7±0.7	6.1±1.0*	58.8±5.8	34.3±5.8	24.5±8.2	2.1±0.2	6.8±0.7	124.2±5.3	20.5±7.0
Amiloride 10^{-3} *M*	9.6±0.5*	7.4±1.5	2.2±1.5*	48.4±4.3*	37.7±2.9	10.7±5.5*	2.2±0.2	6.8±0.6	120.6±5.3	10.6±4.6

Fluxes were measured over two 30-min periods before (control period) and 15 min after amiloride.
J_{ms} = Mucosal to serosal; J_{sm} = serosal to mucosal unidirectional; J^R_{net} = residual ion net flux. Values are means ± SEM, n = 6–15 per group.
* $p < 0.05$ compared to control period.

Table 2. Effects of serosal furosemide 10^{-3} *M* and ouabain 10^{-3} *M* on ion fluxes in normal and adapted rat cecum mucosa

	Na fluxes, μEq/cm²·h			Cl fluxes, μEq/cm²·h			J^R_{net}
	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}	
Controls							
Control period	13.9±0.8	11.9±0.7	2.0±1.0	43.3±2.8	30.4±1.9	12.9±3.4	11.9±3.7
Furosemide	11.7±1.1*	9.7±1.0*	2.1±1.4	26.3±3.3*	16.3±3.6*	10.0±4.6	8.9±5.2
PEG							
Control period	21.0±1.3	10.4±0.6	10.6±1.6	61.3±2.9	34.1±1.9	27.2±3.6	18.9±3.5
Furosemide	13.9±1.3*	8.6±1.4	5.3±2.3*	33.7±6.3*	20.7±3.0*	13.0±7.1*	10.0±8.1
Controls							
Control period	13.2±0.8	11.6±1.3	1.6±1.7	40.8±3.0	26.5±1.6	14.3±3.8	13.6±3.4
Ouabain	11.7±0.6	11.4±1.3	0.3±1.6	27.4±2.7*	23.4±2.2	4.0±3.9*	3.7
PEG							
Control period	19.4±1.0	10.6±0.6	8.8±1.3	58.0±2.3	33.5±2.7	24.5±4.0	17.4±2.3
Ouabain	10.1±1.1*	10.3±0.7	−0.2±1.5*	27.0±5.4*	24.3±6.5	2.7±8.5*	3.2

Fluxes were measured over two 30-min periods before (control period) and 15 min after adding furosemide or ouabain.
Values are means ± SEM, n = 6–12 per group.
* $p < 0.05$ compared to control period.

of scattering of the small readings. The reduction in net transport was due to a decrease in absorptive fluxes. I_{SC} and PD were slightly increased, resistance remained unchanged. In the adapted cecum amiloride 10^{-3} *M* inhibited Na and Cl mucosal to serosal and net movement. A decrease in J^R_{net} just failed to reach statistical significance. Small net fluxes of Na and Cl remained in the presence of amiloride 10^{-3} *M*. Electrical parameters were not altered.

Effects of Mucosal DIDS 5×10^{-5} M, Serosal Furosemide 10^{-3} M and Serosal ouabain 10^{-3} M

DIDS, often used as an inhibitor of Cl/HCO_3 exchange, had no effect at all on ion fluxes and electrical parameters when applied to the mucosal side of the normal or adapted mucosa (data not shown).

The effects of the loop diuretic furosemide, which blocks the coupled Na-K-2Cl cotransporter in intestinal epithelia, are summarized in table 2. In the control group serosal furosemide reduced all unidirectional fluxes, but

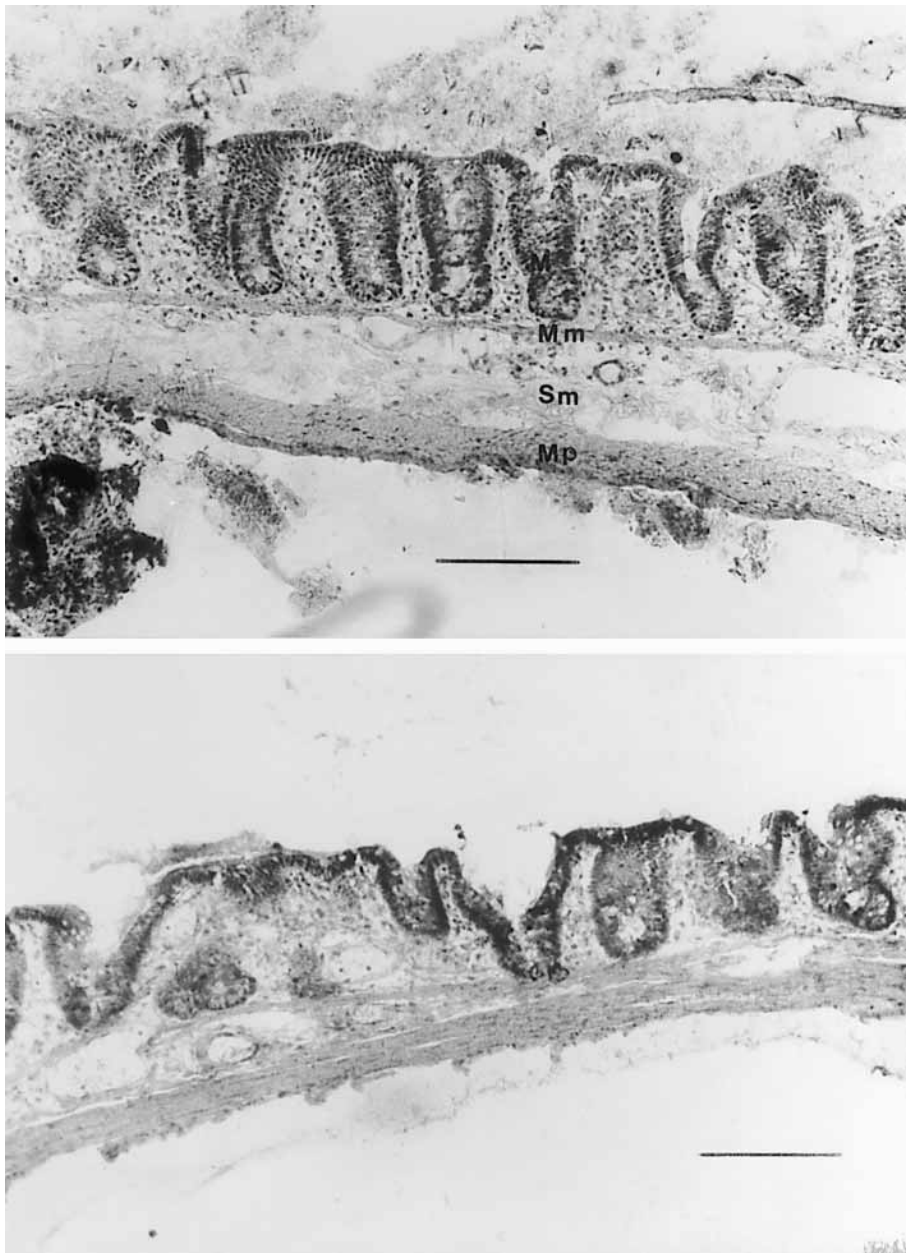

Fig. 1. Light micrographs of the snap-frozen whole cecal wall of the normal (**a**) and PEG rat (**b**). Compare the thickness of wall layers and the configuration of crypts. The bar represents 200 μm. M = Mucosa; Mm = muscularis mucosae; Sm = submucosa; Mp = muscularis propria. HE. × 100.

Na and Cl net transport was unaffected. In the adapted cecum effects on unidirectional fluxes were qualitatively similar. However, since absorptive fluxes were reduced to a larger degree, Na and Cl net transport was diminished. J^{R}_{net} remained unchanged in both control and PEG animals. The same was true for electrical parameters (data not shown).

The Na-K-ATPase-blocking agent ouabain caused a reduction in Na and Cl net flux to near zero in both the normal and adapted mucosa (table 2). The decrease was due to the reduction in mucosal to serosal fluxes. I_{SC} and PD also rapidly approached zero (data not shown).

Cecum Histology

Compared to control tissue, all wall layers were thinner and the crypt shape deformed in the enlarged cecum (fig. 1). The thickness of the mucosa, submucosa and muscularis propria was reduced by 20–33%. Crypt depth was reduced and crypt width increased, whereas the intercryptal distance remained unchanged (table 3).

Table 3. Morphological parameters of normal and enlarged rat cecum

	Controls	PEG
Thickness of		
Mucosa, μm	155 ± 5.3	122 ± 2.9*
Submucosa, μm	30 ± 1.7	20 ± 1.7*
Muscularis propria, μm	44 ± 2.0	35 ± 2.1*
Crypt depth, μm	129 ± 5.6	109 ± 3.9*
Crypt width, μm	15 ± 1.3	49 ± 3.3*
Intercryptal distance, μm	39 ± 2.2	41 ± 2.0

All histological studies were done in longitudinal sections of the whole cecum freeze-fixed in situ. Crypt width and intercryptal distance were measured at the tip. Values are means ± SEM, n = 15 per group.

* $p < 0.05$ compared to controls.

Discussion

As known from previous in vivo studies, a diet containing the poorly absorbable polymer PEG stimulates growth and NaCl absorption of the rat cecum [4–6]. The present in vitro experiments suggest that the transport increase is accomplished by electroneutral ion transporters. The morphological data offer an interesting explanation, namely that the higher transport rates may be related to altered tissue structure.

Under control conditions the short-circuited rat cecum absorbs Na and Cl and secretes an unidentified anion, probably bicarbonate. Compared to the proximal [10] and distal rat colon [9, 11, 12], Na absorption in the cecum is lower and Cl absorption substantially higher. Other investigators using the normal rat cecum in vitro found a similar Na but lower Cl net absorption [13] and bicarbonate secretion [14]. However, in these studies the full-thickness cecal wall was used including the myenteric and submucosal plexus which are known to inhibit absorption or even induce Cl secretion [15]. The electrical parameters I_{SC} and PD are low and correspond to those found in the other segments. Thus, unlike the rabbit cecum [16, 17], the rat cecum does not exhibit the highest I_{SC} within the intestine.

Based upon several observations we suggest that Na and Cl transport in the normal rat cecum is electroneutral and best explained by the presence of a double antiport system of a Na/H and Cl/HCO_3 exchange. First, the insensitivity of I_{SC} and J^{Na} to low-dose mucosal amiloride (10^{-4} *M*) excludes the existence of an amiloride-sensitive electrogenic Na channel [18]. Because of the very low I_{SC} the amiloride analogue phenamil, which inhibits amiloride-insensitive electrogenic Na transport in the rabbit cecum [17], was not tested. Second, high-dose mucosal amiloride (10^{-3} *M*) reduced Na net absorption without decreasing I_{SC}, consistent with the presence of a Na/H exchange [18]. And third, the accompanying reduction in Cl net absorption and residual ion flux indicates a coupling between Na/H and Cl/HCO_3 exchange. Considering the discrepancy in basal transport rates, the antiporter coupling appears to be loose. DIDS, an inhibitor of Cl/HCO_3 exchange in various cell systems including intestinal tissues [19], had no influence on ion fluxes in the normal and adapted cecum. However, such an unexplained lack of effect of mucosal stilbenes is not uncommon in tissues that by other techniques can be shown to contain Cl/HCO_3 exchangers [10, 11, 14, 20]. In particular, even higher mucosal stilbene concentrations were ineffective in rat proximal colon and cecum [10, 14]. The results with furosemide and ouabain will be discussed in context with the adapted mucosa.

The addition of the poorly absorbable polymer PEG to the diet led to a large and approximately equivalent increase in Na and Cl net absorption without an adequate change in I_{SC}. Relative to control values the increase in Na transport was much higher than in Cl transport.

The demonstration that J^{Na}_{net} was substantially greater than I_{SC}, and that low-dose amiloride did not inhibit I_{SC} excludes an electrogenic Na transport also in the adapted mucosa. Rather the data suggest that the predominant effect of adaptation was a stimulation of the basal electroneutral Na and Cl absorptive process. The inhibition of unidirectional mucosal to serosal as well as net Na and Cl fluxes by high-dose amiloride is well compatible with an effect on a double antiport system. That J^{Na}_{net} was decreased by low-dose amiloride is probably due to a partial effect on the large Na/H exchange already at this concentration. The data with ouabain also fit into this proposal. While the previously reported increase in Na-K-ATPase-specific activity [6, 7] is probably secondary to the increased apical Na entry providing an accelerated basolateral Na exit, blocking the Na-K-ATPase rapidly reduced the net transport not only of Na but also of Cl and residual ions to near zero (in both the adapted and normal mucosa). Mucosal DIDS had no effect as discussed above for controls.

PEG feeding stimulated cecal Na and Cl absorption to a roughly equivalent degree (≈11 μEq/cm^2·h), and amiloride 10^{-3} *M* did not completely abolish Na and Cl net

transport in the adapted mucosa. Therefore, the possibility was considered that the adaptive change was not simply an extension of normal transport properties but involved an additional process. There is no evidence of an apical NaCl cotransport in rat intestine [21, 22], but a basolateral Na-K-2Cl cotransport has been described [23–26]. This (group of) transporter(s) is inhibited by furosemide or bumetanide and may be secretory or absorptive in various epithelia [24]. Although usually secretory in the intestine, serosal furosemide reduced Na and Cl absorption in the adapted cecum by predominantly decreasing mucosal to serosal fluxes, while in the normal tissue only unidirectional but no net fluxes were affected. Thus, the adapted transport could comprise a minor NaCl absorptive component perhaps too small to be picked up under the normal condition. Furosemide could inhibit NaCl cotransport from the cell to the serosal compartment and, via a change in the electrochemical driving force, also NaCl influx across the apical membrane into the cell. However, the action of the loop diuretic is not specific enough to prove this possibility, and at least its effect on serosal to mucosal fluxes in both tissues is not consistant with this proposal.

If the adaptive response was merely an augmentation of basal transport properties, one may ask whether it could be explained by altered tissue morphology. Previous studies have clearly shown that transport is stimulated beyond the increase in macrosurface, dry weight or mucosal cell number (as measured per unit DNA), that the Na-K-ATPase specific activity of plasma membranes is doubled and that functional changes and hyperplasia follow divergent time courses [6]. Therefore, transport changes cannot be referred simply to a larger number of otherwise unaffected cells. The present histology data add a new aspect by demonstrating that mucosal geometry is altered at a time when functional changes are complete [6, 7]. Apart from a thinning of all wall layers as similarly reported for the enlarged germ-free cecum [27], crypts became shallower and wider whereas the epithelium between crypts was unchanged in width. Thus crypts were spread out implying that the crypt number per unit macrosurface exposed to the luminal solution was rather diminished. Crypt spreading and wall thinning appear to be due to distension induced by the large luminal volume attracted by a high colloid-osmotic pressure [28, 29]. A direct relationship between luminal distention and absorption of various solutes is known from other studies [30, 31]. In rat ileum, distension reversibly reduced villus height and increased the intervillus space as well as the absorption rate of some solutes [32, 33]. It was proposed that distension may increase the functional rather than absolute absorbing surface area and promote solute access to underutilized intervillus transport sites [33]. Similar phenomena were seen in the enlarged cecum. The argument that crypts are involved exclusively in secretion rather than absorption is probably not valid since recent data suggest that both crypt and surface epithelia are involved in absorption as well as secretion [34, 35]. Thus, the marked histological changes in the adapted cecum may contribute to or even fully account for the stimulation of Na and Cl absorption by facilitating solute access to additional mucosal absorptive sites.

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