J. Membrane Biol. 136, 147–158 (1993)

The Journal of
Membrane
Biology

Segmental Differences in Short-Chain Fatty Acid Transport in Rabbit Colon: Effect of pH and Na

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Received: 10 February 1993/Revised: 1 July 1993

Abstract. Short-chain fatty acids (SCFAs) are the predominant luminal anion in the mammalian colon. Although they are rapidly absorbed in vivo, little is known about the mechanisms of transepithelial transport in vitro. Previous studies have suggested that SCFA transport may be linked to Na absorption or an anion exchange mechanism. We compared the transport of propionate under short-circuit conditions in rabbit proximal and distal colon to determine whether there were segmental differences, how SCFAs may be linked to either Na absorption or anion transport, and whether SCFAs, as weak electrolytes, may be affected by transepithelial pH gradients. In distal colon, propionate transport was not significantly altered by stimulation of electrogenic Na absorption, epinephrine or Cl removal. However, a modest transepithelial pH gradient (luminal 6.8/serosal 7.4) stimulated propionate absorption. In proximal colon, propionate transport was significantly altered by manuevers that either stimulated (lowered [Na] in the bathing media) or inhibited (theophylline) apical Na-H exchange. Neither Cl removal, nor the anion exchange inhibitor DIDS, nor a transepithelial bicarbonate gradient, altered propionate transport. A transepithelial pH gradient inhibited propionate secretion, but not in a manner entirely consistent with the effect of pH on the distribution of a weak electrolyte. These results suggest that there is significant segmental heterogeneity in colonic SCFA transport; that transepithelial propionate fluxes are altered by changes in pH or electroneutral Na absorption (Na-H exchange), but not by chloride removal, bicarbonate gradients or electrogenic Na absorption. Regulation of SCFA transport may be an important factor in the physiology of colonic fluid balance.

Key words: Short-chain fatty acids — pH gradient — Propionate — Na-H exchange — Proximal colon — Distal colon

Introduction

Although short-chain fatty acids (SCFAs) are the predominant luminal anion in colonic fluid and have been implicated as significant factors in several basic colonic functions, little is known about the role of SCFAs in colonic physiology. SCFAs are produced by the bacterial metabolism of carbohydrate passing through the ileocecal valve. At one time, SCFAs were thought to be an important etiologic factor in the diarrheas associated with carbohydrate malabsorption. It is now apparent that SCFAs are rapidly absorbed in vivo [1, 2, 18, 30, 38] and may enhance colonic Na and fluid absorption. SCFAs may be a significant factor in normal colonic function; changes in colonic SCFAs may have both pathophysiological and therapeutic implications [5, 14, 20, 25, 29, 33, 39].

In vitro studies of colonic SCFA transport have, surprisingly, shown either no absorption or SCFA secretion [15, 16, 36]. We have recently demonstrated that propionate absorption in rabbit proximal colon in vitro correlated with factors that altered electroneutral Na absorption [34]. There was limited passive diffusion of propionate in this epithelium. These results suggested that propionate transport (and that of other SCFAs) may be linked to Na-H exchange. In the present study, we examined possible mechanisms relating propionate transport to

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electroneutral Na absorption. Specifically, we examined the role of pH gradients, alterations in Na absorption, and the contribution of Cl and HCO_3 in propionate absorption. We hypothesized that if Na-H exchange has an integral role in SCFA absorption, then an epithelium without an apical Na-H exchanger would exhibit a different pattern of SCFA transport than proximal colon. Therefore, we examined propionate transport in rabbit distal colon, a colonic segment that does not absorb Na by an electroneutral pathway (i.e., Na-H exchange).

SCFAs are weak electrolytes that may exist in either a protonated, neutral form (HA) or as an ionized species (A-); this has important implications because there may be separate pathways across the epithelium for the two species. At physiological pH, the vast majority of SCFAs are ionized. Changes in acid-base balance may have a considerable impact on the absorption of a weak acid by changing the relative proportion of the neutral and ionized species [19]. The colon is subject to considerable variations in pH [24, 25, 27] that may modulate SCFA transport.

These studies demonstrate that transepithelial propionate absorption is closely linked to electroneutral Na absorption, may be modified by pH gradients across the epithelium, but is not dependent on other anions such as Cl and HCO_3. Apical Na-H exchange and luminal pH may be pivotal factors in governing SCFA transport in rabbit colon.

Materials and Methods

New Zealand white male rabbits (2–3 kg) were fed standard rabbit chow and water *ad libitum*. A group of animals were treated with methylprednisolone (40 mg/d IM × 2 days) following a previously described protocol [35] to increase electrogenic Na transport in the distal colon. Rabbits were killed by ear-vein injection with T-61 euthanasia solution. A segment of proximal colon (10 cm in length, beginning 10 cm distal to the cecum) was rapidly excised, opened along its mesenteric border and rinsed in a chilled Ringer solution. Distal colon was obtained from a segment beginning 5 cm above the anus. Before use, tissues were maintained in ice-cold solutions that were bubbled with O_2 and varying amounts of carbon dioxide. The serosa and outer muscle layer were removed by placing the sheet of proximal colon, serosal side up, on a Plexiglass plate and moistening it with Ringer solution. A transverse incision was made through the muscle layers with a razor blade, and the layers were peeled off longitudinally with a fine curved forceps. A standard Ringer solution, as previously described [34], was used. Appropriate adaptations with propionate, HEPES, gluconate and mannitol were made as described in the text. Choline was substituted for Na when appropriate. (Details of specific composition of each solution are shown in Table 1.)

Electrical and Ion Flux Studies

Transepithelial electrical potential difference (PD), total conductance (G_t) and short-circuit current (I_{sc}) were measured as described previously [34]. Pieces of stripped intestinal mucosa were mounted in Ussing chambers (exposed surface area 1.12 cm^2) and bathed with 10 ml of Ringer solution on each side. Solutions were circulated by gas lift and maintained at 37°C in water-jacketed reservoirs. In the pH gradient experiments, the % CO_2 was varied as noted in the text.

Propionate fluxes were measured over two successive periods. Tissues from the same rabbit were mounted and ^{14}C propionate was added to either the mucosal or serosal reservoirs for 30–45 min prior to flux measurements. Tissues were paired by matching resistances. If the resistance of paired tissues differed by > 25% during fluxes, the experiment was rejected. The initial flux period lasted 30 min. After a 20-min equilibration period, a second flux measurement was made over a 40-min period. Test substances were added 10–15 min before a flux period, depending on the protocol of the individual experiment.

Unidirectional mucosal to serosal (m-s) and serosal-to-mucosal (s-m) fluxes and the net flux of propionate were calculated from aliquots taken at the beginning and end of each flux period. To calculate the unidirectional propionate fluxes, the values of the steady-state rates of radioisotope transfer were divided by the value of the specific activity of the initially labeled side and the surface area of the exposed tissue. The net flux is calculated as the difference between oppositely directed unidirectional fluxes of tissue pairs ($J_{net}^{Prop} = J_{m\text{-}s}^{Prop} - J_{s\text{-}m}^{Prop}$). Prior experiments showed that propionate fluxes were stable over the time course of these experiments [34].

Statistics

Results are expressed as mean ± SEM. Student's paired *t*-test was applied when appropriate; otherwise, an unpaired *t*-test was used.

Materials

Epinephrine was obtained from Elkins-Sims (Cherry Hill, NJ); [^{14}C] propionate from ICN Radiochemicals (Irvine, CA). Methylprednisolone was obtained from Schein Pharmaceuticals (Phoenix, AZ). All other chemicals were obtained from Sigma (St. Louis, MO).

Results

Distal Colon

Stimulation of Na Absorption

To determine whether stimulation of Na absorption by a mechanism other than Na-H exchange has an effect on SCFA transport in distal colon, we examined SCFA fluxes in Cl-free Ringer. Substitution of an impermeant anion for chloride stimulates electrogenic Na absorption in distal colon [37]. A gluconate Ringer elicited the expected increase in I_{sc} consistent with stimulation of electrogenic Na absorption (Table 2). However, neither net nor unidirectional fluxes were significantly changed.

Steroids increase electrogenic sodium absorp-

Table 1. Composition of test solutions

Solution	I N1 Ringer	II Ringer (6.8)	III Cl-free/ 5 mM HCO_3	IV Low Na	V Bicarb-free
Na Propionate	20.00	20.00	20.00	20.00	20.00
NaCl	60.10	60.10		1.40	60.10
$NaHCO_3$	25.00	5.00	5.00	5.00	0.00
Na Gluconate	33.50	53.50	113.40		58.30
HEPES					5.00
Choline-Cl				58.90	
Mannitol				106.50	
KCl	5.00	5.00		5.00	5.00
K-Gluconate			5.0		
$CaCl_2$	1.25	1.25		1.25	1.25
$MgCl_2$	1.10	1.10		1.10	1.10
Mg-Gluconate			1.10		
Ca-Gluconate			1.25		
Na_2HPO_4	1.65	1.65	1.65	1.65	1.65
NaH_2PO_4	0.30	0.30	0.30	0.30	0.30
Glucose	10.00	10.00	10.00	10.00	10.00
pH	7.4	6.8	6.8	6.8	6.8/7.4

All values are expressed in mM (except pH).

Table 2. Effect of Cl substitution of SCFA fluxes in distal colon

Experimental condition	J^{prop}			I_{sc}	G_t
	m-s	s-m	Net		
Control	1.62 ± 0.16	1.62 ± 0.14	0.0 ± 0.10	0.1 ± 0.3	4.8 ± 0.4
Cl-free	1.90 ± 0.17	1.89 ± 0.21	0.0 ± 0.08	1.4* ± 0.4	5.1 ± 1.2

Experiments were conducted either in a Cl-Ringer pH 6.8 (Solution II) or in a Cl-free gluconate substituted Ringer solution. (Solution III, Table 1) $N = 5$ animals for each group. Fluxes are expressed in μmol · cm^{-2} · hr^{-1}, I_{sc} μEq · cm^{-2} · hr^{-1} and G_t in mS · cm^{-2}. No statistically significant differences were noted in SCFA fluxes. I_{sc} increased significantly, $^*P < 0.05$.

tion in rabbit distal colon. Using a previously developed protocol [35], we compared propionate fluxes before and after low dose mucosal amiloride (10^{-4} M). The decrease in I_{sc} of 4.24 ± 0.70 μEq · cm^{-2} · hr^{-1} after amiloride is due to a decrease in electrogenic Na absorption. Despite this large change in Na transport, there was no significant change in propionate fluxes (Table 3).

Epinephrine

Epinephrine stimulates K secretion in distal colon, but does not alter Na absorption [12]. In contrast, in proximal colon, epinephrine stimulates apical Na-H exchange activity and SCFA absorption. Therefore, by examining the effect of epinephrine on SCFA fluxes in distal colon, one may be able to determine whether the effect of epinephrine is dependent on the presence of an apical Na-H antiporter or whether it has a more direct effect on SCFA fluxes.

We conducted these experiments in 5 mM HCO_3 Ringer (pH 6.8) because these conditions maximized the epinephrine-induced stimulation of SCFA absorption in proximal colon [34]. In distal colon, epinephrine does not alter either $J^{prop}_{m\text{-}s}$ or J^{prop}_{net}. Therefore, in distal colon, without an apical Na-H exchanger, epinephrine has no effect on propionate fluxes (Table 4).

Anion Substitution

Previous studies have suggested that there may be a SCFA : Cl exchanger operative on the apical membrane mediating colonic SCFA absorption. The Cl-

Table 3. Effect of steroid-stimulated Na absorption on SCFA fluxes

Control	Mean	2.00	1.56	0.45	2.54	11.10
	SEM	0.09	0.13	0.15	0.79	1.15
Amiloride	Mean	1.71	1.66	0.04	−1.70*	11.02
	SEM	0.25	0.207	0.23	0.24	1.29

Methylprednisolone-treated distal colon (n = 8) mounted in Ussing chambers and bathed in Ringer bicarbonate pH 7.4 (Solution I, Table 1). After an initial flux, 10^{-4} M amiloride was added to the mucosal solution and a second flux performed, $^*P < 0.001$ *vs.* control. The negative I_{sc} after amiloride has been described previously and represents Cl absorption. [35].

Table 4. Epinephrine's effect on SCFA fluxes in distal colon

Experimental condition	J^{prop}			I_{sc}	G_t
	m-s	s-m	Net		
Control	1.92 ± 0.09	2.14 ± 0.08	−0.22 ± 0.11	0.3 ± 0.5	5.2 ± 0.4
Epi (n = 5)	1.83 ± 0.13	2.23 ± 0.21	−0.40 ± 0.30	0.4 ± 0.5	5.3 ± 0.5

Experiments were conducted in 5 mM HCO_3 Ringer (pH 6.8), Solution II, in five animals. Unidirectional fluxes are expressed in μmol · cm^{-2} · hr^{-1}, I_{sc} in μEq · cm^{-2} · hr^{-1} in G_t mS · cm^{-2}. No statistical differences were noted between groups.

substitution experiments provided an opportunity to test whether such an antiport system is operative in rabbit distal colon, an epithelium in which Cl absorption is mediated by a presumptive Cl : HCO_3 exchange. The data in Table 2 do not support such a hypothesis.

pH Gradients

Because the partitioning of weak acids and bases across a membrane may be altered by a pH gradient [19], we examined the effect of varying the pH of the mucosal and serosal fluids bathing the proximal colon in vitro. Given a pKa of 4.8, a decrease in pH to 6.8 would increase the proportion of SCFA in the protonated (and readily diffusible) form fourfold to 1%. In distal colon, which does not exhibit electroneutral Na absorption, pH gradients were established with alterations of [HCO_3]. Mucosal acidification reversed a low basal secretory rate when compared to serosal acidification (Fig. 1).

In theory, a lowered solution pH, by increasing the protonated SCFA, would increase the unidirectional flux from the more acidic to the more alkaline reservoir. In distal colon, luminal acidification led to an increase in $J^{prop}_{m\text{-}s}$ (1.87 ± 0.17 *vs.* 1.30 ± 0.17 μmol · cm^{-2} · hr^{-1}, a decrease in $J^{prop}_{s\text{-}m}$, and an increase in J^{prop}_{net} (0.43 ± 0.17 *vs.* −0.78 ± 0.15) compared to serosal acidification (Fig. 1).

To clarify whether this is due to a pH gradient or the decreased luminal pH alone, we performed a

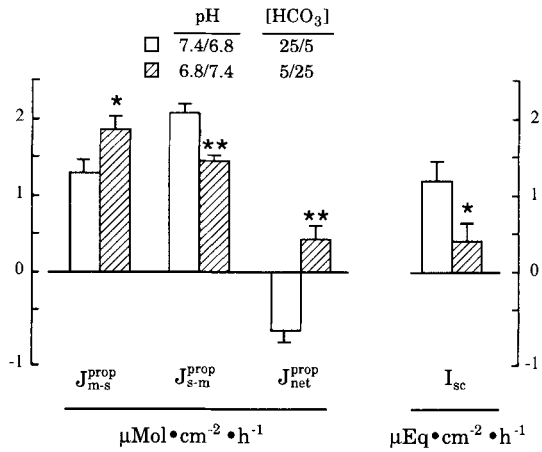

Fig. 1. Effect of a transepithelial pH gradient on propionate fluxes in distal colon. Creation of a pH gradient [mucosal/serosal] and bicarbonate gradient [mucosal/serosal] (Solutions I and II, Table 1) caused a significant change in propionate fluxes. J^{prop} is greater from the more acidic reservoir. $^*P < 0.05$, $^{**}P < 0.01$. N = 8 for 7.4/6.8, 7 for 6.8/7.4 experiments.

further series of fluxes comparing pH 6.8/7.4 with 6.8/6.8 (Table 5). In these studies, the gradient was associated with an increase in $J^{prop}_{m\text{-}s}$ and J^{prop}_{net}, suggesting that the gradient itself is functioning as an absorptive stimulus.

Effect of Changing [SCFA]

Previous studies in rabbit proximal colon [34] and other animal models [9, 10] have not demonstrated

Table 5. pH gradient effects in distal colon

n	pH Gradient	J^{prop}			I_{sc}	G_t
		m-s	s-m	Net		
(6)	6.8/6.8	2.57 ± 0.07	2.07 ± 0.14	0.49 ± 0.13	0.10 ± 0.26	8.0 ± 0.5
(4)	6.8/7.4	3.46* ± 0.32*	1.74 ± 0.34	1.72* ± 0.26*	1.17 ± 0.69	10.6 ± 1.9

Results are expressed in μmol · cm^{-2} · hr^{-1} (fluxes), μEq · cm^{-2} · hr^{-1} (I_{sc}) and mS · cm^{-2} (G_t). Solution II (Table 1) was used for pH 6.8 conditions. Solution II (Table 1) was used for pH 7.4 conditions. Significant increases were noted in $J^{prop}_{m\text{-}s}$ and J^{prop}_{net}, $^*P < 0.05$.

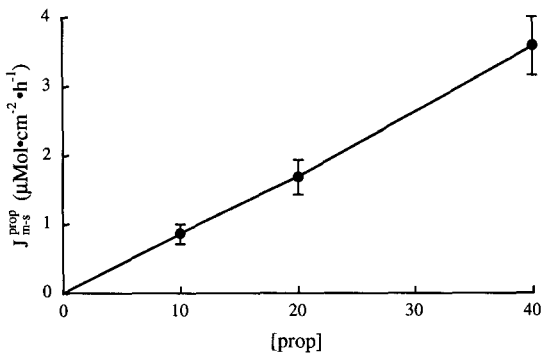

Fig. 2. Increasing concentrations of luminal SCFAs in distal colon are associated with a proportional increase in $J^{prop}_{m\text{-}s}$. (n = 4, pH 6.8). No evidence of saturation noted.

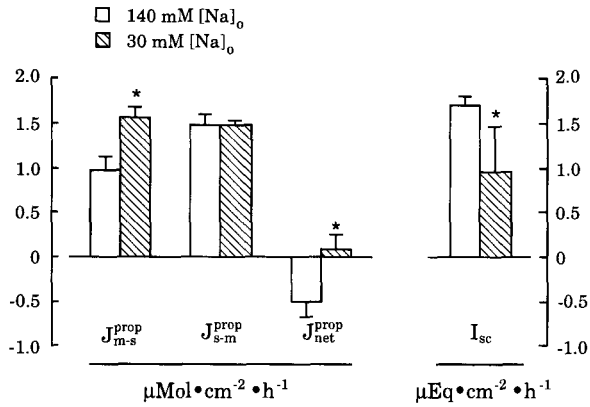

Fig. 3. Lowered [Na]$_o$ stimualtes propionate absorption in proximal colon. In pair-matched controls from the same animals, epithelia bathed in 30 mM [Na]$_o$ (Solution IV, Table 1) exhibited a significantly greater $J^{prop}_{m\text{-}s}$ and J^{prop}_{net} compared to normal (140 mM Na) Ringer. $^*P < 0.05$; n = 5.

any obvious saturation kinetics with increasing luminal concentrations of SCFAs. To determine whether this could be operative in distal colon, we examined the unidirectional flux ($J^{prop}_{m\text{-}s}$) with increasing concentrations of luminal propionate. The increase in flux was linear with concentration up to 40 mM propionate (Fig. 2).

Proximal Colon

Alterations in Na Transport

Our previous studies in proximal colon had linked Na absorption, Na-H exchange and SCFA transport, using a series of pharmacologic agents, including epinephrine, amiloride, and ouabain. To further explore this connection, we used additional maneuvers that alter Na-H exchange.

To determine whether SCFA fluxes are increased specifically by epinephrine-induced stimulation of Na-H exchange, we used an alternative mechanism to stimulate electroneutral Na absorption in proximal colon. Previous studies have shown that rabbit proximal colon responds to a reduced [Na]$_o$ with a paradoxical increase in net Na absorption [32]. Therefore, we measured SCFA fluxes in 30 mM Na Ringer, which predictably stimulates electroneutral Na absorption in proximal colon. As shown in Fig. 3, both $J^{prop}_{m\text{-}s}$ and J^{prop}_{net} increased under these conditions. Thus, two different stimuli of Na absorption in proximal colon both enhance propionate absorption.

To further examine the possible relationship between SCFA transport and other ion transport pathways, we tested the effect of theophylline on propionate fluxes in proximal colon. These experiments were performed under a 6.8/7.4 pH gradient (HEPES buffer) to create a basal absorptive state (Table 6). Theophylline increases intracellular cyclic AMP, inhibits electroneutral NaCl absorption and stimulates electrogenic Cl secretion in various epithelia. In proximal colon, secretagogues have been shown to have primarily an anti-absorptive effect on ion transport, blocking Na-H exchange rather than stimulating electrogenic Cl secretion [31].

Theophylline did not elicit a significant change in I_{sc}, similar to previous observations in the proximal colon [31]. However, it decreased J^{prop}_{net} from 0.68 to

Table 6. Effects of theophylline on propionate fluxes in proximal colon

Experimental condition	J^{prop}			I_{sc}	G_t
	m-s	s-m	Net		
Control	2.83 ± 0.37	2.13 ± 0.28	0.68 ± 0.19	1.0 ± 0.3	15.1 ± 0.8
Theophylline 10^{-3} M(S)	2.45 ± 0.32*	2.26 ± 0.25	0.20 ± 0.26*	1.1 ± 0.3	19.4 ± 0.4

Results are expressed in μmol · cm^{-2} · hr^{-1} ± SE (J^{prop}), μEq · cm^{-2} · hr^{-1} (I_{sc}), and mS · cm^{-2} (G_t) for eight animals. Solution V, Table 1, was used in both mucosal and serosal reservoirs, with a 6.8/7.4 gradient to establish an initial absorptive flux. Theophylline inhibited both $J^{prop}_{m\text{-}s}$ and J^{prop}_{net} (*$P < 0.01$ *vs.* control). The lack of change in I_{sc} is expected in rabbit proximal colon.

0.20 μmol · cm^{-2} · hr^{-1} (Table 6). This was due primarily to a decrease in $J^{prop}_{m\text{-}s}$, consistent with inhibition of Na-H exchange. Theophylline did not alter $J^{prop}_{s\text{-}m}$. Thus, two further maneuvers altering Na-H exchange (low $[Na]_o$ and theophylline) alter propionate transport consistent with a link to Na-H exchange.

Effect of Chloride Removal

Because SCFA transport may be mediated by an anion exchange system localized to the apical membrane, we examined whether replacement of chloride with an impermeable anion, gluconate, would alter propionate fluxes. In proximal colon, basal rates of propionate transport were identical in the Cl-containing and Cl-free solutions (Fig. 4*A*). We next examined whether stimulation of SCFA absorption by epinephrine would be altered by Cl removal. In normal Ringer, epinephrine increased $J^{prop}_{m\text{-}s}$ and J^{prop}_{net} as described previously. The identical response was observed in both Cl-containing and Cl-free Ringer. (Fig. 4*B*) Thus, removal of chloride had no significant effect on transepithelial propionate fluxes, indicating that SCFA transport is not Cl dependent.

Effect of DIDS

Because previous studies had suggested that SCFA transport mediated by an anion exchanger could be inhibited by stilbene derivatives [13], we examined the effects of DIDS on basal and epinephrine-stimulated propionate fluxes (Table 7). Epinephrine increased net propionate absorption by 0.57 μmol · cm^{-2} · hr^{-1} in control tissues and by 0.61 μmol · cm^{-2} · hr^{-1} in the DIDS-treated epithelial (pNS). These experiments did not demonstrate any significant inhibitory effect of mucosal DIDS on propionate absorption.

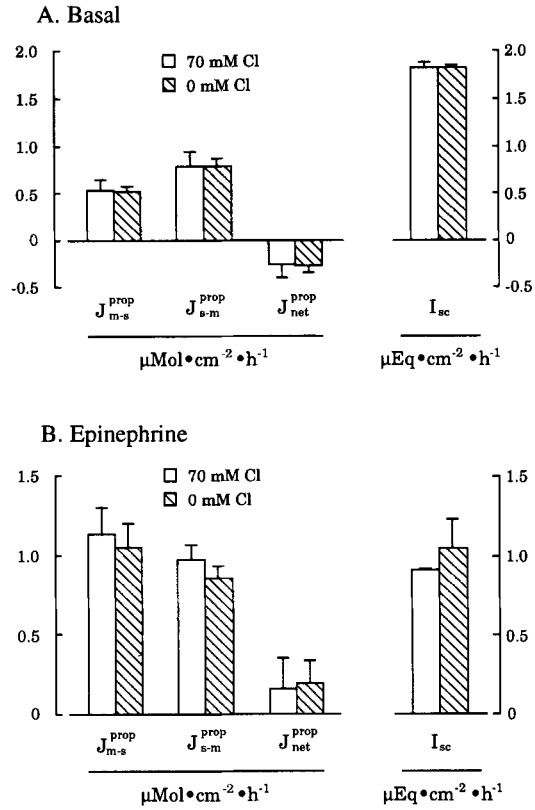

Fig. 4. Cl removal does not alter propionate fluxes in proximal colon. Removal of chloride altered neither basal rates of propionate fluxes (Fig. 4*A*) nor fluxes after stimulation by epinephrine (Fig. 4*B*), $n = 6$. Solutions II and III, Table 1, were used in these studies.

Effect of pH Gradients

We created pH gradients across the proximal colon to determine whether transport of propionate would be altered in a manner consistent with a weak electrolyte. In proximal colon acidification of the mucosal solution to pH 6.8, decreasing $[HCO_3]$m to 5

Table 7. DIDS does not alter propionate fluxes in proximal colon

Experimental condition	$J^{prop}_{m\text{-}s}$	$J^{prop}_{s\text{-}m}$	J^{prop}_{net}	I_{sc}	G_t
Control	0.44	1.08	−0.64	1.68	10.36
	0.11	0.18	0.20	0.28	0.63
+Epi(S)	1.17*	1.24	−0.07*	1.03*	9.56
	0.33	0.10	0.29	0.20	1.21
DIDS(M)	0.79	0.96	−0.17	1.46	11.01
	0.18	0.11	0.19	0.16	0.42
DIDS+ Epi(S)	1.50**	1.06	0.44*	0.94**	9.52
	0.24	0.12	0.20	0.18	0.62

Proximal colon mounted in Ussing chambers was bathed in a 5 mM HCO_3 pH 6.8 solution (Solution II, Table 1). Basal fluxes were performed either under control conditions (n = 6) or with DIDS 10^{-4} M ((*m*) (n = 11). After the initial flux, 10^{-5} M epinephrine was added to the serosal chamber and a second flux performed. Results are expressed in μmol · cm^{-2} · hr^{-1} for propionate fluxes, μEq · cm^{-2} · hr^{-1} for I_{sc} and mS · cm^{-2} (G_t), *P < 0.05, **P < 0.01 *vs.* corresponding first period flux.

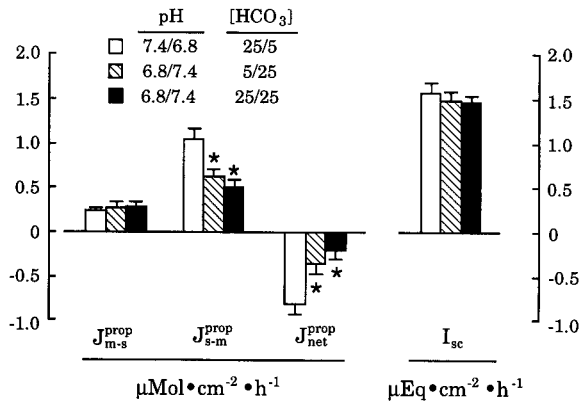

Fig. 5. Effect of transepithelial pH gradients on propionate fluxes in proximal colon. Creation of a pH gradient by altering [HCO_3] caused a significant change in $J^{prop}_{s\text{-}m}$ and J^{prop}_{net} (*P < 0.01). The effect of the pH gradient was independent of a bicarbonate gradient. pH (m/s) and HCO_3 (m/s) describe the conditions in the mucosal and serosal reservoirs. Solutions I and II, Table I, were used. n = 7 (luminal 7.4/serosal 6.8), 10 (pH 6.8/7.4) [HCO_3 5/25]; 8 [HCO_3 25/25] animals.

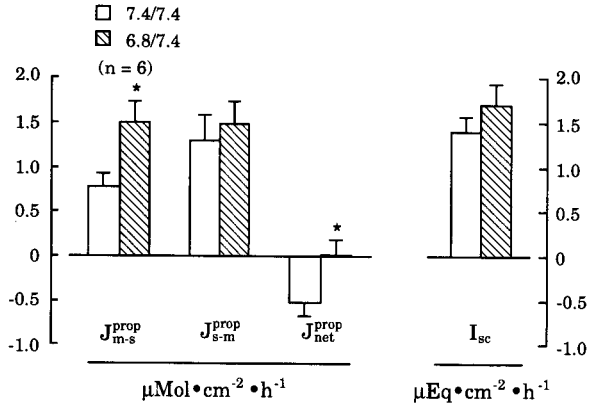

Fig. 6. Bicarbonate-free pH gradients in proximal colon. Creation of a pH gradient in a HEPES-buffered solution gassed with 100% O_2 (Solution V) increased both $J^{prop}_{m\text{-}s}$ and J^{prop}_{net} (*P < 0.05).

mM significantly decreased the rate of spontaneous propionate secretion (Fig. 5). To determine whether this effect was due to the change in pH or creation of a bicarbonate gradient across the epithelium, we performed an additional series of flux studies in which the pH gradient was established by changes in pCO_2 with equimolar mucosal and serosal bicarbonate (Fig. 5). The effect of pH is independent of the presence or absence of a bicarbonate gradient. Because changes in pH and [HCO_3] may alter electroneutral ion transport in the intestine [6, 7, 27] and theoretically exert a secondary effect on propionate transport, we examined the effects of a bicarbonate-free pH gradient (Fig. 6). Under these conditions, spontaneous propionate secretion was abolished.

The effects in proximal colon were not as marked as in distal colon. We considered two confounding variables that may apply to proximal, but not distal, colon: (i) changes in pH of bathing solutions alter intestinal electroneutral Na absorption mediated by Na-H exchange [6, 7], and (ii) rates of transepithelial propionate transport in proximal colon may be altered by changes in bathing solution pH [34].

We, therefore, compared pH 6.8/7.4 gradient to pH 6.8/6.8 conditions (Table 8). Under these conditions in a HEPES-buffered solution we found that there was no significant difference between the rates of propionate transport. This suggests that it is the lowered mucosal pH itself, rather than the gradient, that is the critical factor in stimulation of propionate transport in proximal colon.

Table 8. pH gradient effects in proximal colon

n	pH Gradient	J^{prop}			I_{sc}	G_t
		m-s	s-m	Net		
(5)	6.8/6.8	2.43 ± 0.21	1.93 ± 0.15	0.50 ± 0.18	1.94 ± 0.21	12.7 ± 1.5
(6)	6.8/7/4	2.58 ± 0.38	1.68 ± 0.22	0.90 ± 0.44	1.76 ± 0.24	11.2 ± 2.4

Results are expressed in μmol · cm^{-2} · hr^{-1} (fluxes), μEq · cm^{-2} · hr^{-1} (I_{sc}) and mS · cm^{-2} (G_t). These experiments were conducted in HEPES-buffered solutions (Solution V) gassed with 100% O_2. No changes in SCFA fluxes were observed when gradient conditions were compared to 6.8/6.8 conditions.

Table 9. Bumetanide does not affect propionate transport

Experimental condition	J^{prop}			I_{sc}	G_t
	m-s	s-m	Net		
Control	0.14 ± 0.02	1.03 ± 0.19	−0.89 ± 0.19	1.70 ± 0.27	8.0 ± 0.6
Bumetanide 10^{-4} M (S)	0.09 ± 0.04	1.04 ± 0.21	−0.95 ± 0.24	1.60 ± 0.21	8.2 ± 0.5

Studies were performed in a 20 mM propionate Ringer at pH 7.4 (Solution 1) to maximize a net secretion. Results of fluxes are expressed in μmol · cm^{-2} · hr^{-1}, I_{sc} in μEq · cm^{-2} · hr^{-1} and G_t in mS · cm^{-2} for four experiments. There are no significant changes.

Table 10. The effect of amiloride on propionate fluxes

Experimental condition	J^{prop}			I_{sc}	G_t
	m-s	s-m	Net		
Control	1.57 ± 0.13	1.49 ± 0.05	0.08 ± 0.17	1.0 ± 0.15	6.7 ± 0.40
Amil 10^{-3} (m-s)	1.09 ± 0.10	1.57 ± 0.09	−0.48 ± 0.15	0.7 ± 0.16	5.9 ± 0.41
$P <$	0.012	NS	0.016	0.049	0.027

Experiments were performed in 30 mM Na, 20 mM propionate Ringer pH 6.8. Results are expressed in μmol · cm^{-2} · hr^{-1} (fluxes), μEq · cm^{-2} · hr^{-1} (I_{sc}) and mS · cm^{-2} (G_t) for five experiments. Although amiloride inhibition of apical Na-H exchange decreases $J^{prop}_{m\text{-}s}$, there is no effect on $J^{prop}_{s\text{-}m}$.

Propionate Secretion

Propionate secretion occurs in vitro under short-circuit conditions [15, 34]. Despite its questionable clinical relevance, delineation of a net secretory SCFA pathway may provide a clue to understanding the secretion of bicarbonate and other anions. To explore this pathway, we examined the effects of two transport inhibitors (amiloride and bumetanide) added to the serosal reservoir.

Bumetanide blocks the basolateral Na-K-2Cl entry process integral to chloride secretion [26]. It also may inhibit other anion secretory processes such as bicarbonate [33]. However, bumetanide did not alter SCFA secretory fluxes (Table 9). Given the demonstrated correlation between SCFA fluxes and the activity of the apical Na-H exchanger, an alternative mechanism of basolateral entry of SCFA may be a basolateral Na-H exchanger [8, 21]. To test this possibility, we examined the effects of bilateral addition of amiloride (Table 10). Mucosal amiloride inhibits $J^{prop}_{m\text{-}s}$ as previously reported [34], which serves as a positive control. However, there was no alteration of $J^{prop}_{s\text{-}m}$ with serosal amiloride. These studies do not support a role for the basolateral Na-H antiporter in SCFA secretion.

Discussion

Although SCFAs are the predominant luminal anion in the colon, the mechanisms of epithelial SCFA transport are not yet clearly defined. A limited num-

ber of in vitro studies has demonstrated active SCFA secretion rather than absorption [15, 17, 23] (*see below*). Recently, we found an association in rabbit proximal colon in vitro between Na absorption and propionate fluxes, suggesting an integral role for Na-H exchange in SCFA transport [34]. The present study extends these observations by (i) further demonstrating the linkage between Na-H exchange and SCFA absorption in proximal colon, (ii) documenting significant differences in SCFA transport between proximal and distal colon, and (iii) establishing luminal pH as an important factor in regulating SCFA absorption.

Segmental heterogeneity of ion transport in the colon is well established. Differences in mechanisms of Na absorption are evident as one progresses from cecum to proximal to distal colon [11, 12, 31, 37]. We utilized the differences in proximal and distal colon as regards Na-H exchange to more carefully explore the relation between Na transport and SCFA absorption. These studies establish that it is a stimulation of Na-H exchange specifically, rather than increased Na absorption or an effect of epinephrine directly on SCFA transport, that is involved in the enhancement of SCFA absorption observed in the proximal colon. In distal colon, with no apical Na-H exchanger, epinephrine did not alter propionate fluxes. Stimulation of electrogenic Na absorption in distal colon did not increase J_{net}^{prop}. In contrast, further maneuvers in proximal colon that altered Na-H exchange (low [Na], theophylline) altered propionate transport. Regional differences in SCFA transport have recently been described in guinea pig large intestine, although the mechanisms underlying the differences are not fully delineated [9]. SCFAs enhance electroneutral Na absorption in rat distal colon, but after aldosterone treatment converts this epithelium to an electrogenic Na transporter, the stimulatory effect of SCFA on Na absorption is lost [3]. This suggests another functional linkage between Na : H exchange and SCFA transport. Thus, analogous to differences in Na absorption, there is also a segmental heterogeneity of SCFA transport in the colon.

Acidification of the luminal pH may act as a driving force for SCFA absorption. Net SCFA transport may be considered as the sum of fluxes of the ionized species (A-) and the protonated acid (HA). Given several assumptions, the net transport of a weak electrolyte will occur towards the compartment in which it is more ionized, independent of specific transport mechanisms; for SCFAs this means movement towards a more alkaline environment. Therefore, the relative pHs within the intestinal lumen, the cell and the submucosal space may affect SCFA transport.

Prior in vivo perfusion studies did not demonstrate an effect of luminal acidification on colonic SCFA transport; however, luminal pH changes stimulated butyrate absorption in perfused anuran small intestine [16, 27]. Although potentially problematic, creating a pH gradient across the epithelium in an Ussing chamber may be the experimental model that most closely parallels clinical conditions. Concerns over unstirred water layers during in vivo perfusions, especially in colon, are minimized in vitro and this may explain the variable results. Previous Ussing chamber studies have been generally performed at pH 7.4 and have not fully examined the impact of changes in pH on SCFA fuxes. Studies in apical membrane vesicles define specifically the gradient across a single membrane, but it is not clear how colonic intracellular pH responds to an imposed transepithelial gradient. Given the multiple membranes and compartments in an epithelial preparation, it is difficult to localize an effect of a transepithelial gradient to a specific site with absolute certainty.

This study extends our previous observations on the effects of acid-base variables on SCFA fluxes and demonstrates a significant but complex role for pH and HCO_3 in colonic SCFA transport. We have previously shown that lowered mucosal and serosal pH coupled with bicarbonate removal stimulates basal rates of propionate absorption, while lowered pH alone does not alter basal transport but potentiates the epinephrine response in proximal colon [34].

In distal colon, the changes in propionate flux in response to a pH gradient are consistent with the movement of a weak electrolyte to the more highly ionized compartment. This flux towards the more alkaline environment is theoretically independent of specific membrane transporters.

pH also has an effect in proximal colon, but it differs from distal colon. Although the changes in net fluxes in our initial pH gradient experiments in proximal colon (Fig. 5) were consistent with changes predicted by a weak electrolyte responding to a pH gradient, the vagaries of the unidirectional fluxes led us to pursue several variables including $[HCO_3]$, pCO_2, pH gradients and finally bilateral changes in pH. The results of these studies suggest it is lowered mucosal pH, with or without a gradient, that has a stimulatory effect in proximal colon. This is in contrast to distal colon where the gradient itself has a stimulatory effect (*Compare* Tables 5 and 8). Unlike the small bowel, the colonic lumen is subject to wide variations in luminal pH and, therefore, may be a more dynamic factor in epithelial function. Increases in colonic carbohydrates will increase SCFA production and decrease luminal pH. Measurements of normal colonic luminal pH generally range between

pH 6 and 7.2. Thus, clinically, one may reasonably expect modestly acidic luminal pHs, such as used in these studies, with a constant serosal pH to have an effect on colonic SCFA absorption. This suggests that pH may be a factor in SCFA transport in both proximal and distal colon, although by somewhat different mechanisms.

The presence of electroneutral NaCl transport mediated by dual antiporters in the proximal colon may be a confounding variable that alters the response of propionate to a pH gradient. Changes in pH and HCO_3 alter electroneutral Na absorption in the gut [6, 7]. Lowered [HCO_3] and pH may enhance apical Na-H exchange as occurs in the ileum, potentially resulting in augmented propionate absorption, thus modifying the effect predicted by pH gradients independent of specific membrane transporters.

SCFA transport is linked to Na : H exchange, although defining the mechanism(s) of that linkage is difficult to determine. Substantial evidence exists for two differing models: anion exchange and diffusion of the protonated SCFA. There is a complex family of epithelial anion exchangers in intestinal epithelial with varying ion specificities and kinetics. Binder and Mehta [4] raised the possibility of a Cl : SCFA antiporter. Vesicle studies in human small intestine [13] and rat colon [22] have demonstrated SCFA : HCO_3 exchange. Although vesicle studies have suggested anion exchange of SCFAs, short-circuit experiments have consistently found evidence for nonparacellular diffusion of a protonated SCFA [9, 34].

The present study suggests that, in the short-circuited rabbit colon, the more likely process is diffusion of a protonated acid. There is no Cl dependence, making the SCFA : Cl exchange mechanism less likely. Removal of bicarbonate enhances unidirectional fluxes rather than inhibits them [34]. Anion exchange inhibitors did not significantly block basal or epinephrine-stimulated propionate absorption (Table 7). There is no obvious concentration-dependent saturation of SCFA fluxes (Fig. 2) consistent with prior studies [9, 10, 27, 34]. Saturation kinetics, as found in the vesicle studies, would imply a carrier-mediated transporter rather than diffusion. Prior studies demonstrated that paracellular diffusion of the ionized species is unlikely [9, 34]; thus, the lack of obvious saturability implies either a large capacity transport system or a flux of the protonated SCFA. Creation of a proton gradient across an epithelium that does not exhibit apical Na-H exchange (distal colon) stimulates SCFA absorption, suggesting diffusion of the protonated acid in a manner consistent with pH-dependent distribution of a weak electrolyte.

We searched for, but could not demonstrate evidence for, SCFA transport via an anion exchanger. Neither anion substitution nor the effect of inhibitors was consistent with such a mechanism. However, it is apparent that there is a significant and complex relationship between bicarbonate and SCFA transport. There is evidence that bicarbonate and SCFA may share common transport pathways. Bicarbonate removal with constant pH increases both unidirectional fluxes of propionate in proximal colon, suggesting convergent transport systems for the two anions in passage across the epithelium. Hatch et al. [11] raised a similar possibility, suggesting that HCO_3 and SCFA may have a similar secretory pathway in rabbit cecum. It is well recognized that SCFAs effectively substitute for HCO_3 in promoting electroneutral NaCl absorption [28], implying either the ability to serve as a replacement anion in Cl : HCO_3 exchange or, alternatively, to substitute as an intracellular buffer in maintaining Na-H exchange.

These data form a cohesive picture of a diffusive process in which the protonated SCFA crosses the apical membrane independent of anions. Differing experimental results may be dependent on the experimental methodology or animal model used. SCFA and bicarbonate transport are clearly intertwined, but the specific mechanisms governing the relation between these two weak electrolytes remains to be determined.

The significance of $J_{s\text{-}m}^{prop}$ and SCFA secretion needs to be considered briefly. Conventional in vitro transport studies generally aim to eliminate electrochemical gradients across epithelia to measure "active transport," this despite the fact it may not correlate with the physiological state. For example, most studies of ion transport in distal colon generally use equimolar concentrations of Na despite the fact that luminal [Na] may be an order of magnitude lower than systemic [Na]. In a similar vein, submucosal or serosal SCFA concentrations are not equivalent to those of the colonic lumen and, therefore, measurement of SCFA secretion does not represent a normal biological process. However, it does serve to allow measurement of an active transport process. Additionally, although SCFA secretion may not be physiological, this process may permit a fuller understanding of other ions such as bicarbonate that may share a similar transport pathway.

These studies demonstrate a complexity of colonic SCFA transport with distinct segmental heterogeneity and modulation by both Na-H exchange and luminal pH. Both these factors increase the protonated (and diffusable) species of SCFA. The observation that luminal pH may be a significant factor in regulating SCFA transport is of particular import. Carbohydrate malabsorption leads to generation of

colonic SCFAs and a decrease in luminal pH. If, indeed, a lowered luminal pH promotes SCFA absorption, this may provide a mechanism for balancing rates of production and absorption. The pH gradients used in these studies are relatively modest and often exceeded in the colonic lumen. The recognition that SCFA transport may have segmental variations and be related to active transcellular processes, rather than simple diffusion, is particularly significant because it suggests that the colonic epithelium may regulate SCFA fluxes and may be subject to pathophysiological alterations.

This research has been supported by a grant from the National Institutes of Health, R01 #DK35193, and a grant from the Crohn's & Colitis Foundation of America, Inc. supported by the W.S. Farrish Fund. We would like to thank Patricia Nuckolls for able secretarial assistance and Yu Tao for his technical assistance.

References

1. Argenzio, R.A., Southworth, M., Lowe, L.E., Stevens, C.E. 1977. Interrelationship of Na, HCO_3 and volatile fatty acid transport by equine large intestine. *Am. J. Physiol.* **233:** E469-E478
2. Argenizo, R.A., Whipp, S.C. 1977. Interrelationship of sodium, chloride, bicarbonate and acetate transport by the colon of the pig. *Am. J. Physiol.* **295:**365–381
3. Binder, H.J., Mehta, P. 1990. Characterization of butyrate-dependent electroneutral Na-Cl absorption in the rat distal colon. *Pfluegers Arch.* **417:**365–369
4. Binder, H.J., Mehta, P. 1989. Short-chain fatty acids stimulate active sodium and chloride absorption in vitro in the rat distal colon. *Gastroenterology* **96:**989–996
5. Breuer, R.I., Buto, S.K., Christ, M.L., Bean, J., Vernia, P., Paoluzi, P. DiPaolo, M.C., Caprilli, R. 1991. Rectal irrigation with short-chain fatty acids for distal ulcerative colitis: Preliminary report. *Dig. Dis. Sci.* **36:**185–187
6. Charney A.N., Feldman, G.M. 1984. Systemic acid-base disorders and intestinal electrolyte transport. *Am. J. Physiol.* **247:**G1–G12
7. DeSoignie, R., Sellin, J.H. 1990. Acid-base regulation of ion transport in rabbit ileum in vitro. *Gastroenterology* **99:**132–141
8. Dudeja, P.K., Foster, E.S., Brasitus, T.A. 1989. Na-H antiporter of rat colonic basolateral membrane vesicles. *Am. J. Physiol.* **257:**G624–G632
9. Engelhardt, W., Rechkemmer, G. 1992. Segmental differences of short-chain fatty acid transport across guinea-pig large intestine. *Experimental Physiol.* **77:**491–499
10. Fleming, S.E., Choi, S.Y., Fitch, M.D. 1992. Absorption of short-chain fatty acids from the rat cecum in vivo. *J. Nutr.* **121:**1787–1797
11. Foster, E.S., Budinger, M.E., Hayslett, J.P., Binder, H.J. 1986. Ion transport in proximal colon of the rat. *J. Clin. Invest.* **77:**228–235
12. Halm, D.R., Frizzell, R.A. 1986. Active K transport across rabbit distal colon: relation to Na absorption and Cl secretion. *Am. J. Physiol.* **251:**C252-C267
13. Harig, J.M., Soergel, K.H., Barry, J.A., Ramaswamy, K. 1991. Transport of propionate by human ileal brush border membrane vesicles. *Am. J. Physiol.* **260:**G776–G782
14. Harig, J.M., Soergel, K.H., Komorowski, R.A., Wood, C.M. 1989. Treatment of diversion colitis with short-chain fatty acid irrigation. *New Engl. J. Med.* **320:**23–28
15. Hatch, M. 1987. Short-chain fatty acid transport and its effects on ion transport by rabbit cecum. *Am. J. Physiol.* **253:**G171–G178
16. Hollander, D., Gerand, E.M., Boyd, C.A.R. 1986. Transport of butyric acid in vascularly perfused anuran small intestine: importance of pH and anion transport. *Am. J. Physiol.* **250:**G469–474
17. Horvath, P.J., Weiser, M.M., Duffey, M.E. 1986. Propionate alters ion transport by rabbit distal colon. *Fed. Proc.* **45:**509
18. Hoverstad, T. 1986. Studies of short-chain fatty acid absorption in man. *Scand. J. Gastro.* **21:**257–260
19. Jackson, M.J. 1986. Weak electrolyte transport across biological membranes. General principles. *In*: T.E. Andreoli, J.F. Hoffman, D.D. Fanestil, S.G. Schultz, editors. pp. 235–247
20. Kim, J.R., Kam, W.K., Byrd, J.C. 1987. Effects of sodium butyrate on human colonic adenocarcinoma cells. *J. Biol. Chem.* **262:**1092–1097
21. Knickelbein, R.G., Aronson, P.S., Dobbins, J.W. 1988. Membrane distribution of sodium-hydrogen and chloride-bicarbonate exchangers in crypt and villus cell membranes from rabbit ileum. *J. Clin. Invest.* **82:**2158–2163
22. Mascolo, N., Rajendran, V.M., Binder, H.J. 1991. Mechanism of short chain fatty acid uptake by apical membrane vesicles of rat distal colon. *Gastroenterology* **101:**331–338
23. McNeil, I., Cummings, J.H., James, W.P. 1978. Short chain fatty acid absorption by human large intestine. *Gut* **19:**819–822
24. Mortensen P.B., Holtug, K., Bonnen, H., Clausen, M.R. 1990. The degradation of amino acids, proteins, and blood to short-chain fatty acids in colon is prevented by lactulose. *Gastroenterology* **98:**353–360
25. Newmark, H.L., Lupton, J.R. 1990. Determinants and consequences of colonic luminal pH: Implications for colon cancer. *Nutr. Cancer* **14:**161–173
26. O'Grady, S.M., Palfrey, H.C., Field, M. 1987. Characteristics and functions of Na-K-Cl co-transport in epithelial tissues. *Am. J. Physiol.* **253:**C177–C192
27. Rechkemmer, G., von Engelhardt, W. 1988. Concentration and pH-dependence of short-chain fatty acid absorption in the proximal and distal colon of guinea pig. *Comp. Biochem. Physiol.* **91A:**659–663
28. Reuss, L., Segal, Y., Altenberg, G. 1991. Regulation of ion transport across gallbladder epithelium. *Annu. Rev. Physiol.* **53:**361–373
29. Rodeiger, W.E.W. 1980. The colonic epithelium in ulcerative colitis—an energy deficient disease. *Lancet* **2:**712–715
30. Ruppin, H., Bar-Meir, S., Soergel, K.H., Wood, C., Schmitt, M.G. 1980. Absorption of short-chain fatty acids by the colon. *Gastroenterology* **78:**1500–1507
31. Sellin, J.H., DeSoignie, R. 1984. Rabbit proximal colon: a distinct transport epithelium. *Am. J. Physiol.* **246:**G603–610
32. Sellin, J.H., DeSoignie, R. 1987. Ionic regulation of Na absorption in proximal colon: cation inhibition of electroneutral Na absorption. *Am. J. Physiol.* **252:**G100–G108
33. Sellin, J.H., DeSoignie, R. 1989. Regulation of bicarbonate transport by rabbit ileum: pH stat studies. *Am. J. Physiol.* **257:**G607–G615
34. Sellin, J.H., DeSoignie, R. 1990. Short-chain fatty acid absorption in rabbit colon in vitro. *Gastroenterology* **99:**676–683
35. Sellin, J.H., DeSoignie, R. 1985. Steroids alter ion transport

and absorptive capacity in proximal and distal colon. *Am. J. Physiol.* **249:**G113–G119
36. Soergel, K.H., Georg, K.H., Wood, C.M. 1980. Propionate transport and metabolism by the short-circuited rat colon. *Clin. Res.* **26:**486*A* (*Abstr.*)
37. Turnheim, K., Frizzell, R.A., Schultz, S.G. 1977. Effect of anions on amiloride-sensitive, active sodium transport across rabbit colon in vitro. *J. Membrane Biol.* **37:**63–84
38. Umesake, Y., Yajima, T., Yokokura, T., Masahiko, M. 1979. Effect of organic acid absorption on bicarbonate transport in rat colon. *Pfluegers Arch.* **379:**43–47
39. Vernia, P., Gnaedinger, A., Hauck, W., Breuer, R.I. 1988. Organic anions and the diarrhea of inflammatory bowel disease. *Dig. Dis. Sci.* **33:**1353–1358
40. Yajima, T. 1988. Luminal propionate-induced secretory response in the rat distal colon in vitro. *J. Physiol.* **403:**559–575