Nonionic diffusion of short-chain fatty acids across rat colon

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Charney, Alan N., Ljubisa Micic, and Richard W. Egnor. Nonionic diffusion of short-chain fatty acids across rat colon. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G518–G524, 1998.—Short-chain fatty acid (SCFA) transport across the colon may occur by nonionic diffusion and/or via apical membrane SCFA\(^{-}\)/HCO\(_3\)\(^{-}\) exchange. To examine the relative importance of these processes, stripped segments of rat (Ratus ratus) proximal and distal colon were studied in Ussing chambers, and the unidirectional fluxes of radiolabeled SCFA butyrate, propionate, or weakly metabolized isobutyrate were measured. In N-2-hydroxyethylpiperazine-N\(^-'\)-2-ethanesulfonic acid (HEPES) or 1 or 5 mM HCO\(_3\)\(^{-}\)-Ringer, decreases in mucosal pH stimulated mucosal-to-serosal flux (\(J_{m-s}\)) of all SCFA, decreases in serosal pH stimulated serosal-to-mucosal flux (\(J_{s-m}\)), and bilateral pH decreases stimulated both fluxes equally. These effects were observed whether the SCFA was present on one or both sides of the tissue, in both proximal and distal colon, in the absence of luminal Na\(^{+}\), and in the presence of either luminal or serosal ouabain. Changes in intracellular pH or intracellular [HCO\(_3\)\(^{-}\)] did not account for the effects of extracellular pH.

Luminal Cl\(^{-}\) removal, to evaluate the role of apical membrane Cl\(^{-}\)/SCFA\(^{-}\) exchange, had no effect on \(J_{m-s}\) but decreased \(J_{s-m}\) 32% at pH 6.5 and 22% at 7.2. Increasing SCFA concentration from 1 to 100 mM, at pH 6.4 or 7.4, caused a linear increase in \(J_{m-s}\). We conclude that SCFA are mainly transported across the rat colon by nonionic diffusion.

butyrate; propionate; in vitro; flux; pH; intracellular pH

SHORT-CHAIN FATTY ACIDS (SCFA) are produced by bacterial metabolism of unabsorbed carbohydrates in the mammalian colon. They provide the predominant anions in the colonic lumen and include acetate (60–75%), propionic (15–25%), and butyric acids (10–15%). Once absorbed, SCFA stimulate Na\(^{+}\) and Cl\(^{-}\) absorption, contribute to the maintenance of cell pH and volume, and contribute potential base to the systemic acid-base pool (5). The transport and metabolic pathways by which each of these functions is achieved are well described.

Until recently, the colonic absorption of SCFA was believed to occur through nonionic diffusion. This mechanism of SCFA passage across the luminal and serosal membranes is consistent with the functions noted above and with partial recycling of SCFA across the luminal membrane via Cl\(^{-}\)/SCFA\(^{-}\) exchange (4, 25). This passive transport process is stimulated by decreases in bulk fluid or microclimate luminal pH consistent with the fact that the acid dissociation constant (pK\(a\)) of the most abundant SCFA in the colonic lumen is approximately two pH units lower (6).

Recently, an SCFA\(^{-}\)/HCO\(_3\)\(^{-}\) exchange process was identified in apical brush-border membrane vesicles prepared from the rat colon (24). This process also is stimulated by reductions in luminal pH and exhibits a Michaelis constant (K\(_m\)) for butyrate of 27 mM, near or below typical SCFA concentrations found in the colonic lumen. It was suggested that at least in this segment of this species, the major mechanism by which SCFA are absorbed is luminal membrane anion exchange (24). This mechanism of SCFA absorption had been suggested for the human ileum (21), and in the absence of an identified exchanger, for the rat jejunum (3) and rabbit and guinea pig proximal colon (18, 30). To examine these possibilities in the rat colon, we studied SCFA transport under in vitro conditions designed to test the functional importance rather than the presence of anion exchange. We systematically examined the effects of altering extracellular and intracellular pH (pHe and pHi, respectively) and examined the tenets of carrier-mediated transport. Our intent was to determine to what extent passive movement of SCFA across the luminal and serosal cell membranes could account for transepithelial transport in rat colon.

METHODS

Male Sprague-Dawley rats weighing 250–350 g were maintained on a standard diet with free access to water. Under pentobarbital sodium anesthesia (5 mg/100 g body wt), the proximal or distal 10 cm of colon were removed and rinsed with 0.9% saline. The serosa was stripped while the tissue was mounted on a glass rod.

Ion flux measurements. Details of the method were previously described (9, 10). Briefly, tissue pairs were mounted in modified Ussing half-chambers exposing 1.12 cm\(^2\) surface area. The transepithelial potential difference (PD) was referenced to the mucosal side. Tissues were studied under short-circuit conditions except for 1-s intervals every 100 s, during which bipolar pulses of 0.5 mV yielded electrical current values that were used to calculate tissue conductance (G). Tissues were paired for ion flux studies on the basis of differences in G no greater than 25%. The short-circuit current (I\(_sc\)) divided by G yielded the active transport PD.

The fluxes of SCFA were measured by adding 1 µCi \(^{14}\)C-SCFA (10–20 mCi/mM specific activity; NEN, Boston, MA) to the mucosal side of one member of each tissue pair and to the serosal side of the other. Mucosal-to-serosal (\(J_{m-s}\)) and serosal-to-mucosal (\(J_{s-m}\)) fluxes were measured over a 16-min period after an initial 30-min equilibration period. Twelve minutes were allowed for each new steady state, and 32 min were allowed for the effect of ouabain. Net flux was calculated as \(J_{m-s} = J_{s-m}\).

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various pH values. All solutions were maintained at 37°C.

The solutions were so designed that after the addition of the salt of an SCFA or gluconic acid, similar final osmolality and, where appropriate, Na+ concentration (always <150 mM) were achieved.

In the ion replacement experiments, choline and isethionate replaced Ringer Na+ and Cl−, respectively. SCFA concentration was 25 mM bilaterally in all experiments except in the gradient and transport kinetics studies where 1, 10, 25, 50, or 100 mM Na+ butyrate or Na+ isobutyrate were used on one side and Na+ gluconate on the other. In certain experiments, ouabain (1 mM; Sigma Chemical, St. Louis, MO) was added to either the mucosal or serosal bathing solution.

In several experiments in which butyrate flux was measured, the tops of the fluid reservoirs were sealed and vented either the mucosal or serosal bathing solution.

Table 1. Solutions

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Values in mM. The HEPES Ringer solutions (A and F) were gassed with 100% O2, and pH was titrated using 2 M H2SO4 or 1 M NaOH. HCO3− solutions (B−E) were gassed with 1% CO2 (PCO2 = 7 mmHg), 3% CO2 (PCO2 = 21 mmHg), 5% CO2 (PCO2 = 35 mmHg), 11% CO2 (PCO2 = 75 mmHg), or 14% CO2 (PCO2 = 95 mmHg) (balance O2) to obtain various pH values. All solutions were maintained at 37°C.

Intracellular HCO3− concentration ([HCO3−]) was computed using the Henderson-Hasselbalch equation as described. Intracellular Pco2 was assumed to be equal to the medium Pco2, and the negative log of dissociation (pK') and CO2 solubility were 6.115 and 0.0306, respectively. Bathing solution pH and Pco2 were measured with a Radiometer BMS 3 Mk 2 system with a PHM 73 acid-base analyzer (London Company, Cleveland, OH). Extracellular [HCO3−] was computed using the Henderson-Hasselbalch equation as described.

All data are expressed as means ± SE and were compared by paired Student’s t-test or analysis of variance (ANOVA). Two-tailed P values < 0.05 were considered significant.

RESULTS

Effect of pH on butyrate flux in distal colon. Initially the effect of unilateral and bilateral changes in bathing solution pH on butyrate flux in HEPES Ringer were examined. In these experiments butyrate was present at 25 mM on both sides of the tissue. As shown in Fig. 1, at pH 7.38 the net flux of butyrate was −0.1 ± 0.3 µeq·cm−2·h−1. As mucosal solution pH was decreased in steps from 7.38 to 5.47, Jms increased from 3.4 to 7.2 µeq·cm−2·h−1 and net absorption was observed (3.5 ± 0.3 µeq·cm−2·h−1). As shown when the luminal pH was then increased to 7.38, the increase in Jms was completely reversible. Luminal pH changes had no effect on the Jsm of butyrate.

As shown in Fig. 2, as serosal solution pH was decreased in steps from 7.38 to 5.57 Jsm of butyrate increased from 3.4 to 5.4 µeq·cm−2·h−1. This change caused net butyrate secretion (−2.4 ± 0.5 µeq·cm−2·h−1). When pH was then increased to 7.38 the increase in Jsm was completely reversible. Serosal pH changes had no effect on the Jms of butyrate.
Reversibility of Butyrate Fluxes

The effect of changing the pH of both bathing solutions on butyrate flux is shown in Fig. 3. As solution pH was decreased in steps from 7.39 to 5.58, both $J_{m-s}$ and $J_{s-m}$ of butyrate increased from $\sim 3$ to $7 \mu$eq·cm$^{-2}$·h$^{-1}$. Net butyrate flux was minimal at bilateral pH 7.39 ($-0.8 \pm 0.4 \mu$eq·cm$^{-2}$·h$^{-1}$) and remained minimal at pH 5.53 ($0.1 \pm 0.5 \mu$eq·cm$^{-2}$·h$^{-1}$). When pH was then increased to 7.39, the increases in the unidirectional fluxes were completely reversible.

Reduction in pH of one or both bathing solutions had no effect on G but decreased $I_{sc}$. For example, when mucosal solution pH was reduced from 7.38 to 5.47, $I_{sc}$ decreased from $0.7 \pm 0.2$ to $0.2 \pm 0.3 \mu$eq·cm$^{-2}$·h$^{-1}$, $P < 0.05$. When pH was then increased to 7.38 $I_{sc}$ increased to $0.9 \pm 0.4 \mu$eq·cm$^{-2}$·h$^{-1}$.

Effect of pH on Propionate Flux in Distal Colon

Similar effects of pH changes on propionate fluxes in HEPES Ringer were observed. Propionate was present at 25 mM on both sides of the tissue. Decreases in luminal pH in steps from 7.36 to 5.46 selectively increased $J_{m-s}$ of propionate from $2.7 \pm 0.2$ to $6.3 \pm 0.4 \mu$eq·cm$^{-2}$·h$^{-1}$, $n = 4$, $P < 0.001$. As serosal solution pH was decreased in steps from 7.36 to 5.46, $J_{s-m}$ selectively increased from $4.1 \pm 0.2$ to $5.4 \pm 0.5 \mu$eq·cm$^{-2}$·h$^{-1}$, $n = 4$, $P < 0.05$. Minimal net propionate secretion was observed at bilateral pH 7.36 ($-1.4 \pm 0.3 \mu$eq·cm$^{-2}$·h$^{-1}$, $P < 0.02$), and zero net transport was found at pH 5.46 ($0.9 \pm 0.4 \mu$eq·cm$^{-2}$·h$^{-1}$, $P < 0.01$ compared with flux at pH 7.36).

Effect of Butyrate Gradient on Butyrate Flux

We then examined the effects of unilateral pH changes in HEPES Ringer on butyrate fluxes in the presence of a 25 mM luminal to 0 mM serosal butyrate concentration gradient. As mucosal solution pH was decreased in steps from 7.39 to 5.58, $J_{m-s}$ increased from $1.0 \pm 0.1$ to $2.4 \pm 0.2 \mu$eq·cm$^{-2}$·h$^{-1}$, $n = 4$, $P < 0.005$. When pH was then increased to 7.39, the increase in $J_{m-s}$ was reversed to $1.1 \pm 0.1 \mu$eq·cm$^{-2}$·h$^{-1}$, $P < 0.005$. When serosal pH changes were studied in the presence of a 25 mM serosal to 0 mM luminal butyrate concentration gradient, similar results were obtained. As serosal solution pH was decreased in steps from 7.39 to 5.56, $J_{s-m}$ increased from $2.0 \pm 0.1$ to $4.1 \pm 0.1 \mu$eq·cm$^{-2}$·h$^{-1}$, $n = 4$, $P < 0.007$. When pH was then increased to 7.39, the increase in $J_{s-m}$ was reversed to $2.3 \pm 0.1 \mu$eq·cm$^{-2}$·h$^{-1}$, $P < 0.007$.

Fig. 1. Effect of mucosal pH change on unilateral flux of butyrate. Tissue pairs were bathed in HEPES Ringer, and butyrate was present on both sides of the tissue at 25 mM. As mucosal solution pH was decreased, mucosal-to-serosal flux ($J_{m-s}$) increased and net butyrate absorption was observed. When mucosal pH was then increased, the increase in $J_{m-s}$ was completely reversible. Changes in mucosal pH did not affect serosal-to-mucosal flux ($J_{s-m}$). Values are means ± SE, $n = 4–6$. Increments and decrement in $J_{m-s}$ were significantly different by ANOVA, $P < 0.001$.

Fig. 2. Effect of serosal pH change on unilateral flux of butyrate. Tissue pairs were bathed in HEPES Ringer and butyrate was present on both sides of the tissue at 25 mM. As serosal solution pH was decreased, $J_{s-m}$ increased and net butyrate secretion was observed. When serosal pH was then increased, the increase in $J_{s-m}$ was completely reversible. Changes in serosal pH did not affect $J_{m-s}$. Values are means ± SE, $n = 3–5$. Increments and decrement in $J_{s-m}$ are significantly different by ANOVA, $P < 0.01$.

Fig. 3. Effect of bilateral pH change on unilateral flux of butyrate. Tissue pairs were bathed in HEPES Ringer and butyrate was present on both sides of the tissue at 25 mM. Net butyrate flux was minimal at pH 7.39. As mucosal and serosal solution pH was decreased, both $J_{m-s}$ and $J_{s-m}$ increased and net butyrate flux remained minimal. When solution pH was then increased, the increases in $J_{m-s}$ and $J_{s-m}$ were completely reversible. Values are means ± SE, $n = 3–5$. Increments and decrement in $J_{m-s}$ and $J_{s-m}$ are significantly different by ANOVA, $P < 0.001$. Reductions in pH of one or both bathing solutions had no effect on G but decreased $I_{sc}$. For example, when mucosal solution pH was reduced from 7.38 to 5.47, $I_{sc}$ decreased from $0.7 \pm 0.2$ to $0.2 \pm 0.3 \mu$eq·cm$^{-2}$·h$^{-1}$, $P < 0.05$. When pH was then increased to 7.38 $I_{sc}$ increased to $0.9 \pm 0.4 \mu$eq·cm$^{-2}$·h$^{-1}$.

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Effect of pH on butyrate flux in HCO$_3$ Ringer. The effect of bilateral changes in bathing solution pH on butyrate flux across distal colon was also examined in HCO$_3$ Ringer where pH changes were induced by changing Pco$_2$. In these experiments butyrate was present at 25 mM on both sides of the tissue. In 1 mM HCO$_3$ Ringer at Pco$_2$ = 7 mmHg, pH 6.79, the net flux of butyrate was $0.1 \pm 0.2 \mu$eq·cm$^{-2}$·h$^{-1}$, $n = 6$. As solution pH was decreased in steps to 6.08 by increasing Pco$_2$ to 95 mmHg, net flux was little changed: $-0.5 \pm 0.2 \mu$eq·cm$^{-2}$·h$^{-1}$, J$_{m-s}$ increased from 4.2 ± 0.2 to 4.8 ± 0.3 µeq·cm$^{-2}$·h$^{-1}$ and J$_{s-m}$ increased from 4.1 ± 0.3 to 5.3 ± 0.5 µeq·cm$^{-2}$·h$^{-1}$, $n = 6$, P < 0.05.

In 5 mM HCO$_3$ Ringer at Pco$_2$ = 7 mmHg, pH 7.24, minimal net butyrate secretion was observed ($-1.0 \pm 0.2 \mu$eq·cm$^{-2}$·h$^{-1}$, $n = 5$). As solution pH was decreased to 6.44 in steps by increasing Pco$_2$ to 95 mmHg, net flux was little changed: $-0.4 \pm 0.5 \mu$eq·cm$^{-2}$·h$^{-1}$, J$_{m-s}$ increased from 2.6 ± 0.1 to 4.0 ± 0.3 µeq·cm$^{-2}$·h$^{-1}$ and J$_{s-m}$ increased from 3.6 ± 0.2 to 4.4 ± 0.2 µeq·cm$^{-2}$·h$^{-1}$, $n = 5$, P < 0.02. These flux changes in 1 and 5 mM HCO$_3$ Ringer were completely reversible and were similar in magnitude to flux changes in HEPES Ringer ($-1 \mu$eq·cm$^{-2}$·h$^{-1}$ per pH unit). In addition, in both 1 and 5 mM HCO$_3$ Ringer, reductions in pH did not affect G but reduced lsc from 0.4 µeq·cm$^{-2}$·h$^{-1}$ to near zero.

Effect of butyrate metabolism on butyrate flux. We then examined whether the metabolism of SCFA influenced the pattern of their transepithelial transport. We studied the effect of pH in HEPES Ringer on the flux across distal colon of isobutyrate, a weakly metabolized SCFA (22). Isobutyrate was present at 25 mM on both sides of the tissue. Decreases in luminal pH in steps from 7.35 to 5.45 increased J$_{m-s}$ of isobutyrate from 2.1 ± 0.1 to 4.9 ± 0.1 µeq·cm$^{-2}$·h$^{-1}$, $n = 4$, P < 0.0001. J$_{s-m}$ flux increased slightly from 2.2 ± 0.1 to 2.8 ± 0.1 µeq·cm$^{-2}$·h$^{-1}$, $n = 4$, P < 0.04. In a separate experiment, when pH was reduced in both bathing solutions in steps, net isobutyrate flux remained unchanged: $-1.0 \mu$eq·cm$^{-2}$·h$^{-1}$ at pH 7.34, 0.0 µeq·cm$^{-2}$·h$^{-1}$ at pH 6.68, $-0.8 \mu$eq·cm$^{-2}$·h$^{-1}$ at pH 6.04, and $-0.5 \mu$eq·cm$^{-2}$·h$^{-1}$ at pH 5.55. These changes in unidirectional fluxes were completely reversible.

Effect of luminal Na$^+$ removal and ouabain. To determine whether apical Na$^+$/H$^+$ exchange activity was necessary for SCFA transport, the exchanger was inhibited by substituting choline for Na$^+$ in the mucosal bathing solution. In HEPES Ringer, with butyrate present at 25 mM on both sides of the tissue, bilateral reductions in pH in steps stimulated J$_{m-s}$ and J$_{s-m}$ of butyrate equivalently. At pH 7.42 and 5.68, net flux was unchanged and near zero, and J$_{m-s}$ was 1.3 ± 0.1 and 5.1 ± 0.1 µeq·cm$^{-2}$·h$^{-1}$ and J$_{s-m}$ was 2.1 ± 0.2 and 4.1 ± 0.1 µeq·cm$^{-2}$·h$^{-1}$, respectively, $n = 2$, P < 0.05.

In 5 mM HCO$_3$ Ringer, similar results were obtained. At pH 7.32 and 6.51, net fluxes were unchanged and near zero, and J$_{m-s}$ was 1.5 ± 0.1 and 2.1 ± 0.1 µeq·cm$^{-2}$·h$^{-1}$ and J$_{s-m}$ was 2.7 ± 0.1 and 3.4 ± 0.3 µeq·cm$^{-2}$·h$^{-1}$, respectively, $n = 3$, P < 0.05. The effects of pH in both HEPES and HCO$_3$ Ringer were completely reversible.

The effect of luminal ouabain was tested to determine whether an apical membrane H$^+$-K$^+$-adenosinetriphosphatase (ATPase) participated in the action of luminal pH on SCFA absorption. The experiments were carried out in 5 mM HCO$_3$ Ringer with butyrate at 25 mM on both sides of the tissue. Ouabain (1 mM) did not affect J$_{m-s}$ at pH 7.30 (2.7 ± 0.3 vs. 2.6 ± 0.3 µeq·cm$^{-2}$·h$^{-1}$, n = 6). Luminal ouabain also did not affect the stimulatory action of a luminal pH reduction to 6.36 on J$_{m-s}$ (3.2 ± 0.5 µeq·cm$^{-2}$·h$^{-1}$, n = 6, P < 0.02).

The effect of serosal ouabain was tested to determine if any active transport process was involved in the SCFA response to pH. Fluxes were not measured for 32 min and/or until the lsc was reduced to near zero. The experiments were carried out in 5 mM HCO$_3$ Ringer with butyrate at 25 mM on both sides of the tissue. As shown in Fig. 4, the addition of 1 mM ouabain to the serosal solution did not alter butyrate fluxes. When pH was reduced on both sides of the tissue from 7.29 to 6.45, increases in both J$_{m-s}$ (3.0 ± 0.2 vs. 4.4 ± 0.2 µeq·cm$^{-2}$·h$^{-1}$, n = 4, P < 0.01) and J$_{s-m}$ (3.3 ± 0.2 vs. 4.0 ± 0.1 µeq·cm$^{-2}$·h$^{-1}$, n = 4, P < 0.05) were noted.

Effect of luminal Cl$^-$ removal on butyrate flux. To evaluate the role of apical membrane Cl$^-$/SCFA exchange, we examined the effect of substituting isethionate for Cl$^-$ in the mucosal bathing solution. In 5 mM HCO$_3$ Ringer, the absence of luminal Cl$^-$ at pH 7.21 did not significantly increase J$_{m-s}$ of butyrate (2.6 ± 0.1 vs. 3.2 ± 0.3 µeq·cm$^{-2}$·h$^{-1}$, n = 5, not significant (NS)) but did decrease J$_{s-m}$ 22% (3.6 ± 0.2 vs. 2.4 ± 0.2 µeq·cm$^{-2}$·h$^{-1}$, n = 5, P < 0.05). At pH 6.50, the removal of luminal Cl$^-$ did not increase J$_{m-s}$ (4.0 ± 0.3 vs. 4.7 ± 0.5 µeq·cm$^{-2}$·h$^{-1}$, n = 5, NS) and again decreased J$_{s-m}$ 32% (4.4 ± 0.2 vs. 3.0 ± 0.3 µeq·cm$^{-2}$·h$^{-1}$, n = 5, P < 0.005). The absence of luminal Cl$^-$ also altered the effect of pH on butyrate flux. A reduction in pH from 7.21 to 6.50 stimulated net flux from 0.8 ± 0.2 to 1.7 ±
distal colon during the various experimental conditions. As shown in Table 2, in HEPES Ringer containing 25 mM butyrate and gassed with 100% O2, CO2 tension and therefore [HCO3−], were zero. pHi mirrored pHs whether the pH change was unilateral or bilateral. However, when the pHs decrease was unilateral, the decrease in pHi was less than when the pHs change was bilateral. Furthermore, mucosal changes in pHs seemed to have a somewhat greater effect on pHi than serosal changes.

In 5 mM HCO3− Ringer containing 25 mM butyrate, qualitatively similar effects of bilateral changes in pH were observed. Increasing bathing solution PCO2 from 7 mmHg (pHe 7.43 ± 0.02) to 95 mmHg (pHe 6.48 ± 0.01) decreased pHs from 7.39 ± 0.02 to 6.46 ± 0.03, n = 6, P < 0.001, and increased [HCO3−] from 4.8 ± 0.5 to 5.8 ± 0.4 mM, n = 6, P < 0.05. In 5 mM HCO3− Ringer containing 25 mM isobutyrate, similar effects of PCO2 on pHs and [HCO3−] were observed. When bilateral pHs was decreased from 7.44 to 6.48, pHi decreased from 7.20 ± 0.02 to 6.53 ± 0.02 and [HCO3−] increased from 3.0 ± 0.1 to 7.1 ± 0.3 mM, n = 5, P < 0.001. In both HEPES and HCO3− Ringer, the presence of unilateral or bilateral SCFA did not affect the steady-state value of pHi.

Effect of pH on butyrate flux in proximal colon. We then examined whether the pattern of pH effects on SCFA transport was similar in the proximal colon. Butyrate flux was measured when present at 25 mM on both sides of the tissue in HEPES Ringer. Bilateral decreases in pH from 7.39 to 5.69 in steps increased Jm−s from 2.9 ± 0.2 to 4.5 ± 0.3 µeq·cm−2·h−1, n = 4, P < 0.01, and Js−m from 2.2 ± 0.4 to 3.3 ± 0.4 µeq·cm−2·h−1, n = 4, P < 0.002. Net butyrate fluxes were minimal at bilateral pH 7.39 (0.8 ± 0.5 µeq·cm−2·h−1) and pH 5.69 (1.2 ± 0.5 µeq·cm−2·h−1, n = 4, NS). Reductions in pH also had no effect on Jsc. All of these changes were qualitatively and quantitatively similar to those observed in distal colon.

**DISCUSSION**

The importance of SCFA in colonic energy metabolism (5, 26), ion transport (1, 2, 4, 12, 17, 26, 28, 32), pH regulation (7, 14, 15), and systemic acid-base balance (5) has been recognized for some time. SCFA absorption precedes and is required for these functions. For many

### Table 2. Effect of pHs on colonic pHi in HEPES Ringer

<table>
<thead>
<tr>
<th>Mucosal pHs Change</th>
<th>Serosal pHs Change</th>
<th>Bilateral pHs Change</th>
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<tbody>
<tr>
<td>pHs</td>
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<tr>
<td>7.38 ± 0.01</td>
<td>7.48 ± 0.03</td>
<td>7.38 ± 0.01</td>
</tr>
<tr>
<td>6.67 ± 0.01</td>
<td>7.12 ± 0.03</td>
<td>6.69 ± 0.01</td>
</tr>
<tr>
<td>6.10 ± 0.01</td>
<td>6.84 ± 0.03</td>
<td>6.10 ± 0.01</td>
</tr>
<tr>
<td>5.45 ± 0.02</td>
<td>6.49 ± 0.03</td>
<td>5.48 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. Butyrate was present on both sides of tissue at 25 mM in 21 mM HEPES Ringer. Mucosal and serosal extracellular pH (pHs) were initially equal at 7.38, and pHs was decreased on one or both sides of tissue by addition of 2 M H2SO4. Decreases in pHs reduced intracellular pH (pHi) to a greater degree when the pHs change was bilateral > mucosal > serosal. Mucosal pHs change, n = 6; serosal pHs change, n = 8; bilateral pHs change, n = 5.
Our experiments were designed to determine the relative importance of nonionic diffusion and anion exchange as mechanisms by which SCFA cross the colonic mucosa. We considered the $K_m$ of the apical anion exchanger (27 mM for butyrate in rat colon) (24), the $pK_a$ of the SCFA under consideration (4.8–4.9), metabolism of SCFA by the colonic mucosa, the cell-to-lumen flux of SCFA by apical Cl$^-$/SCFA exchange processes. Indeed, these processes have recently been identified in colonic membrane vesicles (20, 24, 27).

The measurements of pH$_i$ also shed light on the mechanism of SCFA transport. In HEPES Ringer, the effects of pH$_e$ were similar in HEPES and HCO$_3^-$ Ringer, and that transepithelial transport was unsaturated at SCFA concentrations up to 100 mM. None of these findings would be expected or accounted for by a carrier-mediated epithelial transport process and by apical membrane SCFA/HCO$_3^-$ exchange in particular. It is otherwise difficult to explain how SCFA could move at equivalent rates in both directions across colonic tissue if the transport process were not passive, how SCFA could traverse the tissue via a SCFA/HCO$_3^-$ exchange process in the apparent absence of intracellular bicarbonate (in HEPES Ringer), and why transport saturation would not be observed at substrate concentrations almost four times greater than the $K_m$ (observed in brush-border membrane vesicles).

The measurements of pH$_i$ also shed light on the mechanism of SCFA transport. In HEPES Ringer, the effects of pH$_e$ on unidirectional fluxes were equivalent whether the changes in pH$_e$ were unilateral or bilateral. Moreover, the absolute values for the unidirectional fluxes were equivalent at similar values for unilateral pH$_e$ and bilateral pH$_e$. However, in HEPES Ringer (Table 2), the effects of unilateral and bilateral changes in pH$_e$ on pH$_i$ were not equivalent. This suggests that the effect of pH$_e$ on SCFA flux was primarily localized to the mucosal or serosal compartment in which it occurred rather than through the effects of pH$_e$ on pH$_i$. As discussed previously, such an effect would be more compatible with the transport process of nonionic diffusion than anion exchange.

We also found that luminal removal of Na$^+$ to inhibit apical Na$^+$/H$^+$ exchange did not alter SCFA transport in rat colon or the effects of pH$_e$. Furthermore, neither luminal ouabain, which may inhibit colonic apical membrane H$^+$/K$^+$-ATPase (8, 16, 19, 23), nor serosal ouabain, which inhibits all active transport processes, affected SCFA transport or the effects of pH$_e$. Epinephrine stimulation of apical Na$^+$/H$^+$ exchange has been shown to stimulate propionate absorption in rabbit proximal but not distal colon (29, 30). A pH gradient (presumably luminal microclimate pH$_e$ < pH$_i$) was suggested as the mechanism of these effects (29). Because we could not confirm a role for apical Na$^+$/H$^+$ exchange in SCFA absorption, we believe that the reported requirement for this exchanger may be species specific. The presence of a microclimate pH, of course, is consistent with both SCFA absorption by nonionic diffusion and anion exchange.

Metabolism of SCFA by the colonic mucosa certainly occurs, and in preliminary experiments we found that ~7% of the butyrate that entered cells was metabolized to CO$_2$. In the rabbit proximal colon in vitro, from 4 to 7% of absorbed propionate was metabolized to CO$_2$ under similar experimental conditions (29). We do not believe that SCFA metabolism affected our flux measurements or their interpretation. First, in our studies the absolute fluxes and the effects of pH and substrate concentration were similar for butyrate, propionate, and weakly metabolized isobutyrate. Second, the presence of glucose in our studies reduced the fraction of colonic energy derived from SCFA (5). Third, our unidirectional flux calculations were based on the appearance of radiolabeled butyrate in the unlabeled “cold” bathing solution rather than on the disappearance of butyrate from the labeled “hot” side. Thus it is irrelevant to the calculation of unidirectional flux that ~7% more butyrate entered cells than exited into the opposite bathing solution.

In addition to transport across the cell, SCFA may be recycled across the apical membrane. Recycling presumably occurs via the Cl$^-$/SCFA$^-$ exchange process described by Rajendran and Binder (25). In our studies of rat colon, the fraction recycled was estimated by comparing the $J_{m-s}$ of butyrate in the presence and absence of luminal Cl$^-$. We found that $J_{m-s}$ was not significantly affected by the absence of this anion. $J_{m-s}$, however, was decreased 22–32%, depending on pH$_e$. Apparently, under the experimental conditions described (5 mM HCO$_3^-$ Ringer at pH$_e$ between 6.50 and 7.21) apical membrane Cl$^-$/SCFA$^-$ exchange has a greater role in the transcellular secretory flux of SCFA than in apical membrane recycling of absorbed SCFA.

Our findings do not rule out a contribution of anion exchange at the apical and basolateral membranes to SCFA absorption in situ. The effects of SCFA transport of intact tissue layers, blood flow, cell membrane potential, competing substrates, and varying energy stores and demands are unknown. Moreover, the complexity of the in situ environment suggests that the relative
importance of active and passive transport of SCFA may not be fixed. Nevertheless, the experimental conditions examined here do mirror in situ conditions where the [HCO₃⁻] is very low and a lumen-to-blood pH gradient and SCFA concentration gradient exist. Indeed, such conditions favor net absorption of SCFA by nonionic diffusion. Our studies suggest that at least in the rat colon nonionic diffusion is the most important if not the only mechanism of SCFA absorption.

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