Apical Na⁺/H⁺ exchange near the base of mouse colonic crypts

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Chu, Jingsong, Shaoyou Chu, and Marshall H. Montrosse. Apical Na⁺/H⁺ exchange near the base of mouse colonic crypts. Am J Physiol Cell Physiol 283: C358-C372, 2002; 10.1152/ajpcell.01380.2000.—Colonic crypts can absorb fluid, but the identity of the absorptive transporters remains speculative. Near the crypt base, the epithelial cells responsible for vectorial transport are relatively undifferentiated and often presumed to mediate only Cl⁻ vectorial transport are relatively undifferentiated and often presumed to mediate only Cl⁻ secretion. We have applied confocal microscopy in combination with an extracellular fluid marker [Lucifer yellow (LY)] or a pH-sensitive dye (2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein) to study mouse colonic crypt epithelial cells directly adjacent to the crypt base within an intact mucosal sheet. Measurements of intracellular pH report activation of colonicocyte Na⁺/H⁺ exchange in response to luminal or serosal Na⁺. Studies with LY demonstrate the presence of a paracellular fluid flux, but luminal Na⁺ does not activate Na⁺/H⁺ exchange near the base of crypts in the nonepithelial cells of the lamina propria, and studies with LY suggest that the fluid bathing colonicocyte basolateral membranes is rapidly refreshed by serosal perfusates. The apical Na⁺/H⁺ exchange in crypt colonicocytes is inhibited equivalently by luminal 20 μM ethylisopropylamiloride and 20 μM HOE-694 but is not inhibited by luminal 20 μM S-1611. Immunoassay reveals the presence of epitopes from NHE1 and NHE2, but not NHE3, in epithelial cells near the base of colonic crypts. Comparison of apical Na⁺/H⁺ exchange activity in the presence of Cl⁻ with that in the absence of Cl⁻ substitution by gluconate or nitrate revealed no evidence of the Cl⁻-dependent Na⁺/H⁺ exchange that had been previously reported as the sole apical Na⁺/H⁺ exchange in the colonic crypt. Results suggest the presence of an apical Na⁺/H⁺ exchanger near the base of crypts with functional attributes similar to those of the cloned NHE2 isoform.

intracellular pH; NHE2; NHE3; NHE1; sodium absorption; epithelial polarity; laser scanning confocal microscopy; 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; Lucifer yellow; immunofluorescence

IN MAMMALIAN COLON, intracellular pH (pHᵢ) regulation and electroneutral NaCl absorption are closely related functions. Apical Na⁺/H⁺ exchange is the luminal uptake mechanism contributing to transcellular Na⁺ absorption (3, 38), and its activity consequently affects pHᵢ in the colonic epithelium (8, 29, 44, 51). Among the six known Na⁺/H⁺ exchanger isoforms, only NHE1, NHE2, and NHE3 have been found in the colonic epithelium on the basis of mRNA expression and immunological detection (4, 5, 18). NHE1 is ubiquitously expressed in mammalian cells to maintain pH homeostasis of cells (57). In epithelial cells of the intestine and colon, NHE1 is localized in the basolateral membrane (4). In contrast, NHE2 and NHE3 have a more restricted tissue distribution and are apical membrane proteins in intestinal epithelia (4, 5, 26, 54, 55). Within the rat colon, immunostaining detected NHE3 exclusively in the surface cells, whereas NHE2 mRNA was detected predominantly on the surface cells with a diminishing gradient that terminated in the upper third of the colonic crypt (4, 5). In human colon, evidence has suggested that NHE2 mRNA is present deeper in the crypts (18).

The physiological role of different Na⁺/H⁺ exchangers is controversial. In NHE3-knockout mice, basal fluid absorption by the intestine was severely diminished (49), demonstrating that NHE3 plays at least a permissive role in overall salt and water absorption. In complementary experiments, NHE2-knockout mice have no obvious intestinal absorption defect (48). Aldosterone increases abundance of NHE3, but not NHE2, in the rat proximal colon (11). However, it is NHE2, and not NHE3, that is responsible for the enhanced Na⁺ absorption in response to a low-Na⁺ diet in chicken colon (17), and it has been reported that rat colonic NHE2 and NHE3 are affected in parallel by Na⁺ depletion (27). In rat proximal colon, evidence suggests that NHE2 is the predominant contributor to basal Na⁺ absorption (7), although the role of NHE3 seems more dominant in isolated membrane vesicles from the same tissue (11, 27). Adding to the complexity, evidence suggests that rat colonic crypts may contain apical Na⁺/H⁺ exchange activity that requires extracellular Cl⁻ (44, 45). Cl⁻-dependent function has been proposed as evidence of a new Cl⁻-NHE isoform Na⁺/H⁺ exchanger in the colon, but an intracellular Cl⁻ dependence has recently been shown to be a feature of conventional NHE isoforms as well (1).

There is also a controversy about the colonic epithelial cell types contributing to salt and water absorption. In contrast to the classic view of crypts as secre-
atory structures, recent studies have shown that colonic crypts also contribute to fluid absorption (21, 52). Although crypt epithelial cells are less directly exposed to the luminal contents than surface epithelial cells, they are an abundant cell type of the colonic epithelium because of the density and size of crypts in the tissue (23). Therefore, their overall contribution to absorption could be substantial.

The major goal of this study was to question whether apical Na+/H+ exchange was a feature of the cells near the base of colonic crypts and, if so, to characterize that function with respect to known NHE isofoms and the Cl−-dependent NHE that have been reported. We have loaded isolated mouse colonic mucosa with 2′,7′-bis-(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) acetoxymethyl ester (BCECF-AM), a pH-sensitive dye, and measured activity of apical Na+/H+ exchange by confocal microscopy while retaining normal epithelial architecture in the mucosa. Results demonstrate that even cells in the base of colonic crypts have the potential to contribute to Na+ absorption and that known NHE isofoms are sufficient to explain results.

MATERIALS AND METHODS

Tissue preparation. ICR mice (Harlan, Indianapolis, IN) were killed with halothane vapor (Halocarbon Laboratories). The distal colon was excised, flushed with saline, and stripped of muscle layers, as described previously (12). Mucosal sheets were kept in DMEM (GIBCO BRL) on ice and used within 3 h. For experiments, a microscope chamber allowed mounting of mucosal sheets of muscle-stripped mouse colon, such that a physiological saline could be superfused independently at the luminal and serosal surfaces (12). The serosal surface of the tissue was mounted facing the microscope objective lens (Zeiss C-Apo ×40) to facilitate study of crypt epithelium. Dye loading and perfusion were performed on tissue mounted in the microscope chamber. For pH measurements, the solution contained 5 μM BCECF-AM (Molecular Probes) in Na+ medium [in mM: 130 NaCl, 5 KCl, 1 MgSO4, 2 CaCl2, 1 (Na)PO4, 20 HEPES, 25 mannose, and 1 probenecid, titrated to pH 7.4 with NaOH]. Incubation was at room temperature for 30 min. After BCECF dye loading, the chamber was placed on the microscope stage and continuously perfused.

Perfusate solutions. Perfusate solutions were based on the Na+ medium described above. In Na+-free solutions, tetramethylammonium (TMA) chloride replaced all NaCl mole for mole; in ammonium media, 25 mM NH4Cl replaced equimolar NaCl or TMA chloride; and in isobutyrate media, 130 mM gluconate, 2 calcium gluconate, 1 MgSO4, 1 (Na)PO4, and 20 HEPES. When necessary, Ca2+ was reduced by equimolar substitution of the Na+ salt by 130 mM TMA gluconate or TMA nitrate, respectively. To inhibit Na+/H+ exchange activity, 20 μM 5-(N-ethyl-N-isopropyl)amiloride (EIPA; RBI), S-1611, or HOE-694 [3-(methylene sulfonyl-4-piperidinobenzoxy]guanidine methanesulfonate; the two latter compounds were generous gifts from Dr. H. J. Lang, Advenitis Pharma Deutschland, Frankfurt/Main, Gemany] was added to select media. All perfusate and drug solutions were prepared fresh directly before use, and final perfusates were adjusted to pH 7.4.

Confocal microscopy and image analysis. Images were collected using a Zeiss LSM510 confocal microscope. To measure BCECF fluorescence, excitation was alternated between 488- and 458-nm lines of an argon laser, with emission collected at >505 nm at a single photomultiplier tube detector held at constant gain and dark current during the experiment. The LSM510 confocal microscope allowed rapid switching of excitation wavelength after each scan line for excitation ratio imaging, so that <10 ms separated data collected at the two wavelengths. Ratio images of fluorescence at 488 nm to fluorescence at 458 nm were calculated after subtraction of background images at each wavelength, and values from the entire cytosol of all imaged epithelial cells within a crypt were averaged. To measure pH, gradients, 1- to 2-μm2 regions in subapical and subbasal areas of crypts were analyzed as previously described (24, 34). In some experiments, tissue was not loaded with BCECF but was superfused instead with Na+ medium containing 100 μM Lucifer yellow [CH lithium salt (LY); Molecular Probes]. The extracellular LY dye was imaged with 458-nm excitation and >505-nm emission. In each image, average LY fluorescence (minus background) was recorded from crypt lumens, lateral intercellular spaces (LIS), and lamina propria tissue adjacent to the crypts. In BCECF and LY experiments, images were routinely collected at the same focal plane over time, with the confocal pinhole adjusted for 1.5-μm optical section thickness. The plane of focus was selected to be directly adjacent to the base of crypts, such that the crypt lumen was just visible and crypt epithelial cells within the image could be visualized along their apical-to-basal pole. Background images were collected from nontissue areas of the chamber. Post-data-acquisition image analysis was performed (MetaMorph, Universal Imaging) to analyze results from four to six crypts per experiment.

pH, calibration. To avoid problems with poor permeation of nigericin into tissue (data not shown), intracellular dye calibration was performed with isolated mouse colonocytes (28) using high-K+ medium and nigericin (an artificial K+/H+ exchanger), as described previously (36). Colonocytes were loaded with 5 μM BCECF-AM and superfused with 130 mM K+ and 10 μM nigericin solution at pH 6–8 during imaging on the confocal microscope. Excitation ratios of fluorescence at 488 nm to fluorescence at 458 nm at different pH values were obtained (Fig. 1A) from confocal images. A single-site calibration dependency equation was used to fit a pH calibration curve by nonlinear regression to data of eight independent preparations (Prism, Graphpad Software; Fig. 1B). The calibration curve demonstrates a favorable dynamic range of the ratio for pH measurement and was used to calculate pH, of experimental data. After each experiment, a solution containing 25 μM BCECF in pH 7 Na+ medium was imaged on the confocal microscope stage as an internal standard to normalize results of the nigericin calibration curve to daily settings of the confocal microscope. All averaged results are presented as means ± SE of separate experiments.

Immunofluorescence staining. Mouse colon was fixed with 2% paraformaldehyde in PBS through cardiac perfusion of the mouse under thiobutabarbital (Inactin, 100 mg/kg) anesthesia. The excised colon was further fixed for 2 h at 4°C in the same fixative. Fixed tissue was rinsed with PBS twice and transferred to 30% sucrose in PBS for 24–36 h at 4°C. The colon was embedded with tissue-freezing medium.
Fig. 1. Intracellular pH (pH$_i$) calibration of mouse colonocytes. Fresh isolated mouse distal colonocytes were loaded with 5 μM 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-AM in high-K$^+$ medium with 10 μM nigericin. Cells were superfused with high-K$^+$ medium containing 10 μM nigericin, and pH varied between 6 and 8 during imaging on the confocal microscope stage. Background-corrected excitation ratio of fluorescence at 488 nm to fluorescence at 505 nm was calculated from raw fluorescence images. A: results from a representative experiment showing time course of ratio change in response to changes in medium pH. B: results from 8 experiments (means ± SE) are fit to the pH calibration curve used to determine pH, values in subsequent experiments.

RESULTS

As described in MATERIALS AND METHODS, muscle-stripped colonic mucosa was mounted in a microscope chamber and examined by confocal microscopy. Our goal was to examine polarized Na$^+/H^+$ exchange function in crypt epithelial cells, but interpreting those experiments required characterization of how well the luminal and serosal perfusates were separated in the perfusion chamber. Specifically, we needed to test whether the epithelium sustained a barrier between luminal and serosal compartments and whether the LIS between adjacent crypt epithelial cells was a site of restricted access to the serosal perfusates. For these general questions, we superfused tissue with the membrane-impermeant fluorescent dye LY in the luminal or serosal compartment and imaged LY fluorescence by confocal microscopy every 10 s while we focused near the base of colonic crypts.

When LY was added to the serosal compartment, LY fluorescence rapidly equilibrated into LIS [half time ($t_{1/2}$) = 14 ± 3 (SE) s, $n$ = 6 experiments] and the lamina propria tissue between crypts ($t_{1/2}$ = 20 ± 8 s). Some LY bound to collagen fibers in the lamina propria and could not be washed out, but in the majority of lamina propria regions, LY fluorescence was rapidly reversible on removal of LY from the perfusate and washed out with rapid kinetics ($t_{1/2}$ = 30 ± 3 s). LY also appeared in crypt lumens ($t_{1/2}$ = 16 ± 2 s), demonstrating a transepithelial leak of the dye. As shown in Fig. 2, all fluorescence in LIS and crypt lumens was rapidly and completely reversible on removal of serosal LY ($t_{1/2}$ = 11 ± 2 s and 13 ± 2 s, respectively). The levels of steady-state fluorescence in the presence of serosal LY are shown in Table 1, with results normalized to the (reversible) fluorescence in lamina propria. Luminal fluorescence was 10% of fluorescence in the lamina propria. Because the LIS are physically smaller than optical resolution (i.e., any imaged pixel includes regions containing LIS and non-LIS regions), fewer dye molecules are available to report fluorescence, and so LIS are dim, even when equilibrated with LY concentrations in the lamina propria [also observed previously with carboxyseminaphthorhodofluor (SNARF)-1 fluorescence] (12). In addition to demonstrating transepithelial leak of LY, results suggest that the rate of fluid entry and exit at the LIS and surrounding lamina propria tissue is rapid and indistinguishable.

When added to the luminal compartment, LY slowly equilibrated in the lumen ($t_{1/2}$ = 122 ± 12 s, $n$ = 6 experiments; Fig. 2). This is consistent with previous measurements of SNARF-1 fluorescence (13) and shows that fluid in the luminal perfusates only slowly
Fig. 2. Transmucosal permeability of extracellular Lucifer yellow (LY) dye. Tissue in the microscope chamber was superfused with Na+/H+ medium with or without 100 μM LY. Confocal images of tissue were collected over time while tissue was continuously superfused. Superfusates were independently controlled at the luminal (L) or serosal (S) surfaces. Results were compiled separately from the same images for dye accumulation in the crypt lumens and the lateral intercellular spaces (LIS) between adjacent crypt colonocytes. Raw fluorescence values were collected from a representative time course during polarized presentation of LY to the colonic mucosa. Results are representative of 6 preparations.

migrates down the crypt lumen to undergo mixing. Even after luminal fluorescence attained steady-state levels, Table 1 shows that negligible fluorescence (i.e., not significantly different from zero in one-sample t-test, $P > 0.2$) was detected in the LIS and lamina propria (2 and 4% of luminal fluorescence, respectively). Given the known transepithelial leak of LY, defined by the serosal application of dye in the same experiments, results suggest that rapid mixing in the serosal compartment leads to effective control of extracellular fluid composition in this space, despite known transepithelial leakage from the lumen. Conversely, serosally added components will have a more substantial effect to alter composition of the fluid in the crypt lumen because of relatively slow mixing in the luminal compartment. Thus, for studies of polarized functions (i.e., Na+/H+ exchange) activated by extracellular factors (e.g., Na+) in perfusates, results encourage luminal application of those factors to yield the tightest conclusions about the membrane localization of effects.

Visualization of BCECF-loaded colonic mucosa during superfusion. As described in MATERIALS AND METHODS, muscle-stripped colonic mucosa was loaded with BCECF-AM and then continuously superfused with dye-free medium. Figure 3 shows confocal fluorescence images of BCECF-loaded colonic mucosa during superfusion, imaged at 488-nm excitation and >505-nm emission. BCECF loads into crypt epithelial cells as well as nonepithelial cells in the lamina propria surrounding crypts. Because the mucosa was oriented in the chamber with serosal surface adjacent to the objective lens, we could image crypt epithelial cells clearly. The series of images shows the transition between imaging the base of crypts (Fig. 3A) and the crypt opening (Fig. 3C) as focus is advanced in the microscope. In all subsequent experiments, results are reported from the region directly above the base of the crypt, at the point where multiple crypt lumens were visible and individual epithelial cells were aligned along their apical-basal axis in a single focal plane of the image. Because of tissue motion in the dually perfused chamber, the plane of focus could shift along the crypt-surface axis of the crypt during an experiment. The crypt base is used as a landmark to reposition the focal plane as needed during an experiment.

Polarized activation of Na+/H+ exchangers. With the use of confocal microscopy, images of BCECF-loaded tissue were collected every 1 min, and results were analyzed from crypt colonocytes in the field of view. To activate Na+/H+ exchange, we used media without added bicarbonate/CO2 and acidified the tissue by transient exposure to a weak base (25 mM NH4Cl; ammonia medium) in luminal and serosal superfusates. Simultaneous with ammonia exposure, Na+ was then selectively returned to the luminal perfusate (substitution with TMA; TMA medium) to allow adequate time to wash Na+ from this compartment. On removal of ammonia medium, colonocyte pHi alkalized rapidly (Fig. 4A). During this treatment, Na+ was removed from the serosal medium to halt all Na+/H+ exchange activity. Na+ was then selectively returned to the luminal or serosal perfusate to activate apical or basolateral Na+/H+ exchange, respectively. As shown in Fig. 4A, colonocyte pHi alkalinated after cells were exposed to luminal Na+. To activate NHE activity, we added luminal 140 mM Na+, an Na+ concentration much higher than the Michaelis-Menten constant of NHEs for Na+ (55, 57, 59). In this case, full activation of

![Graph](image.png)

**Table 1. Steady-state dye accumulation in response to polarized perfusion with Lucifer yellow**

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<th>Luminal LY</th>
<th>Serosal LY</th>
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<td>Lumen</td>
<td>100%</td>
<td>10 ± 3</td>
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<tr>
<td>LIS</td>
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<td>Lamina Propria</td>
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As described in MATERIALS AND METHODS, colonic mucosal sheets were mounted in the microscope chamber and superfused continuously with Na+ medium during imaging of colonic crypts with confocal microscopy. Lucifer yellow (LY; 100 μM) was added to Na+ medium and perfused through the chamber in either the luminal or serosal LY solution until steady-state fluorescence was attained in the chamber. Using images collected during the steady state, confocal fluorescence was quantified separately in the crypt lumen, lateral intercellular spaces between crypt colonocytes (LIS), and lamina propria tissue between crypts. After removal of LY and the rapid washout of dye (see Fig. 2), images were collected to record background fluorescence values from precisely the same regions. During quantification, we avoided regions of lamina propria with high levels of bound LY. Results were identical if tissue was transiently pre-exposed to serosal LY to saturate binding sites before a second exposure to LY for quantification of the reversible (unbound) fluorescence. To account for differences in confocal settings in different experiments, results were normalized for the value of fluorescence in the cis compartment (the side containing the LY dye). Results are presented as means ± SE for $n = 6$ separate experiments (3–5 crypts analyzed per experiment).
Apical NHE will occur before the crypt lumen is equilibrated with the perfusate Na\(^+\) concentration. On the basis of mixing kinetics in the crypt lumen, we predict that even 2 min after addition of luminal Na\(^+\), the luminal Na\(^+\) concentration is 70 mM, which should maximally stimulate the transporter. This is reasonably well matched with our rate of data collection (every 1 min) and should allow reliable measures of transport activity to be collected. Subsequent serosal Na\(^+\) addition elicited a more rapid pH\(_i\) recovery to return pH\(_i\) to normal resting level. A second round of NH\(_4\)Cl exposure, acidification, and pH\(_i\) recovery showed that the pH\(_i\) recovery was robust and reproducible in our experimental system. Rates of Na\(^+\)-dependent pH\(_i\) recovery were calculated for both rounds of acidification by using the lowest common pH\(_i\) value from the two recoveries as the starting value for rate calculation: a way to account for the known pH\(_i\) sensitivity of Na\(^+\)/H\(^+\) exchange activity and accommodate a data set in which the level of acidification cannot be perfectly controlled. No significant difference between the first and second acidification was detected in the rates of pH\(_i\) recovery in response to luminal Na\(^+\) (Fig. 4B) calculated from 4 min of pH\(_i\) recovery from the lowest common pH\(_i\). Similar calculations were performed for results from the subsequent addition of serosal Na\(^+\) after subtraction of the rate of apical Na\(^+\)/H\(^+\) exchange directly before addition of serosal Na\(^+\) (to estimate rates of basolateral Na\(^+\)/H\(^+\) exchange). There was no significant difference (P = 0.25) in basolateral Na\(^+\)/H\(^+\) exchange after the first vs. second acidification (0.25 ± 0.03 and 0.21 ± 0.04 pH/min, respectively, n = 6). These absolute values calculated from (2–3 min) pH\(_i\) recovery after serosal Na\(^+\) should be viewed with reservation, because linear rates of pH\(_i\) change were sometimes poorly resolved due to rapid pH\(_i\) recoveries.

Results tentatively suggest the presence of apical and basolateral Na\(^+\)/H\(^+\) exchange but required further validation, because transepithelial Na\(^+\) leakage may

**Fig. 3.** Confocal images of BCECF-AM-loaded mouse colonic mucosa. Smooth muscle-stripped mouse distal colonic mucosa was mounted into a microscope chamber, loaded with BCECF-AM, and superfused with Na\(^+\) medium. Mucosa was imaged with a Zeiss LSM510 confocal microscope at 488-nm excitation and ≥500-nm emission. Large, circular multicellular structures are colonic crypts imaged in cross section. Series of images were taken at different focal planes at the base of crypts (i.e., nearest the objective lens; A), focused 5 μm deeper into tissue to the midpoint of the basal cells (B), and focused another 5 μm into tissue to reach the crypt lumen (C). Arrowhead points to crypt epithelium, and arrow points to crypt lumen.

**Fig. 4.** Luminal and serosal Na\(^+\) activate pH\(_i\) recovery in colonocytes. Values are means ± SE from 4 experiments. A: time course of cellular pH\(_i\) response in colonic crypt epithelial cells acidified by NH\(_4\)Cl prepulse and Na\(^+\)-free superfusion. Luminal Na\(^+\) addition activated a pH\(_i\) recovery. Subsequent serosal Na\(^+\) addition accelerated pH\(_i\) rise toward normal resting level. Data show 2 rounds of acidification and subsequent pH\(_i\) recovery. TMA, tetramethylammonium. B: pH\(_i\) recovery rates of initial 4 min after luminal Na\(^+\) addition from 1st and 2nd rounds were calculated starting at lowest common pH\(_i\) between the 2 rounds of acidification.
be different from transepithelial LY leakage. Therefore, we asked whether luminal Na\(^+\) was able to stimulate pH recovery in crypt epithelial cells and cells in the lamina propria close to the crypts. Because cells in the lamina propria are not in direct contact with the luminal compartment, this is a stringent test of whether sufficient Na\(^+\) from the lumen infiltrates the tissue to activate the (presumptive NHE1) Na\(^+\)/H\(^+\) exchanger on these cells. As shown qualitatively in the sequence of ratio images of fluorescence at 488 nm to fluorescence at 458 nm of Fig. 5, A–C, all cells in the colonic mucosa acidified after ammonium prepulse (Fig. 5A). Crypt epithelial cells, but not cells in the lamina propria, alkalinate after addition of 140 mM luminal Na\(^+\) (Fig. 5B). Subsequent addition of 140 mM serosal Na\(^+\) confirmed that the lamina propria cells were able to activate Na\(^+\)/H\(^+\) exchange (Fig. 5C).

These results are substantiated by direct comparison of the time course of BCECF response in the two cell types (Fig. 5D). Results in Fig. 5D are plotted as fluorescence ratio, since we lack pH calibration data for the lamina propria cells. Results document that luminal Na\(^+\) cannot increase Na\(^+\) concentration in the lamina propria sufficiently to activate Na\(^+\)/H\(^+\) exchange.

This observation was refined by questioning how much Na\(^+\) would have been required in the serosal tissue to activate Na\(^+\)/H\(^+\) exchange on colonocytes or lamina propria cells. For this purpose, we added different concentrations of Na\(^+\) to the serosal perfusate and monitored pH recovery in both cell types. Results (Fig. 6) show that 1 mM Na\(^+\) did not stimulate measurable pH recovery, 3 mM Na\(^+\) induced a slow but detectable pH recovery, and \(\geq 10\) mM Na\(^+\) activated significant pH recovery. Importantly, the pH recovery of epithelial and lamina propria cells was equally sensitive to low concentrations of added serosal Na\(^+\), and even low Na\(^+\) concentrations caused a simultaneous and prompt activation of Na\(^+\)/H\(^+\) exchange in both cell types. Results suggested no evidence of a diffusion barrier to Na\(^+\) or sequestering of Na\(^+\) at the colonocyte cell membrane.

Fig. 5. Polarized Na\(^+\)/H\(^+\) addition: comparison of pH response between epithelial and nonepithelial cells in the colonic mucosa. Experiments were performed as described in Fig. 4 legend. A–C: pseudocolor images of intracellular BCECF ratio (488 nm/458 nm), with qualitative correspondence to pH change indicated in the color bar. A: cells in mucosa were acidified by NH\(_4\)Cl prepulse and kept in Na\(^+\)-free TMA medium. Crypt colonocytes (arrows) and nonepithelial cells in the lamina propria (arrowheads) acidified. B: luminal Na\(^+\) addition caused pH recovery of crypt colonocytes but not lamina propria cells. Note lamina propria cell at top left, directly adjacent to colonic crypt base. C: subsequent serosal Na\(^+\) addition caused further pH rise in colonocytes and initiated pH recovery in lamina propria cells. D: direct comparison of BCECF fluorescence ratio time course simultaneously recorded from crypt epithelial cells and lamina propria cells in the same experiment. Results are representative of 4 experiments.

Fig. 6. pH response to different serosal Na\(^+\) concentrations: comparison between epithelial and nonepithelial cells in the colonic mucosa. BCECF fluorescence ratio time course simultaneously recorded from crypt epithelial cells and lamina propria cells was directly compared in the same experiment. Tissue was exposed to indicated Na\(^+\) concentration in the serosal perfusate while images were collected every 30 s.

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basolateral membranes containing Na\(^+\)/H\(^+\) exchangers, and to explain the lack of pH\(_i\) recovery by lamina propria cells in this condition, the direct addition of luminal 140 mM Na\(^+\) must have resulted in <3 mM Na\(^+\) in the lamina propria tissue. On the basis of this estimate, the serosal tissue accumulation of Na\(^+\) is maximally 2% of the added luminal Na\(^+\) concentration, a percentage similar to that directly measured for LY fluorescence (4%) after luminal LY addition.

In all colonocyte measurements (Figs. 4–6), the pH\(_i\) response of entire crypts was averaged. To test the possibility of intercellular heterogeneity of Na\(^+\)/H\(^+\) exchange among the ring of crypt epithelial cells at this focal plane of the crypt, we measured pH\(_i\) of individual cells during Na\(^+\)-dependent pH\(_i\) recovery. With the use of raw fluorescence images, in which individual cell boundaries can be defined (Fig. 3), four to six 4.5-\(\mu\)m-diameter regions were positioned in individual cells of each crypt to record pH\(_i\) from the same cells in the subsequently derived ratio images. Results in Fig. 7 show the average magnitude of pH\(_i\) recovery from a single experiment. Every cell near the crypt base activated Na\(^+\)/H\(^+\) exchange activity in response to luminal and serosal Na\(^+\). Identical results were obtained in four experiments, suggesting that the majority of colonocytes at the crypt base have similar Na\(^+\)/H\(^+\) exchange activity. On the basis of the surprising suggestion of apical Na\(^+\)/H\(^+\) exchange at the base of colonic crypts, we tested different Na\(^+\)/H\(^+\) exchange inhibitors to learn whether known NHE isoforms are responsible for the putative apical Na\(^+\)/H\(^+\) exchange activity.

**Fig. 8.** Luminal ethylisopropylamiloride (EIPA) inhibits colonocyte Na\(^+\)/H\(^+\) exchange activity in response to luminal Na\(^+\). A: after a 1st round of NH\(_4\)Cl prepulse, luminal Na\(^+\)-dependent pH\(_i\) recovery was activated. After a 2nd round of acidification, 20 \(\mu\)M EIPA was added to the luminal superfusate before luminal Na\(^+\) addition. B: pH\(_i\) recovery rates of initial 4 min after luminal Na\(^+\) addition from 1st and 2nd rounds were calculated starting at lowest common pH\(_i\) between the 2 rounds of acidification. **P < 0.001 vs. control.
greater than the nonsignificant 20% decrease reported in the absence of drug and clearly distinct from the 79% inhibition in response to luminal Na\(^+\) activation. Results suggest that the combination of luminal EIPA and luminal Na\(^+\) is an effective pairing for selective analysis of apical Na\(^+\)/H\(^+\) exchange and, combined with earlier results, support the presence of distinct apical and basolateral Na\(^+\)/H\(^+\) exchangers in colonic crypt epithelial cells.

Differential effect of isoform-specific NHE inhibitors. To identify candidate NHE isoforms on the apical membrane of crypt epithelium, we compared two NHE inhibitors. To inhibit NHE3, we used S-1611, a compound that has relatively high specificity for inhibition of NHE3 vs. NHE2 (50). Conversely, we used HOE-694, which is relatively specific for inhibition of NHE2 vs. NHE3 (15). Figure 10A shows qualitatively that, in the presence of luminal 20 \(\mu\)M S-1611, the pH\(_i\) recovery stimulated by luminal Na\(^+\) was similar to control, a result confirmed by quantification in Fig. 10B. In contrast, luminal HOE-694 inhibited the pH\(_i\) recovery stimulated by luminal Na\(^+\) by 89 ± 2\% (\(n = 4\); Fig. 11). Similar to results with serosal EIPA, serosal 20 \(\mu\)M HOE-694 caused inhibition of apical and basolateral NHE (data not shown), suggesting that the drug was also leaky across the epithelial layer. Results are most consistent with NHE2, but not NHE3, as a candidate for the apical Na\(^+\)/H\(^+\) exchanger near the base of colonic crypts.

Short-chain fatty acids. Short-chain fatty acids (SCFAs) are major anions of the colonic lumen and stimulate apical Na\(^+\)/H\(^+\) exchange to promote colonic Na\(^+\) absorption (2, 20, 47). We asked whether luminal SCFA activates the HOE-694-sensitive Na\(^+\)/H\(^+\) exchanger in crypt colonocytes. The pH\(_i\) of crypt epithelial cells was monitored during exposure to luminal 130 mM isobutyrate in the absence and presence of luminal 20 \(\mu\)M HOE-694. Results in Fig. 12 show that isobutyrate acidified crypt epithelial cells and activated an HOE-694-sensitive Na\(^+\)/H\(^+\) exchanger. HOE-694 inhibited 76 ± 2\% (\(n = 4\)) of apical Na\(^+\)/H\(^+\) exchange activated by the SCFA, suggesting that ammonium prepsulate and SCFA activate a similar or identical transporter.

Testing Cl\(^-\) dependence of crypt apical Na\(^+\)/H\(^+\) exchange. Recent studies have reported that all apical Na\(^+\)/H\(^+\) exchange is dependent on extracellular Cl\(^-\) in crypt epithelial cells from rat distal colon (43–45). To test for Cl\(^-\) dependence of apical Na\(^+\)/H\(^+\) exchange in crypt epithelial cells from mouse distal colon, we measured apical Na\(^+\)/H\(^+\) exchange with and without Cl\(^-\) (Fig. 13A). The mucosa was first exposed to 25 mM ammonium in Na\(^-\)free solution. Subsequent removal of ammonium caused rapid cellular acidification. Simultaneously, Cl\(^-\) was removed from the luminal and serosal superfusion (glucenate substitution) for 8–10 min. Returning luminal Na\(^+\) activated pH\(_i\) recovery in the absence of Cl\(^-\), and subsequent luminal Cl\(^-\) addition did not accelerate the pH\(_i\) recovery rate. To test for effects of different anion substitution, Cl\(^-\) was replaced with gluconate.
by gluconate (Fig. 13A) or nitrate. Control experiments with the continuous presence of Cl− were used for comparison with Cl−-free conditions. Results from four experiments using each condition are shown in Fig. 13B, which shows that Na+/H+ exchange activity is similar in the presence of Cl−, gluconate, or nitrate. Results indicate that any apical Na+/H+ exchange in mouse distal colonic crypt epithelium is independent of extracellular Cl−.

Testing for pHi gradient in crypt colonocytes. Using polarized HT29-C1 cells, we had observed more extreme pHi changes in the subapical cytoplasm than in the region adjacent to the basal pole of the cell. This led to pHi gradients being observed in HT29-C1 cells in response to luminal SCFA exposure or NH4Cl prepulse (24, 34). Because the apical-basal axis of the epithelial cells was directly imaged in our native tissue experiments, we asked whether a similar pHi gradient could be observed in mouse crypt colonocytes under comparable conditions. We analyzed small (1–2 μm²) regions of cytoplasm and compared the pHi reported at the subapical and subbasal domains of crypt epithelial cells. In Fig. 14, these subcellular pHi values in the resting state (exposed to Na+/H+ medium), in the presence of luminal SCFA (absence of Na+ in all superfusates), and after NH4Cl prepulse (also in the absence of Na+) in all superfusates are compared. As shown in Fig. 14, pHi was not significantly different between the two subcellular sites under any of the tested conditions.

Immunostaining of NHE isoforms. Figure 15 shows immunofluorescent staining of mouse distal colon with polyclonal antisera raised against the divergent COOH-terminal sequences of NHE1, NHE2, and NHE3. With the use of nuclear staining to mark cellular structures, results show that NHE1 was expressed on the basolateral membrane of epithelial cells along the crypt-surface axis (Fig. 15, a and b). In contrast, NHE3 was expressed on surface but not crypt epithelial cells (Fig. 15, g and h), and the majority of NHE3 was in the apical or subapical domain of the surface cells. The expression of NHE2 in the colon was more complex: it was observed at the apical region of the surface epithelial cells (Fig. 15 d) and also in crypt epithelial cells (Fig. 15, d and e). NHE2 stained the apical membrane of cells near the base of the crypts but, similar to NHE3, also displayed cytosolic staining. For all antisera, preimmune serum confirmed specificity of the signal. Results suggest that NHE2, or an-

Fig. 12. Luminal HOE-694 inhibits isobutyrate-activated colonocyte Na+/H+ exchange activity. Values are means ± SE from 4 experiments. A: luminal TMA-isobutyrate (T-B) medium acidified crypt epithelial cells, and subsequent switch to sodium isobutyrate (Na-B) medium initiated pHi recovery. Apical Na+/H+ exchange in the absence and presence of 20 μM apical HOE-694 is compared using the protocol described in Fig. 8 legend. B: pHi recovery rates of initial 4 min after luminal Na+ addition from 1st and 2nd rounds were calculated starting at lowest common pHi between the 2 rounds of acidification. *P < 0.01 vs. control.

Fig. 11. Luminal HOE-694 inhibits colonocyte Na+/H+ exchange activity in response to luminal Na+. Protocol described in Fig. 8 legend was used to compare results in the absence with results in the presence of 20 μM luminal HOE-694. Values are means ± SE from 4 experiments. A: time course of pHi response in the absence or presence of 20 μM luminal HOE-694. B: pHi recovery rates of initial 4 min after luminal Na+ addition from 1st and 2nd rounds were calculated starting at lowest common pHi between the 2 rounds of acidification. *P = 0.01 vs. control.
other NHE isoform with similar epitopes, is a candidate for the apical exchanger near the base of colonic crypts.

**DISCUSSION**

This study introduces the use of BCECF, the most commonly used fluorescent pH indicator, for ratiometric measurements of living native tissue by confocal microscopy. Recent advances in commercial instrumentation allow rapid switching between appropriate excitation wavelengths (458 and 488 nm) for the use of excitation ratio imaging while minimizing concern about motion artifacts in living specimens. Using the method in conjunction with a chamber that allows separate superfusion of the luminal and serosal compartments of isolated colonic tissue (12), we have applied the method to analyze functional properties of the Na⁺/H⁺ exchangers expressed in the crypt epithelium.

Experiments analyzed the fidelity of the preparation for selective presentation of substances to the luminal vs. serosal compartments and the apical vs. basolateral membrane of the colonocytes. In the serosal compartment, Na⁺ and the structurally unrelated LY dye had rapid access to nonepithelial cells in the lamina propria and basolateral membrane of colonocytes. No evidence was found for slow or restricted mixing of the serosal perfusate in the LIS between colonocytes compared with the directly adjacent lamina propria tissue. Others and we previously observed that pH in the LIS of cultured epithelia and colonocytes can be distinct from that in the serosal tissue and is relatively constant in response to changes in acid/base conditions (9, 12, 25, 34). This was shown to be due to fixed proton buffers on LIS membranes, rather than altered ion diffusion in the LIS (19, 25, 33, 58). This model is supported by our observation of rapid fluid exchange between the lamina propria and the LIS. Our results suggest that LIS Na⁺ concentration can be rapidly altered, even by low concentrations of added Na⁺, suggesting lack of substantial Na⁺ binding or buffering in LIS. Of importance for studies in which Na⁺ is added to luminal or serosal perfusates, results demonstrated that the basolateral membrane of colonocytes was exposed to a fluid that was rapidly and efficiently refreshed by serosal perfusates.

In contrast, multiple results suggested that the crypt lumen has only slow access to luminal perfusates and is thereby subject to greater influence from leakage of fluid and substances from the serosal compartment. The most direct demonstration is that luminal equilibration of an extracellular marker (LY) added to the luminal perfusate required sixfold more time than serosal equilibration of the same marker added to the serosal perfusate. We also observed a marked asymmetry when the transepithelial LY leakage between the luminal and serosal superfusions was compared with the direct presentation of the two compartments.

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**Fig. 13.** Crypt colonocyte Na⁺/H⁺ exchange is not dependent on extracellular Cl⁻. *A:* representative time course experiment showing crypt colonocytes acidified by ammonium prepulse and then exposed to Na⁺-free and Cl⁻-free TMA-gluconate (TMA-G) medium. After pH acidified to a steady state, luminal Na⁺ was returned to luminal Cl⁻-free superfusion [sodium gluconate (Na-G) medium]. Further addition of Cl⁻ to the luminal superfusion did not accelerate pH recovery. Further addition of Cl⁻ to the luminal superfusion did not accelerate pH recovery. Further addition of Cl⁻ to the luminal superfusion did not accelerate pH recovery. Further addition of Cl⁻ to the luminal superfusion did not accelerate pH recovery. Further addition of Cl⁻ to the luminal superfusion did not accelerate pH recovery.

**Fig. 14.** Testing for pH gradients in crypt colonocytes. Measurements from subcellular regions were made in cells exposed to Na⁺ medium (resting pH, n = 8) or in Na⁺-free conditions during exposure to luminal isobutyrate (+isobutyrate, n = 3) or Na⁺-free conditions after transient exposure to NH₄Cl medium (post NH₄, n = 5).
bulk fluid environments of the crypt lumen and the lamina propria was compared as the orientation of LY transepithelial gradients was reversed. In response to serosal LY, 10% of the dye concentration appeared in the crypt lumen. The response to luminal LY addition was significantly less ($P = 0.026$) dye accumulation in the lamina propria, at only 4% (and this value was not significantly different from zero). This suggests that the extracellular fluid in the serosal compartment of the colonic mucosa is more rapidly and efficiently controlled by the perfusate than the extracellular fluid in the luminal compartment.

Experiments unequivocally demonstrated limited leakage of an extracellular fluid marker (LY, 450 g/mol) between the serosal and luminal compartments. Additionally, experiments showed that NHE inhibitors

Fig. 15. Immunofluorescent localization of NHEs in mouse distal colon. Red, nuclear staining with propidium iodide; green, NHE immunofluorescence (see MATERIALS AND METHODS for staining protocols). *, Crypt lumen; arrow, surface epithelium. Images are for NHE1 (a–c), NHE2 (d–f), and NHE3 (g–i). Images a, d, and g exposed crypt-to-surface view of the colonic epithelium. Images b, e, and h are cross sections of crypts near the crypt base. Images c, f, and i are controls using the same dilution of preimmune sera as the primary antisera. Images are representative of results from 3 animals. Scale bars, 20 μm.
The only tenable explanations proposed above that evaluate the in vivo permeability of perfusates to the spaces surrounding colonocytes must be <3 mM. To explain the rapid rate of colonocyte Na+/H+ exchange activated by luminal Na+ as due to erroneous presentation of Na+ at basolateral membranes, the LIS/basal membranes would have to be exposed to a much higher Na+ concentration: ≳10 mM Na+ at basolateral membranes. This more than threefold Na+ gradient over 10–30 μm is clearly incompatible with results being due to 1) gross mixing of the two perfusates via a poorly built microscope chamber or 2) transepithelial Na+ absorption at the surface epithelium adding Na+ to the serosal tissue adjacent to crypts. The only tenable explanations are those in which Na+ appears at the basolateral surface by flux directly across the crypt epithelium. One possibility is a substantial transcellular Na+ flux that loads the LIS/basal extracellular region with Na+. This Na+ absorptive function requires a robust apical Na+ uptake route in the crypt base cells to continually fuel Na+ loading in these well-mixed extracellular spaces, and the apical route would have to be EIPA and HOE-694 insensitive and a transport reaction other than Na+/H+ exchange. The only other apical transport mechanism contributing to mammalian Na+ absorption is Na+ channels, and evidence suggests that these are solely expressed at the surface epithelium (31). The second possibility is a paracellular Na+ flux locally activating LIS Na+/H+ exchangers. Because of the localized entry of Na+ across tight junctions and rapid fluid mixing in the LIS, a gradient of decreasing Na+/H+ exchange activity along the apical-basal axis of the LIS is predicted. No gradient of pH or Na+ concentration has been observed along the apical-basal axis of the LIS (9, 10, 25, 32). In contrast, our observations would require a steep Na+ gradient characterized by <3 mM Na+ near the basal pole of colonocytes and an average 10 mM Na+ along the length of the LIS. Although such a gradient may seem unlikely, LY leakage ensures some paracellular Na+ flux. Results suggest that the crypt colonocyte tight junctions must be relatively impermeable to Na+, since transepithelial accumulation of the 450 g/mol LY is greater (4% on the basis of results in Table 1) than the maximal accumulation of Na+ that is predicted from study of lamina propria cells (<2% on the basis of results in Fig. 6). In our analysis of the apical Na+/H+ exchange, we have optimized conditions for selectively detecting apical transport by adding Na+ and inhibitors only to the luminal compartment. In addition to the studies described above that evaluate the influence and accessibility of perfusates to the spaces surrounding colonocytes, the suitability of this approach is suggested by experiments with EIPA. Luminal application of EIPA produces only slight inhibition of basolateral NHE (presumptive NHE1) compared with apical NHE, despite using an EIPA concentration (20 μM) that is ~1,000-fold greater than the inhibition constant (K_i) of NHE1 for the inhibitor (39, 59) and 100-fold greater than the IC_{50} in the presence of 145 mM Na+ (42). For all the reasons cited above, we believe that our analysis has resolved an Na+/H+ exchanger localized to the apical membrane of crypt colonocytes.

Absorptive transporter in relatively undifferentiated colonic crypt epithelium. In mouse colonic crypts from distal colon, epithelial cells near the base of colonic crypts are proliferating and are only separated by a few cell divisions from the stem cells positioned at the crypt base (6, 41). Although our experiments are clearly not studying the stem cells, the expression of more differentiated functions (such as absorptive transporters) is believed to start nearer the colonic surface. With the use of isolated, microperfused crypts from rat distal colon, it has been observed that crypts (in the absence of secretory stimuli) absorb fluid in an Na+-dependent manner and express an (Cl–dependent) apical Na+/H+ exchange (21, 43–45, 52). In these experiments, the need to cannulate the upper portion of the crypt and puncture/cannulate the crypt base allowed analysis in the midregion of the crypt. In contrast, our measurements preserve normal epithelial architecture while allowing study of colonocytes directly adjacent to the crypt base. In this site, we also observe apical Na+/H+ exchange and report that this transport can be activated by physiological luminal stimuli such as Na+ and SCFAs. These results strongly suggest that even cells near the base of the crypt express an absorptive-type transporter and, for this reason, have at least the potential to contribute to Na+ absorptive function. Given the high abundance of the Cl– secretory channel cystic fibrosis transmembrane conductance regulator in the lower crypt (56) and the observation that all cells near the crypt base express apical Na+/H+ exchange (Fig. 7), we speculate that individual crypt epithelial cells in the colon may mediate Na+ absorption and Cl– secretion.

Candidate NHEs at the crypt base. Recent studies on isolated, microperfused rat distal colonic crypts and apical membrane vesicles have shown a Cl–-dependent Na+/H+ exchange activity (43–45). Using protocols designed to closely parallel those applied previously to resolve Cl–NHE (44, 45), we were unable to demonstrate any Cl– dependence of apical Na+/H+ exchange in mouse distal colonic crypts. We compared a relatively permeant (nitrate) and impermeant (gluconate) anion substitution with no difference in results. The discrepancy between our results and previous studies may be due to species differences (rat vs. mouse) or site of analysis (crypt base vs. midcrypt region). It should be noted that known NHE isoforms can be Cl– dependent in some cases due to an (incompletely understood) effect of intracellular Cl– (1). However, our results clearly demonstrate that the Cl–NHE as previously analyzed in rat distal colonocytes is not a ubiquitous function of colonic crypt epithelia.

Known NHE isoforms are sufficient to explain our observations in crypt colonocytes. The basolateral ex-
changer identified by function is presumed to be NHE1 (4), a conjecture made more likely by demonstration of the presence of NHE1 in the basolateral membrane of epithelial cells near the crypt base (Fig. 15). However, our main focus was to discriminate among potential NHE isoforms at the apical membrane. Experiments were designed to discriminate among the three cloned NHE isoforms (NHE1, NHE2, and NHE3) expressed in small intestine and colon (4, 5, 18). It was important to include NHE1 in the functional analysis because of reports that NHE1 can be expressed apically in some cultured intestinal epithelia (40) and because of the general lack of information about cells near the crypt base.

Our experimental design used luminal addition of inhibitors with differential activity against NHE isoforms, using the absence of serosal Na+ to block activity of basolateral Na+/H+ exchange. EIPA, able to inhibit all three NHEs at the applied concentration of 20 μM (K<sub>i</sub> = 0.02–2.4 μM) (39, 42, 59), blocked >75% of apical Na+/H+ exchange activity. A similar level of inhibition was observed with addition of 20 μM HOE-694, which was predicted to inhibit NHE1 and NHE2 (K<sub>i</sub> = 0.16–5 μM) but not NHE3 (K<sub>i</sub> = 650 μM) (15). No inhibition was observed with 20 μM S-1611, which was predicted to inhibit NHE1 and NHE3 (K<sub>i</sub> = 0.7–5 μM) but not NHE-2 (K<sub>i</sub> = 89 μM) (50). These functional studies suggested that NHE1 and NHE3 do not contribute to apical Na+/H+ exchange near the crypt base. In addition, they suggested that ~75% of the response to luminal Na+ may be due to NHE2. Results were supported by immunostaining, which suggested that epithelial cells near the crypt base did not express NHE3 but did express NHE2-like epitopes.

Our results cannot unequivocally assign NHE2 as the apical Na+/H+ exchanger in the crypt. Other undiscovered NHE isoforms may have a pharmacological profile and an epitope signature similar to NHE2. However, it seems clear that the crypt apical NHE will have properties closely related to those of NHE2. Similar conclusions were made about HT29-C1 cells, where NHE2 and NHE1 (but not NHE3) are expressed, and use of HOE-694 also suggested that NHE2 was apical and NHE1 was basolateral (24). Interestingly, the HT29-C1 cells also express cystic fibrosis transmembrane conductance regulator (37), as shown for colonic epithelial cells near the base of the crypt. Together, results suggest a close functional correlation between the cells near the crypt base and HT29-C1 cells.

In native tissue and HT29-C1 cells, physiological gradients of SCFAs cause selective activation of Na+/-H+ exchange in the apical membrane (16, 34, 46, 51). It has been proposed that heterogeneity of intracellular and extracellular pH may explain why apical exchange activity is activated in preference to basolateral exchange activity (16, 34, 46, 51). In support of this model, qualitatively similar changes in extracellular pH were observed around HT29-C1 cells and crypt colonocytes exposed to transepithelial SCFA gradients (13, 14, 22, 34). HT29-C1 cells also reported a pH gradient in which the cytoplasm near the apical membrane was significantly more acidic than the cytoplasm near the basal pole of the cell (24, 34). It has been observed that mouse small intestinal enterocytes can demonstrate cytosolic pH gradients (53). Therefore, we analyzed whether pH gradients were measurable in crypt colonocytes under the tested conditions. Because cytosolic pH gradients were not observed, we assume that if any pH heterogeneity exists in colonocytes under these conditions, it must be below our level of spatial resolution. In the future, use of near-membrane pH probes might be helpful, inasmuch as they have resolved pH microdomains near the apical membrane of colonocytes exposed to SCFAs (22, 30). Nevertheless, there seems a clear divergence between the observations made in HT29-C1 cells and crypt colonocytes with respect to pH gradients that can be detected with cytosolic pH indicators.

In summary, several unexpected findings have come from this first report of polarized epithelial cell function from cells adjacent to the colonic crypt base. First, apical Na+/-H+ exchange function is detected even among the relatively undifferentiated cells in this region of the mouse crypt. Second, this apical Na+/-H+ exchanger has functional attributes of NHE2, although the presence of NHE2 mRNA was not detected in this region of the rat crypt (5). Our transport and immunostaining results more closely mirror a report from the human colon, where NHE2 mRNA was detected deeper in crypts (18). Third, there is no evidence of the Cl−/HCO<sub>3</sub>− exchanger that has been reported as the sole apical Na+/-H+ exchange activity in the rat colonic crypt (43–45). Results suggest that the colonic crypt has even broader expression of Na+ absorptive transporters than was previously recognized and that our understanding of cellular function in the crypt epithelium remains incomplete.

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