The alveolar epithelium is generally considered to be a tight barrier capable of restricting the movement of salt and water between air space and blood (5, 20). Although most of the daily metabolic acid production passes through it in the form of CO₂, little is known about its ability to form a barrier for acid/base equivalents per se. The alveolar space is lined by a thin, continuous layer of fluid [alveolar lining fluid (ALF)], the aqueous subphase pH of which has been measured to be acidic (6.9) relative to blood (7.4) (2, 23). The mechanism by which this gradient is maintained is undetermined, although its existence is presumed to depend primarily on transport and permeability properties of the alveolar epithelium.

The alveolar epithelium has luminal (apical) and serosal (basolateral) aspects functionally compartmentalized by cell-cell junctions. This polarity, which is the structural and functional hallmark of epithelia, makes vectorial transport across the alveolar epithelium possible (13). Apically situated epithelial Na⁺ channels and basolateral Na⁺ pumps have been shown to provide the mechanisms for active transepithelial Na⁺ transport across the alveolar epithelium (5, 20). Other polarized transport mechanisms, including the water..
channel aquaporin-5 located in the apical membrane of alveolar type I cells (3), are also present in alveolar epithelial cells (AEC). In addition, differences in the composition and functional properties of apical vs. basolateral cell membranes have been shown to exist in epithelia (15, 26), although little is directly known about these differences in AEC.

Several acid/base transport mechanisms have been described in alveolar epithelium that could contribute to regulation of ALF pH (6, 14, 16–19). These acid/base transport mechanisms, which include Na+/H+ exchange (NHE1), Na+/HCO3− cotransport, and Cl−/HCO3− exchange, have been shown to contribute to intracellular pH (pHi) homeostasis in AEC. However, little information is available concerning their contribution to the permeability of AEC to acid/base equivalents or, more generally, to the response of pHi to changes in extracellular pH (pHe) (1, 21, 25, 27). None of these acid/base transporters have specifically been localized to the apical membrane of these cells. These basolateral transporters are therefore unlikely to be solely responsible for regulation of ALF pH, although they could do so in conjunction with a mechanism present in the apical membrane that directly faces the extracellular alveolar milieu.

The low paracellular permeability of the alveolar epithelium, which is of similar magnitude to other tight epithelia (e.g., toad urinary bladder), is thought to be primarily due to the integrity of the intercellular complexes that link the cells (5). Tight junctions are responsible for maintaining and regulating the paracellular permeability of the alveolar epithelial barrier. The molecular basis by which paracellular permeability is regulated, which probably depends on the expression of specific tight junction proteins that confer relative impermeability to one or more ions, has only recently begun to be understood (11). Although generally assumed to be impermeable to cations, including protons, neither the role of tight junctions in maintaining a transepithelial acid-base gradient across the alveolar epithelium nor the specific junctional proteins involved have been determined.

The goal of the present study is to describe mechanisms whereby the alveolar epithelium could maintain a transepithelial pH gradient. To this end, we used a model of the alveolar epithelium in which primary cultured AEC were grown as electrically resistive monolayers on permeable supports, permitting independent access to apical and basolateral aspects of the cells. First, we assessed the ability of these monolayers to acutely maintain a transepithelial pH gradient. Second, we studied the differential effects of apical vs. basolateral changes in pHi on pHe in AEC monolayers. Third, we examined basolateral acid/base entry pathways in AEC. These first three sets of experiments were performed under CO2− and HCO3−-free conditions. This made it possible to eliminate the effects of CO2−, which is freely permeable across AEC membranes, on pHi. Fourth, we studied the differential effects of apical vs. basolateral changes in pHe on transepithelial transport by AEC monolayers. Finally, we studied the effects of adaptation to acidosis or alkalosis on the relationship between pHe on pHi. Our results indicate that alveolar epithelial cell monolayers are capable of maintaining a transepithelial pH gradient because of the relative impermeability of the apical cell membrane and intercellular junctions to acid/base equivalents and, furthermore, suggest that chronic basolateral acid exposure impairs transepithelial Na+ transport via the effects of intracellular acidosis.

**METHODS**

**Preparation of AEC monolayers.** Alveolar type II epithelial cells were isolated from 125- to 150-g male Sprague-Dawley rat lungs by a modified differential adherence method, as previously described (8). Briefly, elastase (2–3 U/ml) was used to disaggregate the type II cells from the supporting lung matrix. The cells were plated on tissue-culture-treated Nuclepore filters (Snapwell; Corning Costar, Acton, MA) at 1.5 × 10⁶ cells/cm² and maintained in primary culture in medium consisting of DMEM-Ham’s F-12 medium (1:1), BSA (1.25 mg/ml), 10 mM HEPES, 0.1 mM nonessential amino acids, 2.0 mM glutamine, sodium penicillin G (1,000 U/ml), and streptomycin (1,000 μg/ml). For experiments in which cells were exposed to altered pH, in culture (see Effects of extracellular acid and base on AEC bioelectric properties), cells were cultured in media of identical composition supplemented with 10% newborn bovine serum and titrated to pH ~6.0 or ~8.2 using 1 N HCl or KOH for acid or base exposures, respectively. Transepithelial tissue resistance (Rt), a measure of monolayer confluency and intercellular junction integrity, and spontaneous potential difference (SPD) across the monolayer were determined daily using a rapid screening device (Millicell-ERS; Millipore, Bedford, MA). Equivalent short-circuit current (Isc), a measure of active transepithelial ion transport, was calculated from the following relationship: Isc = SPD/Rt. Experiments are performed 3–4 days after monolayers were plated, coincident with the development of tissue resistance ≥2,000 Ω cm², except where otherwise indicated.

**Modified Ussing chamber cuvette for fluorescence measurements.** AEC monolayers were mounted in a custom Plexiglas cuvette (California Institute of Technology Machine Shop, Pasadena, CA) designed for fluorescence measurements in a fluorescence spectrophotometer (model LS5B; Perkin-Elmer, Pasadena, CA) designed for fluorescence measurements in a fluorescence spectrophotometer (model LS5B; Perkin-Elmer, Norwalk, CT), as previously described (16–18). The cuvette is diagonally bisected by a Plexiglas slide machined to form a tight seal with the floor and side corners of the cuvette. Before each monolayer was mounted, the filter was detached from its supporting plastic struts. The filter (with the adherent cell monolayer) was then secured in a window in the Plexiglas slide by an O ring, and the slide was positioned in the cuvette. The cuvette is fixed in its position in the spectrophotometer at 45° to the light source to optimize fluorescence signal from the monolayers. Tissue resistance across the monolayer was determined before and after each experiment using the Millicell screening device by placing the tips of its electrodes on opposite sides of the filter positioned in the cuvette and measuring Rt.

**Intracellular loading and fluorescence measurements with a pH-sensitive fluorescent probe.** Changes in pH were monitored using the pH-sensitive fluorescent probe 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-AM by methods similar to those used in previous studies (16–18). Briefly, cells were loaded with this lipophilic esterified form of the dye by addition of BCECF-AM to the solution bathing the
apical surface of the monolayer from a stock in dimethyl sulfoxide at a 1:1,000 dilution to give a final dye concentration of 15 μM. Loading proceeded in the cuvette at ambient temperature for 30 min.

Previous experiments have confirmed that BCECF is maximally fluorescent in the AEC grown on Nuclepore filters at an emission wavelength of 530 nm and excitation wavelength of 503 nm, with an isosbestic point at 440 nm. Changes in fluorescence intensity were monitored continuously in the fluorescence spectrophotometer at ambient temperature. Cell and filter autofluorescence were noted at 440 and 503 nm before dye loading for each monolayer and subtracted from all fluorescence values when pH<sub>i</sub> was calculated. Initial change in pH<sub>i</sub> per unit time (dpH<sub>i</sub>/dt) was calculated by measuring the slope of a line tangent to the initial deflection of the fluorescence curve. The dpH<sub>i</sub>/dt was converted to initial H<sup>+</sup> flux (J<sub>H<sup>+</sub></sup>) using previously reported values for intrinsic buffer capacity (β<sub>i</sub>), as described elsewhere (17).

**Calibration of fluorescence vs. pH**. To correlate rates of fluorescence values at 503- and 440-nm excitation wavelengths to pH<sub>i</sub>, a calibration curve was constructed at ambient temperature using the K<sup>+</sup>-nigericin technique (31). AEC monolayers were exposed to BCECF in the presence of buffer containing (in mM) 140 NaCl, 5 KCl, 1 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 5 glucose, and 6 HEPES at pH 7.4. After the cells were loaded, the dye-containing buffer was evacuated and replaced by dye-free medium composed of 130 mM KCl, 12 mM NaCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 5 mM glucose, 6 mM HEPES, and K<sup>+</sup>-H<sup>+</sup> ionophore nigericin (1 μg/ml). Because intra- and extracellular K<sup>+</sup> concentrations are approximately equal, the nigericin pathway will allow pH<sub>i</sub> and pHe to become equal. Monolayers are then incubated for 20 min in bathing solutions titrated to a series of different (extracellular) pH values using 1 N KOH or 1 N HCl. In this manner, a series of different pH values were obtained and the fluorescence ratio (F<sub>exc</sub>/F<sub>em</sub>) was calculated by fluorescence vs. pH i.

**Statistical analysis**. Values are means ± SE. Significance (P < 0.05) of differences among experimental conditions was determined by ANOVA, except where otherwise indicated.

**RESULTS**

**Impermeability of AEC monolayers to acid and base equivalents**. Monolayers were mounted in the fluorescence chamber and bathed in HEPES buffer in apical (A) and basolateral (B) fluid compartments. At time 0, fluid in the apical or basolateral compartment was replaced with fluid of otherwise identical composition at pH 6.4 or 8.0. Replacement of apical fluid with HEPES buffer at pH 6.4 or 8.0 resulted in minimal changes in pH<sub>i</sub>. In contrast, replacement of the basolateral fluid with HEPES buffer at 6.4 or 8.0 resulted in a significant shift in pH<sub>i</sub> in the same direction as pHe. Transmembrane acid/base fluxes, expressed as dpH<sub>i</sub>/dt, were -0.08 ± 0.01/min (mean ± SE) for experiments where basolateral pH<sub>e</sub> was 6.4 and +0.06 ± 0.01/min for experiments where basolateral pHe was 8.0 (n ≥ 3 for each condition). On the basis of β for AEC monolayers, previously reported to be 27 mM/pH unit for HCO<sub>3</sub>-free conditions in this pH<sub>i</sub> range (17), these results can also be expressed as initial J<sub>H<sup>+</sub></sub> of -21.6 and 16.2 mM/min, respectively. These results indicate that the apical membranes of AEC are relatively impermeable and the basolateral membranes are relatively permeable to acid or base equivalents.

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**Fig. 1. Impermeability of alveolar epithelial cell (AEC) monolayers to acid and base equivalents.** Monolayers were mounted in the fluorescence chamber and bathed in HEPES buffer in apical (A) and basolateral (B) fluid compartments. At time 0, fluid in the apical or basolateral compartment was replaced with fluid of otherwise identical composition at pH 6.4 or 8.0. Replacement of apical fluid with HEPES buffer at pH 6.4 or 8.0 resulted in a significant shift in pH<sub>i</sub> in the same direction as pHe. Transmembrane acid/base fluxes, expressed as dpH<sub>i</sub>/dt, were -0.08 ± 0.01/min (mean ± SE) for experiments where basolateral pH<sub>e</sub> was 6.4 and +0.06 ± 0.01/min for experiments where basolateral pHe was 8.0 (n ≥ 3 for each condition). On the basis of β for AEC monolayers, previously reported to be 27 mM/pH unit for HCO<sub>3</sub>-free conditions in this pH<sub>i</sub> range (17), these results can also be expressed as initial J<sub>H<sup>+</sub></sub> of -21.6 and 16.2 mM/min, respectively. These results indicate that the apical membranes of AEC are relatively impermeable and the basolateral membranes are relatively permeable to acid or base equivalents.
None of these agents reduced the rate of pH decrease. The buffer added to the basolateral fluid compartment(s) changed the pH of the fluid, whereas basolateral membranes are relatively permeable to acid or base equivalents. Results indicate that apical membranes of AEC are impermeable, loaded with BCECF as described above, except 100 μM dimethylamiloride (DMA), 100 μM DIDS, 100 μM ouabain, 1 mM BaCl₂, or 1 mM ZnCl₂ was dissolved in the buffer added to the basolateral fluid compartment. As illustrated in Fig. 3A (n ≥ 3 for each condition), none of these agents reduced the rate of pH decrease due to acid entry when buffer in the basolateral fluid compartment was exchanged for acidic fluid (pH 6.4). In contrast, DMA alone inhibited the base equivalent entry step that occurs when basolateral fluid pH is returned to pH 7.4. As shown in Fig. 3B, DMA similarly blocked the increase in pH observed when HEPES buffer at pH 8.0 was substituted into the basolateral fluid compartment but did not prevent the fall in pH observed when pH 7.4 buffer was replaced in the basolateral fluid. These results suggest that net base entry (or acid extrusion) occurred at least in part via basolateral Na⁺/H⁺ antiport, whereas net proton uptake occurred via a different (DMA-insensitive) pathway.

Effects of extracellular acid and base on AEC bioelectric properties. To determine whether prolonged incubation in a transmembrane acid-base gradient would affect AEC function preferentially via the basolateral surface, AEC monolayers were incubated in the presence of acidic (pHe ~ 6.0) or basic (pHe ~ 8.2) medium on the apical and/or basolateral side of the monolayer from day 3 in culture, and bioelectric properties were measured daily thereafter. As indicated in Fig. 4A, Iₜₜ for AEC monolayers grown in apical acidic medium was not different from that for monolayers grown in pH 7.4 medium, whereas Iₜₜ for monolayers grown in basolateral acidic medium or in acidic medium on both sides was significantly lower than that for monolayers grown in pH 7.4 medium.
grown in pH 7.4 medium on days 4–7. As indicated in Fig. 4B, $I_{SC}$ for AEC monolayers grown in apical basic medium was not different from $I_{SC}$ for monolayers grown in pH 7.4 medium, whereas $I_{SC}$ for monolayers grown in basolateral basic medium or basic medium on both sides was significantly higher than $I_{SC}$ for monolayers grown in pH 7.4 medium on days 6 and 7.

Effects of adaptation to acidosis and alkalosis on the relationship between $pH_i$ and $pH_e$. To determine whether prolonged exposure to acidosis or alkalosis reduced the effects of $pH_e$ on $pH_i$, via an adaptive mechanism, AEC monolayers were grown at pH 7.4 for 3 days and then further cultivated in medium (apical and basolateral) at pH 6.0 (acidosis), 7.4 (control), or 8.0 (alkalosis) for 24 h. AEC monolayers were then mounted in the cuvette for $pH_i$ measurements (see METHODS) in HEPES buffer at pH 7.4. The monolayers were subjected to a series of changes in $pH_e$ by exchange of apical and basolateral fluid compartments with HEPES buffer titrated to pH 6.0–8.0, $pH_i$ was allowed to reequilibrate for ~15 min, and a new steady-state value was recorded. As illustrated in Fig. 5, control monolayers responded to changes in $pH_e$ with changes in $pH_i$ of similar direction but less magnitude. This relationship was changed only minimally after 24 h of exposure to acidic or basic medium, suggesting only limited adaptation in defense of $pH_i$ to extracellular acidosis or alkalosis under the conditions of these experiments ($n \geq 3$ for each condition).
DISCUSSION

In this study, we present evidence that AEC monolayers are relatively impermeable to acid/base equivalents under acute and chronic conditions and that they are capable of maintaining an apical-to-basolateral pH gradient in vitro. The ability of these epithelial monolayers to maintain a proton gradient depends primarily on the relative impermeability of the apical membrane and the intercellular junctions to acid/base equivalents. In contrast, basolateral AEC membranes are relatively permeable to net base influx, at least partly via \( \text{Na}^+ / \text{H}^+ \) exchange, as well as acid influx, occurring by another undetermined mechanism. Our results also suggest that intracellular acidosis impairs, and intracellular alkalosis stimulates, active transepithelial Na\(^{+}\) flux, as indicated by the differential effects of apical vs. basolateral exposure on \( I_{SC} \). Finally, we have shown that basolateral pH\(_i\) substantially affects pH\(_i\) in AEC monolayers and that this relationship is only minimally affected after 24–48 h of adaptation to altered pH\(_i\).

Several lines of evidence indicate that AEC monolayers are impermeable to acid/base equivalents because of the impermeability of intercellular junctions and the relative and differential impermeability of the apical cell membranes compared with the basolateral cell membranes. First, as shown in Fig. 1, a and b, minimal transepithelial movement of acid/base equivalents occurs across AEC monolayers grown on filters when they are incubated in the presence of a pH gradient for \( \geq 30 \) min. Neither the porous polycarbonate filters nor other barriers (e.g., unstirred layer effects) block acid/base entry at the basolateral membrane (Fig. 2), indicating that the only impediments to the movement of acid/base equivalents are the AEC monolayers themselves. Therefore, the intercellular junctions (paracellular pathway) and the cells forming the monolayers (transcellular pathway) display minimal permeability for acid/base equivalents.

Second, as shown in Fig. 2, exposure of the basolateral surface of AEC monolayers to relatively acidic or basic buffer solutions results in significant changes in pH\(_i\). Apical exposure results in minimal change in pH\(_i\). Transmembrane movement of acid/base equivalents, across the basolateral membrane itself or via specific membrane transporters, provides the likely explanation for these observed effects. The relative impermeability of the apical membrane correlates with the absence of any acid/base transport mechanisms specifically localized therein and suggests that the relative impermeability of the membrane itself is an adequate explanation for the lack of changes in pH\(_i\) in the presence of a transapical gradient for pH.

Transepithelial acid/base fluxes occur across the basolateral membranes of AEC, at least in part via membrane transporters. As shown in Fig. 3, intracellular alkalinization due to the presence of a transmembrane gradient across the basolateral membrane (pH\(_o\) < pH\(_i\)) is reduced when DMA is present in basolateral fluid. These results suggest that pH\(_i\) is increased by acid exit (or base entry) via the Na\(^+\)/H\(^+\) exchanger (NHE1), previously shown to be present on the basolateral membrane of these cells (17). Acid efflux (i.e., base loading) is characteristic of Na\(^+\)/H\(^+\) exchange in AEC, although reversal of Na\(^+\)/H\(^+\) exchange resulting in net acid influx is also possible in the presence of a large outwardly directed gradient for Na\(^+\) across the basolateral membrane (17). Nonetheless, acid entry across the basolateral membrane occurs by a mechanism that is not inhibitable by DMA or by a panel of inhibitors of other potential acid entry pathways. DIDS, an inhibitor of HCO\(_3\)\(^-\) transport pathways such as Cl\(^-\)/HCO\(_3\)\(^-\) exchange and Na\(^+\)-HCO\(_3\)\(^-\) cotransport known to be present on the basolateral membrane of AEC (17, 18), had no effect on acid influx. Our results further suggest that other related acid/base transport processes known to be inhibited by DIDS (e.g., Cl\(^-\)/OH\(^-\) and Cl\(^-\)/base exchange) are also not present or do not contribute to acid influx (10, 27). Despite literature suggesting that other transporters such as Na\(^+\) pumps and channels, K\(^+\) channels, and H\(^+\) channels could provide an entry pathway for acid equivalents in these or other cells (6, 7, 12, 32), their respective inhibitors or blockers (e.g., ouabain, BaCl\(_2\), and ZnCl\(_2\)) also had no effects. Taken together, these results are consistent with proton permeability directly across the basolateral membrane or, more likely, net acid efflux primarily via Na\(^+\)/H\(^+\) exchange and acid influx via some other as yet undetermined mechanism(s) in the presence of an acid-base gradient.

A third line of evidence for the differential permeability of apical and basolateral membranes of AEC to acid/base equivalents and the ability of their intercellular junctions to prevent transepithelial acid/base fluxes comes from experiments performed on cells maintained in media of various pH. Electrically resistive AEC monolayers maintained in media titrated to acidic or alkaline pH facing apical or basolateral sides of the monolayer on days 4–7 in culture show no significant loss of the pH gradient for the entire time they are maintained in this fashion (data not shown). Nonetheless, cells grown in the presence of a basolateral transmembrane gradient for pH develop changes in bioelectric properties that are not evident in the presence of an apical gradient. As shown in Fig. 4, A and C, \( I_{SC} \) (a measure of active transepithelial Na\(^+\) transport) across AEC monolayers is reduced within 24 h in the presence of basolateral acid (or apical and basolateral acid) relative to cells exposed only to apical acid (or no pH gradient). As also indicated in Fig. 4, B and C, \( I_{SC} \) is increased in the presence of basolateral (or apical and basolateral) alkalinization relative to cells exposed only to apical base (or no pH gradient). In neither case did exposure of the cells on days 4–7 to acid or alkaline medium significantly change \( R_t \) (a measure of monolayer confluence or integrity of intercellular junctions; data not shown), consistent with the ability of the AEC monolayers to maintain a pH gradient (and to transport Na\(^+\) in a vectorial fashion) over several days in culture.

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Direct effects of \( p_{H_e} \) on basolateral membrane active transport properties (e.g., \( Na^+ \) pump activity) and \( I_{SC} \) cannot be completely separated from effects mediated via changes in \( p_{H_i} \) on the basis of our results. The permeability of the basolateral membranes of AEC to acid/base equivalents makes it difficult to distinguish effects of \( p_{H_i} \) from \( p_{H_e} \). \( p_{H_i} \) in AEC cannot be chronically changed by exposure to altered \( p_{H_e} \), as described here if basolateral medium is restored to \( p_{H_e} \) 7.4, for example, in the absence of a specific inhibitor of acid entry via the basolateral membrane (Fig. 3). Further distinction between the effects of intra- and extracellular acidosis will therefore require a method, presently unavailable, of chronic acidifying and alkalinizing AEC while the membranes are maintained in a pH 7.4 medium.

Interpretation of experiments where medium pH was changed at the apical membrane (where changes in \( I_{SC} \) were not observed and alterations in \( p_{H_e} \) were not anticipated) is somewhat more straightforward. The lack of effect of chronic changes in apical medium pH on \( I_{SC} \) indicates that active transport is not altered by changes in \( p_{H_i} \) at the apical membrane and is further compatible with a lack of effect of \( p_{H_e} \) on \( p_{H_i} \) via apical acid/base transport. Taken together, the results illustrated in Fig. 4 are most consistent with the concept that chronic changes in \( p_{H_i} \) result from basolateral (but not apical) acid/base transport. Furthermore, although direct effects of \( p_{H_e} \) on basolateral membrane active transport properties cannot be excluded, it appears likely that at least some of the observed effects of basolateral \( p_{H_e} \) are mediated via changes in \( p_{H_i} \).

Although the cellular mechanisms by which changes in \( p_{H_i} \) (and/or \( p_{H_e} \)) alter transepithelial transport in AEC cannot be determined at this time, several possibilities exist. The permeability of epithelial \( Na^+ \) channels (ENaC) and activity of \( Na^+ \) pumps are potentially altered by pH changes (4, 29), with direct changes occurring over a short time course (i.e., minutes). The observed changes in \( I_{SC} \) in the presence of basolateral pH gradients occurred over a time course of several hours (data not shown), suggesting that an indirect mechanism (e.g., change in cellular ATP levels) could be responsible. Alternatively, a change in gene expression or protein turnover of one or more \( Na^+ \) transporters could have contributed to the observed changes in \( I_{SC} \). Measurements of steady-state levels of \( Na^+ \) pump \( \alpha \)-subunits by immunoblot (data not shown) showed no change in the presence of an increase or decrease in \( I_{SC} \) induced by basolateral alkalosis or acidosis, however, suggesting that other mechanism(s) are operative.

Whatever the mechanism of altered \( Na^+ \) transport in the presence of a basolateral proton gradient, it appears very likely that differential effects on \( p_{H_i} \) occur in chronic experiments in a manner similar to those that occur acutely (Figs. 2 and 3). Because the \( p_{H_i} \) of AEC monolayers could not be measured directly while monolayers were being cultivated in 5% \( CO_2 \) over several days and given the known ability of several different kidney cell lines to adapt to changes in \( p_{H_i} \), by changing transporter activity and/or intracellular buffer capacity (24, 30), experiments were performed after 24 h of incubation in the presence of acidic or alkaline medium to determine whether adaptation occurs that could limit pH changes in chronically exposed cells. Monolayers were subjected to a series of different \( p_{H_e} \) buffers (apical and basolateral), \( p_{H_i} \) was determined, and, as indicated in Fig. 5, little difference in the response of \( p_{H_i} \) to \( p_{H_e} \) was observed. These data suggest that minimal adaptation occurred over 24 h of culture in the presence of altered \( p_{H_e} \). Whereas it is possible, perhaps likely, that such adaptation could occur over longer periods of time to protect cells from the effects of, for example, prolonged acidosis, the absence of significant adaptation is consistent with the development of changes in \( p_{H_i} \) with chronic experimental exposure to basolateral (but not apical) pH gradients similar to those found acutely.

Taken together, these data indicate that AEC monolayers restrict the movement of acid/base equivalents because of the impermeability of the apical membrane of AEC and the intercellular junctions between them to protons (or their equivalent). The apparent lack of specific acid/base permeability pathways (i.e., channels, carriers, or transporters) active at the apical membrane and the impermeability of the apical membrane itself to acid/base equivalents are probably sufficient to explain the results of acute and chronic experiments showing a lack of effect of apical pH gradients on \( p_{H_i} \) or \( I_{SC} \), respectively. In contrast, there is insufficient information at this time to fully explain the mechanisms by which intercellular junctions restrict acid/base movement across AEC monolayers. These effects most likely result from specific properties of the tight junctions present in these electrically resistive monolayers (11).

One implication of these results is that \( p_{H_i} \) of AEC may be more closely related to plasma or interstitial pH than to alveolar pH. This was first proposed in 1969 by Effros and Chinard (9) on the basis of a study in which they estimated the extravascular pH of the lung in dogs by measuring the pH-sensitive distribution of nicotine across the pulmonary vasculature. Changes in pulmonary extravascular pH occurred more closely in parallel to changes in arterial pH than to alterations in \( PCO_2 \) at constant arterial pH. These findings led the authors to suggest that relative insensitivity of tissue pH to changes in \( PCO_2 \) at constant plasma pH could be advantageous in the lung, which is exposed to rapid variations in \( PCO_2 \). The authors further concluded that the stability of pulmonary tissue pH could be linked to blood (and thereby whole body) acid-base balance, rather than to local buffering mechanisms alone, the latter being particularly vulnerable to changes in alveolar ventilation. Although our present findings do not specifically address the issue of the role of \( PCO_2 \) in regulation of alveolar epithelial cell \( p_{H_i} \), they are consistent with the concept that these cells are sensitive to changes in basolateral (i.e., plasma or interstitial), rather than apical (i.e., alveolar), pH in a fashion similar to that described in situ by these authors.
Our present results are not consistent with the hypothesis of DeCoursey (7), in which a role for proton channels in the excretion of CO₂ by the lung has been proposed. In a recent review on the subject (7), the author suggested the possibility that CO₂ could be at least partly excreted across the apical membrane of AEC as H⁺ and HCO₃⁻, which would subsequently recombine in the ALF to produce CO₂ and H₂O. Although DeCoursey and colleagues have presented extensive evidence for the presence of voltage-gated proton channels that could provide an H⁺ exit pathway across the apical membrane of AEC, we were not able to find a physiological function for these channels or define their cellular location (i.e., apical or basolateral) in the present study. Our own preliminary data (21) also do not reveal a significant apical conductance or permeability for HCO₃⁻ or any evidence for impermeability for CO₂ across the apical membrane of AEC. Thus, although further study could reveal conditions under which, for example, proton transport via apical channels does contribute to net CO₂ excretion across the alveolar epithelium, our present data do not support DeCoursey’s hypothesis in its present form.

In summary, we have shown that AEC monolayers grown in primary culture on polycarbonate filters are relatively impermeable to acid/base equivalents because of the impermeability of the apical membrane of the AEC and the intercellular junctions between them. The basolateral membranes of AEC are freely permeable to acid/base equivalents that enter and exit the cells via one or more known basolateral acid/base transporters in addition to other as yet undetermined mechanisms. Chronic exposure of AEC monolayers to basolateral (but not apical) pH gradients results in changes in transepithelial Na⁺ transport likely due to changes in pHi that appear not to be blunted by intracellular adaptation to acidosis or alkalosis. These results suggest that acid/base impermeability is a fundamental property of the alveolar epithelium and are consistent with the ability of the alveolar epithelium to maintain an air space-to-blood pH gradient as observed in situ. They also suggest that chronic changes in systemic pH, as occur in chronic metabolic acidosis, could affect the ability of the alveolar epithelium to transport Na⁺ for maintenance of alveolar fluid balance and resolution of alveolar edema.

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