Resistence of the pulmonary epithelium to movement of buffer ions

R. M. Effros, L. Olson, W. Lin, S. Audi, G. Hogan, R. Shaker, K. Hoagland, and B. Foss. Resistance of the pulmonary epithelium to movement of buffer ions. Am J Physiol Lung Cell Mol Physiol 285: L476–L483, 2003; 10.1152/ajplung.00398.2002.—Exposure of the apical surfaces of alveolar monolayers to acidic and alkaline solutions has been reported to have little influence on intracellular pH compared with basolateral challenges (Joseph D, Tirmizi O, Zhang X, Crandall ED, and Lubman RL. Am J Physiol Lung Cell Mol Physiol 282: L675–L683, 2002). We have used fluorescent pH indicators and a trifurcated optical bundle to determine whether the apical surfaces are less permeable to ionized buffers than the membranes that separate the vasculature from the tissues in intact rat lungs. In the first set of experiments, the air spaces were filled with perfusate containing FITC-dextran (mol wt 60,000) or 2,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF). Air space pH fell progressively from 7.4 to 6.6 ± 0.3 mean ± SE, n = 11, air space buffers at 10 mM). Perfusion for 2 min with 2 mM NH4Cl increased air space pH by 0.142 ± 0.019 unit, without a subsequent acidic overshoot. Infusions of NaHCO3 and sodium acetate reduced pH without a subsequent alkaline overshoot. In the second set of experiments, cellular pH was monitored in air-filled lungs after perfusion with BCECF-AM. Injections of NH4Cl caused a biphasic response, with initial alkalinization of the cellular compartment followed by acidification after the NH4Cl was washed from the lungs. Subsequent return of pH to normal was slowed by infusions of 1.0 mM dimethyl amiloride. These studies suggest that lung cells are protected from air space acidification by the impermeability of the apical membranes to buffer ions and that the cells extrude excess H+ through basolateral Na+/H+ exchangers.

air space acidification; intracellular pH; ammonium; bicarbonate; acetate

RELENTIVELY LITTLE IS KNOWN about acid-base balance across the membranes that separate the blood and the small amount of fluid that lines the air spaces. Before birth, the fluid in the air spaces is typically acidic (pH 6.27) in fetal lambs (1). Utilizing microelectrodes, Nelson et al. (12) found that the pH of the alveolar surface liquid of rat alveoli averages 6.92. They suggested that the relatively low pH of this fluid might play a role in protein activity, surfactant properties, and macrophage function. Kyle et al. (10) reported that the pH of the airway surface fluid of the ferret trachea averaged 6.85. They proposed that the low pH of the airways might influence ciliary activity and the interaction of bacteria with the airway mucosa. Using an in vivo fluorescent technique, Jayaraman et al. (8) obtained a pH of 6.95 in the surface fluid of tracheas in anesthetized mice. Lubman and Crandall (11) reviewed a variety of mechanisms that could acidify the air spaces.

Joseph et al. (9) used a fluorescent pH sensitive dye, 2,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF), to conduct studies of intracellular pH on alveolar epithelial cell monolayers. These monolayers were bathed with a nonbicarbonate solution buffered with 6 mM HEPES. Replacement of apical fluid with acidic (pH 6.4) or basic (pH 8.0) solutions had little effect on intracellular pH. In contrast, changes in basolateral fluid pH caused rapid responses in intracellular pH. Intracellular alkalinization was blocked ≥80% by dimethyl amiloride, an inhibitor of the Na+/H+ exchanger. No measurements were provided for the movement of specific buffers such as HCO3- across the apical membranes, leaving the possibility that alteration of air space pH could result in rapid changes in cellular pH in vivo. Furthermore, movement of HEPES buffer across basolateral membranes was not ruled out.

We have investigated whether the polarity in permeability of the apical cells to the movement of acid-base equivalents observed in vitro can be demonstrated in intact lungs. The pH of the air space and cellular compartments of the lung were monitored with a surface fluorescence approach modified from that introduced by Carter et al. (4). These studies support the conclusions of Joseph et al. (9), by showing that the apical membranes of the pulmonary epithelium are less permeable to ionized buffers than the basolateral surfaces. The apical barrier presumably permits maintenance of relatively acid fluid in the air space compartment and may also serve to protect the underlying pulmonary tissues from transient acid challenges.

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buffers provided quite linear buffering capacity over the range of pH values that were observed in the air spaces of these experiments (Fig. 2A). The amount of buffer was intentionally increased in one group of experiments to 25 mM HEPES. As indicated in Fig. 2, this solution was better buffered than the HEPES-MES solution. Although the buffer curve was more curvilinear, it was fairly linear between pH 7.4 and 7.0, values encountered in these experiments. The pH of the perfusion solutions was adjusted to 7.4 with small volumes of 1 N NaOH.

**Calculation of Alveolar pH**

When fluorescein-dextran (pK_a = 6.4) or BCECF (pK_a = 6.98) is excited at 490 nm, the emission at 515 nm decreases as pH decreases. In contrast, emission from FITC-dextran is insensitive to pH when it is excited at 420 nm, and emission of BCECF is insensitive to pH when it is excited at 440 nm. As indicated in Figs. 3–5, the excitation signal at 490 nm exceeded that at 420 nm, making it possible to distinguish the emission signals at these two wavelengths. The lung was illuminated alternately at 490 and 420 nm for 2-s intervals, and the recorded fluorescent signals were interpolated to provide values every 2 s. The voltage of the signal at 490 nm

**METHODS**

**Protocol**

The protocol was approved by the Animal Studies Subcommittee (Institutional Animal Care and Use Committee) of the Zablocki Veterans Affairs Medical Center.

**Fluid-filled lungs (air space pH)**. Sprague-Dawley rats (n = 26, 390 ± 71 g body wt) were anesthetized with an intraperitoneal injection of 0.7 ml of a 50 mg/ml pentobarbital sodium solution. The chest was opened, and catheters were placed in the trachea, pulmonary artery, and left atrium. Blood was flushed from the vasculature at 37°C with 20 ml of perfusion fluid (see below), and the air spaces were filled with 5 ml of perfusate containing 0.2 mg/ml FITC-dextran (mol wt 2 × 10^6) or 0.1 μg/ml BCECF. The lungs were then perfused at 10 ml/min for ~1 h. A trifurcated fiber-optic bundle (Oriel) was placed against the surface of the lungs (Fig. 1). The surface of the lungs was alternately illuminated at 2-s intervals with monochromatic excitation beams at wavelengths of 490 and 420 nm, and the emission from the surface of the lungs was monitored at 515 nm. When BCECF was used, the lungs were illuminated alternately at 490 and 440 nm, and emission was monitored at 515 nm. The change in excitation wavelength was accomplished by alternately opening the shutters of two monochromatic light sources at these wavelengths at 2-s intervals. The fluorescent signals were monitored with a photomultiplier (Hamamatsu), the output of which was amplified, filtered, and recorded on a spreadsheet in a computer. No change in the drift of the fluorescence was observed when the light source was kept off during most of the experiment, indicating that exposure to light did not affect air space pH measurements.

**Air-filled lungs (intracellular pH)**. In these experiments, the lungs were inflated with air at a constant pressure of 5 cmH_2O, and the lungs were perfused for 10 min with a solution of 2 μg/ml BCECF-AM. Per fusate containing 20 mM NH_4Cl was infused for 2 min as described above.

**Perfusion Solutions**

The lungs were perfused with solutions containing the following solutes: 30 g/dl dextran (mol wt 7 × 10^6), 1 g/l glucose, 118 mM NaCl, 4.7 mM KCl, 1.1 mM KH_2PO_4, 2.5 mM CaCl_2, and 1.15 mM MgSO_4. In all but one group of experiments, 5 mM HEPES (pK_a = 7.55) and 5 mM MES (pK_a = 6.15) were included in the solution. This mixture of

**Fig. 1.** Experimental apparatus. A trifurcated, randomized fiber-optic bundle is used to deliver monochromatic light with wavelengths set alternately at 2-s intervals at 490 nm and 420 or 440 nm. This is made possible by opening and closing shutters in front of the 2 halogen light sources. A third branch of the bundle conducts light emitted by the lung to the photomultiplier through an interference filter at 515 nm. Signal from the photomultiplier is amplified and recorded in computer spreadsheet.

**Fig. 2. A:** buffering properties of perfusate solutions were measured by addition of small volumes of 1 N HCl to solutions. HCl concentrations that would be present in the solutions are indicated. pH of perfusate containing low concentrations of buffer (5 mM HEPES-5 mM MES) decreased linearly with added HCl. pH of high-buffer solution (25 mM HEPES) fell more slowly and remained fairly linear to pH 7.0. **B:** FITC-dextran was infused into pulmonary vasculature at different pH levels. Calculated effect on whole lung pH measured from fluorescent signals is indicated: acidification of perfusate decreased pH of lungs calculated from fluorescent emission. Effect of acidification was less when lungs were perfused with higher concentrations of buffer (25 mM HEPES).
was then divided by that at 420 nm at each point along the curve to yield the fluorescence ratio (R). R fell as pH decreased. R₀ at the beginning of each experiment was assumed to be representative of pH 7.4, because the pH of the FITC-dextran solution that was originally instilled into the lungs was 7.4. Similarly, the fluorescence ratio at the end of the experiment (Rₚₑₚ) was assumed to be representative of the pH that was measured in the air space fluid recovered from the trachea at the end of the experiment. A linear relation was assumed between fluorescence and air space pH between the beginning and end of the studies, and the values of air space pH were calculated from the ratios on the basis of the linear equation

\[ \text{pH}_t = 7.4 - m(\text{R}_0 - \text{R}_t) \]

where pHₜ is pH at time t, 7.4 is the initial pH, and m was calculated as follows

\[ m = (7.4 - \text{pH}_{\text{end}})/(\text{R}_0 - \text{R}_{\text{end}}) \]

To test the linearity of the relation between the calculated pH and R in the lung preparations, the lungs were perfused in four consecutive intervals for 2 min at the end of the studies with solutions containing the same concentration of FITC-dextran that was present in the air space solution (0.2 mg/ml). These solutions were adjusted with small volumes of 1 N HCl to pH 6.0, 6.4, 7.0, and 7.4 (Figs. 3–5). Injections of FITC-dextran (at pH 7.4 and 7.0) increased the observed fluorescence, because the vasculature as well as the air spaces now contained the fluorescent dye (see below). The change in the calculated pH of the lung (air spaces and vasculature) varied linearly with the pH of the FITC-dextran that was injected (Fig. 2B).

Selection of the maximum pH changes after each of the infusions of weak acids and bases, hypotonic solutions, and FITC-dextran was facilitated by using the PeakFit program (Jandel).

Detection of Directional Changes in Intracellular pH

BCECF-AM is the esterified, nonfluorescent form of BCECF. BCECF-AM is lipophilic and readily crosses cell membranes. Once inside the cells, it is rapidly deesterified to BCECF, which is fluorescent and lipophobic. It remains.
trapped within the cells, where it can be used to monitor changes in intracellular pH. Standardization of intracellular pH with this dye in isolated cell preparations is conventionally accomplished by treating the cells with nigericin and 100–150 mM K\(^+\)/H\(^+\), which equalizes intracellular and extracellular pH. It was not possible to perfuse lungs with these solutions without disrupting the pulmonary vasculature. Although absolute values of intracellular pH could not be calculated, directional changes in pH after injections of NH\(_4\)Cl could be determined by following changes in fluorescence of lungs related to 2-min infusions of perfusate altered in the following fashion: 0.5 mg/ml FITC-dextran, pH 7.4 (A); 25 mM NaHCO\(_3\), pH 7.4 (B); 25 mM NaHCO\(_3\), pH 8.0 (C); 25 mM NaHCO\(_3\) and 0.5 mM acetazolamide, pH 7.4 (D); perfusate pH 6.0 (E); and 0.5 mg/ml FITC-dextran added to perfusate at pH 6.0, 6.4, 7.0, and 7.4 (F, G, H, and I, respectively).

**Chemical Analysis**

Na\(^+\), K\(^+\), and Cl\(^-\) concentrations were measured with ion-specific electrodes. Glucose concentrations were determined by the glucose oxidase Trinder method (catalog no. 315, Sigma, St. Louis, MO), and lactic acid was determined with lactic dehydrogenase (catalog no. 726-UV/826-UV, Sigma). These determinations were made in air space and perfusate samples at the beginning and end of the studies.

**Statistical Analysis**

The significance of differences between mean values was calculated with a one-way analysis of variance, and Tukey’s test was used to compare individual mean values.
RESULTS

Fluid-Filled Lungs

Changes in baseline fluorescence, pH, and solute concentrations of the air space compartment. There was initially a tendency for baseline fluorescence to increase when the lungs were excited at 490 nm, followed by a decline in baseline values (Figs. 3–5). Baseline fluorescence at 420 nm consistently increased in the FITC-dextran but not the BCECF experiments. Calculated, ratiometric values of pH invariably fell. Direct measurements of the pH of the air space fluid indicated that air space pH fell from 7.40 to 6.61 ± 0.03 (mean ± SE) over the course of the 1 h when the buffer in the solutions was kept low (5 mM HEPES-5 mM MES). This is equivalent to an increase of 2.02 ± 0.07 μeq of H⁺ into each milliliter of the air space fluid. Acidification of the air spaces was not due to accumulation of lactic acid, which remained <1 mM in all but one of these experiments.

K⁺ concentrations in the air spaces exceeded those in the perfusate by 41 ± 10% at the end of 1 h (P < 0.05). In contrast, no differences were observed in airway and perfusate concentrations of Na⁺ or Cl⁻. Glucose concentrations of the air space solution fell to 60 ± 2% of those in the perfusate (P < 0.05).

Response of air space fluorescence and pH to transient infusions of buffers. After a baseline infusion of unlabeled perfusate, the lungs were perfused for 2 min with a total of 20 ml of each solution (Table 1, Figs. 3–6). Infusions of perfusate containing FITC-dextran increased the emission signal when the lungs were illuminated at 490 and 420 nm. However, the signal at

![Graph showing fluorescence and pH changes over time](image-url)
490 nm decreased when the pH of the injection solutions was decreased. Injections of NH₄Cl increased the lung fluorescence at 490 nm but had no effect at 420 or 440 nm, and an increase in air space pH was calculated. This response was greater when the perfusate contained lower concentrations of buffer (5 mM HEPES-5 mM MES, rather than 25 mM HEPES), despite the fact that more NH₄Cl was injected in the latter experiments (Fig. 5). Injections of NaHCO₃ decreased the fluorescence at 490 nm but had no effect at 420 nm and decreased the calculated air space pH. This effect was less (P < 0.05) when the injection pH was increased from 7.4 to 8.0. Acetazolamide, an inhibitor of carbonic anhydrase, had no effect on air space fluorescence or pH. The effects of sodium acetate injections were similar to those of sodium bicarbonate: fluorescence at 490 nm decreased, but there was no effect at 420 nm, and the calculated air space pH decreased. In all the experiments with NH₄Cl, NaHCO₃, and sodium acetate, the change in pH was "monotonic," in other words, after the injection fluid had been washed from the vasculature, pH returned to normal without an overshoot in the opposite direction. Furthermore, the effects of these weak acids and bases was less pronounced when the air space fluid contained more buffer (25 mM HEPES, rather than 5 mM HEPES-5 mM MES; cf. Fig. 6; P < 0.05). Injections of acidified perfusate (pH 6.0) had little effect on air space pH. Hypotonic injections decreased the fluorescence of the air space fluid at 490 and 420 nm but had no effect on the air space pH.

Similar observations were made when the extracellular pH indicator BCECF (1 μg/ml), rather than FITC-dextran, was instilled into the air spaces. Inusions of NH₄Cl were followed by monophasic increases in the fluorescence at 490 nm, and infusions of NaHCO₃ resulted in monophasic decreases in fluorescence (Figs. 4 and 5).

**Air-Filled Lungs: Intracellular pH**

The intracellular compartments of the isolated lungs were labeled by perfusion with BCECF-AM. Injections of 20 mM NH₄Cl for 2 min yielded a "biphasic" response in fluorescence (at 490 nm excitation): fluorescence increased above baseline levels and then decreased below baseline levels after the NH₄Cl had been flushed from the lungs (see Fig. 8). No change in fluorescence was observed when the lungs were illuminated at 440 nm, the pH-insensitive wavelength for this indicator. The return of fluorescence to baseline levels after injections of NH₄Cl was slowed in each of five studies (by >90% in 4 of 5 experiments, P < 0.05) after the lungs were perfused for 5 min with perfusate containing the Na⁺-H⁺ inhibitor dimethyl amiloride at 0.1 mM (Fig. 7).

**DISCUSSION**

**Baseline Acidification of the Air Space Fluid and Electrolyte Concentrations (Fluid-Filled Lungs)**

The initial pH of the air spaces fell progressively in these experiments. Acidification of the baseline pH of the air space fluid was initially rapid but subsequently slowed (Figs. 3–5). This slowing could be due to backdiffusion of acid out of the air spaces. Backdiffusion could also be responsible for the observation that the net movement of H⁺ into the air spaces was greater.

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**Table 1. Response of fluorescence and air space pH to infusions (2 minutes, 20 ml)**

<table>
<thead>
<tr>
<th>Infusion</th>
<th>pH*</th>
<th>Excitation Wavelength</th>
<th>490 nm</th>
<th>420 nm</th>
<th>ΔpH†</th>
<th>Figs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC-dextran, BCECF</td>
<td>6–7.4</td>
<td></td>
<td>+</td>
<td>+</td>
<td>NC</td>
<td>3–5</td>
</tr>
<tr>
<td>NH₄Cl (2 and 5 mM)</td>
<td>7.4</td>
<td>+,+ +</td>
<td>NC</td>
<td>+,+ +</td>
<td>3,5</td>
<td></td>
</tr>
<tr>
<td>NaHCO₃ (25 mM)</td>
<td>7.4</td>
<td>-,-,-</td>
<td>NC</td>
<td>-,-,-</td>
<td>4,5</td>
<td></td>
</tr>
<tr>
<td>NaHCO₃ (25 mM) + Acetaz (0.5 mM)</td>
<td>7.4</td>
<td></td>
<td>NC</td>
<td>-</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Sodium acetate (10 mM)</td>
<td>7.4</td>
<td></td>
<td>NC</td>
<td>-</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Acidified perfusate (20% water)</td>
<td>6.0</td>
<td></td>
<td>NC</td>
<td>NC</td>
<td>3, 4</td>
<td></td>
</tr>
<tr>
<td>Hypotonic perfusate (20% water)</td>
<td>7.4</td>
<td></td>
<td>NC</td>
<td>NC</td>
<td>3–5</td>
<td></td>
</tr>
</tbody>
</table>

* +, increase; −, decrease; NC, no change; Acetaz, acetazolamide; BCECF, 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein. †pH of infusion. ‡Calculated change in air space pH.
when the air spaces were more effectively buffered with 25 mM HEPES than when less buffer was used (5 mM HEPES-5 mM MES). Decreases in air space pH could not be attributed to accumulation of lactic acid in the preparation. There was an increase in $K^+$ of the air space fluid that was consistent with previous observations (1, 2). Secretion of $K^+$ and $H^+$ into the air spaces could play a role in the reabsorption of $Na^+$ from the air spaces (11). Glucose concentrations in the air space fell significantly relative to those in the plasma. This could represent local consumption or transport out of the air spaces (3). Consistent increases in fluorescence of FITC-dextran at 420 nm presumably reflect fluid reabsorption from the air spaces. These increases were not reproducible in the studies with BCECF, which is a smaller molecule that may leak out of the air spaces during the course of the experiments.

**Monophasic Response of Air Space pH to Infusions of Acids and Bases (Fluid-Filled Lungs)**

Exchange of weak acids and bases across the barriers separating the perfusate and the air spaces was virtually confined to the nonionized forms of the acid-base pairs. For example, alkalinization of the air spaces with infusions of $NH_4Cl$ at pH 7.4 was presumably due to the selective diffusion of $NH_3^-$ rather than $NH_4^-$, into the air spaces. The initial alkalinization with $NH_4Cl$ has also been reported in individual cells loaded with pH-sensitive dyes (13), but there is an acidic overshoot after the exposure to $NH_4Cl$ ends. This was not observed in air space pH, which returned to normal without an acidic overshoot after the $NH_4Cl$ injection ended. In this respect, the pH response of the air spaces appears to differ from that of the cellular pH. As indicated in Fig. 7, intracellular pH (as judged from fluorescence after cellular loading with BCECF-AM) initially increased after the injections of $NH_4Cl$ but then fell below baseline levels. This overshoot has been attributed to some movement of $NH_2^+$ into the cells when they are exposed to $NH_4Cl$ (Fig. 8). After the $NH_4Cl$ fluid is washed out of the vasculature, $NH_2^+$ remaining in the cells dissociates into $H^+$ and $NH_3$. $NH_3$ diffuses out of the cell, leaving excess $H^+$. Excess $H^+$ within the cells are then extruded by transport from the cells. The rate at which intracellular pH returned to normal was significantly slowed when the lungs were perfused with 0.1 mM amiloride, an inhibitor of $Na^+/H^+$ exchange. This observation is in accord with the observations of Joseph et al. (9).

Failure to observe an overshoot when the pH-sensitive indicators were placed in the air spaces suggests that the membranes separating the vasculature and air spaces are more impermeable to $NH_2^+$ than the basolateral surfaces. Because the $pK_a$ of $NH_2^+$ is 9.23, the amount of $NH_3$ in the perfusate exceeds that of $NH_3$ by a factor of 67 at pH 7.4. It can therefore be concluded that the barrier is $=67$ times less permeable to $NH_2^+$ than to $NH_3$.

Infusions of 10 mM sodium acetate ($pK_a = 5.23$) and 25 mM $NaHCO_3$ ($pK_a = 6.1$) acidified the air space solution, and no alkaline overshoot was observed with either of these solutions. This again suggests that the nonionized forms of the acid-base pairs diffuse through the membranes much more rapidly than the ionized forms. In the case of acetate, the membranes were

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*Fig. 7. Response of intracellular pH to infusions of $NH_4Cl$. Cells were loaded with BCECF by perfusion of lungs for 10 min with BCECF-AM. Infusion of $NH_4Cl$ resulted in an increase in fluorescence when lungs were illuminated at 490 nm (sensitive to pH) but had little effect on the signal when they were illuminated at 440 nm (insensitive to pH). After $NH_4Cl$ was washed from the lungs, fluorescence fell below baseline level. This is consistent with an acidic overshoot after the exposure to $NH_4Cl$ ends. This overshoot has been attributed to some movement of $NH_2^+$ into the cells when they are exposed to $NH_4Cl$ (Fig. 8). After the $NH_4Cl$ fluid is washed out of the vasculature, $NH_2^+$ remaining in the cells dissociates into $H^+$ and $NH_3$. $NH_3$ diffuses out of the cell, leaving excess $H^+$. Excess $H^+$ within the cells are then extruded by transport from the cells. The rate at which intracellular pH returned to normal was significantly slowed when the lungs were perfused with 0.1 mM amiloride, an inhibitor of $Na^+/H^+$ exchange. This observation is in accord with the observations of Joseph et al. (9).*
FLUOROMETRIC MEASUREMENTS OF AIR SPACE pH

\( \geq 148 \) times more permeable to acetic acid than to acetate. Acidification of the air spaces with \( \text{NaHCO}_3 \) was greater at pH 7.4 than 8.0. This presumably reflects the fact that the concentration of \( \text{CO}_2 \) is 1.2 mM at pH 7.4 and only 0.31 mM at pH 8.0. The observation that, even at pH 8.0, only acidification is observed indicates that the air space-perfusate barrier must be \( \geq 79 \) times more permeable to \( \text{CO}_2 \) than to \( \text{HCO}_3^- \). Differences between permeability of the barrier to ionized and neutral forms of these weak acids are probably much greater than the minimum values calculated from the fluorescent data. In studies using \( ^{14}\text{CO}_2 \) and \( ^{14}\text{HCO}_3^- \) with more extreme changes in pH, it was possible to show that the barrier is \( \geq 600 \) times more permeable to \( \text{CO}_2 \) than to \( \text{HCO}_3^- \) (7). Acetazolamide failed to cause a significant decrease in acidification of the air spaces. This suggests that the delivery of \( \text{CO}_2 \) in the perfusate is sufficiently rapid that \( \text{CO}_2 \) production from \( \text{HCO}_3^- \) does not significantly limit \( \text{CO}_2 \) movement into the air spaces.

DeCoursey (5) recently suggested that movement of \( \text{CO}_2 \) from the blood to the air spaces is facilitated by the secretion of acid across alveolar epithelial cells through \( \text{H}^+ \) channels. This hypothesis assumes that the diffusion of \( \text{HCO}_3^- \) into the epithelial lining fluid is rapid compared with that of \( \text{CO}_2 \). The present study and an earlier study with \( ^{14}\text{HCO}_3^- \) suggest that the transport of \( \text{HCO}_3^- \) across the apical membranes of the lung is considerably slower than that of \( \text{CO}_2 \) (6). Any facilitation of \( \text{CO}_2 \) transport by \( \text{H}^+ \) secretion would presumably be modest.

An infusion of only 2 mM \( \text{NH}_4\text{Cl} \) resulted in a pH increase of 0.142 unit, whereas 10 mM sodium acetate increased by 0.089 unit. In other words, for equal concentrations of \( \text{NH}_4\text{Cl} \) and sodium acetate, changes in pH are 8 times greater with the \( \text{NH}_4\text{Cl} \) infusions than with sodium acetate. This suggests that the membranes separating the vasculature from the air spaces are more permeable to \( \text{NH}_3 \) than to acetic acid.

Our observations concerning air space pH differed in some respects from those reported by Carter et al. (4) in the original work describing the surface fluorescence in one mouse. They observed a rapid decrease in air space fluorescence after 5-min infusions of perfusate at pH 5.6. Infusion of bicarbonate caused an initial acidification that was followed by alkalinization. Neither of these phenomena were observed in the present study. It is possible that the more prolonged infusion of a more acidic solution resulted in leakage of ions across the epithelium in this species. However, it is difficult to interpret the data, because the lung was illuminated at a single wavelength, and alterations in pH cannot be directly calculated.

Intracellular pH is commonly measured by fluorescent microscopy in individual cells with a variety of indicators. Alternatively, it can be measured in suspensions of cells using conventional fluorometry (13). As indicated in this study, it is also possible to measure changes in compartmental pH (air space and cellular) with these indicators by using an appropriate optical bundle placed on the surface of an organ. This approach permits verification of measurements made in cellular monolayers with observations made in intact organs. The studies of Joseph et al. (9) in monolayers and these studies in intact lungs show that the apical membranes of the lungs are more impermeable to ionized buffers than the basolateral membranes. This may maintain the normally acidic environment in the air spaces and insulate the lung parenchyma from abrupt changes in pH induced by aspiration of small amounts of acid from the lungs or inflammatory cells in the air spaces. These studies are also in accord with earlier studies of the extravascular pH of the lung, which indicated that tissue pH is more closely linked to vascular than to air space pH (6). This barrier presumably fails when larger amounts of acid are aspirated. The present study and the study of Joseph et al. (9) also suggest that the \( \text{Na}^+/{\text{H}^+} \) exchanger mediates extrusion of excess acid from basolateral surfaces of the lung cells.

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DISCLOSURES

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REFERENCES