pH$_i$ responses to osmotic cell shrinkage in the presence of open-system buffers

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Heming, Thomas A., Gregory Boyarsky, Divina M. Tuazon, and Akhil Bidani. pH$_i$ responses to osmotic cell shrinkage in the presence of open-system buffers. J Appl Physiol 89: 1543–1552, 2000.—Changes in plasma volume in vivo cause rapid changes in extracellular pH by altering the plasma bicarbonate concentration at a constant PCO$_2$ (Garella S, Chang BS, and Kahn SI. Kidney Int 8: 279, 1975). Few studies have examined the possibility that changes in cell volume produce comparable changes in intracellular pH (pH$_i$). In the present study, alveolar macrophages were exposed to hyperosmotic medium in the absence or presence of the open-system buffers CO$_2$-HCO$_3^-$, propionic acid-propionate, or NH$_3$-NH$_4^+$. In the absence of open-system buffers, exposure to twice-normal osmolarity (2T) produced a slow cellular alkalinization [change in pH$_i$ (ΔpH$_i$) = 0.38; exponential time constant (τ) ≈ 120 s]. In the presence of 5% CO$_2$, 2T caused a biphasic pH$_i$ response: a rapid increase (ΔpH$_i$ = 0.10, τ ≈ 15 s) followed by a slower pH$_i$ increase. Identical rapid pH$_i$ increases were produced by 2T in the presence of propionic acid (20 mM). Conversely, 2T caused a rapid pH$_i$ decrease (ΔpH$_i$ = −0.21, τ ≈ 10 s) in the presence of NH$_3$ (20 mM). Thus osmotic cell shrinkage caused rapid pH$_i$ changes of opposite direction in the presence of a weak acid buffer (contraction alkalosis with CO$_2$ or propionic acid) vs. a weak base buffer (contraction acidosis with NH$_3$). Graded ΔpH$_i$ were produced by varying extracellular osmolarity in the presence of open-system buffers; osmolarity increases of as little as 5–10% produced significant ΔpH$_i$. The rapid pH$_i$ responses to 2T were insensitive to inhibitors of membrane H$^+$ transport (ethylisopropylamiloride and bafilomycin A$_1$). The results are consistent with shrinkage-induced disequilibrium in the total cellular buffer system (i.e., intrinsic buffers plus added weak acid-base buffer).

alveolar macrophage; cell volume

REGULATION OF CELL VOLUME and regulation of intracellular pH (pH$_i$) are important for normal cell function (5, 19, 21). In many cells, the two regulatory processes involve common membrane ion transporters, specifically the Na$^+$/H$^+$ exchanger and Cl$^-$/HCO$_3^-$ exchanger (7, 11). As a result, regulation of cell volume can entail changes in pH$_i$, whereas regulation of pH$_i$ can entail changes in cell volume (7, 11). This interdependence raises interesting questions about the hierarchy of cell volume regulation vs. pH$_i$ regulation, especially in cells (e.g., macrophages) that exhibit coordinated changes in pH$_i$ and cell volume during cell activation.

The relationship between cell volume and pH$_i$ is complicated further by the potential effects of osmotically induced changes in cell volume on the equilibrium state of intracellular buffers. Osmotically induced dilution or concentration of a closed-system cytosolic buffer (i.e., a buffer such as intracellular protein, which has a cytosolic content that is essentially fixed) should exert similar effects on the concentrations of the buffer, its conjugate species, and H$^+$. This would produce little, if any, change in pH$_i$. However, the situation is different for open-system cytosolic buffers (i.e., buffers that readily permeate the plasma membrane and with a total cytosolic content that can vary rapidly). For example, consider cells that are shrunken by exposure to a hyperosmotic medium in the presence of physiological concentrations of CO$_2$-HCO$_3^-$, an open-system buffer. One expects that osmotic cell shrinkage (reflecting a net loss of cytosolic water) would produce comparable increases in the cytosolic concentrations of HCO$_3^-$ and H$^+$ with little or no change in the cytosolic PCO$_2$ (and hence CO$_2$ concentration). In this instance, cell shrinkage would produce an intracellular CO$_2$-HCO$_3^-$-H$^+$ disequilibrium, leading to the net formation of CO$_2$ from HCO$_3^-$ and H$^+$ and an increment in pH$_i$ (i.e., an intracellular contraction alkalosis). This change in pH$_i$ is comparable to the effect on plasma pH of reducing extracellular fluid volume in vivo (10). Weak acids (e.g., CO$_2$) and bases are, by definition, buffers. Thus one might also expect osmotic changes in cell volume to disrupt pH$_i$ in the presence of other membrane-permeant weak acids or bases (e.g., lactic acid or NH$_3$).

We have shown previously that osmotic shrinkage of resident alveolar macrophages activates the plasma-lamellar Na$^+$/H$^+$ exchanger and, under CO$_2$-free conditions, causes an increase in the steady-state pH$_i$ (15). In the present study, we determined the effects of osmotic cell shrinkage on the pH$_i$ of single alveolar macrophages in the presence of CO$_2$. Macrophage exp-

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1543
posure to hyperosmotic medium in the presence of CO₂ caused a biphasic rise in pHᵢ (i.e., an initial rapid increase, followed by a slower increment in pHᵢ), whereas osmotic shrinkage in the absence of CO₂ caused a slow monotonic rise in pHᵢ. We then conducted studies with other open-system buffers, specifically a membrane-permeant weak acid (propionic acid) or weak base (NH₃), to test the hypothesis that changes in pHᵢ with doubling of extracellular osmolarity (Table 1). The effect was reversible. When the solution was returned to the original osmolarity, pHᵢ slowly recovered toward the initial starting value (Fig. 1). In 18 cells, the mean ΔpHᵢ after 2T removal was −0.34 ± 0.04 (Table 1).

The time courses of the pHᵢ responses to 2T were well described by single exponentials. For 18 cells, the mean exponential time constant (τ) was 121 s for the

<table>
<thead>
<tr>
<th>Experiment</th>
<th>n</th>
<th>pHᵢ before</th>
<th>pHᵢ after</th>
<th>ΔpHᵢ</th>
<th>pHᵢ before</th>
<th>pHᵢ after</th>
<th>ΔpHᵢ</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPES</td>
<td>18</td>
<td>6.92 ± 0.03</td>
<td>7.30 ± 0.04</td>
<td>0.38 ± 0.04*</td>
<td>7.29 ± 0.05</td>
<td>6.95 ± 0.05</td>
<td>−0.34 ± 0.04*</td>
</tr>
<tr>
<td>CO₂-HCO₃</td>
<td>20</td>
<td>7.00 ± 0.04</td>
<td>7.10 ± 0.04</td>
<td>0.10 ± 0.01*</td>
<td>7.24 ± 0.05</td>
<td>7.10 ± 0.04</td>
<td>−0.14 ± 0.02*</td>
</tr>
<tr>
<td>Propionic acid-propionate</td>
<td>17</td>
<td>6.91 ± 0.04</td>
<td>7.03 ± 0.04</td>
<td>0.12 ± 0.01*</td>
<td>7.07 ± 0.04</td>
<td>6.96 ± 0.03</td>
<td>−0.10 ± 0.01*</td>
</tr>
<tr>
<td>+15 μM EIPA</td>
<td>6</td>
<td>6.93 ± 0.09</td>
<td>7.05 ± 0.09</td>
<td>0.13 ± 0.02*</td>
<td>7.08 ± 0.08</td>
<td>6.98 ± 0.07</td>
<td>−0.10 ± 0.04*</td>
</tr>
<tr>
<td>+5 μM bafilomycin A₁</td>
<td>7</td>
<td>6.72 ± 0.05</td>
<td>6.81 ± 0.05</td>
<td>0.09 ± 0.03*</td>
<td>6.82 ± 0.05</td>
<td>6.74 ± 0.05</td>
<td>−0.08 ± 0.03*</td>
</tr>
<tr>
<td>NH₃-NH₄Cl + 300 mM mannitol</td>
<td>28</td>
<td>7.02 ± 0.02</td>
<td>6.81 ± 0.03</td>
<td>−0.21 ± 0.01*</td>
<td>6.80 ± 0.03</td>
<td>7.01 ± 0.02</td>
<td>0.21 ± 0.01*</td>
</tr>
<tr>
<td>+300 mM sucrose</td>
<td>6</td>
<td>7.00 ± 0.06</td>
<td>6.79 ± 0.06</td>
<td>−0.21 ± 0.01*</td>
<td>6.78 ± 0.06</td>
<td>6.99 ± 0.06</td>
<td>0.20 ± 0.01*</td>
</tr>
<tr>
<td>+150 mM NaCl</td>
<td>3</td>
<td>7.02 ± 0.08</td>
<td>6.84 ± 0.09</td>
<td>−0.19 ± 0.01*</td>
<td>6.84 ± 0.10</td>
<td>7.04 ± 0.09</td>
<td>0.20 ± 0.01*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of cells. Data for CO₂-HCO₃ are for initial, rapid intracellular pH (pHᵢ) responses. EIPA, ethylisopropylamiloride. *Significantly different from zero, P < 0.0001.
On phase and 163 s for the Off phase (Table 2). The magnitudes of the pH_i responses to 2T (ΔpH_i during On and Off phases) were not significantly dependent on the starting pH_i (i.e., pH_i at which 2T was applied or removed). Figure 2 shows the ΔpH_i vs. starting pH_i. The slopes of lines fitted to the data were not significantly different from zero (P 0.27 for On data and 0.08 for Off data).

Effects of increased extracellular osmolarity in the presence of CO_2. In the presence of CO_2, exposure to 2T caused a biphasic rise in pH_i. There was a rapid initial alkalinization from a baseline value of 7.00 ± 0.04 to a shoulder value of 7.10 ± 0.04, followed by a slower rise over the course of >5 min to a new steady-state value of 7.24 ± 0.05 (n = 20 cells) (Fig. 3, Table 1). In 20 cells, the mean ΔpH_i was 0.10 ± 0.01 for the initial rapid portion of the On phase and 0.14 ± 0.04 for the subsequent slower portion. The Off response was monitored for only 1–2 min (Fig. 3). Macrophage pH_i declined rapidly during that period of time to an apparently stable value (ΔpH_i ~ -0.14; Table 1, Fig. 3).

The pH_i responses to 2T in the presence of CO_2 occurred more rapidly than those in the absence of CO_2. The mean τ was 15 s for the initial On response in CO_2 and 24 s for the Off response (Table 2), considerably shorter than those in CO_2-free solution. Furthermore, in contrast to the case in CO_2-free solution, the rapid ΔpH_i produced by 2T (On and Off phases) in CO_2 was dependent on the starting pH_i value, increasing with increments in the starting pH_i (Fig. 4).

Effects of increased extracellular osmolarity in the presence of propionic acid. If the rapid pH_i response to 2T in CO_2 reflected a disequilibrium in the total cellular buffer system, then other membrane-permeable weak acids would be expected to mimic the effects observed in CO_2. Furthermore, alveolar macrophages have HCO_3^-dependent plasmalemma acid-base transporters (3) that could be operating during the osmotic challenge in CO_2. Propionic acid was selected as a membrane-permeant weak acid (4) that is unlikely to support HCO_3^-dependent transport.

Figure 5 illustrates an experiment in which a cell was exposed to 20 mM propionic acid-propionate in the nominal absence of CO_2. The initial exposure to propionic acid caused a rapid cell acidification consistent with the entry of propionic acid, a fraction of which dissociated to form intracellular propionate and H^+ (4).
After the rapid acidification, pH recovered to a value near the original baseline, presumably through the actions of the plasmalemma H⁺ extruders (2, 4). The cell was then briefly exposed to solutions of altered extracellular osmolarity, ranging from 110 to 300% of normal (1.1T to 3T). Changes in extracellular osmolarity under these conditions produced rapid, reversible changes in pH.

The pH responses to osmotic cell shrinkage in propionic acid were quantitatively identical to those in CO₂. The magnitudes of the responses to 2T in propionic acid (ΔpH ≈ 0.12 during the On phase and −0.10 during the Off phase) were not significantly different from those in CO₂ (Table 1). Furthermore, the ΔpH produced by 2T in propionic acid was sensitive to the starting pH value (data not shown). The regression slopes (±SD) of ΔpH vs. starting pH were 0.27 ± 0.07 (R² = 0.67) for the On phase and 0.19 ± 0.05 (R² = 0.67) for the Off phase (each slope was significantly different from zero). The regression slopes in propionic acid were not significantly different from those in CO₂ (Table 1). Furthermore, the pH responses to 2T (ΔpH during the On and Off phases) were not significantly different from those in CO₂ (Table 1). The pH responses to 2T (ΔpH during the On and Off phases) were not significantly different from those in CO₂ (Table 1).

Effects of increased extracellular osmolarity in the presence of NH₃. If the rapid pH response in the presence of a weak acid was due to changes in the equilibrium characteristics of the total cellular buffer system, then a different response should be observed in the presence of a membrane-permeant weak base. Now the total cellular buffer system consists of a weak base-conjugate acid buffer pair (e.g., NH₃-NH₄⁺) plus the intrinsic buffers. Figure 7A illustrates an experiment in which a cell was exposed to 20 mM NH₃-NH₄⁺ in the nominal absence of CO₂. This solution caused a rapid alkalization, consistent with the entry of NH₃, a fraction of which combined with intracellular H⁺ to form NH₄⁺. The pH subsequently recovered to near the original baseline value, reflecting a compensatory net (i.e., osmolarity relative to 300 mosM). The best-fit relationship indicated that ΔpH varies as 0.35 log₂ + 0.32.246 on June 5, 2017 http://jap.physiology.org/ Downloaded from
H+ loading of the cell. The cell was then briefly exposed to NH3 solutions of altered osmolarity. In marked contrast to the results with a weak acid buffer (CO2 or propionic acid), increases in extracellular osmolarity caused cellular acidifications in the presence of NH3. In the case with 2T, ΔpHi was 2.01 for the On response in NH3 and 0.21 for the Off response (Table 1).

We visually confirmed that the hyperosmotic challenges produced the intended cell shrinkage. Using a reticle in the microscope eyepiece, we observed comparable decreases in cell diameter during exposures to hyperosmotic medium with or without CO2, propionic acid, or NH3. We also checked that similar pHi responses were observed when the osmolality of the NH3 solution was altered with 300 mM sucrose or 150 mM NaCl instead of 300 mM mannitol (Fig. 7B, Table 1).

The pHi responses to 2T in NH3 were well fit by single exponentials (data not shown). The mean τ was 10 s for the On phase and 15 s for the Off phase (Table 2), similar to the results with CO2 and propionic acid. Figure 8 illustrates the pHi dependence of ΔpHi produced by 2T in NH3. Neither On nor Off data showed a demonstrable dependence on starting pHi, unlike the case for weak acids. The slopes of lines fitted to the data were not significantly different from zero (P > 0.0001). The observed ΔpHi values produced during both the On and Off phases (On data plotted with positive sign) as a function of the relative extracellular osmolarity. The absolute ΔpHi increased with increments in relative osmolarity between 1.05T and 3T. The curve in Fig. 9 is a fit of the data to the equation A + B log10s. The observed ΔpHi values are means ± SE; n = 20 cells. *Significantly different from zero, P < 0.0001. †Significantly different from zero, P < 0.01.

### Table 3. Changes in pHi with variations in external extracellular osmolarity in the presence of propionic acid

<table>
<thead>
<tr>
<th>Relative Osmolarity</th>
<th>pHi before</th>
<th>pHi after</th>
<th>ΔpHi</th>
<th>pHi before</th>
<th>pHi after</th>
<th>ΔpHi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>6.94 ± 0.02</td>
<td>6.96 ± 0.03</td>
<td>0.02 ± 0.004†</td>
<td>6.97 ± 0.03</td>
<td>6.95 ± 0.02</td>
<td>−0.02 ± 0.005†</td>
</tr>
<tr>
<td>1.25</td>
<td>6.96 ± 0.02</td>
<td>7.01 ± 0.03</td>
<td>0.05 ± 0.006*</td>
<td>7.02 ± 0.03</td>
<td>6.97 ± 0.03</td>
<td>−0.05 ± 0.004*</td>
</tr>
<tr>
<td>1.5</td>
<td>6.97 ± 0.03</td>
<td>7.04 ± 0.03</td>
<td>0.08 ± 0.007*</td>
<td>7.06 ± 0.04</td>
<td>6.99 ± 0.03</td>
<td>−0.07 ± 0.007*</td>
</tr>
<tr>
<td>2.0</td>
<td>6.98 ± 0.04</td>
<td>7.10 ± 0.05</td>
<td>0.12 ± 0.01*</td>
<td>7.12 ± 0.05</td>
<td>7.01 ± 0.04</td>
<td>−0.11 ± 0.01*</td>
</tr>
<tr>
<td>3.0</td>
<td>7.01 ± 0.04</td>
<td>7.19 ± 0.06</td>
<td>0.18 ± 0.02*</td>
<td>7.21 ± 0.06</td>
<td>7.03 ± 0.04</td>
<td>−0.17 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 20 cells. *Significantly different from zero, P < 0.0001. †Significantly different from zero, P < 0.01.

Fig. 6. Effect of magnitude of osmotic challenge on ΔpHi in the presence of propionic acid-propionate. The mean ΔpHi values produced during both the On and Off phases (Off plotted with positive sign) for 20 cells are shown as a function of the relative osmolarity of the pulse. The curve is a fit of the data to A + B log10s, where s represents an ideal shrinkage factor (i.e., extracellular osmolality relative to 300 mosM). Best-fit values (±SD) were 0.009 ± 0.005 for A and 0.35 ± 0.02 for B in the equation.

Fig. 7. Representative effects on pHi of osmotic challenges in the presence of NH3-NH4+. A: initial exposure to 20 mM total NH3-NH4+ produced a rapid alkalinization, as NH3 entered the cell and combined with H+ to form NH4+. In the continued presence of ammonium, the pHi recovered back toward the starting value over a period of 10–15 min. The cell was then pulsed with differing amounts of added mannitol to alter extracellular osmolarity. B: similar responses to 2T in NH3 were produced when extracellular osmolarity was altered using 300 mM mannitol, 300 mM sucrose, or 150 mM NaCl.

Fig. 8. pHi dependence of ΔpHi produced by 2T in NH3. Neither On nor Off data showed a demonstrable dependence on starting pHi, unlike the case for weak acids. The slopes of lines fitted to the data were not significantly different from zero (P = 0.77 for On data and 0.20 for Off data). Figure 9 plots the mean ΔpHi during the On and Off phases in 24 cells (On data plotted with positive sign) as a function of the relative extracellular osmolarity. The absolute ΔpHi increased with increments in relative osmolarity between 1.05T and 3T. The curve in Fig. 9 is a fit of the data to the equation A + B log10s. The observed ΔpHi values are means ± SE; n = 20 cells. *Significantly different from zero, P < 0.0001. †Significantly different from zero, P < 0.01.
base buffer (NH$_3$) caused the pH$_i$ to decrease rapidly
ing extracellular osmolarity in the presence of a weak
the extracellular pH change (10). Conversely, increas-
ers playing an important role in determining the size of
volume on plasmalemma acid-base transport (7, 11,
ined in this study. Other investigators have deter-
13). However, the present results suggest that changes
of the expected
D
CO$_2$ or propionic acid.

**DISCUSSION**

Increases in extracellular osmolarity were accompa-
nied by changes in pH$_i$ under all circumstances exam-
inied in this study. Other investigators have deter-
mained the effects of osmotically induced changes in cell
volume on plasmalemma acid-base transport (7, 11,
13). However, the present results suggest that changes
in pH$_i$ also can result from alterations in the equilib-
rium characteristics of the total cellular buffer system
(intrinsic buffers plus added weak acid-base buffers)
during changes in extracellular osmolarity and cell

In the presence of weak acid buffers (CO$_2$ or propi-
onic acid), exposure to 2T caused a rapid, reversible
alkalinization, the size of which was dependent on the
starting pH$_i$ (see Figs. 3 and 4). Varying the magnitude
of the osmotic challenge produced corresponding changes in the ΔpH$_i$ (see Fig. 6). This “intracellular
contraction alkalosis” is comparable to the plasma
acid-base disturbance described in vivo, wherein con-
traction of the extracellular fluid volume produces an
alkalosis associated with concentrating HCO$_3^-$ at a
constant CO$_2$, with intrinsic (i.e., nonbicarbonate) buff-
ers playing an important role in determining the size of the
extracellular pH change (10). Conversely, increas-
ing extracellular osmolarity in the presence of a weak
base buffer (NH$_3$), caused the pH$_i$ to decrease rapidly
(see Fig. 7), that is, an “intracellular contraction acido-
sis”. Changes in pH$_i$ in the presence of NH$_3$ were
graded with the size of the osmotic challenge (see Fig.
9) but did not correlate with the starting pH$_i$ (see Fig.
8). In absolute terms, the ΔpH$_i$ produced by osmotic
cell shrinkage in NH$_3$ was larger than that observed in
CO$_2$ or propionic acid.

The **APPENDIX** presents a simple qualitative analysis
of the expected ΔpH$_i$ elicited by cell shrinkage in the

Some of these issues have been addressed previously
with alveolar macrophages (15). In suspensions of al-
veolar macrophages under CO$_2$-free conditions, addi-
tion of 320 mM sucrose to the external solution pro-
duced an approximate halving of cell volume, and
macrophage volume remained at the smaller level for
(at least) 10 min. The shrinkage was associated with
an approximate doubling of $\beta_{\text{int}}$ at a given pH$_i$. Na$^+$/H$^+$
exchange (amiloride-sensitive and Na$^+$-dependent re-
covery from acid loads) was activated by osmotic cell
shrinkage. This is consistent with the slow but large
pH$_i$ increase observed in the present study in CO$_2$-free
solution without added weak acid-base (see Fig. 1). In
the presence of CO$_2$, additional acid-base transporters
(e.g., Cl$^-$/HCO$_3^-$ exchangers) could contribute to
changes in pH$_i$ after cell volume perturbations. For
this reason, we chose propionic acid as a model weak
acid; it rapidly permeates the macrophage plasma
membrane (4) and is unlikely to support plasmalemma
HCO$_3^-$-dependent transport. Osmotic cell shrinkage
produced similar rapid pH$_i$ transients in the presence

![Fig. 8. pH$_i$ dependence of responses to 2T in the presence of NH$_3$-NH$_4^+$ for 28 cells.](http://example.com/fig8)

![Fig. 9. Effect of magnitude of osmotic challenge on ΔpH$_i$ in the presence of NH$_3$-NH$_4^+$ for 28 cells.](http://example.com/fig9)
of CO₂ or propionic acid. Thus it is unlikely that the responses were mediated via Cl⁻/HCO₃⁻ exchange. The rapid pHᵢ responses in propionic acid were insensitive to EIPA or bafilomycin A₁. Hence, it also is unlikely that the responses were mediated via Na⁺/H⁺ exchange or the H⁺ pump. Furthermore, osmotic challenges caused pHᵢ to change in opposite directions in the presence of a weak acid buffer (i.e., contraction alkalosis) vs. a weak base buffer (i.e., contraction acidosi). It is difficult to imagine how shrinkage-activated acid-base transport could produce such divergent pHᵢ responses. These findings suggest that the rapid pHᵢ responses were not dependent on the effects of osmotic cell shrinkage on membrane ion transporters.

The simplest explanation is that the rapid pHᵢ responses reflected disequilibria in the total cellular buffer system due to osmotic changes in cell volume.

This interpretation of the data is consistent with all salient features of the pHᵢ responses. As shown in the APPENDIX, a shrinkage-induced disequilibrium in the total cellular buffer system is predicted to produce a cytosolic alkalization in the presence of a weak acid buffer (e.g., CO₂ or propionic acid) and a cytosolic acidification in the presence of a weak base buffer (e.g., NH₃). Such paradoxical changes in pHᵢ were observed experimentally. The analysis in the APPENDIX also predicts that the magnitude of the pHᵢ response (ΔpHᵢ) should be dependent on the size of the osmotic challenge (s) and βᵢₑ, reaching a maximum value of + log₁₀s when βᵢₑ is zero (direction of change in pHᵢ determined by presence of weak acid vs. weak base). The observed ΔpHᵢ values varied directly with s (see Figs. 6 and 9) and were 35–74% of the predicted maximum, in keeping with the presence of intrinsic buffers in alveolar macrophages (15).

If cell shrinkage induced a disequilibrium in the total cellular buffer system, then reequilibration should be determined by competing reactions between intrinsic buffers and the added weak acid-base buffer (i.e., CO₂·HCO₃⁻, propionic acid-propionate, or NH₃·NH₄⁺). Although the kinetics of these competing reactions are not known, we can compare the relative contribution of each reaction to the final equilibrium state by examining the buffering powers of the individual buffers. Alveolar macrophages in isotonic solution have an βᵢₑ of ~20 mM/pH at pH 7.1 (15). At the same pHᵢ in the presence of 5% CO₂, the intracellular HCO₃⁻ concentration is calculated to be ~12 mM, yielding an intracellular bicarbonate buffering power (βbicarb) of ~28 mM/pH (assuming the apparent CO₂·HCO₃⁻ equilibrium constant and CO₂ concentration are the same inside and outside the cell and that CO₂·HCO₃⁻ behaves as an open-system buffer such that βbicarb is 2.303 times the concentration of HCO₃⁻). In the presence of 20 mM propionic acid-propionate or NH₃·NH₄⁺, the intracellular propionate buffering power (βprop) is calculated to be ~23 mM/pH and the intracellular ammonium buffering power (βammonium) to be ~92 mM/pH (using similar assumptions as above). The absolute ΔpHᵢ elicited by osmotic cell shrinkage was larger in the presence of NH₃ than weak acids, whereas similar ΔpHᵢ values were detected in experiments with CO₂ and propionic acid, in keeping with βammonium > βbicarb > βprop.

The approach of comparing buffering powers can also be used to explain the different sensitivities of ΔpHᵢ to starting pHᵢ in experiments with weak acids vs. weak bases. βbicarb, βprop, βammonium, and βint are all sensitive to pH. In experiments with CO₂ or propionic acid, the conjugate base concentration (and hence, βbicarb or βprop) increased with increments in pHᵢ, following the law of mass action. In contrast, the βint of alveolar macrophages decreased at higher pHᵢ values (15). Thus the relationship between the buffering powers of intrinsic buffers vs. the added weak acid buffer (hence, the magnitude of the pHᵢ responses to osmotic cell shrinkage) should be relatively sensitive to starting pHᵢ in the presence of CO₂ or propionic acid. On the other hand, in experiments with NH₃, the conjugate acid concentration (hence, βammonium) decreased with increments in pHᵢ, along with the pH-induced decrements in βint. Thus the relationship between the buffering powers of intrinsic buffers vs. the added weak base buffer (hence, ΔpHᵢ) should be relatively insensitive to starting pHᵢ in the presence of NH₃. In keeping with this interpretation of the data, ΔpHᵢ was dependent on the starting pHᵢ value in weak acid experiments but was not correlated with the starting pHᵢ in NH₃ experiments.

The present findings are probably not unique to alveolar macrophages. We have previously observed rapid changes in pHᵢ during changes in extracellular osmolarity in the presence of CO₂·HCO₃⁻ in renal mesangial cells (Boyarsky, unpublished results). Conditions exist in vivo in which cells are exposed to diverse osmotic microenvironments in the presence of multiple open-system buffers (e.g., CO₂ and NH₃), particularly in the kidney. The effects on pHᵢ of alterations in extracellular osmolarity in a multiple buffer system are unclear. Furthermore, although the present studies focussed on the responses to hyperosmotic cell shrinkage, the pHᵢ effects were reversed On return to the original tonicity (with consequent cell swelling to recover macrophage volume). This observation suggests that “expansion” acidoses and alkaloses might occur during hypotonic cell swelling in the presence of weak acids and weak bases, respectively.

The experimental cells were highly sensitive to increases in extracellular osmolarity. Statistically significant changes in pHᵢ occurred with only 5–10% increases in extracellular osmolarity (see Figs. 6 and 9). This raises the possibility that the responses have physiological/pathophysiological relevance. A large body of evidence indicates that recovery of cell volume following osmotic cell shrinkage involves the activation of plasmalemma acid-base transporters and often is accompanied by transporter-mediated changes in pHᵢ with a time course of several minutes (8, 9, 12–15, 18, 20). The present results show that cell shrinkage under physiological conditions (5% CO₂) can produce rapid increases in pHᵢ with a time course of several seconds, most likely due to disequilibria in the total cellular buffering system. The duration of these pHᵢ shifts was
not determined. Nonetheless, such rapid responses will define the initial changes in pH that occur after osmotic cell shrinkage under physiological conditions. It is worthwhile to consider, therefore, that rapid buffer-associated pH changes are involved in the allosterical activation of the acid-base transporters that play a role in the subsequent recovery of cell volume.

Regardless of the specific mechanisms involved (transporter-mediated or buffer-associated), any change in pH that accompanies changes in cell volume will effectively reset the relationship between pH and extracellular pH. As such, pH responses to cell volume changes will influence the functioning of physiological systems that track extracellular acid-base status. For example, in animal studies, Kasserra and co-workers (16, 17) found that intravenous infusion of a hyperosmotic solution caused a prolonged blood acidosis (dilution acidosis) and hypercarbia without eliciting a compensatory increase in ventilation. The hyperosmotic challenge also produced alkaline shifts in the tissue pH of skeletal muscle (i.e., a tissue contraction alkalosis). Assuming that a similar pH shift occurred in chemoreceptive cells (intracellular alkalosis), the authors suggested that aniosmotic-induced changes in pH caused the chemoreceptors to mistrack blood acid-base status, and this impaired respiratory compensation of the acid-base disturbance. The present data suggest that uncoupling of intracellular and extracellular pH can have a rapid onset, before the involvement of volume-activated plasmalemma acid-base transporters.

**APPENDIX: ANALYSIS OF pH CHANGES PRODUCED BY CHANGES IN CELL VOLUME**

The assumptions and simplifications of this analysis are outlined above. Volume changes are expected to produce little change in pH in the presence of a closed-system buffer (i.e., a situation in which the total amount of cytosolic buffer remains constant). In this case, we assume that equilibrium of the buffer (Buf) is described as follows (subscript 1 denotes initial conditions)

\[
\text{Buf} + H^+ \leftrightarrow \text{HBuf}^+ \quad K_{eq} = \frac{[\text{HBuf}^+][H^+]}{[\text{Buf}]} \tag{1}
\]

where \(K_{eq}\) is the equilibrium constant. If the cell volume is shrunk by a factor \(s\), then, initially, the concentrations of all constituents increase by the same factor (subscript 2). Now, the buffer is no longer in equilibrium (dropping concentration brackets and superscripts)

\[
\frac{\text{Buf}_2 H_2}{\text{HBuf}_2} = \frac{(s\text{Buf})(sH_2)}{(s\text{HBuf})} = sK_{eq} \tag{2}
\]

and the following reaction occurs until reestablishment of equilibrium (subscript 3)

\[
\text{Buf} + H \rightarrow \text{HBuf} \quad K_{eq} = \frac{\text{Buf}_3 H_3}{\text{HBuf}_3} \tag{3}
\]

The extent of an equilibrating reaction \((x)\) is given by the following relationships

\[
\text{Buf}_x = \text{Buf}_2 - x \quad \text{HBuf}_x = \text{HBuf}_2 + x \\
H_3 = H_2 - x \tag{4}
\]

Equation 4 assumes that there is no other buffer present, which obligates a 1:1 consumption of \(H^+\) along with Buf in producing HBuf. One approach to understanding why the pH returns to normal (i.e., \(H_3 \approx H_1\)) is to plug into Eq. 3 from Eqs. 1, 2, and 4

\[
\frac{(s\text{Buf}_1 - x)(sH_1 - x)}{(s\text{HBuf}_1 + x)} = K_{eq} \tag{5}
\]

Rearrangement yields

\[
(sH_1 - x) = K_{eq} \frac{(s\text{HBuf}_1 + x)}{(s\text{Buf}_1 - x)} \tag{6}
\]

Ignoring the \(s\) terms on the right (because \(s\) is only nM compared with the mM of Buf and HBuf) and canceling the \(s\) terms leaves the following

\[
(sH_1 - x) = K_{eq} \frac{\text{HBuf}_1}{\text{Buf}_1} = H_1 \tag{7}
\]

The last equation results from the original equilibrium definition (Eq. 1). Thus, by rearrangement

\[
x = (s - 1)H_1 \tag{8}
\]

Plugging into the value for \(H_3\) (Eq. 4) and \(H_2\) (Eq. 2), one obtains the following

\[
H_3 = H_2 - x = sH_1 - (s - 1)H_1 = H_1 \tag{9}
\]

**Rapid Loss of Cellular Water in the Presence of a Weak Acid**

The response is different in the presence of an open-system buffer (i.e., a situation in which the total amount of cytosolic buffer can vary). We will assume that \(CO_2-HCO_3^-\) is present and that \(CO_2\) rapidly equilibrates across the cell membrane. Then, the \(CO_2-HCO_3^-\) buffer pair obey the following equilibrium

\[
H^+ + HCO_3^- \leftrightarrow CO_2 + H_2O \quad K_{eq} = \frac{[H^+][HCO_3^-]}{[CO_2][H_2O]} \tag{10}
\]

Assuming there is a pure loss of water producing cell shrinkage by \(s\), we can consider two extreme cases. In the first case, there are no intrinsic buffers \((\beta_{int} = 0)\). The concentrations of \(HCO_3^-\) and \(H^+\) then change by \(s\). \(CO_2\) freely permeates the plasma membrane and, consequently, remains constant. A disequilibrium is created

\[
HCO_3^- \rightarrow sHCO_3^- \quad CO_2 = CO_2 \tag{11}
\]

The following reaction occurs until a new equilibrium is reached (subscript 3)

\[
H^+ + HCO_3^- \rightarrow CO_2 \uparrow \quad \left(\frac{H_3}{CO_2}\right) = \frac{K_{eq}}{sK_{eq}} \tag{12}
\]

Inasmuch as \(\beta_{int} = 0\), the \(H^+\) concentration decreases with little change in the \(HCO_3^-\) concentration because stoichiometric coupling of the changes in \(H^+\) (nM) and \(HCO_3^-\) (mM) force reequilibration with nanomolar changes in each. Ignor-
ing the nanomolar change in HCO₃⁻ concentration, Eq. 12 can be rearranged as follows

\[
(H_3) = \frac{(HCO_3^-)}{(HCO_3^-)}(H_1)
\]

\[
HCO_3^- = HCO_3^- = sHCO_3^- (13)
\]

\[
H_3 = (1/\text{s})H_1
\]

Thus, with \( \beta_{\text{int}} = 0 \), the H⁺ concentration decreases by a factor 1/s or pH increases by \( \log_{10} s \). In the second extreme case, \( \beta_{\text{int}} \) is assumed to be infinite. Here, we expect to see no change in the H⁺ concentration but large changes in the HCO₃⁻ concentration. Shrinkage produces changes in the HCO₃⁻ concentration, which result in disequilibrium

\[
HCO_3^- = sHCO_3^- (14)
\]

\[
(CO_2^-) = \frac{(H_3)(HCO_3^-)}{(CO_2^-)} = sK_{\text{eq}}
\]

Reestablishment of equilibrium is again accomplished by the reaction in Eq. 12

\[
(H_3) = \frac{(HCO_3^-)}{(HCO_3^-)}(H_1)
\]

\[
H_3 = H_2 = H_1
\]

\[
HCO_3^- = HCO_3^- (15)
\]

Thus osmotic cell shrinkage in the presence of a weak acid causes a ΔpH, that ranges in magnitude from zero (infinite \( \beta_{\text{int}} \)) to a maximum of \( \log_{10} s \) (\( \beta_{\text{int}} = 0 \)). In experiments with CO₂ and propionic acid, hyperosmotic challenges produced alkalinizations equivalent to 0.35 \( \log_{10} s \) (i.e., 35% of the expected maximum), consistent with the modest \( \beta_{\text{int}} \) of alveolar macrophages (15).

**Rapid Loss of Cellular Water in the Presence of a Weak Base**

We will assume that NH₃-NH₄⁺ is present and that NH₃ rapidly equilibrates across the cell membrane. Then, the NH₃-NH₄⁺ buffer pair obey the following equilibrium

\[
\text{H}^+ + \text{NH}_3 \leftrightarrow \text{NH}_4^+
\]

\[
K_{\text{eq}} = \frac{[\text{H}^+][\text{NH}_3]}{[\text{NH}_4^+]} (16)
\]

We again consider that the cell loses water to undergo shrinkage by \( s \) and that this produces proportional increases in all constituents except NH₃ (which freely permeates the cell membrane)

\[
\text{NH}_3^+ = s(\text{NH}_4^+),
\]

\[
H_2 = sH_1, \quad \text{NH}_3^+ = s\text{NH}_4^+ (17)
\]

\[
\frac{(H_3)(\text{NH}_3^+)}{(H_4^+)} = \frac{(H_1)(\text{NH}_4^+)}{(s\text{NH}_4^+)} = K_{\text{eq}}
\]

During osmotic cell shrinkage, the ammonium buffer pair remains in equilibrium with the increase in \( \text{H}^+ \) proportionally balanced by the increase in \( \text{NH}_4^- \). Thus, in the extreme case with \( \beta_{\text{int}} = 0 \), the \( \text{H}^+ \) concentration remains elevated at \( s[H_1] \) and pH changes by \( \log_{10}s \), that is, pH decreases. On the other hand, in the presence of intrinsic buffers (i.e., \( \beta_{\text{int}} > 0 \)), although the ammonium buffer pair remains in equilibrium during osmotic cell shrinkage, the intrinsic buffers do not

\[
\text{Buf}_2 = s\text{Buf}_1
\]

\[
\text{HBuf}_2 = s\text{HBuf}_1 \quad H_2 = sH_1
\]

\[
\frac{(H_1)(\text{HBuf}_1)}{(H_2)} = \frac{(H_1)(\text{HBuf}_1)}{(s\text{HBuf}_1)} = sK_{\text{eq}}
\]

The following reactions then occur until equilibrium is reestablished (subscript 3)

\[
\text{Buf} + \text{H}^+ \rightarrow s\text{HBuf}^+ \quad \text{NH}_4^+ \rightarrow \text{NH}_3 + \text{H}^+
\]

\[
\frac{(H_3)(\text{NH}_3^+)}{(H_4^+)} = \frac{(H_3)(\text{NH}_3^+)}{(s\text{NH}_4^+)} = K_{\text{eq}} = \frac{(H_3)(\text{NH}_3^+)}{(H_4^+)} (19)
\]

Thus cell shrinkage in the presence of a weak base causes a decrease in pH that ranges in magnitude from \( \log_{10} s \) (\( \beta_{\text{int}} = 0 \)) to zero (infinite \( \beta_{\text{int}} \)). In experiments with NH₃, hyperosmotic challenges produced acidifications equivalent to \( -0.74 \log_{10} s \) (i.e., 74% of the expected maximum), consistent with the modest \( \beta_{\text{int}} \) of alveolar macrophages (15).

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