Acetate Transport in the S3 Segment of the Rabbit Proximal Tubule and its Effect on Intracellular pH

NAZIH L. NAKHOUL and WALTER F. BORON

From the Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut 06510

ABSTRACT We monitored intracellular pH (pHᵢ) in isolated perfused S3 segments of the rabbit proximal tubule, and studied the effect of acetate (Ac⁻) transport on pHᵢ. pHᵢ was calculated from the absorbance spectrum of 4',5'-dimethyl-5-(and 6) carboxyfluorescein trapped intracellularly. All solutions were nominally HCO₃⁻-free. Removal of 10 mM Ac⁻ from bath and lumen caused pHᵢ to rapidly rise by ~0.2, and then to decline more slowly to a value ~0.35 below the initial one. Removal of only luminal Ac⁻ caused pHᵢ changes very similar to those resulting from bilateral removal of Ac⁻. When Ac⁻ was removed from bath only, pHᵢ rose rapidly at first, and then continued to rise more slowly. Readdition of Ac⁻ to bath caused pHᵢ to rapidly fall to a value slightly higher than the one prevailing before the removal of Ac⁻ from the bath. In experiments in which Ac⁻ was first removed from both bath and lumen, readdition of 10 mM Ac⁻ to only lumen caused a rapid but small acidification, followed by a slower alkalinization that brought the pHᵢ near the value that prevailed before the bilateral removal of Ac⁻. The alkalinizing effects caused by the readdition of 10 or 0.5 mM Ac⁻ were indistinguishable. When Ac⁻ was returned to only lumen in the absence of luminal Na⁺, there was a small and rapid pHᵢ decrease, but no pHᵢ recovery. Removal of Na⁺ from bath did not affect the pHᵢ transients caused by the addition of Ac⁻ to lumen. In experiments in which Ac⁻ was first removed bilaterally, readdition of Ac⁻ to only bath caused a large and sustained drop in pHᵢ whereas the subsequent removal of Ac⁻ from the bath caused a slight alkalinization. These pHᵢ changes caused by readdition or removal of Ac⁻ from baths were unaffected by the absence of external Na⁺. We conclude that there is a Na⁺/Ac⁻ cotransporter at the luminal membrane, and pathways for acetic acid transport at both luminal and basolateral membranes. The net effect of Ac⁻ transport on pHᵢ is to alkalinize the cell as a result of the luminal entry of Na⁺/Ac⁻, which is followed by the luminal and basolateral exit of acetic acid.

INTRODUCTION

Nearly 70% of the reabsorptive work of the kidney involves the transport of solutes by the proximal tubule. Monocarboxylic acids and other metabolic substrates that...
are taken up from the lumen by cells of the proximal tubule can be handled in one of three ways. First, some of these organic solutes may be metabolized in the proximal-tubule cell to produce energy. It is known that proximal-tubule cells metabolize lactate, pyruvate, and a variety of Kreb's cycle intermediates (Mandel, 1985). Moreover, work on whole kidneys has established that both acetate (Ac-) (Mudge, 1951) and acetoacetate (Guder et al., 1986) are excellent fuels. The second possible fate of some of these organic solutes is the conversion from one substrate to another; for example, from lactate to glucose (Gullans et al., 1984). Finally, some of the organic solutes taken up from the lumen can be transferred to the basolateral solution. Evidence for transcellular reabsorption comes from whole kidney experiments in which it has been found that the oxidation of organic substrates such as lactate is a small fraction of the amount reabsorbed (Cohen and Barac-Nieto, 1973). In the isolated tubule preparation of the rabbit S2 segment (Schafer and Andreoli, 1976), evidence was provided for an energy-dependent process responsible for the reabsorption of Ac- from the lumen.

In spite of its potential importance, relatively little is known about monocarboxylic-acid transport in intact proximal tubules or proximal-tubule cells. Major difficulties are the metabolism of these substrates and the lack of nonmetabolized analogues (see Wright, 1985). For these reasons, much of what is known is derived from studies using vesicle preparations. Four distinct mechanisms for the transport of monocarboxylates have been demonstrated or proposed. The first is a Na+-coupled monocarboxylate cotransporter. For example, evidence for luminal Na+/lactate cotransport in the proximal tubule is derived from studies on brush border vesicles of rat (Barac-Nieto et al., 1980), rabbit (Nord et al., 1983), and horse (Mengual and Sudaka, 1983). In a study on intact tubules, Siebens and Boron (1987) have reported evidence for luminal Na+/lactate cotransport in the isolated proximal tubule of the salamander. A second proximal-tubule monocarboxylate transport mechanism, identified in membrane-vesicle studies, exchanges Cl- for either urate (Kahn and Aronson, 1983) or formate (Schild et al., 1986). Formate and urate are not substrates for the Na+-coupled monocarboxylate system, nor are the other monocarboxylates substrates for the Cl- exchange system. A third example of monocarboxylate transport is H+/monocarboxylate cotransport (or monocarboxylate/base exchange). H+/lactate cotransport has been reported in Ehrlich ascites tumor cells (Spencer and Lehninger, 1976), cardiac Purkinje fibers (DeHemptinne et al., 1983), and the basolateral membrane of the salamander proximal tubule (Siebens and Boron, 1987). Finally, one might expect the neutral, protonated form of weak monocarboxylic acids to move across plasma membranes by simple nonionic diffusion.

The studies summarized above indicate that there is limited information at the cellular level concerning monocarboxylate transport. In particular for epithelial cells, it is crucial to understand how the transport of monocarboxylates at the luminal membrane interacts with the transport at the basolateral membrane. Inasmuch as monocarboxylates are weak bases, one might expect their transport to affect and to be affected by pH. However, although pHi as well as extracellular pH affect the net efflux of lactate from the rat proximal-tubule lumen (Ullrich et al., 1982), it is not yet known how the transport of monocarboxylic acids might affect pHi in mammalian tubules.
In the preceding paper (Nakhoul et al., 1988) we found, in the isolated perfused S3 segment of the rabbit proximal tubule, that a major component of the pH recovery from intracellular acid loads requires both luminal Na+ and luminal Ac−. The latter is a common component of mammalian Ringer’s solutions. In the present study, we have investigated the ionic mechanism of this Na+ and Ac−-dependent acid-extrusion process by studying the effect of Ac− transport on pH. To minimize the effects of HCO3− transport, experiments were performed in nominally HCO3−-free solutions. pH was calculated from the absorbance spectrum of a pH-sensitive dye incorporated into the proximal-tubule cells. We found that: (a) there is a potent Na+/Ac− cotransport system at the luminal membrane; (b) acetic acid (HAc) is transported across both the luminal and basolateral membranes, either by nonionic diffusion of HAc, a H+/Ac− cotransporter, or by an Ac−/base exchanger; and that (c) the combined effect of the Na+/Ac− and HAc transport systems is a net extrusion of acid from the cell, and the likely net reabsorption of Ac−. The former leads to a substantial increase in steady-state pH. Thus, at least in the nominal absence of HCO3-, these Ac− transport systems have a major effect on pH regulation.

METHODS

The details of the methodology are contained in the preceding paper (Nakhoul et al., 1988). The experiments were performed on isolated perfused S3 segments of the rabbit proximal tubule. Tubule cells were loaded with the pH-sensitive dye 4',5'-dimethyl-5-(and 6) carboxyfluorescein. The absorbance spectrum of this dye was determined by illuminating a tubule with a 10-μm diam spot of white light, and focusing the transmitted light on a 1024-element photodiode array. pH was calculated from the absorbance spectrum, employing a calibration obtained with nigericin/high-K+ solutions (see Thomas et al., 1979). The compositions of the basolateral (i.e., bath) and luminal solutions are given in Table I. All experiments were performed in the nominal absence of HCO3− to minimize the contribution of HCO3−-dependent acid/base transporters to the measurement of pH. The temperature in the chamber was maintained at 38°C.

Statistics and Data Analysis

Means are reported ± SE. Statistical significance was judged from paired and unpaired Student’s t test, as indicated in the text. Rates of pH change were determined by using a computer to fit the pH vs. time data to a straight line.

RESULTS

Bilateral Removal of Ac− and Phosphate

We first examined the effect on pH of removing Ac− and inorganic phosphate (Pi), the only two buffers (other than HEPES) normally present in our standard HCO3−-free solution. As shown in Fig. 1, bilaterally exposing the tubule to solutions lacking both Ac− and Pi (solution 2, Table I) results in a transient rise in pH (ab) followed by a slower decline to a value far below the initial one (bc). In a series of similar experiments, the average transient rise of pH was 0.19 ± 0.01 (n = 32), whereas the average decline below the initial value (comparing points a and c) was 0.35 ± 0.03 (n = 17). When Ac− and Pi are returned to the luminal and basolateral solutions, the pH transients are similar but opposite in direction to those elicited by removal of...
FIGURE 1. Effect on pH$_i$ of bilateral removal and readdition of external Ac$^-$ and phosphate. Removal of Ac$^-$ and phosphate resulted in a rapid rise in pH$_i$ (ab), followed by a slower decline (bc). Readdition of Ac$^-$ and phosphate bilaterally caused a small and rapid decrease in pH$_i$ (cd), followed by a slower increase (de). Eight similar experiments were performed.

TABLE I

| Component | 1 Standard HEPES | 2 0 Ac$^-$, 0 Pi, HEPES | 3 0 Pi, HEPES | 4 0 Ac$^-$, 0 Pi, HEPES | 5 0 Na$^+$, 0 Ac$^-$, HEPES | 6 Citrate, HEPES |
|-----------|-----------------|------------------------|-------------|------------------------|--------------------------|------------------|
| Na$^+$    | 146.4           | 145.8                  | 146.4       | 146.4                  | 0                        | 146.4            |
| K$^+$     | 5.0             | 5.0                    | 5.0         | 5.0                    | 5.0                      | 5.0              |
| NMDG$^+$  | 0               | 0                      | 0           | 0                      | 145.8                    | 0                |
| Mg$^{2+}$ | 1.2             | 1.2                    | 1.2         | 1.2                    | 1.2                      | 1.2              |
| Ca$^{2+}$ | 1.0             | 1.0                    | 1.0         | 1.0                    | 1.0                      | 1.0              |
| meq (+)   | 155.8           | 155.2                  | 155.8       | 155.8                  | 155.2                    | 155.8            |
| Cl$^-$    | 120.0           | 135.0                  | 125.6       | 132.0                  | 131.2                    | 132.0            |
| H$_2$PO$_4^-$ | 0.4 | 0                      | 0.4         | 0.4                    | 0.4                      | 0.4              |
| HEPS$^-$  | 1.6             | 0                      | 1.6         | 1.6                    | 1.6                      | 1.6              |
| Ac$^-$    | 10.0            | 0                      | 10.0        | 0                      | 0                        | 0                |
| HEPES     | 17.8            | 17.8                   | 17.8        | 17.8                   | 17.8                     | 17.8             |
| SO$_4^{2-}$ | 1.2          | 1.2                    | 1.2         | 1.2                    | 1.2                      | 1.2              |
| meq (-)   | 155.8           | 155.2                  | 155.8       | 155.8                  | 155.2                    | 155.8            |
| Glucose   | 5.5             | 5.5                    | 5.5         | 5.5                    | 5.5                      | 5.5              |
| Alanine   | 5.0             | 5.0                    | 5.0         | 5.0                    | 5.0                      | 5.0              |
| HEPES     | 14.4            | 14.4                   | 14.4        | 14.4                   | 14.4                     | 14.4             |
| pH        | 7.4             | 7.4                    | 7.4         | 7.4                    | 7.4                      | 7.4              |

All concentrations are given in millimolar. NMDG$^+$, N-methyl-D-glucamine.
Ac− and Pi; there is first a transient pHi decline (cd), although this is barely evident in the experiment of Fig. 1, and then a slower but sustained pHi increase (de) to the value that prevailed before the removal of Ac− and Pi.

**Bilateral Removal of Ac−**

To determine which of the two buffer species, Ac− or Pi, was responsible for these pHi changes, we next removed either Ac− or Pi bilaterally. In the experiment of Fig. 2, bilateral removal of Pi only (solution 3) results in a very slight decrease in pHi (ab) that is reversed (bc) when Pi is returned to the external solution. In contrast, bilateral removal of Ac− only (solution 4) results in a transient rise in pHi (cd), followed by a slower but profound decrease in pHi (de). The pHi changes are reversed upon returning external Ac− (efg). Four similar experiments were performed.

![Diagram](image)

**FIGURE 2.** Effect on pHi of bilateral removal of either Ac− or phosphate from the external solutions. Bilateral removal of only phosphate caused a very slight acidification (ab and gh), which was reversed when phosphate was restored (bc and hi). Bilateral removal of only Ac− caused a rapid, transient rise in pHi (cd), followed by a slower decline (de). Readdition of Ac− produced the opposite changes in pHi (efg). Four similar experiments were performed.

by a slower but profound decrease in pHi (de). The pHi changes are reversed upon returning external Ac− (efg). These patterns of pHi changes are very similar to those caused by the bilateral removal of both Ac− and Pi (see Fig. 1). Later in the experiment of Fig. 2, Pi is removed and restored a second time (gh). Comparison of the separate effects of Pi and Ac− on pHi in this experiment leads us to conclude that, under the conditions of these experiments, Ac− transport has a much greater ability to affect pHi than Pi transport. Moreover, the biphasic pHi transients observed by simultaneously altering both [Ac−] and [Pi] (see Fig. 1) can be reproduced by altering only [Ac−]. The rapid but small spiking changes in pHi that accompany Ac− removal (cd) and readdition (ef) are probably due to the net movement of HAc out of or into the cell, respectively. The slow but substantial pHi changes that accom-
pany Ac\textsuperscript{−} removal (de) or readdition (fg) are presumably due, respectively, to the blockade and reinitiation of an Ac\textsuperscript{−}-dependent acid-extruding process.

**Unilateral Removal or Readdition of Ac\textsuperscript{−}**

**Unilateral removal of Ac\textsuperscript{−}**. To determine the sidedness of Ac\textsuperscript{−} transport, we removed Ac\textsuperscript{−} from either the lumen or the bath. As shown in Fig. 3, removal of Ac\textsuperscript{−} from the lumen only (solution 4) causes a transient rise in pH\textsubscript{i} (ab) that is followed by a large and sustained fall, very similar to the changes caused by removal of Ac\textsuperscript{−} from both lumen and bath (see Figs. 1 and 2). Restoration of luminal Ac\textsuperscript{−} causes pH\textsubscript{i} to recover promptly (cd). One might expect the readdition of luminal Ac\textsuperscript{−} to cause a small transient acidification, as observed for the bilateral addition of Ac\textsuperscript{−} in the experiment of Fig. 2. However, such an acidification generally was not observed when the pH\textsubscript{i} prevailing before the addition of Ac\textsuperscript{−} was relatively low (<6.8). This is to be expected if the transient acidification is due to the influx of HAc, inasmuch as the dissociation of intracellular HAc into H\textsuperscript{+} and Ac\textsuperscript{−} is reduced at a low pH\textsubscript{i}. Note that the pH\textsubscript{i} prevailing before the removal of luminal Ac\textsuperscript{−} (point a) is higher than the final pH\textsubscript{i} after luminal Ac\textsuperscript{−} is returned (d). This implies that, during the period of luminal Ac\textsuperscript{−} removal (ac), when Ac\textsuperscript{−} was still present in the bath, there was a net accumulation of acid by the proximal-tubule cells.

In the same tubule, when Ac\textsuperscript{−} is removed from the bath only, there is a rapid rise in pH\textsubscript{i} (de), followed by a very slow upward drift (ef). Readdition of Ac\textsuperscript{−} to the bath
solution reverses this effect, causing pH_i to quickly fall (fg) and then stabilize (gh). Note that the pH_i prevailing before the removal of basolateral Ac^- (d) is lower than that prevailing after the return of basolateral Ac^- (gh). This implies that during the period of basolateral Ac^- removal, when Ac^- was still present in the lumen, there was a net depletion of acid from the proximal-tubule cells.

Provisional model. The data presented thus far can be accounted for by the following working model. We propose that the proximal-tubule cell has a pathway for HAc transport at both the luminal and basolateral membranes. This pathway could permit simple nonionic diffusion of HAc, or be a transporter (e.g., a H^+/Ac^- cotransporter or an Ac^-/base exchanger). In addition, we propose that the proximal-tubule cell possesses a Na^+-dependent pathway for Ac^- transport at the luminal membrane only, as suggested by the data of the preceding paper (Nakhoul et al., 1988). Under normal conditions, the action of this luminal Na^+/Ac^- cotransporter and the HAc pathways would be to alkalinize the cell. According to this model, the rapid pH_i increase caused by the removal of luminal Ac^- in the experiment of Fig. 3 (ab) is due to the efflux of HAc across the luminal membrane. The slower fall in pH_i (bc) is caused by two factors. First, in the absence of luminal Ac^-, the Na^+/Ac^- cotransporter can no longer participate in alkalinizing the cell. The underlying production of acid thus causes pH_i to fall. Second, HAc continues to enter across the basolateral membrane, and dissociate into Ac^- and H^+. The former leaves the cell via the luminal Na^+/Ac^- cotransporter, whereas the latter is trapped inside, thereby further acidifying the cell. Thus, there is a net accumulation of acid within the cell during the period of luminal Ac^- removal.

Returning Ac^- to the lumen causes a small (inapparent) acidification due to the entry of HAc, followed by a pH_i recovery (ad). This intracellular alkalinization can occur only as the result of two separate transport processes. The entry of Ac^- across the luminal membrane can by itself raise pH_i only by a trivial amount, inasmuch as the pK for the reaction Ac^- + H^+ ~ HAc (i.e., ~4.8) is far lower than the prevailing pH_i (i.e., ~7.0). However, the entering Ac^- can exit the cell together with a H^+ across both the luminal and basolateral membranes, thereby causing the pH_i increase observed during ad. The effects of basolateral Ac^- removal can be similarly explained. The rapid pH_i increase (de) is due to the efflux of HAc across the basolateral membrane. However, intracellular HAc is replenished by the entry of Ac^- across the luminal membrane and its subsequent protonation. Thus, HAc continues to slowly exit across the basolateral membrane, and the cell slowly alkalinizes (ef).

Unilateral Readdition of Ac^- To examine this hypothesis further, we performed an additional set of experiments in which Ac^- was first removed from both the lumen and bath, and then returned either to the lumen or to the bath. As shown in Fig. 4, bilateral removal of Ac^- leads to the usual rapid alkalinization (ab), followed by a slower acidification (bc). Readdition of Ac^- to only the bath causes a sustained drop in pH_i (cd). This acidification should have two components. First, the basolateral influx and dissociation of HAc causes a small, rapid acidification that is difficult to discern. Second, the continuing basolateral influx of HAc, its intracellular dissociation into H^+ and Ac^-, and the luminal exit of Ac^- causes a sustained fall of pH_i. The removal of Ac^- from the bath causes a slight alkalinization (de), which is caused by the basolateral efflux of HAc. Note that the pH_i prevailing before the addition of basolateral Ac^- (c) is substantially higher than the final pH_i value after the removal
of the \( \text{Ac}^- \) (e). This reflects the intracellular accumulation of acid during the exposure to basolateral \( \text{Ac}^- \) (cd).

When \( \text{Ac}^- \) is added only to the lumen, there is a rapid but small acidification (fg), followed by a slower but sustained alkalinization (gh). The transient \( \text{pH}_i \) decrease is due to the luminal influx of \( \text{HAc} \), whereas the sustained \( \text{pH}_i \) increase presumably is caused by the luminal entry of \( \text{Ac}^- \), followed by the bilateral exit of \( \text{HAc} \). Thus, the data from the experiments of Figs. 3 and 4 are consistent with the model of a \( \text{Na}^+ / \text{Ac}^- \) cotransporter at the luminal membrane, and bilateral pathways for \( \text{HAc} \) transport.

\[
\begin{align*}
\text{LUMEN} & \quad [\text{Ac}] & 10 & 0 & 10 \\
\text{BATH} & \quad [\text{Ac}] & 10 & 0 & 10 & 0
\end{align*}
\]

**Figure 4.** Effect on \( \text{pH}_i \) of unilateral addition of \( \text{Ac}^- \). As a preliminary step, \( \text{Ac}^- \) was removed bilaterally, causing the usual sequence of \( \text{pH}_i \) changes (abc). Addition of \( \text{Ac}^- \) to only the bath caused a sustained drop in \( \text{pH}_i \) (cd). Removal of \( \text{Ac}^- \) from the bath caused a rapid but slight rise in \( \text{pH}_i \) (de), followed by a very slight alkalinization (ef). Adding \( \text{Ac}^- \) to only the lumen caused a rapid, small decrease in \( \text{pH}_i \) (fg), followed by a slower increase (gh). Three similar experiments were performed.

\( \text{Na}^+ \) Dependence of the Luminal \( \text{Ac}^- \) Transport System

Our working model makes several predictions concerning experiments of the type shown in Figs. 3 and 4. First, the rapid spiking changes in \( \text{pH}_i \) caused by changes in luminal or basolateral [\( \text{Ac}^- \)], which we attribute to \( \text{HAc} \) fluxes, should not depend upon the presence of external \( \text{Na}^+ \). Second, the \( \text{pH}_i \) recovery caused by the addition of \( \text{Ac}^- \) to the lumen should not depend upon the presence of \( \text{Na}^+ \) in the bath, as long as \( \text{Na}^+ \) is present in the lumen. Third, the \( \text{pH}_i \) recovery caused by the addition of \( \text{Ac}^- \) to the lumen should be completely blocked by removal of \( \text{Na}^+ \) from the lumen, whether or not \( \text{Na}^+ \) is present in the bath. In the following experiments we have examined each of these predictions.

**The effect of adding basolateral \( \text{Ac}^- \) in the bilateral absence of \( \text{Na}^+ \).** In the experiment of Fig. 5, both \( \text{Na}^+ \) and \( \text{Ac}^- \) are removed from both the lumen and the bath.
The resulting transient alkalinization \((ab)\) can be attributed to the bilateral efflux of \(\text{HAc}\). The sustained pH decline \((bc)\) is probably due to the removal of both the \(\text{Ac}^-\) (i.e., blockade and reversal of luminal \(\text{Na}^+ / \text{Ac}^-\) cotransport) and the \(\text{Na}^+\) (i.e., blockade and reversal of \(\text{Na}^+ / \text{H}^+\) exchange). When \(\text{Ac}^-\) is then returned only to the bath, in the continued bilateral absence of external \(\text{Na}^+\), pH rapidly falls by a small amount \((cd)\), recovers somewhat, and then levels off \((de)\). The subsequent removal of basolateral \(\text{Ac}^-\) produces a rapid, but small alkalinization \((ef)\). Finally, when \(\text{Na}^+\) is returned to the lumen only, in the continued bilateral absence of external \(\text{Na}^+\), pH rapidly increases \((gh)\), but does not fully recover to its initial value \((a)\). The addition of \(\text{Na}^+\) to the bath is not shown, but produced only a small pH shift. The complete recovery of pH occurs only when \(\text{Ac}^-\) is returned to the lumen in the presence of \(\text{Na}^+\) \((ij)\).

The rapid pH changes accompanying the application \((cd)\) and removal \((ef)\) of basolateral \(\text{Ac}^-\) in the experiment of Fig. 5 presumably are due to the fluxes of \(\text{HAc}\) across the basolateral membrane, and are very similar to those elicited by changes in basolateral \(\text{Ac}^-\) in the presence of external \(\text{Na}^+\) (e.g., see \(de\) of Fig. 4). Thus, as
predicted by the model, these HAc fluxes are not Na⁺-dependent. On the other hand, the relative stability of pHᵢ during segment de in the experiment of Fig. 5, conducted in the absence of Na⁺, is in marked contrast to the rapid pHᵢ decline observed in experiments in which the cells were exposed to basolateral Ac⁻ in the presence of Na⁺ (see segment cd of Fig. 4). Thus, these results are consistent with the hypothesis that the mechanism for luminal Ac⁻ exit is blocked by the bilateral removal of Na⁺. The pHᵢ recovery elicited by the addition of luminal Na⁺ in the absence of Ac⁻ (gh) is probably due to luminal Na⁺/H⁺ exchange (Nakhoul et al., 1988). The pHᵢ recovery that accompanies the addition of Ac⁻ to the lumen in the presence of Na⁺ (ij) probably reflects the effect of luminal Na⁺/Ac⁻ influx in combination with bilateral HAc efflux.

The effect of adding luminal Ac⁻ in the bilateral absence of Na⁺. We also performed four experiments similar to that of Fig. 5 in which 10 mM Ac⁻ was added to and removed from the lumen in the absence of Na⁺ (data not shown). When Ac⁻ is added to the lumen only, pHᵢ abruptly declines and then fails to recover (similar to cde of Fig. 5). When the Ac⁻ is removed, pHᵢ rapidly rises a small amount (similar to ef). The abrupt pHᵢ changes that occur in the absence of Na⁺ provide further evidence that luminal HAc transport is Na⁺-independent, whereas the failure of luminal Ac⁻ to elicit a pHᵢ recovery in the absence of Na⁺ indicates that the luminal uptake of Ac⁻ is Na⁺ dependent.

The effect of adding luminal Ac⁻ in the absence of bath Na⁺. In another series of experiments, we added Ac⁻ only to the lumen when Na⁺ was present in the lumen.
but not in the bath. As shown in Fig. 6, bilateral removal of external Ac$^-$ results in
the usual pH$_i$ changes (abc). The removal of Na$^+$ from only the bath causes an addi-
tional small pH$_i$ decrease (cd). This acidification could reflect blockade or reversal of
a Na$^+$-dependent acid-extruding process (e.g., Na$^+$/H$^+$ exchange) at the basolateral
membrane. When Ac$^-$ is then returned only to the lumen, in the continued absence
of bath Na$^+$, pH$_i$ transiently falls (de, due to luminal HAc influx), but then recovers
(ef). These pH$_i$ changes (def) are very similar to those that are routinely observed in
the presence of bath Na$^+$ (see cd of Fig. 3 and fgh of Fig. 4), and indicate that the
pH$_i$ recovery caused by the addition of luminal Ac$^-$ does not require the presence
of Na$^+$ in the bath. Note that in the experiment of Fig. 6, the intracellular Na$^+$

![Figure 7](image_url)

**Figure 7.** Luminal-Na$^+$ dependence of the pH$_i$ increase induced by addition of luminal
Ac$^-$. Initially, the bilateral removal of Ac$^-$ and the luminal removal of Na$^+$ caused the usual
pH$_i$ changes (abc). Readdition of Ac$^-$ to only the lumen, in the continued absence of luminal
Na$^+$, caused a rapid but slight pH$_i$ decrease (cd), but no recovery (de). These changes were
reversed by removal of the Ac$^-$. Four similar experiments were performed.

activity is likely to be lower than normal, due to the absence of bath Na$^+$; this does
not appear to have a major effect on the luminal Ac$^-$-induced pH$_i$ recovery (ef).

**The effect of adding luminal Ac$^-$ in the absence of luminal Na$^+$.** To determine
whether the luminal Ac$^-$-induced pH$_i$ recovery is specifically blocked by the removal
of luminal Na$^+$, we performed the experiment of Fig. 7. Bilateral removal of Ac$^-$,
and the luminal removal of Na$^+$, cause the usual pH$_i$ changes (abc). Na$^+$ is main-
tained in the bath solution throughout the experiment. When Ac$^-$ is returned to the
luminal perfusate, in the continued absence of luminal Na$^+$, pH$_i$ rapidly falls by a
small amount (cd) and remains at that level (de). Removal of luminal Ac$^-$ had the
opposite effect (ef). Note that the usual pH$_i$ recovery that follows the rapid luminal
Ac$^-$-induced acidification (e.g., fgh of Fig. 4 and def of Fig. 6) is completely abol-
ished in the absence of luminal Na⁺. All that remains are the rapid pHᵢ changes (cd and ef) that we attribute to HAc fluxes across the luminal membrane.

The above observations are consistent with our hypothesis that there are two components of Ac⁻ transport in the S3 segment of the rabbit proximal tubule. The first is the movement of HAc across the luminal and basolateral membranes. This component is independent of Na⁺, and causes rapid but small changes in pHᵢ. The second component is a Na⁺-dependent pathway for Ac⁻ across only the luminal membrane. We propose that when the combined gradient for Na⁺ and Ac⁻ are in the inward direction, this transporter promotes a sustained pHᵢ increase, whereas if this combined gradient is outward, it promotes a sustained pHᵢ decrease.

**Dependence of the Ac⁻-dependent pHᵢ Changes on Ac⁻ Concentration**

Having established that the addition and removal of 10 mM Ac⁻ (a concentration commonly used in experiments on mammalian renal tubules) has a profound effect on pHᵢ, we next tried to determine whether lower concentrations of Ac⁻ have similar effects. In the following experiments, Ac⁻ was removed from the lumen and the bath, and then varied concentrations of Ac⁻ were added back to the lumen only. As shown in Fig. 8 A, bilateral removal of Ac⁻ resulted in the usual pHᵢ changes (abc). Readdition of 1 mM Ac⁻ to the lumen produced a prompt pHᵢ recovery (cd), which was reversed upon removal of luminal Ac⁻ (de). Note the absence at point c of the transient pHᵢ fall that normally accompanies addition of 10 mM Ac⁻, as well as the absence of the transient pHᵢ increase at point d that normally accompanies removal of 10 mM Ac⁻. These rapid pHᵢ transients, which we attribute to fluxes of HAc, presumably are absent in these 1-mM-Ac⁻ experiments, inasmuch as the prevailing extracellular [HAc] is only one tenth as great as in solutions containing 10 mM Ac⁻. Indeed, when 10 mM Ac⁻ is added to the lumen of the same tubule, the usual transient fall in pHᵢ is observed (ef) and is followed by a pHᵢ recovery that is no faster than in the presence of 1 mM Ac⁻ (fg). Removal of 10 mM luminal Ac⁻ causes the usual transient pHᵢ increase (gh), followed by a sustained acidification (hi).

In the experiment of Fig. 8 B, we examined the effect of adding even lower concentrations of Ac⁻ to the lumen of a tubule from which Ac⁻ had previously been removed from both the lumen and bath. The initial rate of alkalinization produced by 0.2 mM Ac⁻ (ab) was only about 40% as great as that produced by 1.0 mM Ac⁻ (cd) when both are measured at pHᵢ of 7.0. In four paired experiments the rate of alkalinization produced by 0.2 mM Ac⁻ was 46 ± 7% of that produced by 1 mM Ac⁻. We conclude that, to avoid the alkalinizing effect of luminal Ac⁻ in nominally HCO₃⁻-free solutions, extracellular [Ac⁻] would have to be lowered substantially below 1 mM, and even concentrations as low as 0.2 mM can be expected to produce a substantial steady-state alkalinization.

**Effect of Luminal Citrate on pHᵢ**

In an attempt to find a metabolizable carboxylate that does not produce a substantial alkalinization in the nominal absence of HCO₃⁻, we tested citrate, a tricarboxylate. Evidence from membrane vesicle experiments indicates that citrate is not transported by the Na⁺/monocarboxylate cotransporter that mediates fluxes of Ac⁻, but rather by a Na⁺-dependent transporter highly specific for intermediates of the tri-
FIGURE 8. Effect of lower concentrations of luminal Ac\textsuperscript{−} on the Ac\textsuperscript{−}-induced alkalization. (A) Comparison of 1 and 10 mM luminal Ac\textsuperscript{−}. After the initial bilateral removal of Ac\textsuperscript{−}, which caused the usual pH\textsubscript{i} changes (abc), adding 1 mM Ac\textsuperscript{−} to the lumen caused the cell to alkalize (ad) at a rate that was as fast as the alkalization induced by the addition of 10 mM Ac\textsuperscript{−} (fg). Note that the addition of 10 mM Ac\textsuperscript{−} caused an initial pH\textsubscript{i} decrease (ef), whereas the addition of 1 mM Ac\textsuperscript{−} did not. Seven similar experiments were performed. (B) Comparison of 0.2 and 1 mM Ac\textsuperscript{−}. After the bilateral removal of Ac\textsuperscript{−} (not shown), the addition of 0.2 mM Ac\textsuperscript{−} caused a pH\textsubscript{i} recovery (ab) that, in five similar experiments, was only about 40\% as fast as that elicited by the addition of 1 mM Ac\textsuperscript{−} (ad). The experiments of A and B were performed on different tubules.
carboxylic acid cycle (Kippen et al., 1979). This latter system does not appear to transport monocarboxylates (Wright et al., 1980), and its effects on pH$_i$ are unclear. In the experiment of Fig. 9, Ac$^-$ is first removed from both the lumen and the bath, which results in the usual pH$_i$ changes (abc). When 5 mM citrate (solution 6) is added to the lumen, pH$_i$ increases only slightly (cdel). Removal of the citrate produces no discernible change in pH$_i$ (ef). Addition (fg) and then removal (gh) of 5 mM citrate to/from the bath produced, at most, slight pH$_i$ changes. Finally, addition of 10 mM Ac$^-$ to the lumen produced the usual series of pH$_i$ changes (hij).

**Figure 9.** Effect of unilateral addition of citrate on pH$_i$. The initial bilateral removal of Ac$^-$ caused the usual pH$_i$ changes (abc). Addition of 5 mM citrate to the lumen caused a slight increase in pH$_i$ (cdel), whereas the removal had a minimal effect (ef). Addition of citrate to the bath (fg), or its removal (gh), also had a negligible effect on pH$_i$. Six similar experiments were performed.

**DISCUSSION**

In the preceding paper (Nakhoul et al., 1988) on the S3 segment of the rabbit proximal tubule, we found that 65% of the Na$^+$-dependent acid extrusion taking place in the nominal absence of HCO$_3^-$ requires Ac$^-$ and is insensitive to amiloride. A possible explanation for this finding was suggested by the results of a study on the effects of lactate transport on pH$_i$ in *Ambystoma* proximal tubules, by Siebens and Boron (1987). These authors showed that there is an electroneutral Na$^+$/lactate cotransporter at the luminal membrane, and a H$^+$/lactate cotransporter (or an equivalent process, such as lactate/base exchange) at the basolateral membrane. Because the normal direction of net transport in the *Ambystoma* cells is apparently a net uptake of Na$^+$ and lactate at the luminal membrane, and a net exit of H$^+$ and lactate at the basolateral membrane, the net effect would be the transepithelial reabsorption of lactate. In addition, there would be a net uptake of Na$^+$ across the luminal membrane and net acid extrusion from the cell across the basolateral membrane. We
undertook the present study to determine if a similar mechanism that involved Ac− might be operative in the rabbit S3 proximal tubule and if it could account for the Na+/Ac−-dependent acid extrusion previously observed.

The results of the present study indicate that the cells of the S3 segment of the rabbit proximal tubule have a Na+/Ac− cotransporter at the luminal membrane, and HAc transport pathways at both the luminal and basolateral membranes (see Fig. 10). It should be noted that the latter could be accounted for by either the nonionic diffusion of HAc, a H+/Ac− cotransporter, or an Ac−/base exchanger. We have made no attempt to distinguish among these. Our model predicts that the addition of Ac− to the lumen only should cause two phases of pHi change. The first is a rapid acidification caused by the influx of HAc across the luminal membrane and the subsequent dissociation of this intracellular HAc into H+ and Ac−. This net influx of HAc (or an equivalent process) should continue until [HAc]i is equal to [HAc]o.

The second predicted phase of pHi change induced by the addition of Ac− to the lumen is a slower but sustained pHi increase that reaches a level higher than that prevailing in the absence of Ac−. This pHi increase should occur as the luminal Na+/Ac− cotransporter mediates a net uptake of Na+ and Ac−, while luminal and basolateral pathways permit the exit of the protonated weak acid HAc. Note that the pHi change requires the activity of two kinds of transport processes. The luminal entry of Ac− by itself is not expected to cause a substantial pHi increase, inasmuch as the pK governing the dissociation of HAc is much lower than the prevailing pHi. On the other hand, by themselves, the luminal and basolateral HAc pathways are expected to mediate a rapid, net acidification. Only when the two transporters operate in concert does the luminal application of Ac− result in a pHi increase. We cannot rule out the possibility that a small amount of the pHi increase elicited by the application of luminal Ac− is due to the metabolic consumption of Ac− and H+, rather than to the efflux of HAc. However, our observation, that the application of Ac− to the bath only causes a sustained fall in pHi suggests that HAc fluxes are far more important for determining pHi than is metabolism.

The net effect of these transport processes can be analyzed from the perspectives.
of both pH$_i$ regulation and transepithelial transport. Consider a cell possessing luminal Na$^+/\text{Ac}^-$ and bilateral HAc cotransport pathways, but no other acid-base transporters. In the steady state, there would be a net influx of Na$^+$ across the luminal membrane, and a net efflux of H$^+$ across both luminal and basolateral membranes. As far as pH$_i$ regulation is concerned, these transporters would appear to be an Ac$^-$-dependent, amiloride-insensitive (see Nakhoul et al., 1988) Na$^+$ for H$^+$ exchange process that would function as an acid extruder. Indeed, we find that the steady-state pH$_i$ is substantially higher in the presence than in the absence of Ac$^-$. The observation that the cells of the rabbit S3 proximal tubule possess two Na$^+$-dependent acid-extrusion mechanisms, an amiloride-sensitive Na$^+/\text{H}^+$ exchanger (Nakhoul et al., 1988) and an Ac$^-$-dependent mechanism, raises the question of the relative roles played by each of these mechanisms in pH$_i$ homeostasis.

As far as transepithelial transport is concerned, the net effect of Na$^+/\text{Ac}^-$ and HAc transport processes depends upon the extent to which HAc exits across the basolateral as opposed to the luminal membrane. First, consider a cell with a luminal Na$^+/\text{Ac}^-$ cotransporter, an HAc pathway only at the basolateral membrane, and no other acid-base transporters. Together, the luminal and basolateral Ac$^-$ transporters would have three effects: (a) there would be net transepithelial Ac$^-$ reabsorption, and (b) an uptake of Na$^+$ across the luminal membrane. This is the luminal step of Na$^+$ reabsorption; the basolateral step would be mediated by a Na$^+$-extrusion process, such as the Na-K pump. Finally, (c) there would be a net extrusion of H$^+$ across the basolateral membrane. This latter effect would oppose the basolateral efflux of HCO$_3^-$, and therefore reduce net acid secretion. This scheme is the one that apparently prevails in the salamander proximal tubule (Siebens and Boron, 1987), where Na$^+/\text{lactate}$ cotransport is confined to the luminal membrane, and H$^+/\text{lactate}$ cotransport, to the basolateral membrane.

Now consider a cell with a luminal Na$^+/\text{Ac}^-$ cotransporter, an HAc pathway confined to the luminal membrane, and no other acid-base transporters. Together, these luminal Ac$^-$ transporters would have three effects: (a) there would be no net transport of Ac$^-$; (b) there would be a net uptake of Na$^+$, which would represent the luminal step of Na$^+$ reabsorption, and (c), there would be extrusion of H$^+$ across the luminal membrane, which would represent the luminal step of H$^+$ secretion and/or HCO$_3^-$ reabsorption. This scheme of monocarboxylate shuttling across the luminal membrane was suggested on theoretical grounds by Aronson (1983).

Our data indicate that HAc transport in the rabbit S3 segment occurs at both the luminal and basolateral membranes. Thus, the influence of Ac$^-$ on the transepithelial transport of Na$^+$, H$^+$, and Ac$^-$ would be expected to lie between the two extremes described above. Our model predicts that, in the nominal absence of HCO$_3^-$, the addition of Ac$^-$ should: (a) alkalinate the cell, (b) promote transepithelial Ac$^-$ reabsorption, and (c) promote the secretion of acid into the tubule lumen. Indeed, a preliminary report (Geibel et al., 1987) indicates that, in the nominal absence of HCO$_3^-$, Ac$^-$ promotes the secretion of acid into the lumen of the rabbit S3 segment.

The profound effect of Ac$^-$ transport on both steady-state pH$_i$ and the rate of Na$^+$-dependent pH$_i$ changes suggests that Ac$^-$ transport may have important implications for other studies on proximal tubules. Ac$^-$ has been employed in several studies on isolated perfused mammalian proximal tubules (Schafer and Andreoli,
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1976; Sasaki and Berry, 1984; Biagi, 1985). Our results indicate that Ac\(^-\) concentrations in the submillimolar range could have a substantial effect on pH\(_i\) and acid extrusion rates, at least in the nominal absence of HCO\(_3^-\). This suggests that the effective K\(_m\) for Ac\(^-\)-dependent acid extrusion must be rather low. This could be particularly important for the late proximal tubule if there is substantial reabsorption of monocarboxylates earlier in the proximal tubule. Although, in this study, we examined only Ac\(^-\), it is likely that other monocarboxylates (e.g., lactate and pyruvate) have effects on pH\(_i\) similar to those of Ac\(^-\).

Finally, this study demonstrates a novel way of assessing Ac\(^-\) transport. Although using pH\(_i\) measurements to study Ac\(^-\) transport is a somewhat indirect approach, the results obtained in this manner reveal information on Ac\(^-\) transport pathways that would be otherwise inaccessible.

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