Effect of Electroneutral Luminal and Basolateral Lactate Transport on Intracellular pH in Salamander Proximal Tubules

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ABSTRACT We used microelectrodes to examine the effects of organic substrates, particularly lactate (Lac⁻), on the intracellular pH (pHi) and basolateral membrane potential (VBl) in isolated, perfused proximal tubules of the tiger salamander. Exposure of the luminal and basolateral membranes to 3.6 mM Lac⁻ caused pHi to increase by ~0.2, opposite to the decrease expected from nonionic diffusion of lactic acid (HLac) into the cell. Addition of Lac⁻ to only the lumen also caused alkalinization, but only if Na⁺ was present. This alkalinization was not accompanied by immediate VBl changes, which suggests that it involves luminal, electroneutral Na/Lac cotransport. Addition of Lac⁻ to only the basolateral solution caused pHi to decrease by ~0.08. The initial rate of this acidification was a saturable function of [Lac⁻], was not affected by removal of Na⁺, and was reversibly reduced by α-cyano-4-hydroxycinnamate (CHC). Thus, the pHi decrease induced by basolateral Lac⁻ appears to be due to the basolateral entry of H⁺ and Lac⁻ mediated by an H/Lac cotransporter (or a Lac-base exchanger). Our data suggest that this transporter is electroneutral and is not present at the luminal membrane. A key question is how the addition of Lac⁻ to the lumen increases pHi. We found that inhibition of basolateral H/Lac cotransport by basolateral CHC reduced the initial rate of pHi increase caused by luminal Lac⁻. On the other hand, luminal CHC had no effect on the luminal Lac⁻-induced alkalinization. These data suggest that when Lac⁻ is present in the lumen, it enters the cell from the lumen via electroneutral Na/Lac cotransport and then exits with H⁺ across the basolateral membrane via electroneutral H/Lac cotransport. The net effect is transepithelial Lac⁻ reabsorption, basolateral acid extrusion, and intracellular alkalinization.

INTRODUCTION

Intracellular pH (pHi) in nearly all animal cells has been found to be higher than predicted if H⁺ were passively distributed across the cell membrane (see Roos and Boron, 1981). To maintain pHi at the levels normally observed, the cell

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must actively extrude acid. Acid-extruding transport systems can be divided into three general groups: primary, secondary, and tertiary active-transport systems. Two acid-extruding primary active-transport systems, both involving ATP hydrolysis, have been identified in plasma membranes: a K-H exchanger found in the stomach (see Forte and Machen, 1986) and an H+ pump found in the kidney (see Steinmetz, 1985). Two acid-extruding secondary active-transport systems, both involving Na+ transport, have been identified in plasma membranes. The first is an Na-dependent Cl-HCO3 exchanger that obligatorily exchanges what appears to be internal H+ and Cl- for external Na+ and HCO3- (see Roos and Boron, 1981; Thomas, 1984). The second mechanism is an Na−H exchanger, which extrudes acid as Na+ enters the cell in exchange for H+ (see Roos and Boron, 1981; Aronson, 1985). In principle, acid extrusion could also occur by means of a tertiary active-transport system, as has been suggested by Aronson (1983) for the renal proximal tubule. For example, an Na monocarboxylate cotransporter could mediate Na+ and monocarboxylate influx across the luminal membrane (secondary active transport). The efflux of monocarboxylate with a proton (or the exchange of monocarboxylate for luminal base) would then result in the extrusion of acid from the cell (tertiary active transport).

The two classes of monocarboxylate transporters mentioned above have been studied separately in a variety of preparations. Na/monocarboxylate cotransport has been identified in both renal proximal tubule cells and small-intestine cells (see Wright, 1985). The data have been obtained by measuring the disappearance of labeled lactate from the lumen of renal tubules (Ullrich et al., 1982a), by monitoring induced changes in membrane voltage in intact cells (Samarzija et al., 1981), and by studying the effect of various monocarboxylates on lactate transport in membrane vesicles (see Wright, 1985). The evidence indicates that this Na/monocarboxylate cotransporter carries net positive charge and is capable of transporting a wide variety of monocarboxylates (Wright, 1985). The second class of transporters mediates the cotransport of a monocarboxylate and an H+, or an analogous process (e.g., monocarboxylate-OH or monocarboxylate-HCO3 exchange), and is thought to be electroneutral (Deuticke, 1982). Isotopic flux and pH studies on several nonepithelial preparations indicate that the cotransporter accepts a wide variety of monocarboxylates (Deuticke, 1982), transports lactate and H+ with a transport ratio of approximately unity (Spencer and Lehninger, 1976), and is reversibly and competitively inhibited by a-cyano-4-hydroxycinnamate (CHC), an aromatic monocarboxylic acid (Halestrap and Denton, 1974; Spencer and Lehninger, 1976).

We became interested in lactate transport because of our observation that lactate plays a critical role in the intracellular alkalinization induced by depolarization in isolated, perfused Ambystoma proximal tubules (Siebens and Boron, 1985). The purpose of the present study was to examine the effects of lactate transport on pH in nondepolarized proximal tubules. Lactate transport was assessed by using microelectrodes to monitor lactate-induced changes in pH and basolateral membrane potential. Our results indicate that, in the presence of bilateral lactate, lactate enters the cell across the luminal membrane via an electroneutral Na/lactate cotransporter and exits across the basolateral membrane via an H+/lactate cotransporter (or a lactate-base exchanger). The net
Effect of Lactate Transport on pH

Portions of this work have been published in preliminary form (Siebens and Boron, 1986a, b).

METHODS

General

Female tiger salamanders (Ambystoma tigrinum) in the neotenic phase were obtained from Mr. Charles Sullivan (Nashville, TN) and maintained in aquaria at 4°C. The aquarium water contained 1 mM NaCl, 0.5 mM KCl, and 0.5 mM CaCl₂. The methods used for isolating and perfusing the salamander proximal tubules were the same as those described by Sackin and Boulpaep (1981) and Boron and Boulpaep (1983a). After the animals were decapitated, the kidneys were removed and cut transversely into slices ~2 mm thick. The slices were stored and dissected in chilled solution 1 (see Table I) to which 1.5 g% polyvinyl pyrrolidone was added to minimize adherence of the tubules to the dissecting instruments. A 700-1,000-μm segment of the proximal tubule immediately distal to the glomerulus and neck segment was dissected with fine forceps, drawn into a 10-μl pipette, and transferred to the perfusion chamber. The isolated perfused tubule apparatus was similar to that originally described by Burg et al. (1966). Briefly, the outside of the tubule was held at both ends by glass micropipettes and cannulated at both ends by smaller pipettes through which the luminal perfusate was introduced and collected. The volume of the chamber was ~150 μl, and the bath flow rate was 7–10 ml/min. The time constants for exchange of luminal and basolateral solutions, ~5 and ~3 s, respectively, were estimated from changes in basolateral membrane potential (Vₜₙ) that accompanied solution changes. All experiments were performed at ambient temperature (21–25°C). Our methods for obtaining microelectrode measurements of membrane potential and pH, were similar to those described previously (Sackin and Boulpaep, 1981; Boron and Boulpaep, 1983a).

Solutions

The compositions of the solutions are given in Table I. Solution 1 is the same as the standard HEPES solution used by Boron and Boulpaep (1983a). The concentrations of lactate and other organic solutes were based on the formulation of Forster et al. (1980). The other solutions were made by making one or more of the following modifications to solution 1: (a) omitting all organic substrates (lactate, glucose, and amino acids); (b) omitting only glucose and amino acids; (c) increasing the lactate concentration; (d) omitting Na+. All solutions had osmolalities within 4 mosmol of that of solution 1 (i.e., ~201 mosmol), with any adjustments being made with water, with NaCl, or with N-methyl-D-glucammonium (NMDG) and HCl. Solutions were delivered to the chamber or pipettes by gravity through CO₂-impermeable Saran tubing (Clarkson Equipment and Controls, Detroit, MI). HEPES, CHCl, NMDG, L- and D-lactate, glucuronic acid, and cyclamic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Amiloride was a gift from Merck, Sharp & Dohme Research Laboratories (West Point, PA), and 3-mercaptopicolinate was a gift from Smith, Kline & French (Philadelphia, PA). The lactate used in the standard solution was an unspecified mixture of L- and D-lactate (Ca-lactate, J.T. Baker Chemical Co., Phillipsburg, NJ). The NMDG was titrated with HCl to produce NMDG⁺.

Electrodes and Electronics

We used single-barreled, liquid-membrane pH microelectrodes containing the neutral-carrier–based ligand described by Ammann et al. (1981) and obtained from Fluka.
| Component  | 1 | 2 | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 |
|------------|---|---|----|----|----|----|----|----|----|----|----|
| Standard saline | 97.7 | 97.8 | 97.7 | 97.8 | 97.8 | 97.8 | 97.8 | 0 | 0 | 0 | 0 |
| 0 substrate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 97.2 | 97.2 | 97.2 |
| 3.6 Lac⁻ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.5 | 2.5 | 2.5 |
| 7.2 Lac⁻ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 36.0 | 36.0 | 36.0 |
| 36 Lac⁻ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 36.0 | 36.0 | 36.0 |
| 0 Na⁺ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 Na⁺ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 Na⁺ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 Na⁺ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 Na⁺ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Na⁺ | 106.0 | 105.9 | 106.0 | 105.9 | 105.9 | 105.9 | 105.9 | 105.9 | 105.9 | 105.9 | 105.9 |
| NMDG⁺ | 94.7 | 98.3 | 98.3 | 94.7 | 91.1 | 62.2 | 62.3 | 97.7 | 94.1 | 61.7 | 61.7 |
| K⁺ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 36.0 | 36.0 | 36.0 |
| Mg⁺⁺ | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Ca⁺⁺ | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Lac⁻ | 0.6 | 0.6 | 0.6 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Glu⁻ | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| HEPES | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |
| meq(+) | 106.0 | 105.9 | 106.0 | 105.9 | 105.9 | 105.9 | 105.9 | 105.9 | 105.9 | 105.9 | 105.9 |
| Cl⁻ | 94.7 | 98.3 | 98.3 | 94.7 | 91.1 | 62.2 | 62.3 | 97.7 | 94.1 | 61.7 | 61.7 |
| X⁻ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 36.0 | 36.0 | 36.0 |
| H₂PO₄⁻ | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| HPO₄²⁻ | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Lac⁻ | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 |
| Glu⁻ | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| HEPES | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |
| meq(−): | 106.0 | 105.9 | 106.0 | 105.9 | 105.9 | 105.9 | 105.9 | 105.9 | 105.9 | 105.9 | 105.9 |
| Glucose | 2.2 | 2.2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gin | 0.5 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ala | 0.5 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| HEPES | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |

* Compositions are given in millimolar unless otherwise noted. X⁻ is glucuronate or cyclamate.
Chemical Corp. (Hauppauge, NY). Micropipettes for Ling-Gerard (i.e., voltage) and pH microelectrodes were pulled identically from 1.0-mm-o.d. borosilicate glass (Frederick Haer & Co., Brunswick, ME) on a horizontal microelectrode puller (either Brown-Flaming model P-77, Sutter Instrument Co., San Francisco, CA, or model PD5, Narashige Scientific Instrument Laboratory, Tokyo, Japan). Voltage electrodes were filled with 3 M KCl and had resistances of 8–30 MΩ and tip potentials <5 mV.

The pH electrodes were made in the following manner. Pulled pipettes were dried by placing them vertically into holes in an aluminum block and baking them in an oven at 200°C for at least 2 h. Pipettes were stored in the oven for up to 1 wk. The electrodes were silanized, filled, and used on the day of the experiment. The following protocol produced a yield of ~80% usable electrodes. The electrodes were silanized by adding 10 μl of tri-n-butyl chlorosilane into a 500-ml borosilicate glass vessel containing the aluminum block and pipettes, and allowing the fumes to contact the pipettes for 2 min (Boron and Boulpaep, 1983a). After baking the pipettes for an additional 30 min at 200°C, we placed the silanized electrodes horizontally on a plasticine support in a small plastic box containing CaSO₄ (W. A. Hammond Drierite Co., Xenia, OH). The electrodes were filled from the back with the Fluka proton cocktail by passing a glass capillary injector through a narrow slit in the plastic box. The slit was otherwise covered with tape to prevent entry of moist air into the box. The electrodes then were backfilled with a pH 7 solution containing 0.04 M KH₂PO₄, 0.023 M NaOH, and 0.015 M NaCl (Ammann et al., 1981), and inserted into Ag/AgCl half-cell microelectrode holders (World Precision Instruments, Inc., New Haven, CT). The microelectrode tips were not broken or beveled. The pH electrodes were calibrated in standards made from solution 1 titrated to pH 6 and pH 8. The mean slope was 56.9 ± 0.1 mV/pH unit (n = 37). The calibration was performed immediately before the impalement and immediately after withdrawal of the electrodes from the cells. After a typical 1-h experiment, the second calibration yielded an electrode slope ~4 mV/pH unit lower than the first, and an absolute electrode voltage at pH 7.5 that corresponds to a pH ~0.1 more acid. All of our analyses are based on the initial calibration. The pH electrodes had resistances of 3–5 × 10¹² Ω and responded to solution changes with a time constant of 1–4 s.

The arrangement of the electronics was the same as previously described (Boron and Boulpaep, 1983a). Briefly, the pH electrode and a calomel half-cell in the drain line of the chamber were connected to an electrometer (model 223, World Precision Instruments, Inc., New Haven, CT), the voltage electrode and a calomel half-cell in the drain line of the perfusion assembly were connected to an electrode amplifier (model 750, World Precision Instruments, Inc.), and the bath was grounded through a platinum wire. The voltage due to pH was determined by electronically subtracting the potential recorded by the voltage microelectrode from that recorded by the pH microelectrode and filtering with a time constant of 1 s. The basolateral membrane potential (Vₒ) was the electronically obtained difference between the potentials of the voltage microelectrode and the bath calomel half-cell. The gross pH electrode voltage (i.e., that due to both pH and Vₒ) was the difference between the voltages of the pH microelectrode and the bath calomel half-cell. The transepithelial potential (Vₚₑ) was the difference between the voltages of the perfusion calomel half-cell and the bath calomel half-cell. These four signals were plotted on a strip-chart recorder (model L650, Linseis, Inc., Princeton Junction, NJ). In the figures, we present the pH and Vₒ records. Changes in Vₚₑ, where noteworthy, are described in the text.

**Experimental Protocol**

When the tubule was first perfused, it was exposed bilaterally to solution 1. The luminal solution also contained 0.1% Hercules green dye no. 1 (H. Kohnstamm, New York, NY).
If the dye was excluded from all cells, we impaled a cell with a voltage electrode. The criteria for successful impalements were similar to those discussed by Biagi et al. (1981). The pH electrode was then inserted and a period of several minutes was allowed for the $V_{bl}$ and $pHi$ records to stabilize. The luminal and basolateral solutions were then changed as indicated in the figures.

**Analysis of Data**

The rate of $pHi$ change was determined by drawing a line through the linear portion of the $pHi$ vs. time record. Unless otherwise indicated, data were analyzed using the two-tailed paired Student's $t$ test. Results are given as means ± SEM.

**RESULTS**

**Control Values**

The mean initial values of $pHi$, $V_{bl}$, and $V_{te}$ for tubules bathed bilaterally with solution 1 were $7.47 \pm 0.02$, $-68.0 \pm 1.7$ mV, and $-3.4 \pm 0.2$ mV, respectively ($n = 37$). These values are similar to the values of 7.43 for $pHi$, $-57$ mV for $V_{bl}$, and $-3.8$ mV for $V_{te}$ reported by Boron and Boulpaep (1983a) for these tubules under identical conditions in a study in which glass pH microelectrodes were used rather than liquid-membrane pH microelectrodes.

**Effects of Organic Substrate Removal on $pHi$ and $V_{bi}$**

The initial step in the present study was to determine whether organic substrate transport plays an important role in determining $pHi$. Fig. 1 shows the results of an experiment in which the solution bathing the luminal and basolateral membranes was changed from one containing the organic substrates normally present in *Ambystoma* Ringer's (i.e., lactate as well as glucose and amino acids) to an organic substrate-free solution. We found that organic substrate removal caused a reversible $pHi$ decrease (segment $ab$), the magnitude of which averaged $0.18 \pm 0.02$. The mean maximal acidification rate was $0.182 \pm 0.016$/min ($n = 23$). Organic substrate removal also caused the cells to hyperpolarize (point $a'$) by $13.9 \pm 1.2$ mV ($n = 23$), which presumably reflects electrogenic luminal Na/substrate cotransport, such as Na/glucose (Frömter, 1981; Morgunov and Boulpaep, 1987) and Na/amino acid cotransport (Frömter, 1981). The removal of all organic substrates also caused $V_{bi}$ to approach zero (not shown), as previously reported for this (Morgunov and Boulpaep, 1987) and other proximal tubule preparations (Burg et al., 1976).

The decrease in $pHi$ produced by organic substrate removal suggests that at least one of the organic substrates contained in solution 1 plays an important role in determining $pHi$. We therefore performed six experiments similar to that shown in Fig. 1 in which we compared the $pHi$ change caused by the removal of only lactate (segment $cd$) with that caused by the removal of all organic substrates (segment $ab$) in the same tubule. Removal of lactate ($Lac^{-}$) alone caused $pHi$ to decrease by $0.21 \pm 0.04$, with a maximal rate of $0.167 \pm 0.034$/min. Neither of these values is statistically different from the paired values of $0.181 \pm 0.071$ and $0.200 \pm 0.046$/min observed when all substrates were removed. Note that the initial $pHi$ change observed after removal of either all organic substrates (point
or only Lac− (point c) is a small but consistently observed transient alkanization of ≈0.05. The mechanism of this transient alkanization will be considered below.

After the removal of only Lac− in the experiment of Fig. 1, we removed glucose and amino acids to determine whether these solutes also affect pHi. A small further pH decrease of 0.03 ± 0.01 (P < 0.01) was observed (segment de), and this was reversed upon restoration of glucose and amino acids (segment ef). The mechanism of these slight pH effects was not investigated further in this study. The above findings suggest that the acidification caused by removing all organic substrates (segment ab) is specifically due to the removal of Lac−. This conclusion is further supported by the finding that if glucose and amino acids are simultaneously removed from tubules bilaterally exposed to Lac−, pHi actually rises by ≈0.2 (Siebens, A. W., and W. F. Boron, manuscript in preparation).

When Lac− was restored at the end of the experiment of Fig. 1 (segment fg), an alkanization was observed that was similar to, but slightly slower than (22 ± 7%, P < 0.05, n = 4), that observed when all organic substrates were restored (segment bc). If Lac− crossed the cell membranes via nonionic diffusion of lactic acid (HLac), then addition of Lac− should have acidified the cell owing to dissociation of entering HLac into Lac− and H+. Our observation that exposure...
to Lac\textsuperscript{−} causes alkalinization suggests that a mechanism other than nonionic diffusion of HLac is involved.

Note that whenever glucose and amino acids were removed or restored, there were immediate (usually \( \leq 30 \) s) and large changes in \( V_{bl} \). In the six experiments with the protocol of Fig. 1, the immediate \( V_{bl} \) changes at points \( a' \), \( b' \), \( d' \), and \( e' \) were \(-12.0 \pm 2.9\), \(+16.5 \pm 4.8\), \(-16.6 \pm 2.5\), and \(+25.5 \pm 2.8\) mV, respectively. In contrast, removal or readdition of Lac\textsuperscript{−} in the same tubules did not cause immediate \( V_{bl} \) changes. The mean changes were \(-0.2 \pm 0.1\) mV at \( c' \) (\( n = 6 \)) and \(-1.1 \pm 0.4\) mV at \( f' \) (\( n = 4 \)), neither of which differs significantly from zero. Indeed, rather than the sustained depolarization observed after addition of glucose and amino acids (\( e' \)), a gradual hyperpolarization was observed after addition of Lac\textsuperscript{−} (\( f' \)). During the 3 min after point \( f' \), the basolateral membrane hyperpolarized by \( 5.7 \pm 0.9\) mV (\( n = 4 \)). The absence of immediate \( V_{bl} \) changes with alterations in extracellular [Lac\textsuperscript{−}] suggest that there is no substantial conductive pathway for Lac\textsuperscript{−} at either the luminal or the basolateral membrane under these conditions. The slow \( V_{bl} \) changes could be secondary to Lac\textsuperscript{−}-induced alterations in cell composition (e.g., pH\textsubscript{i}).

Because these data suggest that Lac\textsuperscript{−} is responsible for nearly all of the acidification observed when organic substrates are removed, we undertook a systematic examination of the mechanisms of luminal and basolateral Lac\textsuperscript{−} transport in this preparation.

**Lactate Transport across the Luminal Membrane**

**Effect of luminal Lac\textsuperscript{−} in the presence of Na\textsuperscript{+}**. In the experiment of Fig. 2, all organic substrates were first removed from the luminal and basolateral solutions. The lumen was then exposed to a solution containing 3.6 mM l-Lac\textsuperscript{−}. Rather than causing the acidification expected to result from Lac\textsuperscript{−} entry by nonionic diffusion of HLac, luminal addition of Lac\textsuperscript{−} caused cell alkalinization (\( ab \)). In 13 similar experiments, addition of l-lactate to only the lumen resulted in a mean pH\textsubscript{i} increase of \( 0.38 \pm 0.05 \), with a mean maximal rate of \( 0.41 \pm 0.04 \) min\textsuperscript{−1}. The maximal rate of the pH\textsubscript{i} decline after removal of luminal l-Lac\textsuperscript{−} (\( be \)) was \( 0.27 \pm 0.03\) min\textsuperscript{−1}, \( \sim 35\% \) lower than the corresponding rate of alkalinization (\( P < 0.001 \)). There was no immediate change in \( V_{bl} \) when Lac\textsuperscript{−} was added (\( a' \)) or removed (\( b' \)). As in the experiment of Fig. 1, the addition of Lac\textsuperscript{−} produced a small, gradual hyperpolarization, which averaged \( 1.8 \pm 0.6\) mV (\( n = 12 \), \( P < 0.02 \)) after 1 min.

In *Ambystoma* proximal tubules (Morgunov and Boulpaep, 1987) and rabbit proximal convoluted tubules (Burg et al., 1976), electrogenic Na/glucose cotransport causes \( V_{ec} \) to become several millivolts more negative. In experiments similar to that of Fig. 2, we found no significant change (\( 0.02 \pm 0.02, n = 13 \)) in \( V_{ec} \) after addition of Lac\textsuperscript{−} to the lumen. Our observations that the addition of Lac\textsuperscript{−} to the lumen produces no immediate change in either \( V_{bl} \) or \( V_{ec} \) suggest that luminal Lac\textsuperscript{−} transport is electroneutral in *Ambystoma* proximal tubules under these conditions. Thus, the *Ambystoma* cotransporter appears to differ from the electrogenic Na/Lac cotransporter identified in mammalian renal preparations (see Wright, 1985).
In the experiment of Fig. 2, we also examined the stereospecificity of luminal \( \text{Lac}^- \) transport. The luminal addition of \( \text{D-Lac}^- \) caused \( \text{pH}_i \) to increase (cd) with an initial rate of 0.171 pH units/min. This is \( \sim 97\% \) as great as the initial rates of alkalinization for the preceding (0.182 pH units/min) and succeeding (0.171 pH units/min) exposures to \( \text{L-Lac}^- \). In six experiments, the ratio of the initial rate for \( \text{D-Lac}^- \) to that for \( \text{L-Lac}^- \) was 69 \( \pm \) 9\% \( (P < 0.05) \). It has long been known that the kidney reabsorbs \( \text{D-Lac}^- \) nearly as well as \( \text{L-Lac}^- \) (Craig, 1946).

In preliminary experiments (not shown), we found that luminal addition of acetate or pyruvate also causes an alkalinization. The alkalinization induced by

\[
\begin{align*}
\text{LUMEN:} & \quad \frac{[\text{Na}^+]}{[\text{Lac}]} \\
\text{BATH:} & \quad \frac{[\text{Na}^+]}{[\text{Lac}]} \\
\end{align*}
\]

\[98 \quad 98 \quad 0 \quad 0 \quad 0 \quad 0 \quad 0 \]

**Figure 2.** Effect of luminal \( \text{L-} \) or \( \text{D-lactate} \) in the presence of \( \text{Na}^+ \). After all substrates were removed (solution 2), the lumen was exposed to 3.6 mM \( \text{L-} \) or \( \text{D-lactate} \) (solution 4). Substrates were removed 20 min before the time represented in this record. Six similar experiments were performed. 072485A.

4 mM \( \text{Lac}^- \) was mimicked by 4 mM pyruvate, whereas 16 mM acetate was required to cause similar alkalinization.

**Effect of luminal \( \text{Lac}^- \) in the absence of \( \text{Na}^+ \).** To determine whether the \( \text{pH}_i \) and voltage effects of luminal \( \text{Lac}^- \) addition depend on \( \text{Na}^+ \), we performed the experiment of Fig. 3. The data are from a later part of the experiment of Fig. 2, which permits direct comparison of data obtained in the presence and absence of \( \text{Na}^+ \). When \( \text{Na}^+ \) was removed from the lumen, we observed a slight acidification (ab) and a marked hyperpolarization (a'b'). The majority of this hyperpolarization presumably resulted from noncoupled \( \text{Na}^+ \) exit via an \( \text{Na}^+ \) conductance, inasmuch as the tubule had been exposed to an organic substrate–free
solution for 5.4 min before the removal of Na⁺ (point a). When Na⁺ was removed from the bath, pHᵢ gradually decreased (be) and the basolateral membrane gradually depolarized (b′c′). The mean pHᵢ decrease observed upon bilateral replacement of Na⁺ with NMDG (a vs. c) was 0.51 ± 0.07 (n = 7), so that pHᵢ declined to a mean value of 6.90 ± 0.08 (n = 6). This acidification probably has two major causes. First, reversal of Na-H exchange (Boron and Boulpaep, 1983a) is expected to acidify the cell as H⁺ enters the cell in exchange for exiting Na⁺.

Second, in the absence of Na-dependent acid-extrusion processes, the cell is expected to be acidified by various other processes, such as passive H⁺ influx, HCO₃⁻ efflux, and cellular metabolism.

When the lumen of the tubule in the experiment of Fig. 3 was perfused with a solution containing either D-Lac⁻ (cd) or L-Lac⁻ (ef), in the continued absence of Na⁺, there was little, if any, change in pHᵢ. This contrasts with the large pHᵢ changes observed in the same tubule in the presence of Na⁺ (see cd and ab in Fig. 2). In five experiments, removal of Na⁺ reduced the maximal rate of the
alkalinization induced by luminal L- or D-Lac− by 95 ± 5 and 96 ± 3%, respectively. This suggests that the mechanism responsible for the alkalinization observed in Fig. 2 involves Na-dependent transport of Lac−. The tubule was then exposed to luminal Na+ in the absence of Lac−. In this example, this maneuver caused a 0.05 increase in pHᵢ (gḥ) and a depolarization of 20 mV (g').

The pHᵢ response in six experiments was variable, with luminal Na+ addition producing an unequivocal alkalinization in only two tubules (pHᵢ increases of 0.15 and 0.09). The mean pHᵢ increase in these six tubules was 0.03 ± 0.01, which is not statistically significant. Because organic substrates were absent, the marked depolarization at g′ presumably results from noncoupled Na+ entry via an Na+ conductance. The magnitude of this depolarization was variable. In three tubules, the depolarization was <2 mV, and in three others it was 11, 20, and 22 mV.

The final maneuver in the experiment of Fig. 3 was the addition of D-Lac− to the lumen in the continued presence of luminal Na+. This produced a dramatic alkalinization (h) and a slow hyperpolarization (h'). In four experiments, the maximal rate of alkalinization after luminal addition of D- or L-Lac− in the presence of Na+ was 0.24 ± 0.06/min. pHᵢ increased by an average of 0.31 ± 0.05, to a value of 7.29 ± 0.08. This final pHᵢ is not statistically different from the pHᵢ observed before removal of Na+ in the same four tubules (7.38 ± 0.08). Thus, luminal addition of Na+ and Lac− in combination, but not separately, produces a marked alkalinization. The finding that the pHᵢ increase elicited by Na+ and Lac− in combination is more than an order of magnitude greater than that induced by Na+ alone indicates either that luminal Na-H exchange is Lac− dependent, or that luminal Na/Lac cotransport is a more important determinant of pHᵢ than luminal Na-H exchange, at least under the conditions of our experiments.

As shown at point h′ in Fig. 3, the addition of Lac− in the presence of luminal Na+ causes no immediate change in Vbh. The gradual hyperpolarization at h′ is similar to that observed in the portion of this experiment shown in Fig. 2 at a′ and c′. Possible explanations for the hyperpolarization induced by Lac− in the presence of Na+ include: (i) an increased basolateral K+ conductance secondary to the increase in pH, (Steels and Boulpaep, 1976; Cook et al., 1984), and/or (ii) stimulation of the Na-K pump (Sackin and Boulpaep, 1983) secondary to increased Na+ entry via the Na/Lac cotransporter.

The Na+ dependence of the alkalinization induced by addition of luminal Lac−, as well as the absence of an immediate change in Vbh, strongly suggest that luminal Lac− entry is mediated by an electroneutral Na/Lac cotransporter.

**Effect of an inhibitor of gluconeogenesis on the luminal Lac−-induced alkalinization.** Lac− entry itself is not expected to cause a detectable change in pHᵢ, because >99.9% of entering Lac− will remain dissociated (pKᵢₗ₉₉₇ = 3.7). Given the previously determined buffering power of Ambystoma proximal tubule cells under the conditions of our experiments, 36 mM (Boron and Boulpaep, 1983), ~128 mol of Lac− would have to enter per liter of cell water to cause the observed pHᵢ increase of 0.38. One possible mechanism for the luminal Lac−-induced alkalinization is that the entering Lac− undergoes a metabolic reaction that consumes protons. For example, the formation of glucose from Lac− via gluco-
neogenesis involves the consumption of two cytoplasmic H\(^+\) for every glucose formed. Another possibility is that the observed pH\(_i\) increase results from the exit of Lac\(^-\) via H/Lac cotransport (or an equivalent process such as Lac\(^-\)-base exchange or nonionic diffusion of HLac).

The possibility that gluconeogenesis is involved was examined in four experiments (not shown) in which we used the phosphoenolpyruvate-carboxykinase inhibitor 3-mercaptopicolinate (MPA) to inhibit gluconeogenesis (Kostos et al., 1975). The protocol was similar to that used in the experiment of Fig. 2. After removing all organic substrates from the lumen and the bath, we exposed the lumen several times to 3.6 mM L-Lac\(^-\) in either the absence (control) or presence (experimental) of bilateral 1 mM MPA. The relative initial rate (experimental/control) of the luminal Lac\(^-\)-induced alkalinization was 1.00 \(\pm\) 0.09 when Lac\(^-\) was added after 4 min in MPA, and 0.90 \(\pm\) 0.03 \((P < 0.05)\) after 10 min. 10 min exposure to 1 mM MPA is sufficient for inhibition of gluconeogenesis in rabbit proximal tubules in suspension (Gullans et al., 1984). Therefore, gluconeogenesis does not seem to play a major role in the luminal Lac\(^-\)-induced alkalinization.

**Lactate Transport across the Basolateral Membrane**

**Effect of basolateral Lac\(^-\) on pH\(_i\).** In this series of experiments, we investigated whether the basolateral membrane of these tubules possesses an H/Lac cotransport mechanism (or a Lac-base exchanger) similar to that found in nonepithelial cells. In the experiment of Fig. 4, all substrates were first removed from the lumen and bath, resulting in the acidification (ab) and hyperpolarization (a') described above (Fig. 1). The tubule was then subjected to 1-min basolateral exposures to 3.6 mM L-Lac\(^-\). As seen at b in Fig. 4, exposure of the basolateral membrane to Lac\(^-\) caused a rapid decrease in pH\(_i\), consistent with the coupled uptake of H\(^+\) and Lac\(^-\). The effect was immediately reversed upon removal of Lac\(^-\) (c). In eight experiments, the acidification rate observed upon exposure to Lac\(^-\) was 0.33 \(\pm\) 0.04/min, and the alkalinization rate when Lac\(^-\) was removed was 0.39 \(\pm\) 0.04/min. These rates are not statistically different.

**Effect of CHC on basolateral Lac\(^-\) transport.** If the equilibration of HLac across the basolateral membrane is mediated by an H/Lac cotransporter, then it is expected to be sensitive to CHC, a known inhibitor of H/Lac cotransport in nonepithelial cells (Halestrap and Denton, 1974; Spencer and Lehninger, 1976). In five experiments similar to that of Fig. 4, the addition of 2 mM CHC to only the bath in the absence of Lac\(^-\) (d) produced no statistically significant change in pH\(_i\), which suggests that CHC does not substitute for Lac\(^-\) in transporting acid equivalents into the cell. This is consistent with the finding that CHC does not enter Ehrlich cells (Spencer and Lehninger, 1976), but is at variance with reports that CHC enters red cells and cells of renal cortical slices (Halestrap and Denton, 1974) and that CHC causes a pH\(_i\) decrease in Purkinje strands (De Hemptinne et al., 1983).

Addition of 3.6 mM L-Lac\(^-\) in the continued presence of CHC caused acidification (ef), but at a much slower rate than in the absence of CHC. In six experiments, CHC reduced the maximal rate of acidification by 75 \(\pm\) 3%. Similarly, CHC reduced by 76 \(\pm\) 3\% \((n = 5)\) the rate of alkalinization observed
when \( \text{Lac}^- \) was removed. The inhibitory effect of \( \text{CHC} \) was completely reversed after removal of \( \text{CHC} \) from the basolateral solution, as indicated in the final two \( \text{Lac}^- \) pulses of Fig. 4. Inasmuch as the presence of a competitive inhibitor of \( \text{Lac}^- \) transport would not be expected to affect nonionic diffusion of \( \text{HLac} \) through the membrane, the above data suggest that the basolateral membrane of these tubules possesses an \( \text{H/Lac} \) cotransporter (or \( \text{Lac-base} \) exchanger).

Biphasic \( \text{pH}_i \) changes after bilateral \( \text{Lac}^- \) removal. As seen at point \( a \) in Fig. 4, bilateral removal of all organic substrates typically causes a small, transient alkalization, followed by a much larger acidification. As noted in regard to Fig. 1, a similar biphasic \( \text{pH}_i \) change is observed when only \( \text{Lac}^- \) is removed. We confirmed that the transient alkalization is not an artifact of switching the bath solution before the luminal solution. If \( \text{Lac}^- \) (or all substrates) is removed from only the bath or only the lumen, there is monotonic alkalization (\( c \) in Fig. 4) or acidification (\( b \) in Fig. 2), respectively. This suggests that the transient alkalization is due to early predomination of the basolateral effect, whereas the sustained acidification is due to late predomination of the luminal effect.

Effects of basolateral \( \text{Lac}^- \) and \( \text{CHC} \) on \( V_{bl} \). Two aspects of the \( V_{bl} \) data of Fig. 4 are noteworthy. First, basolateral addition of 3.6 mM \( \text{Lac}^- \) (\( b' \)) caused a

\[
\text{LUMEN:} \begin{bmatrix} \text{L-Lac}^- \\ \text{CHC} \end{bmatrix} 3.6, \quad 0, \quad 0
\]

\[
\text{BATH:} \begin{bmatrix} \text{L-Lac}^- \\ \text{CHC} \end{bmatrix} 3.6, \quad 0, \quad 3.6, \quad 0, \quad 3.6, \quad 0, \quad 3.6, \quad 0, \quad 3.6, \quad 3.6, \quad 0, \quad 0
\]

\[
\text{pH}_i \quad 7.8
\]

\[
V_{bl} \quad (\text{mV}) \quad -80
\]
slight depolarization that averaged 0.8 ± 0.3 mV (n = 8, P < 0.05); a possible mechanism for this is presented in the Discussion. Second, the addition of CHC (d') depolarizes the cell. In six tubules, the mean CHC-induced depolarization was 6.0 ± 1.3 mV. The V_h recovery (i.e., hyperpolarization) upon removal of CHC was 2.5 ± 0.5 mV. We did not investigate the mechanism of the CHC-induced depolarization.

**Dependence of basolateral lactate-induced acidification on [Lac-].** The hypothesis that basolateral Lac^- transport is carrier-mediated is supported by our observation that the initial rate of basolateral Lac^- induced acidification is a saturable function of basolateral [Lac^-]. This is illustrated in Fig. 5. The estimated

\[
V_{\text{max}} = 1.4 \text{ pH units/min}
\]

\[
K_m = 11.5 \text{ mM}
\]

\[
V_{\text{max}} = 1.40 \text{ pH/min}
\]

**Figure 5.** Initial rate of basolateral lactate-induced acidification, and its dependence on the bath lactate concentration. After all organic substrates were removed from the lumen and bath (solution 2), the basolateral membrane was exposed to different concentrations of L-Lac^- Data are from unpaired experiments, including the four series of experiments of Figs. 4, 6, 7, and 11. Data were fitted to the Michaelis-Menten equation using nonlinear least-squares analysis. The number of experiments for each [Lac^-] was as follows: 8 at 3.6 mM, 7 at 7.2 mM, 4 at 8.0 mM, 3 at 16 mM, and 4 at 36 mM. Best-fit values were 11.5 mM for \( K_m \) and 1.4 min^-1 for \( V_{\text{max}} \).

\( V_{\text{max}} \) is 1.4 pH units/min. The estimated \( K_m \) is 11.5 mM, a value in excellent agreement with the value of 13.4 reported for erythrocytes by Dubinsky and Racker (1978). We found that, for a given [Lac^-]_b, the rate of acidification upon application of Lac^- was not statistically different from the rate of alkalinization upon removal of Lac^-.

The magnitude of the pH decrease induced by basolateral Lac^- also increased as [Lac^-]_b increased. At [Lac^-]_b values of 3.6, 7.2, and 36 mM, the observed pH changes were not statistically different from those predicted for the equilibration of HILac across the basolateral membrane. For example, for a [Lac^-]_b of 3.6 mM, the observed acidification was 0.08 ± 0.01 (n = 8) and the predicted acidification (see Appendix) was 0.07.
Stereospecificity of basolateral H/Lac cotransport. In the experiment of Fig. 6, we examined the stereospecificity of the proposed basolateral H/Lac cotransporter. We applied 7.2 mM Lac\(^-\) rather than 3.6 mM in order to exaggerate the pH changes. In four experiments, the acidification caused by addition of D-Lac\(^-\) (c) was 0.84 ± 0.11 times as rapid as that caused by L-Lac (a), and the alkalinization caused by removal of D-Lac\(^-\) (d) was 0.81 ± 0.11 times as rapid as that caused by removal of L-Lac\(^-\) (b). Neither of these values was statistically different from unity. In some preparations, the affinity of the H/Lac cotransporter for D-Lac\(^-\) has been found to be nearly identical to that for L-Lac\(^-\) (Spencer and Lehninger, 1976), whereas in others, the cotransporter has a much higher affinity for L-Lac\(^-\) (Deuticke et al., 1978).

Note that the application of 7.2 mM basolateral Lac\(^-\) not only caused larger pH\(_i\) changes than did 3.6 mM Lac\(^-\), but it also produced larger V\(_{bl}\) changes (compare Figs. 4 and 6).

Effect of SITS on basolateral Lac\(^-\) transport. Among the inhibitors of H/Lac cotransport are the disulfonic stilbenes, which produce a progressive inhibition in erythrocytes (Deuticke, 1982). We examined the effect of 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonate (SITS) on the basolateral Lac\(^-\)–induced acidification in the experiment of Fig. 7. The first three 7.2 mM Lac\(^-\) pulses
were applied in the absence of SITS. 1 min after the introduction of SITS, the initial acidification rate was already reduced in this example and it continued to fall progressively. In a series of similar experiments, the mean inhibition was 15 ± 8% (n = 4, NS) 1–2.5 min after the introduction of SITS, and increased to 30 ± 9% (n = 5, P < 0.05) after 2.5–5 min, 49 ± 8% (n = 5, P < 0.01) after 5–10 min, and 65 ± 5% (n = 5, P < 0.001) after 10–15 min.

Interaction between Luminal and Basolateral Lactate Transport

Effect of basolateral CHC on the luminal Lac–induced alkalinization. Given the evidence for a basolateral H/Lac cotransporter, we tested the hypothesis that the alkalinization caused by addition of Lac− to the lumen is the result of a two-step process. In the first step, Lac− enters with Na+ across the luminal membrane via the Na/Lac cotransporter, and in the second, Lac− exits across the basolateral membrane with H+ via the H/Lac cotransporter (or in exchange for a base such as OH− or HCO3−). If this model is correct, then inhibition of basolateral H/Lac cotransport by bath CHC should inhibit the luminal Lac−–induced alkalinization. As shown in the experiment of Fig. 8, the alkalinization induced by luminal addition of L-Lac− is substantially slower (de) in the presence of 2 mM basolateral CHC than in its absence (ab and fg). In five similar experiments, 2 mM basolateral CHC reduced the initial alkalinization rate by 69 ± 4%. As indicated by the third luminal Lac− pulse of Fig. 8, the inhibitory effect of basolateral CHC is fully reversible. In preliminary studies (not shown), we found that basolateral CHC
similarly inhibits the alkalinization induced by addition of luminal pyruvate or acetate. These data strongly support the view that the alkalinization resulting from luminal addition of Lac$^-$ (or pyruvate or acetate) results from basolateral Lac$^-$ exit via a CHC-sensitive H-monocarboxylate cotransporter (or monocarboxylate-base exchanger).

Effect of luminal CHC on the luminal Lac$^-$-induced alkalinization. To determine whether CHC can directly inhibit luminal Na/Lac cotransport, we repeated the protocol of Fig. 8, but added the drug to the lumen instead of the bath. In the experiment of Fig. 9, in which the lumen was exposed to 3.6 mM L-Lac$^-$ in the absence (ab) and presence (de) of 2 mM CHC, the drug had only a small effect on the rate of the luminal Lac$^-$-induced alkalinization. In six experiments, the mean inhibition was 10 ± 4% (NS). Thus, CHC does not have a substantial effect on luminal Na/Lac cotransport.

Effect of basolateral CHC on steady state pH$_i$. The above CHC data support the previously mentioned Na/Lac:H/Lac model, one implication of which is that transepithelial Lac$^-$ reabsorption causes cell alkalinization. If this is true, then inhibition of Lac$^-$ reabsorption should be associated with a decrease in steady state pH$_i$. One way of inhibiting Lac$^-$ reabsorption is to remove Lac$^-$ bilaterally. As was evident in the experiment of Fig. 1, this does indeed produce a sustained
Figure 9. Effect of luminal CHC on luminal lactate-induced alkalinization. After all organic substrates were removed from the lumen and bath (solution 2), the lumen was exposed to 3.6 mM \( L \)-lactate (solution 4) with and without 2 mM CHC in the lumen. A solution-change artifact in the voltage trace was deleted at point \( a' \). The broken lines represent the initial slopes of the increases in \( pHi \), and the associated numbers are the values of these slopes in pH units per minute. Six similar experiments were performed.

Another way of inhibiting \( Lac^- \) reabsorption is to add CHC to the bath in the continued presence of bilateral \( Lac^- \). As shown in the experiment of Fig. 10, the addition of 2 mM CHC to the bath caused a sustained but reversible fall in \( pHi \). In five experiments, the mean \( pHi \) decrease was 0.12 ± 0.02, and the initial rate of the CHC-induced acidification was 0.15 ± 0.02 pH units per minute.

Figure 10. Effect of basolateral CHC on steady state \( pHi \). Tubules were bathed bilaterally with solution 1 (containing all substrates) and exposed to CHC in the bath. The broken lines represent the initial slopes of the \( pHi \) changes, and the associated numbers are the values of these slopes in pH units per minute. Five similar experiments were performed.
units/min. The rate of pHr increase upon CHC removal was substantially greater, 0.36 ± 0.04 pH units/min (P < 0.01). pHr may increase twice as fast as it decreases because [Lac\(^-\)] is higher when CHC is removed than when it is added. This would be expected if inhibition of basolateral H/Lac cotransport by CHC allowed intracellular Lac\(^-\) to accumulate as a result of luminal Na/Lac cotransport.

**Na\(^+\)** Independence and Sidedness of H/Lac Cotransport

**Na\(^+\)** independence of basolateral H/Lac cotransport. The experiment of Fig. 11 was performed in order to address the following questions: (a) Is basolateral H/Lac cotransport Na\(^+\) dependent? (b) Does H/Lac cotransport occur at the luminal as well as at the basolateral membrane? At the outset of the experiment, Lac\(^-\) as well as glucose and amino acids were removed from lumen and bath, causing a fall in pHr (ah), similar to that shown in Fig. 1. We then exposed the tubule to 36 mM L-Lac\(^-\), first in the bath (bc) and then in the lumen (de), all in the presence of Na\(^+\). 36 mM Lac\(^-\), 10 times the usual concentration, was used in order to exaggerate the pHr changes. Extracellular [Cl\(^-\)] was fixed at 62 mM throughout the experiment by replacing 36 mM glucuronate or cyclamate with 36 mM Lac\(^-\). As expected, the pHr changes caused by addition of 36 mM Lac\(^-\) to the lumen or bath were faster and larger than those caused by similar additions of 3.6 mM Lac\(^-\). The dependence of the initial rate of acidification on basolateral [Lac\(^-\)] is presented in Fig. 5. The initial rate of alkalinization with 36 mM luminal Lac\(^-\) was 0.82 ± 0.17/min (n = 4), twice as great as that caused by addition of 3.6 mM Lac\(^-\).

After these basolateral and luminal pulses of 36 mM Lac\(^-\), Na\(^+\) was removed bilaterally, causing pHr to increase transiently (fg; see discussion below) and then decline slowly (gh) to a value below the initial one. When Lac\(^-\) was added to the bath again (hi), this time in the absence of Na\(^+\), pHr declined rapidly. In four paired experiments, the initial acidification rate was 84 ± 15% as great in the absence of Na\(^+\) as in its presence (not significantly different from unity). Although the mean initial pHr in the absence of Na\(^+\) (h) was 0.17 ± 0.03 (n = 4, P < 0.05) lower than in the presence of Na\(^+\) (b), this difference in the initial pHr is not expected to affect the initial rate as long as [Lac\(^-\)] is initially zero. On the other hand, this difference in initial pHr is probably why the magnitude of the acidification was significantly less in the absence of Na\(^+\) (0.21 ± 0.03) than in its presence (0.34 ± 0.04; P < 0.02). As discussed in the Appendix, the magnitude of the acidification is expected to decrease as the initial pHr decreases, regardless of the mechanism (e.g., nonionic diffusion of HLac, H/Lac cotransport) that equilibrates HLac across the cell membrane. Thus, basolateral H/Lac cotransport is independent of Na\(^+\).

As noted above (see Figs. 4 and 6), application of basolateral Lac\(^-\) elicits a depolarization. In four experiments similar to that of Fig. 11, this depolarization was unaffected by Na\(^+\) removal. The mean depolarization induced by the addition of 36 mM Lac\(^-\) to the bath was 5.0 ± 1.0 mV in the presence of Na\(^+\) and 6.2 ± 1.4 mV in its absence (compared b' and h').

**Absence of luminal H/Lac cotransport.** An H/Lac cotransporter (or a monocarboxylate-base exchanger) has been described in luminal membrane vesicles
FIGURE 11. Effect of 36 mM L-lactate in the bath or lumen in the presence and absence of Na⁺. All organic substrates were first removed from the bath and lumen, and 36 mM Cl⁻ was replaced with glucuronate (solution 7). Thus, [Cl⁻] was kept constant during subsequent exposure of the tubule to 36 mM Lac⁻ (solution 6), in which the lactate replaced glucuronate. This precaution was taken to minimize Vₑ changes in this tubule, for which the paracellular shunt is Cl⁻ selective. The tubule was then exposed to 36 mM L-Lac⁻ in the lumen or bath (solution 6). Na⁺ was then removed from the lumen and bath (solution 11) and the tubule was exposed to 36 mM Lac⁻ in the absence of Na⁺ (solution 10). Finally, Na⁺ was restored to the bath and then the lumen in the absence of Lac⁻ (solution 7). A solution-change artifact in the voltage trace was deleted at point d'. The two gaps in the record represent periods of 8.4 and 7.7 min, respectively. A total of four similar experiments were performed; in two, the Cl⁻ substitute was glucuronate; in two others, it was cyclamate. 030486A.
from rabbit renal cortex (Guggino et al., 1983). If such a mechanism were present at the luminal membrane of the *Ambystoma* proximal tubule, addition of Lac\(^-\) to the lumen would be expected to cause a decrease in pH, provided Na/Lac cotransport was blocked by Na\(^+\) removal. However, as indicated at *j* in Fig. 11, addition of 36 mM Lac\(^-\) to the lumen caused no detectable change in pH. In three experiments, the mean pH change was an increase of 0.01 ± 0.01 (NS), which indicates minimal H/Lac cotransport or nonionic diffusion of HLac at the luminal membrane. A lack of significant luminal H/Lac cotransport is also suggested by the observation (see Fig. 9) that luminal CHC did not inhibit the alkalinization induced by luminal Lac\(^-\). If substantial recycling of Lac\(^-\) via H/Lac cotransport had occurred across the luminal membrane, alkalinization should have been inhibited by luminal CHC.

**pH changes induced by altering [Na\(^+\)]\(_b\)**. In the final part of the experiment of Fig. 11, we examined the effect of returning Na\(^+\) to only the bath in the continued absence of Lac\(^-\). The basolateral addition of Na\(^+\) caused a transient acidification (tm) followed by a rapid alkalinization (mn). In seven experiments,

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**Figure 12.** Effect of amiloride on the basolateral Na\(^+\)-induced pH\(_i\) recovery. After bilateral removal of Na\(^+\) and organic substrates (solution 11), the basolateral membrane was exposed to Na\(^+\) in the absence and presence of 1 mM amiloride (solution 7). The record shows the pH recoveries after addition of bath Na\(^+\). The first and second gaps in the record represent periods of 17 and 7 min, respectively. Four similar experiments were performed, three with a [Cl\(^-\)]\(_o\) of 62 mM, and one with a [Cl\(^-\)]\(_o\) of 98 mM (solutions 8 and 2). 030786A.
the average rate of this alkalinization was 0.86 ± 0.12/min, 3.6-fold greater than the luminal Na⁺ and Lac⁻-dependent pHi recovery rate (see Fig. 3, hi). The increase in steady state pHi (l vs. n) produced by addition of basolateral Na⁺ was 0.60 ± 0.07. The subsequent addition of Na⁺ to the lumen had little effect on pHi (no).

The transient acidification observed upon basolateral Na⁺ addition (lm), previously recorded by Boron and Boulpaep (1983a, their Fig. 5), is opposite to the transient alkalinization observed upon bilateral Na⁺ removal in the present experiment (fg). These rapid transients, which were ~0.2 in magnitude, are in the direction opposite to that expected for Na-H exchange or for Na/Lac cotransport in combination with H/Lac cotransport. These pHᵢ transients may be caused by changes in extracellular [NMDG⁺] rather than in extracellular [Na⁺]. For example, they could be mediated by an organic cation-H exchanger, such as that found at the luminal membrane of canine proximal tubules (Sokol et al., 1985). In experiments such as that of Fig. 11, which were carried out in 62 mM Cl⁻, the magnitudes of these pHᵢ transients were approximately threefold greater than in experiments carried out in 98 mM Cl⁻ (unpaired data). The transients were not observed upon alterations in luminal [Na⁺], which suggests that they are produced at the basolateral membrane.

**Basolateral Na-H exchange.** The rapid and sustained alkalinization induced by returning Na⁺ to the bath in the absence of Lac⁻ (mn in Fig. 11) is presumably mediated by the same basolateral Na-H exchanger previously identified by Boron and Boulpaep (1983a). Those experiments were conducted using full-substrate solutions on tubules that were acid-loaded by pretreating with NH₄. In order to determine the amiloride sensitivity of the basolateral Na⁺-induced alkalinization, we performed four experiments similar to that of Fig. 12. The protocol was that of segment lmn of Fig. 11, with the nominal [Na⁺]ᵢ raised from 0 to 98 mM, either in the absence or the presence of 1 mM amiloride. The initial rate of the Na-induced alkalinization was inhibited 67 ± 7% by amiloride (e.g., segment ef vs. bc and ij). In the experiment shown, removal of amiloride permitted a further pHᵢ increase (fg), so that pHᵢ at point g was nearly the same as at points c and j.

**DISCUSSION**

**General**

A major finding of this study is that exposure of salamander proximal tubules to Lac⁻ from only the lumen, or from both the lumen and the bath, causes an intracellular alkalinization rather than the acidification expected to result from the nonionic permeation of lactic acid. The alkalinization induced by luminal Lac⁻ is Na⁺ dependent and is not accompanied by Vₒa changes, which suggests that luminal Lac⁻ entry is via an electroneutral Na/Lac cotransport mechanism. In contrast, exposure to Lac⁻ from only the bath causes Na-independent intracellular acidification, the rate of which is rapidly and reversibly reduced by CHC, is progressively reduced by SITS, and is a saturable function of [Lac⁻]ᵢ. These observations suggest that basolateral Lac⁻ transport is via H/Lac cotransport (or the equivalent, Lac-base exchange). As discussed below, this H/Lac transporter
is probably electroneutral. Three lines of evidence indicate that Lac\textsuperscript{−} entry across the luminal membrane leads to an intracellular alkalinization as Lac\textsuperscript{−} exits with H\textsuperscript{+} (or is exchanged for base) across the basolateral membrane. First, addition of Lac\textsuperscript{−} to the lumen causes a sustained increase in pH\textsubscript{i}. Second, basolateral CHC reduces the rate of this luminal Lac\textsuperscript{−}-induced alkalinization. Finally, in the presence of bilateral Lac\textsuperscript{−}, inhibition of basolateral H/Lac cotransport by CHC produces a decrease in the steady state pH\textsubscript{i}.

**Model**

The luminal and basolateral Lac\textsuperscript{−} transporters (i.e., the Na/Lac:H/Lac system), as well as other transporters relevant to pH\textsubscript{i} regulation in *Ambystoma* proximal tubule cells, are depicted in the model of Fig. 13. The Na-K pump establishes a luminal Na\textsuperscript{+} gradient that provides the energy for luminal Lac\textsuperscript{−} entry via electroneutral Na/Lac cotransport. Inasmuch as [Na\textsuperscript{+}]\textsubscript{l} > [Na\textsuperscript{+}]\textsubscript{i}, the secondary active transport of Lac\textsuperscript{−} with Na\textsuperscript{+} could theoretically drive [Lac\textsuperscript{−}]\textsubscript{i} to a value considerably greater than [Lac\textsuperscript{−}]\textsubscript{l} (see below). Lac\textsuperscript{−} then exits down its chemical gradient across the basolateral membrane, via electroneutral H/Lac cotransport (or Lac-base exchange). This basolateral exit of H\textsuperscript{+}, driven by the Lac\textsuperscript{−} gradient, is an example of tertiary active transport (Aronson, 1981). Lac\textsuperscript{−} transport at both membranes is expected to be voltage insensitive, inasmuch as our data indicate that the luminal Na/Lac cotransport is electroneutral and that the basolateral H/Lac cotransport is probably also electroneutral (see below).

In principle, Lac\textsuperscript{−} entering across the luminal membrane could produce a pH\textsubscript{i} increase as a result of metabolic conversion of Lac\textsuperscript{−}. However, our MPA data suggest that the observed pH\textsubscript{i} increase is not due to the conversion of Lac\textsuperscript{−} and H\textsuperscript{+} to glucose. Although conversion of Lac\textsuperscript{−} to another monocarboxylate would increase pH\textsubscript{i} if this monocarboxylate exited with H\textsuperscript{+} across the basolateral...
membrane, Brand and Stansbury (1980) found that Lac− does not undergo detectable metabolic conversion in snake proximal tubules.

According to our model, the interaction between luminal and basolateral Lac− transport has three major effects: (a) Na+ uptake at the luminal membrane, which could be important in Na§ reabsorption; (b) H§ extrusion across the basolateral membrane, which could play a role in pH§ regulation; and (c) trans-epithelial Lac− reabsorption. It has long been known that the kidney reabsors nearly all filtered L- and D-Lac− (Craig, 1946). Although we are unaware of any studies on Lac− reabsorption in Ambystoma proximal tubules, the proximal tubule is the site of Lac− reabsorption in the mammalian kidney (Hohmann et al., 1974), and luminal Na− dependent active Lac− reabsorption has been demonstrated in reptilian proximal tubules (Brand and Stansbury, 1980, 1981).

Fig. 13 also includes three other transporters that affect pH§ regulation in this preparation. The basolateral electrogenic Na/HCO3− cotransporter (Boron and Boulpaep, 1983b) has an HCO3−:Na+ coupling ratio (n) greater than unity. It was inhibited in the present study by using nominally HCO3−-free solutions. The possible interaction between this mechanism and Lac− transport is discussed below. The luminal Na-H exchanger (Boron and Boulpaep, 1983a), at least under the conditions in our experiments (see Fig. 3), is considerably less important in acid extrusion than the Na/Lac:H/Lac system. The results of Figs. 11 and 12 confirm the presence of basolateral Na-H exchange (Boron and Boulpaep, 1983a) and indicate that this is the most potent mechanism of acid extrusion in these cells.

**Luminal Lactate Transport**

Our data indicate that an electroneutral Na/Lac cotransporter is present at the luminal membrane of the Ambystoma proximal tubule. Earlier work on mammalian renal proximal tubule preparations suggests that the Na/Lac cotransporter is relatively nonspecific for monocarboxylates, accepting aliphatic monocarboxylic acids such as pyruvate and acetate, as well as aromatic monocarboxylic acids such as benzoate (Ullrich et al., 1982b, c; Nord et al., 1983; Wright, 1985). Our observation that luminal addition of L-Lac−, D-Lac−, pyruvate, or acetate raised pH§ indicates that the luminal Na/Lac cotransporter of Ambystoma is also relatively nonspecific.

The data on the effect of CHC on luminal Na/Lac cotransport in mammalian preparations are conflicting. In rat small intestine, CHC inhibited Na-dependent Lac− uptake in microvillus vesicles (Storelli et al., 1980). In microperfused rat proximal tubules, luminal CHC inhibited Lac− reabsorption (Ullrich et al., 1982c), whereas CHC did not affect Na-dependent Lac− uptake in rabbit renal microvillus vesicles (Aronson, P. S., et al., manuscript in preparation). Our observation that luminal CHC does not inhibit the luminal Lac−–induced alkalization (Fig. 9) is therefore consistent with the rabbit vesicle data.

In contrast to our evidence that luminal Na/Lac cotransport is electroneutral in Ambystoma proximal tubules, data from mammalian proximal tubule preparations indicate that Na/Lac cotransport is electrogenic. In rat proximal tubules, luminal addition of 5 mM L-Lac− causes a basolateral depolarization of 3.2 mV
8IE BENS AND BORON  Effect of Lactate Transport on pH;
823
(Samarzija et al., 1981). Renal microvillus vesicle studies indicate that Na/Lac
cotransport both causes and responds to changes in the membrane potential (see
Wright, 1985). The Na:Lac coupling coefficient was 2 when estimated from a
Hill plot (Mengual et al., 1983) and ranged from 1.4 to 1.7 when estimated from
equilibrium-exchange studies (Mengual et al., 1983). One possible explanation
for the non-integer values is that two Na/Lac cotransporters are present: an
electroneutral one with a coupling coefficient of 1 (similar to that of the
Ambystoma), and an electrogenic one with a coupling coefficient of 2. The
electroneutral cotransporter might be difficult to detect because of the activity
of the electrogenic system. Electroneutral Na/Lac cotransport has also been
reported in the rabbit small intestine (Hildmann et al., 1980).

In preliminary experiments on Ambystoma proximal tubules, Morgunov and
Boulpaep (unpublished data), using conditions different from those of our study,
have found that luminal addition of 3.6 Lac− in produces a 10-mV depolarization.
Their experiments were performed with 10 mM HCO3− and a full complement
of substrates (including Lac−) in the bath. Among the possible explanations for
the difference in results are the following. (a) HCO3− converts the electroneutral
cotransporter to an electrogenic one. (b) A second, electrogenic Na/Lac cotrans-
porter becomes functional only in the presence of HCO3−. However, because
electrogenic Na/Lac cotransport occurs in vesicles under nominally HCO3−-free
conditions (Barac-Nieto et al., 1980), HCO3− does not appear to be a general
requirement for electrogenic Na/Lac cotransport. (c) Luminal Na+ influx via
electroneutral Na/Lac cotransport increases basolateral Na+ efflux via electro-
genic Na/HCO3− cotransport, producing a depolarization. Blocking the
Na/HCO3− cotransporter with SITS would be expected to prevent this depolar-
ization.

Basolateral Lactate Transport

Our data indicate that an electroneutral H/Lac cotransporter is present at the
basolateral membrane of the Ambystoma proximal tubule. Note that we cannot
distinguish an H/Lac cotransporter from a Lac-OH exchanger, or from a Lac-
HCO3− exchanger functioning with the small amounts of HCO3− present in our
nominally HCO3−-free system.

Data from a number of nonepithelial cells indicate that transport of Lac− or
HLac is mediated by an H/Lac cotransporter (or its equivalent, a monocarbox-
ylate-base exchanger) that is sensitive to CHC. We are unaware of any studies
showing that the cotransporter is independent of Na+. On the other hand, studies
on epithelia have demonstrated that basolateral Lac− transport is electroneutral
and Na+ independent (Storelli et al., 1980; Barac-Nieto et al., 1982), but have
not demonstrated coupling to H+. Furthermore, the effect of CHC on basolateral
Lac− transport has not been examined in proximal tubules. In one study on
small-intestine vesicles (Storelli et al., 1980), CHC (2 mM) inhibited basolateral
transport of Lac− (1 mM) by <20%. To our knowledge, the present study is the
first to demonstrate, in a single preparation, that presumed H/Lac cotransport
mediates H+ fluxes that are Na+ independent and CHC sensitive. Although we
did not apply basolateral acetate or pyruvate, our observation that luminal
addition of either compound causes a CHC-sensitive pH$_i$ increase implies that both are transported across the basolateral membrane by the H/Lac cotransporter.

As noted in the Results, basolateral Lac$^-$ causes an Na-independent depolarization, the magnitude of which increases with increasing [Lac$^-$] (see Figs. 4, 6, and 11). In principle, a $V_m$ change could be produced directly by electrogenic Lac$^-$ transport, three possible mechanisms of which will be considered. The first is a simple basolateral Lac$^-$ conductance. However, this model predicts that basolateral Lac$^-$ would cause hyperpolarization rather than the observed depolarization. The second is an Na/Lac cotransporter carrying net positive charge, analogous to the basolateral Na/glutamate and Na/aspartate cotransporters of the rat proximal tubule (Samarzija and Fromter, 1976). However, the depolarization we observed was Na$^+$ independent. The third is an H/Lac cotransporter carrying net positive charge (i.e., with an H$^+$/Lac$^-$ coupling coefficient greater than unity). However, thermodynamic arguments based on this model predict that unreasonable Lac$^-$ gradients would be required. The data of Fig. 1 (fg) indicate that the bilateral addition of Lac$^-$ causes a sustained pH$_i$ increase. This is presumably the result of luminal Na/Lac entry, which causes [Lac$^-$]$_i$ to increase sufficiently to produce basolateral H/Lac efflux. For an H$^+$/Lac$^-$ coupling ratio of 2, the calculated [Lac$^-$]$_i$ necessary to drive basolateral Lac$^-$ exit would be $>40$ mM, whereas the maximal [Lac$^-$]$_i$ that could be generated by luminal electroneutral Na/Lac cotransport$^3$ is only $~15$ mM. On the other hand, for a coupling ratio of 1, the calculated$^3$ [Lac$^-$]$_i$ would only have to be 3.4 mM. Thus, transepithelial Lac$^-$ transport is most easily explained if the basolateral H/Lac cotransporter, like that of nonepithelial cells, is electroneutral.

The net free energy change with the influx of two H$^+$ and one Lac$^-$ is:

$$-FV_m + 2.3RT \log \left( \frac{[\text{Lac}^-][\text{H}^+]}{[\text{Lac}^-][\text{H}^+]^{2}} \right)$$

If it is assumed that [Lac$^-$]$_o$ = 3.6 mM and pH$_o$ = 7.5 (the values in our experiments), and that pH$_i$ = 7.47 and $V_m$ = $-68$ mV (the mean values in our experiments), then an H/Lac cotransporter with an H$^+$/Lac$^-$ coupling ratio of 2 is in equilibrium when the calculated [Lac$^-$]$_i$ is 46.5 mM.

The net free energy change with the influx of one Na$^+$ and one Lac$^-$ is:

$$2.3RT \log \left( \frac{[\text{Lac}^-][\text{Na}^+]}{[\text{Lac}^-][\text{Na}^+]^{1}} \right)$$

If it is assumed that [Lac$^-$]$_o$ = 3.6 mM, [Na$^+$]$_o$ = 98 mM (the value in our experiments), the activity coefficient for Na$^+$ is 0.75, and the intracellular Na$^+$ activity is 17.8 mM (Morgunov and Boulpaep, 1987), then an Na/Lac cotransporter with an Na$^+$/Lac$^-$ coupling ratio of 1 is in equilibrium when the calculated [Lac$^-$]$_i$ is 14.9 mM. The maximal [Lac$^-$]$_i$ that can be generated by this transporter under these conditions is therefore 14.9 mM.

The net free energy change with the influx of one H$^+$ and one Lac$^-$ is:

$$2.3RT \log \left( \frac{[\text{Lac}^-][\text{H}^+]}{[\text{Lac}^-][\text{H}^+]^{1}} \right)$$

If it is assumed that [Lac$^-$]$_o$ = 3.6 mM, pH$_o$ = 7.5 (the value in our experiments), and pH$_i$ = 7.47 (the mean value in our experiments), then an H/Lac cotransporter with an H$^+$/Lac$^-$ coupling ratio of 1 is in equilibrium when the calculated [Lac$^-$]$_i$ is 3.4 mM.
The above analysis, which indicates that the depolarization induced by basolateral Lac\(^-\) is probably electroneutral, agrees with the conclusions of others on similar transporters in other cells. The observed depolarization upon application of basolateral Lac\(^-\) is probably an indirect result of the accompanying acidification. A fall in pH\(_i\) is expected to cause depolarization by reducing the K\(^+\) conductance (Steels and Boulpaep, 1976; Cook et al., 1984). In support of the hypothesis that pH\(_i\) and V\(_b\) are inversely related, we have observed that the rise in pH\(_i\) caused by luminal Lac\(^-\) is accompanied by hyperpolarization (see Figs. 2 and 3).

**Interaction between Luminal and Basolateral Lactate Transport**

**Limits on [Lac\(^-\)]\(_i\).** \([\text{Lac}^-]_i\) depends upon the balance between luminal Na\(^+\)/Lac entry and basolateral H/Lac exit. Making reasonable assumptions for pH\(_i\) and intracellular Na\(^+\) activity, we calculate that \([\text{Lac}^-]_i\) would be \(~15\text{ mM}\) if the basolateral Lac\(^-\) exit rate were zero (i.e., \([\text{Lac}^-]_i\) determined by equilibration across the luminal membrane via Na/Lac cotransport; see footnote 2). On the other hand, \([\text{Lac}^-]_i\) would be \(~3.4\text{ mM}\) if the rate constant for the equilibration of Lac\(^-\) across the basolateral membrane via H/Lac cotransport were infinite (see footnote 3). The actual \([\text{Lac}^-]_i\) is expected to be between these values. In the experiment of Fig. 10, the basolateral application of CHC (i.e., inhibition of basolateral H/Lac cotransport) is expected to increase \([\text{Lac}^-]_i\) as well as produce the pH\(_i\) decrease observed.

**Rates of Na\(^+\)/Lac and H/Lac cotransport.** The experiment of Fig. 10 also provides information on rates of Na/Lac and H/Lac cotransport. The rate of the acidification induced by CHC in the presence of Lac\(^-\), 0.15/min in Fig. 10, is an index of the rate of acid extrusion mediated by the basolateral H/Lac cotransporter before the CHC exposure. CHC has no significant effect on pH\(_i\) in the absence of Lac\(^-\) (see Fig. 4). If the intracellular buffering power is 36 mM (Boron and Boulpaep, 1983a), the acid-extrusion rate is 0.15 \times 36 = 5.4 mM/min. Because CHC only inhibits H/Lac cotransport by \(~75\%\) under these conditions, the H/Lac cotransport rate may be as high as 5.4/0.75 = 7.2 mM/min. Because the model of Fig. 13 predicts that, in the steady state, luminal Na\(^+\)/Lac and basolateral H/Lac cotransport rates are equal, the steady state influx of Na\(^+\) and Lac\(^-\) via luminal Na/Lac cotransport is expected to be \(~7.2\text{ mM/min}\) under the conditions of our experiments.

**Effects of Dual Monocarboxylate Transport System on pH\(_i\) in Other Epithelia**

The general features of the model of Fig. 13 are not restricted to the salamander. Subsequent to our demonstration of the effects of Lac\(^-\) transport on pH\(_i\) in *Ambystoma* proximal tubules, Nakhoul and Boron (1986), measuring pH\(_i\) with a pH-sensitive dye, showed that similar effects can be demonstrated in the S\(_s\) segment of the rabbit proximal tubule. Their data indicate that an Na/acetate cotransport system is confined to the luminal membrane. The major difference between Lac\(^-\) transport in the *Ambystoma* proximal tubule and acetate transport in the rabbit S\(_s\) segment is that the pathway for acetic acid transport exists at both luminal and basolateral membranes of this mammalian preparation. It is
not known whether this acetic acid transport is mediated by nonionic diffusion or the equivalent of an H/acetate cotransporter.

**Implications for Acid-Base Transport and pH, Regulation**

**Effect on transepithelial acid secretion.** According to the model of Fig. 13, transepithelial Lac⁻ reabsorption in the intact animal would be accompanied by the transfer of acid from the proximal tubule cell to the blood. To the extent that the acid equivalents extruded across the cell's basolateral membrane are derived from the lumen, the net effect would be a direct decrease in transepithelial acid secretion (e.g., HCO₃⁻ reabsorption) or an increase in acid reabsorption. If the acid is derived from the blood (e.g., by basolateral H⁺ influx or HCO₃⁻ efflux), the basolateral efflux of acid via H/Lac cotransport would simply produce a recycling of acid. However, this would amount to an indirect inhibition of transepithelial acid secretion, inasmuch as basolateral acid extrusion would compete with luminal acid extrusion for acid equivalents entering across the basolateral membrane. One might imagine that for the amphibian proximal tubule, which has a low acid-secretion rate, one of the functions of basolateral HCO₃⁻ efflux (Boron and Boulpaep, 1983b) might be to promote monocarboxylate reabsorption by providing the intracellular acid necessary for basolateral H/Lac efflux. If the acid equivalents extruded by the basolateral H/Lac cotransporter initially arise from within the cell, such as by the metabolic production of acid, there would be, strictly speaking, no direct effect on transepithelial acid-base transport. The net effect would be an acidification of the blood, such as that produced by any nonepithelial cell that extrudes acid to regulate its pHᵢ. However, transepithelial acid-base transport could be indirectly affected by basolateral H/Lac cotransport, because of changes in pHᵢ.

The effect of monocarboxylic acid transport on HCO₃⁻ reabsorption has been examined recently in the S₁ segment of the rabbit proximal tubule, in light of the present study and that of Nakhoul and Boron (1986). It was found that HCO₃⁻ reabsorption is reduced by the presence of acetate in the lumen and/or the bath (Geibel et al., 1987).

**Effect on pHᵢ regulation.** Our data indicate that, at least in the nominal absence of HCO₃⁻, Lac⁻ transport plays a substantial role in determining pHᵢ in *Ambystoma* proximal tubules. The steady state pHᵢ is ~0.2 higher in the presence of 3.6 mM bilateral Lac⁻ than in its absence, which suggests that the combination of Na/Lac and H/Lac cotransport is a major acid-extruding mechanism in this preparation. Another implication is that other acid-base transport mechanisms, most notably basolateral Na-H exchange, do not adjust their activity to prevent changes in steady state pHᵢ when Lac⁻ is either added or removed. Thus, the Na-H exchanger does not “clamp” pHᵢ at a particular value, but rather seems to set a limit on how far pHᵢ can fall when Lac⁻ is removed (e.g., see Fig. 1). It was previously observed in *Ambystoma* proximal tubules that switching from HCO₃⁻-free to HCO₃⁻-containing solutions causes a decrease of 0.17 in steady state pHᵢ (Boron and Boulpaep, 1983a). The application of CO₂/HCO₃⁻ introduces a chronic intracellular acid load (owing to HCO₃⁻ exit via Na/HCO₃ cotransport) that causes pHᵢ to fall. According to the general model of pHᵢ
regulation (Roos and Boron, 1981), this fall in pH causes the acid-extrusion rate via Na-H exchange to increase and it may also reduce the rate of acid loading. The pH decline continues until acid loading and acid extrusion come into balance. Similar reasoning suggests that removal of Lac− blocks a potent acid-extruding mechanism. pH would be expected to fall until the gradually increasing rate of Na-H exchange comes into balance with that of acid loading.

It is possible that, at least in the nominal absence of HCO3−, Lac− transport drives pH to a value close to or even above the pH threshold for activation of Na-H exchange. Thus, Na-H exchange may not be the dominant acid-extruding mechanism when Lac− is present. It remains to be determined whether acid extrusion via the Na/Lac:H/Lac system has a pH dependence similar to that of classic pHi regulators, for which the acid-extrusion rate increases as pH decreases (Roos and Boron, 1981). Inasmuch as pH is lower in the presence of CO2/HCO3− (Boron and Boulpaep, 1983a), it is likely that Na-H exchange makes a greater contribution to acid extrusion in the presence than in the absence of CO2/HCO3−. A complete model of pH homeostasis in Ambystoma proximal tubule cells obviously must await analysis of Lac− transport in tubules exposed to HCO3−.

In summary, our data suggest that transepithelial Lac− transport in the Ambystoma proximal tubule involves the tertiary active transport of acid outward across the basolateral membrane. The proposed sequence is the electroneutral uptake of Lac− via a luminal Na/Lac cotransporter, followed by the efflux of H+ and Lac− via an electroneutral H/Lac cotransporter at the basolateral membrane.

APPENDIX

Predicted pH Changes Caused by the Equilibration of Lactic Acid across the Plasma Membrane

Consider a cell containing no lactate (Lac−) and exposed to a Lac−-free solution. Assume that the plasma membrane (a) is freely permeable to the neutral lactic acid (HLac), (b) is impermeant to the charged Lac−, and (c) possesses no other acid-base transporters. If the cell is exposed to a solution containing Lac− and HLac (HLac ⇌ H+ + Lac−), the HLac rapidly enters, whereupon it dissociates into H+ and Lac−. The entry of HLac and the ensuing fall in pH continue until [HLac]i = [HLac]o. The magnitude of the pH decline depends upon (a) the initial pH, (b) [HLac]o, (c) the apparent pK a (pK') of the equilibrium HLac ⇌ H+ + Lac−, and (d) the non-lactate buffering power of the cell. The final pH can be determined mathematically by solving two simultaneous equations. The first describes the equilibrium between intracellular Lac− and HLac, and is analogous to a rearrangement of the Henderson-Hasselbalch equation for CO2/HCO3−:

\[
[Lac^{-}]_i = [HLac]_i \times 10^{pH_i - pK_a}. \tag{A1}
\]

Inasmuch as [HLac] = [HLac]o, this equation can be rewritten:

\[
[Lac^{-}]_i = [HLac]_o \times 10^{pH_i - pK_a}. \tag{A2}
\]

The second equation describes the titration of the cell’s non-lactate buffers by the entering HLac, as the HLac dissociates into H+ and Lac−:

\[
[Lac^{-}]_i = \beta \cdot (pH'_i - pH_i), \tag{A3}
\]
where $\beta$ is the non-lactate buffering power, and $\mathrm{pH}_i'$ is the $\mathrm{pH}_i$ before the cell is exposed to external HLaC (i.e., at which time $[\text{Lac}^-] = 0$).

The $\mathrm{pH}_i$ and $[\text{Lac}^-]$ that prevail at equilibrium (i.e., when $[\text{HLaC}] = [\text{HLaC}]_o$) can be obtained either from a numerical solution to Eqs. A2 and A3, or from a graphic approach analogous to that used to solve problems in a CO$_2$/HCO$_3^-$ buffer system (see Fig. 14). Eq. A2 is used to generate a plot of $[\text{Lac}^-]$ vs. $\mathrm{pH}_i$ for a single value of $[\text{HLaC}]$. This HLaC isopleth is analogous to the CO$_2$ isopleth of Davenport (1958). Similarly, Eq. A3 is used to generate a plot that describes the non-lactate buffering power, and is analogous to Davenport's non-HCO$_3^-$ buffering-power line. The intersection of this buffering-power line with the abscissa is the initial $\mathrm{pH}_i$ (i.e., $\mathrm{pH}_i'$) when the system is Lac$^-$ free. The intersection of the buffering-power line and the HLaC isopleth describes the $\mathrm{pH}_i$ and $[\text{Lac}^-]$ that prevail when HLaC has equilibrated across the plasma membrane. The mechanism by which HLaC equilibrates (e.g., nonionic diffusion, H/LaC cotransport, Lac$^-$/OH exchange) is immaterial.

In Fig. 14, it is assumed that the initial $\mathrm{pH}_i$ (point a) is 7.43, the average $\mathrm{pH}_i$ in seven experiments (e.g., Fig. 4) before tubules were exposed to a pH 7.5 solution containing 3.6 mM total Lac$^-$ (i.e., Lac$^-$ plus HLaC). Assuming a $\mathrm{pK}_a$ of 3.7 for the HLaC/Lac$^-$ equilibrium (Martell and Smith, 1977), $[\text{HLaC}]_o$ is 0.57 $\mu$M. As HLaC enters the cell, it dissociates and titrates non-lactate buffers, causing $\mathrm{pH}_i$ to fall. The model predicts that as HLaC approaches $[\text{HLaC}]_o$, $\mathrm{pH}_i$ should fall to 7.358 (point b). The $\mathrm{pH}_i$ change is thus $-0.072$, not statistically different from the average change observed under these conditions, $-0.08$. If the total $[\text{Lac}^-]$ is raised to 7.2 mM (i.e., $[\text{HLaC}]_o = 1.14$ $\mu$M) rather than 3.6 mM, the equilibrium is described by point c and the predicted $\mathrm{pH}_i$ change is 0.125. The magnitude of the Lac$^-$-induced $\mathrm{pH}_i$ decrease depends not only on the total $[\text{Lac}^-]$, but on the initial $\mathrm{pH}_i$ as well. Thus, if the starting $\mathrm{pH}_i$ is 7.30 (point a') instead of 7.43, the size of the predicted acidification elicited by 3.6 mM Lac$^-$ (point b') is reduced from 0.072 to 0.056, and that by 7.2 mM Lac$^-$ (c') is reduced from 0.125 to 0.100. Thus, the
lower the initial pH, the smaller the fraction of incoming H\text{Lac} that dissociates to H\(^+\) and Lac\(^-\).

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