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

ARTHUR W. SIEBENS and WALTER F. BORON

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

ABSTRACT We used microelectrodes to examine the effects of organic substrates, particularly lactate (Lac^-), on the intracellular pH (pH_i) and basolateral membrane potential (V_{bl}) in isolated, perfused proximal tubules of the tiger salamander. Exposure of the luminal and basolateral membranes to 3.6 mM Lac^- caused pH_i 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 V_{bl} changes, which suggests that it involves luminal, electroneutral Na/Lac cotransport. Addition of Lac^- to only the basolateral solution caused pH_i 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 pH_i 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 pH_i. We found that inhibition of basolateral H/Lac cotransport by basolateral CHC reduced the initial rate of pH_i 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 (pH_i) 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 pH_i at the levels normally observed, the cell

Address reprint requests to Dr. Walter F. Boron, Dept. of Cellular and Molecular Physiology, Yale University School of Medicine, B-106 SHM, P.O. Box 3333, New Haven, CT 06510. Dr. Siebens' present address is Laboratory of Kidney and Electrolyte Metabolism, National Institutes of Health, Bethesda, MD 20298.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/87/12/0799/33 $2.00

Volume 90 December 1987 799–831

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-HCO_3 exchanger that obligatorily exchanges what appears to be internal H^+ and Cl^- for external Na^+ and HCO_3^- (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., 1982*a*), 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-HCO_3 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 α-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_i in nondepolarized proximal tubules. Lactate transport was assessed by using microelectrodes to monitor lactate-induced changes in pH_i 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 these two transporters acting in series is the luminal uptake of Na^+ and the tertiary active extrusion of acid across the basolateral membrane. In addition, these transporters presumably mediate transepithelial lactate reabsorption.

Portions of this work have been published in preliminary form (Siebens and Boron, 1986*a*, *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 $CaCl_2$. 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 (1983*a*). 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 (V_{bl}) 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_i were similar to those described previously (Sackin and Boulpaep, 1981; Boron and Boulpaep, 1983*a*).

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 (1983*a*). 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 CO_2-impermeable Saran tubing (Clarkson Equipment and Controls, Detroit, MI). HEPES, CHC, 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

TABLE I

Solutions

Component	1 Standard saline	2 0 sub-strate	3 0 Lac^-	4 3.6 Lac^-	5 7.2 Lac^-	6 36 Lac^- 62 Cl^-	7 36 X^- 62 Cl^-	8 0 Na^+ 0 substrate	9 0 Na^+ 3.6 Lac^-	10 0 Na^+ 36 Lac^-	11 0 Na^+ 36 X^-
Na^+	97.7	97.8	97.7	97.8	97.8	97.8	97.8	0	0	0	0
$NMDG^+$	0	0	0	0	0	0	0	97.2	97.2	97.2	97.2
K^+	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mg^{++}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ca^{++}	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Lys^+	0.2	0	0.2	0	0	0	0	0	0	0	0
meq(+):	106.0	105.9	106.0	105.9	105.9	105.9	105.9	105.3	105.3	105.3	105.3
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	36.0	0	0	0	36.0
$H_2PO_4^-$	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
HPO_4^{--}	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	0	0	3.6	7.2	36.0	0	0	3.6	36.0	0
Glu^-	0.05	0	0.05	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
meq(−):	106.0	105.9	106.0	105.9	105.9	105.9	105.9	105.3	105.3	105.3	105.3
Glucose	2.2	0	2.2	0	0	0	0	0	0	0	0
Gln	0.5	0	0.5	0	0	0	0	0	0	0	0
Ala	0.5	0	0.5	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, 1983*a*). 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 $CaSO_4$ (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 KH_2PO_4, 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 $3–5 \times 10^{11}$ 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, 1983*a*). 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$_i$ 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 (V_{bl}) 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 V_{bl}) was the difference between the voltages of the pH microelectrode and the bath calomel half-cell. The transepithelial potential (V_{te}) 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$_i$ and V_{bl} records. Changes in V_{te}, 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 pH_i records to stabilize. The luminal and basolateral solutions were then changed as indicated in the figures.

Analysis of Data

The rate of pH_i change was determined by drawing a line through the linear portion of the pH_i 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 pH_i, V_{bl}, and V_{te} for tubules bathed bilaterally with solution 1 were 7.47 ± 0.02, −68.0 ± 1.7 mV, and −3.4 ± 0.2 mV, respectively ($n = 37$). These values are similar to the values of 7.43 for pH_i, −57 mV for V_{bl}, and −3.8 mV for V_{te} reported by Boron and Boulpaep (1983*a*) 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 pH_i and V_{bl}

The initial step in the present study was to determine whether organic substrate transport plays an important role in determining pH_i. 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 pH_i decrease (segment *ab*), the magnitude of which averaged 0.18 ± 0.02. The mean maximal acidification rate was 0.182 ± 0.016/min ($n = 23$). Organic substrate removal also caused the cells to hyperpolarize (point *a'*) by 13.9 ± 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_{te} 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 pH_i produced by organic substrate removal suggests that at least one of the organic substrates contained in solution 1 plays an important role in determining pH_i. We therefore performed six experiments similar to that shown in Fig. 1 in which we compared the pH_i 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 pH_i to decrease by 0.21 ± 0.04, with a maximal rate of 0.167 ± 0.034/min. Neither of these values is statistically different from the paired values of 0.181 ± 0.071 and 0.200 ± 0.046/min observed when all substrates were removed. Note that the initial pH_i change observed after removal of either all organic substrates (point

a) or only Lac^- (point *c*) is a small but consistently observed transient alkalinization of ~0.05. The mechanism of this transient alkalinization 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 pH_i. A small further pH_i 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_i effects was not investigated further in this study. The above findings suggest that the acidification caused by removing all

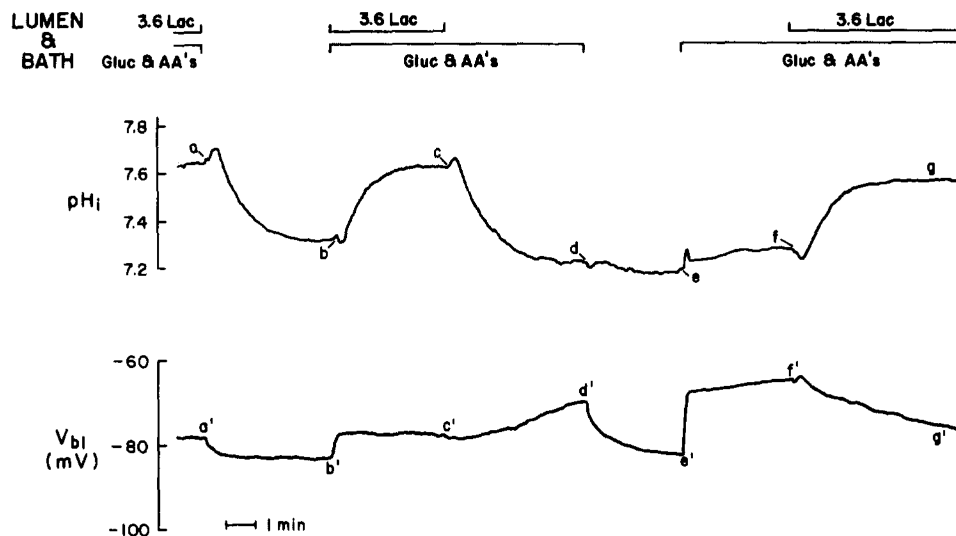

FIGURE 1. Effect of removing all organic substrates or only lactate on pH_i and V_{bl}. At the points indicated in the figure, the lumen and bath solutions were changed simultaneously. The three solutions used were the standard solution containing all organic substrates (solution 1), the zero-substrate solution (solution 2), and the zero-lactate solution containing all organic substrates other than lactate (solution 3). The lactate used was a mixture of L- and D-lactate. In two of the six experiments performed, the pH electrode impalement was lost near the end of the experiment, so that $n = 4$ for the final recovery period (segment *fg*). Preparation 022086A.

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^-, pH_i 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 alkalinization 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^- 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 ≤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 ± 2.9, +16.5 ± 4.8, −16.6 ± 2.5, and +25.5 ± 2.8 mV, respectively. In contrast, removal or readdition of Lac^- in the same tubules did not cause immediate V_{bl} changes. The mean changes were −0.2 ± 0.1 mV at c' ($n = 6$) and −1.1 ± 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^- (f'). During the 3 min after point f', the basolateral membrane hyperpolarized by 5.7 ± 0.9 mV ($n = 4$). The absence of immediate V_{bl} changes with alterations in extracellular [Lac^-] suggest that there is no substantial conductive pathway for Lac^- at either the luminal or the basolateral membrane under these conditions. The slow V_{bl} changes could be secondary to Lac^--induced alterations in cell composition (e.g., pH_i).

Because these data suggest that Lac^- 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^- transport in this preparation.

Lactate Transport across the Luminal Membrane

Effect of luminal Lac^- in the presence of Na^+. 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^-. Rather than causing the acidification expected to result from Lac^- entry by nonionic diffusion of HLac, luminal addition of Lac^- caused cell alkalinization (*ab*). In 13 similar experiments, addition of L-lactate to only the lumen resulted in a mean pH_i increase of 0.38 ± 0.05, with a mean maximal rate of 0.41 ± 0.04/min. The maximal rate of the pH_i decline after removal of luminal L-Lac^- (*bc*) was 0.27 ± 0.03/min, ~35% lower than the corresponding rate of alkalinization ($P < 0.001$). There was no immediate change in V_{bl} when Lac^- was added (a') or removed (b'). As in the experiment of Fig. 1, the addition of Lac^- produced a small, gradual hyperpolarization, which averaged 1.8 ± 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_{te} to become several millivolts more negative. In experiments similar to that of Fig. 2, we found no significant change (0.02 ± 0.02, $n = 13$) in V_{te} after addition of Lac^- to the lumen. Our observations that the addition of Lac^- to the lumen produces no immediate change in either V_{bl} or V_{te} suggest that luminal Lac^- 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 Lac^- transport. The luminal addition of D-Lac^- caused pH_i to increase (*cd*) with an initial rate of 0.171 pH units/min. This is ~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 L-Lac^-. In six experiments, the ratio of the initial rate for D-Lac^- to that for L-Lac^- was 69 ± 9% ($P < 0.05$). It has long been known that the kidney reabsorbs D-Lac^- nearly as well as 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

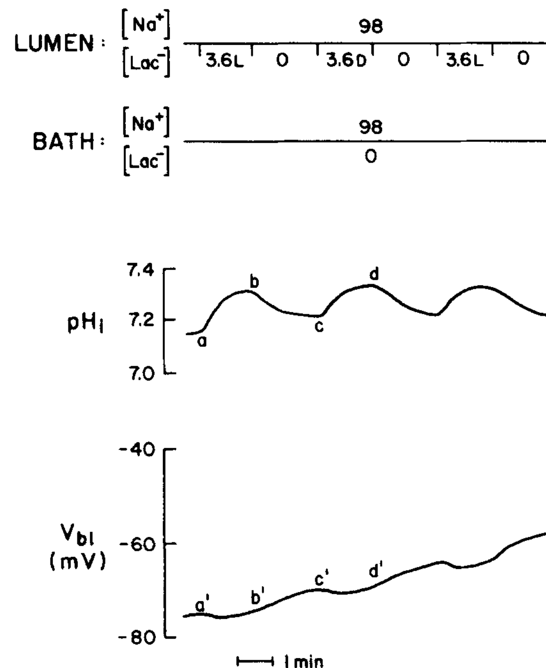

FIGURE 2. Effect of luminal L- or D-lactate in the presence of Na^+. After all substrates were removed (solution 2), the lumen was exposed to 3.6 mM L- or D-lactate (solution 4). Substrates were removed 20 min before the time represented in this record. Six similar experiments were performed. 072485A.

4 mM Lac^- was mimicked by 4 mM pyruvate, whereas 16 mM acetate was required to cause similar alkalinization.

Effect of luminal Lac^- *in the absence of* Na^+. To determine whether the pH_i and voltage effects of luminal Lac^- addition depend on 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 Na^+. When 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 Na^+ exit via an 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, pH_i gradually decreased (*bc*) and the basolateral membrane gradually depolarized (*b'c'*). The mean pH_i decrease observed upon bilateral replacement of Na^+ with NMDG (*a* vs. *c*) was 0.51 ± 0.07 ($n = 7$), so that pH_i 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, 1983*a*) is expected to acidify the cell as H^+ enters the cell in exchange for exiting Na^+.

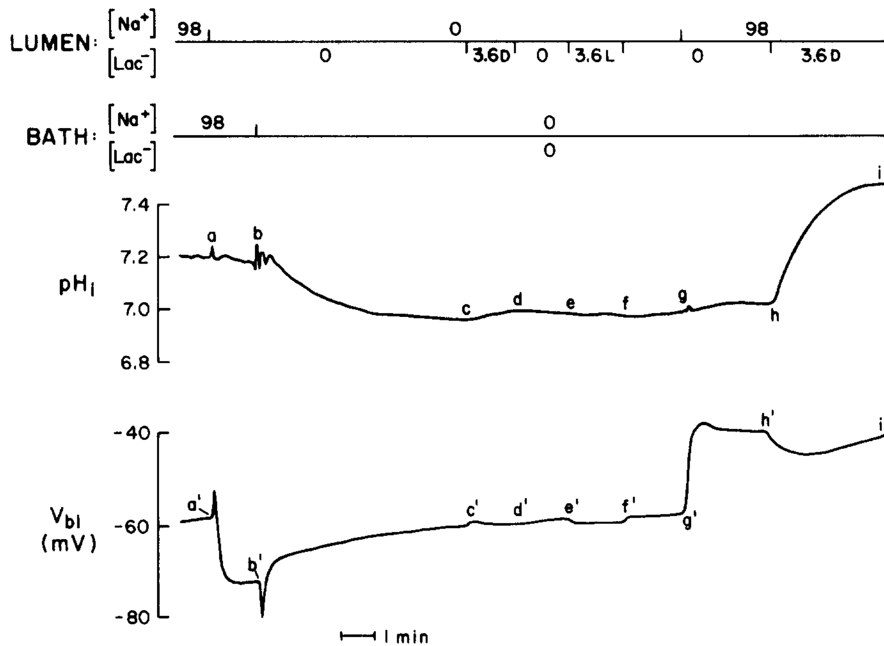

FIGURE 3. Effect of luminal L- or D-lactate in the absence of Na^+. After all Na^+ was removed from lumen and bath (Na^+ replaced with NMDG, solution 8), the lumen was exposed to 3.6 mM D- or L-lactate in the continued absence of Na^+ (solution 9). The lumen was then perfused with a zero-substrate, Na^+-containing solution (solution 2). Finally, 3.6 mM D-lactate was added to the lumen in the continued presence of luminal Na^+ (solution 4). This record is a later portion of the same experiment as that of Fig. 2. The gap between the two records is 11 min. The portion of the protocol in which Lac^- was added in the absence of Na^+ (*cd* and *ef*) was performed on five tubules; the luminal addition of Na^+ (*gh*), on six; and the luminal addition of lactate in the presence of Na^+ (*hi*), on four. 072485A.

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, HCO_3^- 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 pH_i. This contrasts with the large pH_i 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 pH$_i$ (*gh*) and a depolarization of 20 mV (*g'*). The pH$_i$ response in six experiments was variable, with luminal Na$^+$ addition producing an unequivocal alkalinization in only two tubules (pH$_i$ increases of 0.15 and 0.09). The mean pH$_i$ 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 (*hi*) 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. pH$_i$ increased by an average of 0.31 ± 0.05, to a value of 7.29 ± 0.08. This final pH$_i$ is not statistically different from the pH$_i$ 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 pH$_i$ 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 pH$_i$ 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 V_{bl}. 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$_i$ (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 V_{bl}, 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 pH$_i$, because >99.9% of entering Lac$^-$ will remain dissociated (pK$_{lactate}$ ~ 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 pH$_i$ 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 ± 0.09 when Lac^- was added after 4 min in MPA, and 0.90 ± 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 ± 0.04/min, and the alkalinization rate when Lac^- was removed was 0.39 ± 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 Lac^- was removed. The inhibitory effect of CHC was completely reversed after removal of CHC from the basolateral solution, as indicated in the final two Lac^- pulses of Fig. 4. Inasmuch as the presence of a competitive inhibitor of Lac^- transport would not be expected to affect nonionic diffusion of HLac through the membrane, the above data suggest that the basolateral membrane of these tubules possesses an H/Lac cotransporter (or Lac-base exchanger).

Biphasic pH_i *changes after bilateral* Lac^- *removal.* As seen at point *a* in Fig. 4, bilateral removal of all organic substrates typically causes a small, transient

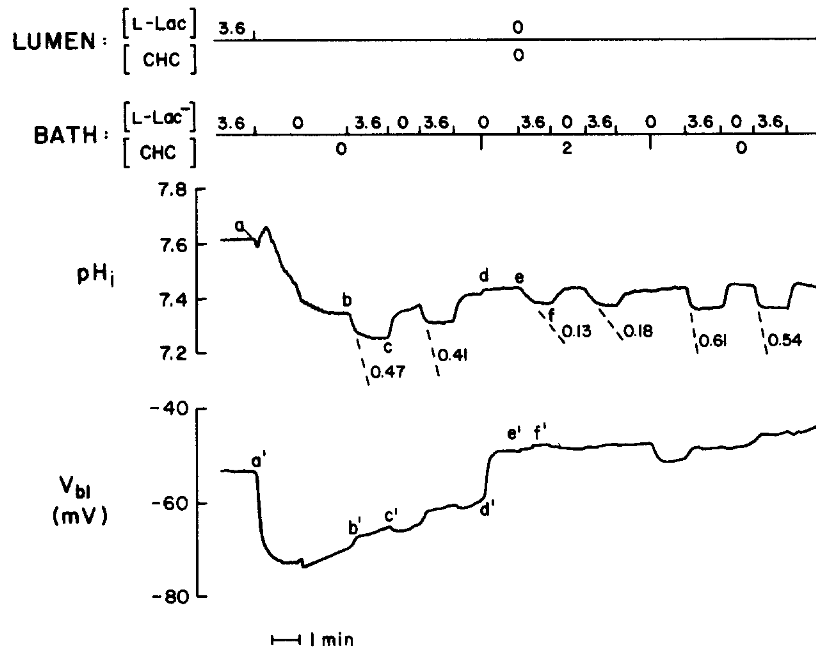

FIGURE 4. Effect of basolateral lactate in the presence and absence of CHC. All organic substrates were first removed from the lumen and bath (solution 2). The basolateral membrane was then exposed to 3.6 mM L-Lac^- in the absence and the presence of 2 mM CHC (solutions 2 and 4, without and with 2 mM added CHC). The broken lines represent the initial slopes of the decreases in pH_i, and the associated numbers are the values of these slopes in pH units per minute. Six similar experiments were performed. 080885A.

alkalinization, followed by a much larger acidification. As noted in regard to Fig. 1, a similar biphasic pH_i change is observed when only Lac^- is removed. We confirmed that the transient alkalinization is not an artifact of switching the bath solution before the luminal solution. If Lac^- (or all substrates) is removed from only the bath or only the lumen, there is monotonic alkalinization (*c* in Fig. 4) or acidification (*b* in Fig. 2), respectively. This suggests that the transient alkalinization 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 Lac^- *and CHC on* V_{bl}. Two aspects of the V_{bl} data of Fig. 4 are noteworthy. First, basolateral addition of 3.6 mM Lac^- (b') caused a

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_{bl} 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 $[Lac^-]_b$. 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

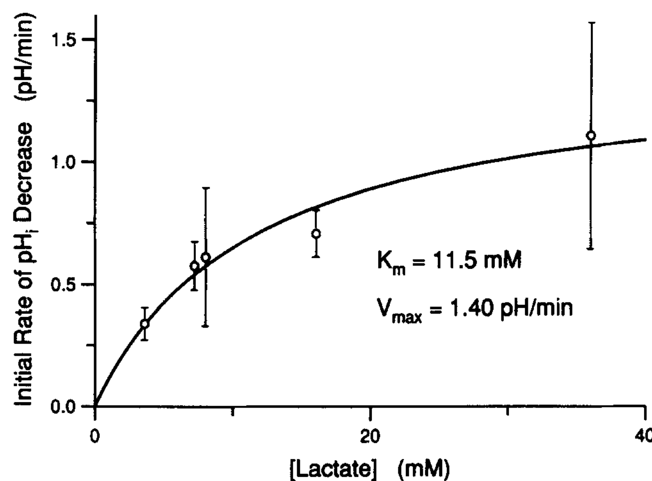

FIGURE 5. Initial rate of basolateral lactate-induced acidification, and its dependence on the bath lactate concentration. After all organic substrates were removed from the lumen and bath (solution 2), the basolateral membrane was exposed to different concentrations of L-Lac^-. Data are from unpaired 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_{max}.

V_{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_i 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_i changes were not statistically different from those predicted for the equilibration of HLac 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$_i$ 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,

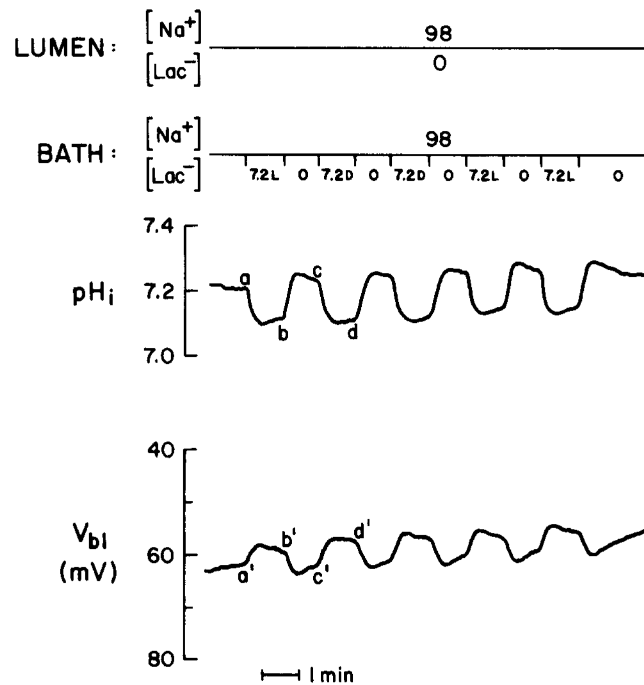

FIGURE 6. Effect of basolateral L- or D-lactate. After all organic substrates were removed from the lumen and bath (solution 2), the basolateral membrane was exposed to 7.2 mM L- or D-Lac$^-$ (solution 5). Four similar experiments were performed. 031986B.

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-

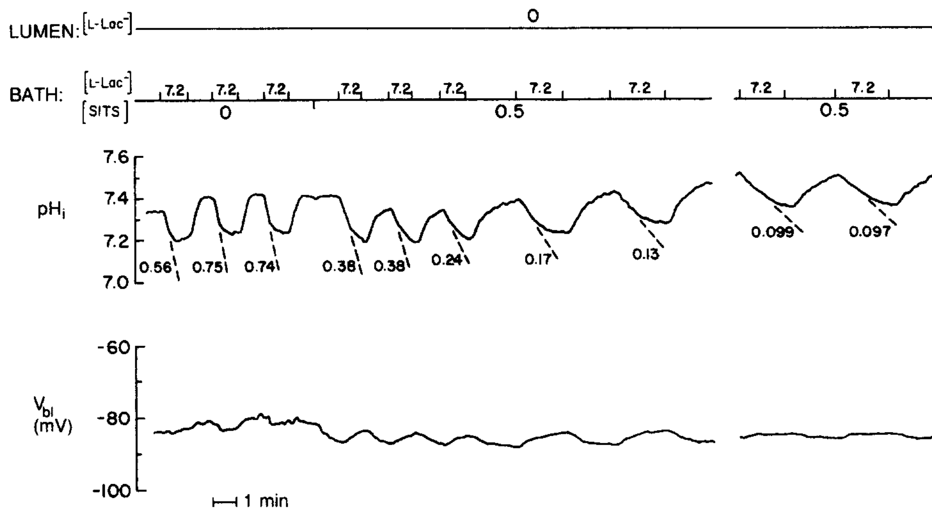

FIGURE 7. Effect of basolateral SITS on the basolateral lactate-induced acidification. After all organic substrates were removed from lumen and bath (solution 2), the basolateral membrane was exposed to 7.2 mM L-Lac (solution 5) in the absence or presence of 0.5 mM SITS. The gap in the record represents a period of 16 min. The broken lines represent the initial slopes of the decreases in pH_i, and the associated numbers are the values of these slopes in pH units per minute. Five similar experiments were performed. 091986B.

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 HCO_3^-). 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

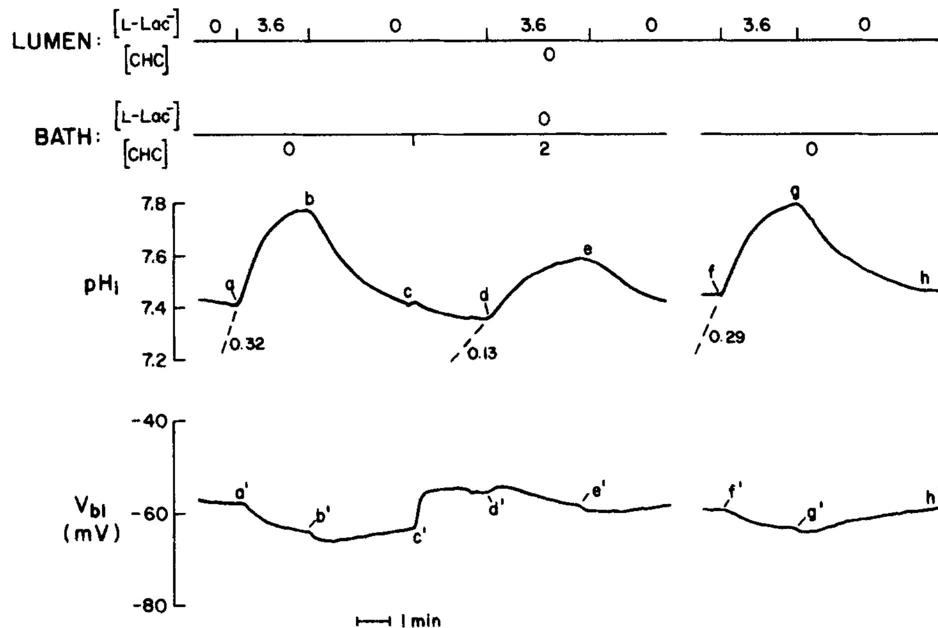

FIGURE 8. Effect of basolateral CHC on luminal lactate–induced alkalinization. After all organic substrates were removed from lumen and bath (solution 2), the lumen was exposed to 3.6 mM L-Lac$^-$ (solution 4) with and without 2 mM CHC in the bath. The gap in the record represents a period of 9.5 min. The broken lines represent the initial slopes of the increases in pH$_i$, and the associated numbers are the values of these slopes in pH units per minute. Five similar experiments were performed. 081385A.

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

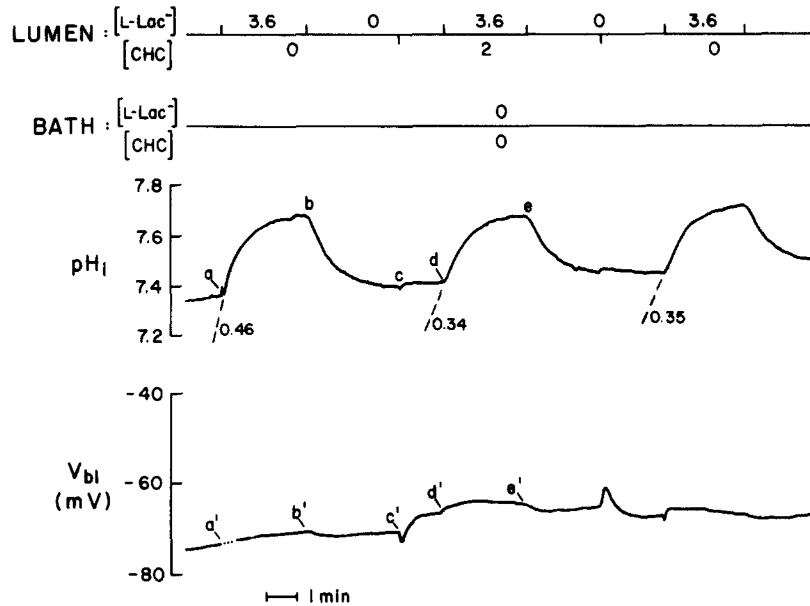

FIGURE 9. Effect of luminal CHC on luminal lactate–induced alkalinization. After all organic substrates were removed from the lumen and bath (solution 2), the lumen was exposed to 3.6 mM L-lactate (solution 4) with and without 2 mM CHC in the lumen. A solution-change artifact in the voltage trace was deleted at point *a'*. The broken lines represent the initial slopes of the increases in pH_i, and the associated numbers are the values of these slopes in pH units per minute. Six similar experiments were performed. 021886A.

decrease in pH_i. 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 pH_i. In five experiments, the mean pH_i decrease was 0.12 ± 0.02, and the initial rate of the CHC-induced acidification was 0.15 ± 0.02 pH

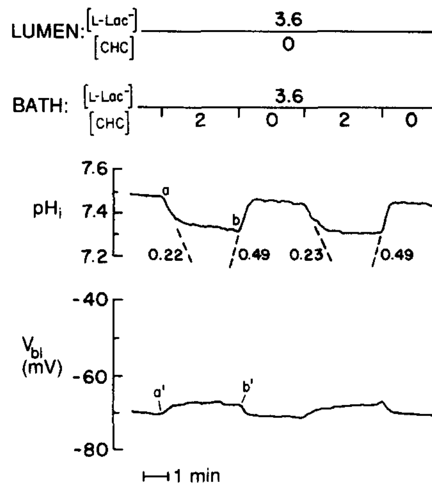

FIGURE 10. Effect of basolateral CHC on steady state pH_i. Tubules were bathed bilaterally with solution 1 (containing all substrates) and exposed to CHC in the bath. The broken lines represent the initial slopes of the pH_i changes, and the associated numbers are the values of these slopes in pH units per minute. Five similar experiments were performed. 081786A.

units/min. The rate of pH_i increase upon CHC removal was substantially greater, 0.36 ± 0.04 pH units/min ($P < 0.01$). pH_i may increase twice as fast as it decreases because $[Lac^-]_i$ 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 pH_i (*ab*), 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 pH_i changes. Extracellular $[Cl^-]$ was fixed at 62 mM thoughout the experiment by replacing 36 mM glucuronate or cyclamate with 36 mM Lac^-. As expected, the pH_i 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 pH_i 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^+, pH_i 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 pH_i 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 pH_i is not expected to affect the initial rate as long as $[Lac^-]_i$ is initially zero. On the other hand, this difference in initial pH_i 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 pH_i 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

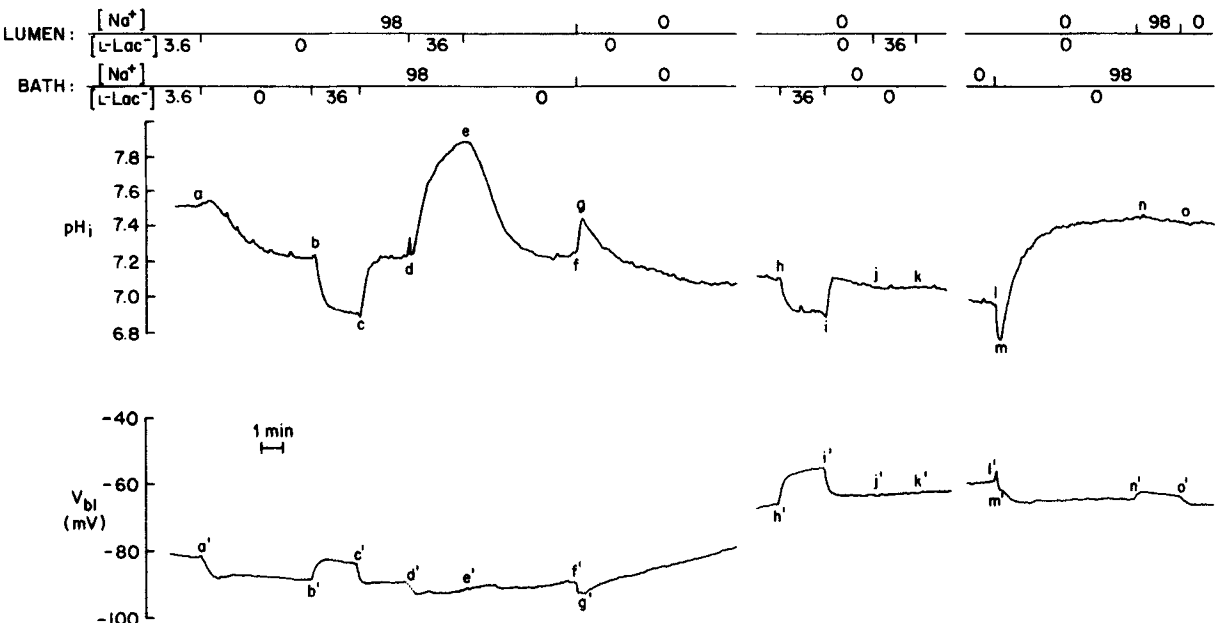

FIGURE 11. Effect of 36 mM L-lactate in the bath or lumen in the presence and absence of Na^+. All organic substrates were first removed from the bath and lumen, and 36 mM Cl^- was replaced with glucuronate (solution 7). Thus, $[Cl^-]$ was kept constant during subsequent exposure of the tubule to 36 mM Lac^- (solution 6), in which the lactate replaced glucuronate. This precaution was taken to minimize V_{te} 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_i, 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_i. In three experiments, the mean pH_i change was an increase of 0.01 ± 0.01 (NS), which indicates minimal H/Lac cotransport or nonionic diffusion of HLac at the

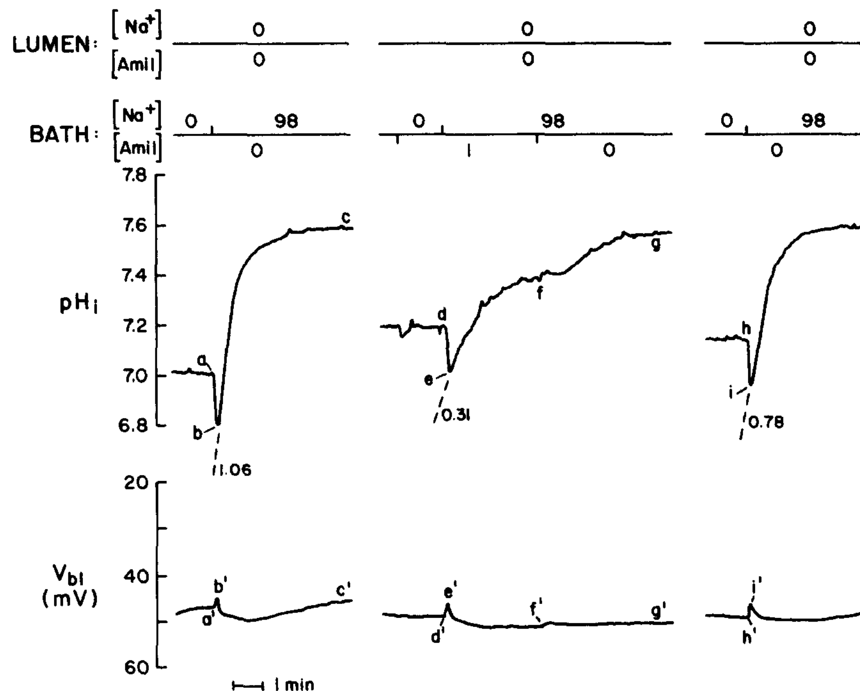

FIGURE 12. Effect of amiloride on the basolateral Na^+-induced pH_i recovery. After bilateral removal of Na^+ and organic substrates (solution 11), the basolateral membrane was exposed to Na^+ in the absence and presence of 1 mM amiloride (solution 7). The record shows the pH_i recoveries after addition 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.

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_i 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 (*lm*) followed by a rapid alkalinization (*mn*). In seven experiments,

the average rate of this alkalinization was 0.86 ± 0.12/min, 3.6-fold greater than the luminal Na^+- and Lac^--dependent pH_i recovery rate (see Fig. 3, *hi*). The increase in steady state pH_i (*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 pH_i (*no*).

The transient acidification observed upon basolateral Na^+ addition (*lm*), previously recorded by Boron and Boulpaep (1983*a*, 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 pH_i 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 pH_i 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 (1983*a*). Those experiments were conducted using full-substrate solutions on tubules that were acid-loaded by pretreating with NH_4^+. 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 $[Na^+]_b$ 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 pH_i increase (*fg*), so that pH_i 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 V_{bl} 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 $[Lac^-]_b$. 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^- entry across the luminal membrane leads to an intracellular alkalinization as Lac^- exits with H^+ (or is exchanged for base) across the basolateral membrane. First, addition of Lac^- to the lumen causes a sustained increase in pH_i. Second, basolateral CHC reduces the rate of this luminal Lac^--induced alkalinization. Finally, in the presence of bilateral Lac^-, inhibition of basolateral H/Lac cotransport by CHC produces a decrease in the steady state pH_i.

Model

The luminal and basolateral Lac^- transporters (i.e., the Na/Lac:H/Lac system), as well as other transporters relevant to pH_i regulation in *Ambystoma* proximal tubule cells, are depicted in the model of Fig. 13. The Na-K pump establishes a

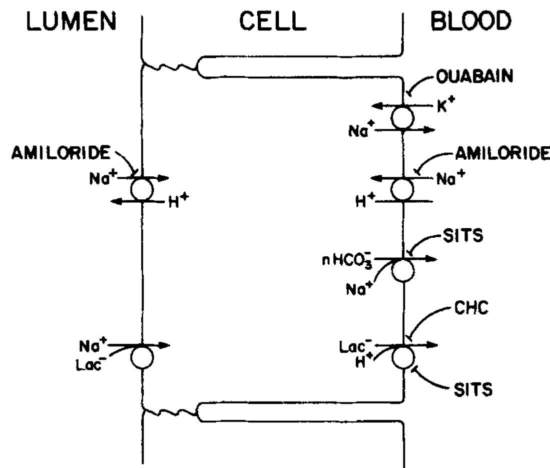

FIGURE 13. Model of acid-base transport in the *Ambystoma* proximal tubule. See text for further description.

luminal Na^+ gradient that provides the energy for luminal Lac^- entry via electroneutral Na/Lac cotransport. Inasmuch as $[Na^+]_l > [Na^+]_i$, the secondary active transport of Lac^- with Na^+ could theoretically drive $[Lac^-]_i$ to a value considerably greater than $[Lac^-]_l$ (see below). Lac^- then exits down its chemical gradient across the basolateral membrane, via electroneutral H/Lac cotransport (or Lac-base exchange). This basolateral exit of H^+, driven by the Lac^- gradient, is an example of tertiary active transport (Aronson, 1981). Lac^- 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^- entering across the luminal membrane could produce a pH_i increase as a result of metabolic conversion of Lac^-. However, our MPA data suggest that the observed pH_i increase is not due to the conversion of Lac^- and H^+ to glucose. Although conversion of Lac^- to another monocarboxylate would increase pH_i if this monocarboxylate exited with H^+ 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_i regulation; and (*c*) transepithelial Lac^- reabsorption. It has long been known that the kidney reabsorbs 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_i regulation in this preparation. The basolateral electrogenic Na/HCO_3^- cotransporter (Boron and Boulpaep, 1983*b*) has an $HCO_3^-:Na^+$ coupling ratio (*n*) greater than unity. It was inhibited in the present study by using nominally HCO_3^--free solutions. The possible interaction between this mechanism and Lac^- transport is discussed below. The luminal Na-H exchanger (Boron and Boulpaep, 1983*a*), 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, 1983*a*) 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., 1982*b*, *c*; Nord et al., 1983; Wright, 1985). Our observation that luminal addition of L-Lac^-, D-Lac^-, pyruvate, or acetate raised pH_i 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., 1982*c*), 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 alkalinization (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

(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 HCO_3^- and a full complement of substrates (including Lac^-) in the bath. Among the possible explanations for the difference in results are the following. (*a*) HCO_3^- converts the electroneutral cotransporter to an electrogenic one. (*b*) A second, electrogenic Na/Lac cotransporter becomes functional only in the presence of HCO_3^-. However, because electrogenic Na/Lac cotransport occurs in vesicles under nominally HCO_3^--free conditions (Barac-Nieto et al., 1980), HCO_3^- 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 electrogenic Na/HCO_3^- cotransport, producing a depolarization. Blocking the Na/HCO_3^- cotransporter with SITS would be expected to prevent this depolarization.

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-HCO_3 exchanger functioning with the small amounts of HCO_3^- present in our nominally HCO_3^--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 monocarboxylate-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_{bl} 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[1] would be >40 mM, whereas the maximal $[Lac^-]_i$ that could be generated by luminal electroneutral Na/Lac cotransport[2] 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.

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

$$-FV_{bl} + 2.3RT \log\left(\frac{[Lac^-]_o[H^+]_o^2}{[Lac^-]_i[H^+]_i^2}\right).$$

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 $V_{bl} = -68$ 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.

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

$$2.3RT \log\left(\frac{[Lac^-]_o[Na^+]_o}{[Lac^-]_i[Na^+]_i}\right).$$

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.

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

$$2.3RT \log\left(\frac{[Lac^-]_o[H^+]_o}{[Lac^-]_i[H^+]_i}\right).$$

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_{bl} 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$^-$]$_i$. [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 [Lac$^-$]$_i$ would be ~15 mM if the basolateral Lac$^-$ exit rate were zero (i.e., [Lac$^-$]$_i$ determined by equilibration across the luminal membrane via Na/Lac cotransport; see footnote 2). On the other hand, [Lac$^-$]$_i$ would be ~3.4 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 [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 [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, 1983*a*), 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 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$_3$ 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$_3$ 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_i 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., HCO_3^- reabsorption) or an increase in acid reabsorption. If the acid is derived from the blood (e.g., by basolateral H^+ influx or HCO_3^- 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 HCO_3^- efflux (Boron and Boulpaep, 1983*b*) 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 pH_i. However, transepithelial acid-base transport could be indirectly affected by basolateral H/Lac cotransport, because of changes in pH_i.

The effect of monocarboxylic acid transport on HCO_3^- reabsorption has been examined recently in the S_3 segment of the rabbit proximal tubule, in light of the present study and that of Nakhoul and Boron (1986). It was found that HCO_3^- reabsorption is reduced by the presence of acetate in the lumen and/or the bath (Geibel et al., 1987).

Effect on pH_i regulation. Our data indicate that, at least in the nominal absence of HCO_3^-, Lac^- transport plays a substantial role in determining pH_i in *Ambystoma* proximal tubules. The steady state pH_i 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 pH_i when Lac^- is either added or removed. Thus, the Na-H exchanger does not "clamp" pH_i at a particular value, but rather seems to set a limit on how far pH_i can fall when Lac^- is removed (e.g., see Fig. 1). It was previously observed in *Ambystoma* proximal tubules that switching from HCO_3^--free to HCO_3^--containing solutions causes a decrease of 0.17 in steady state pH_i (Boron and Boulpaep, 1983*a*). The application of CO_2/HCO_3^- introduces a chronic intracellular acid load (owing to HCO_3^- exit via Na/HCO_3 cotransport) that causes pH_i to fall. According to the general model of pH_i

regulation (Roos and Boron, 1981), this fall in pH_i causes the acid-extrusion rate via Na-H exchange to increase and it may also reduce the rate of acid loading. The pH_i 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_i 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 HCO_3^-, Lac^- transport drives pH_i to a value close to or even above the pH_i 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_i dependence similar to that of classic pH_i regulators, for which the acid-extrusion rate increases as pH_i decreases (Roos and Boron, 1981). Inasmuch as pH_i is lower in the presence of CO_2/HCO_3^- (Boron and Boulpaep, 1983*a*), it is likely that Na-H exchange makes a greater contribution to acid extrusion in the presence than in the absence of CO_2/HCO_3^-. A complete model of pH_i homeostasis in *Ambystoma* proximal tubule cells obviously must await analysis of Lac^- transport in tubules exposed to HCO_3^-.

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_i *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 ($HLac \rightleftharpoons H^+ + Lac^-$), the HLac rapidly enters, whereupon it dissociates into H^+ and Lac^-. The entry of HLac and the ensuing fall in pH_i continue until $[HLac]_i = [HLac]_o$. The magnitude of the pH_i decline depends upon (*a*) the initial pH_i, (*b*) $[HLac]_o$, (*c*) the apparent pK_a (pK_a') of the equilibrium $HLac \rightleftharpoons H^+ + Lac^-$, and (*d*) the non-lactate buffering power of the cell. The final pH_i 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 CO_2/HCO_3^-:

$$[Lac^-]_i = [HLac]_i \times 10^{(pH_i - pK_a')}. \quad (A1)$$

Inasmuch as $[HLac]_i = [HLac]_o$, this equation can be rewritten:

$$[Lac^-]_i = [HLac]_o \times 10^{(pH_i - pK_a')}. \quad (A2)$$

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^-:

$$[Lac^-]_i = \beta \cdot (pH_i' - pH_i), \quad (A3)$$

where β is the non-lactate buffering power, and pH_i' is the pH_i before the cell is exposed to external HLac (i.e., at which time $[Lac^-]_i = 0$).

The pH_i and $[Lac^-]_i$ that prevail at equilibrium (i.e., when $[HLac]_i = [HLac]_o$) 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 $[Lac^-]_i$ vs. pH_i for a single value of $[HLac]_i$. 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 pH_i (i.e., pH_i') when the system is Lac^- free. The intersection of the buffering-power line and the HLac isopleth describes the pH_i and $[Lac^-]_i$ 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.

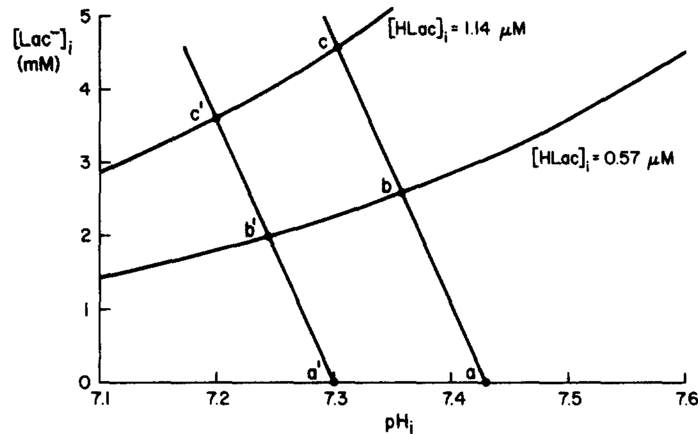

FIGURE 14. Dependence of $[Lac^-]_i$ on pH_i at a fixed $[HLac]_i$. Values of $[Lac^-]_i$ were calculated as a function of pH_i at two fixed values of $[HLac]_i$, 0.57 and 1.14 μM. 0.57 μM is the calculated $[HLac]_o$ at a total $[Lac^-]_o$ of 3.6 mM (assuming a pH of 7.5 and a pK of 3.7), and 1.14 μM is the calculated $[HLac]_o$ at a total $[Lac^-]_o$ of 7.2 mM. The buffer lines *abc* and *a'b'c'* were drawn with slopes of −36 mM/pH unit, corresponding to the intracellular buffering power of 36 mM observed by Boron and Boulpaep (1983*a*).

In Fig. 14, it is assumed that the initial pH_i (point *a*) is 7.43, the average 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 pK_a' of 3.7 for the HLac/Lac^- equilibrium (Martell and Smith, 1977), $[HLac]_o$ is 0.57 μM. As HLac enters the cell, it dissociates and titrates non-lactate buffers, causing pH_i to fall. The model predicts that as $[HLac]_i$ approaches $[HLac]_o$, pH_i should fall to 7.358 (point *b*). The pH_i change is thus −0.072, not statistically different from the average change observed under these conditions, −0.08. If the total $[Lac^-]_o$ is raised to 7.2 mM (i.e., $[HLac]_o = 1.14$ μM) rather than 3.6 mM, the equilibrium is described by point *c* and the predicted pH_i change is 0.125. The magnitude of the Lac^--induced pH_i decrease depends not only on the total $[Lac^-]$, but on the initial pH_i as well. Thus, if the starting 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$_i$, the smaller the fraction of incoming HLac that dissociates to H^+ and Lac^-.

Dr. Siebens was supported by a postdoctoral fellowship from the Connecticut Affiliate of the American Heart Association, and later by a National Research Service Award from the National Institutes of Health (AM-07062). Dr. Boron was supported by a Research Career Development Award from the National Institutes of Health (KO1-DK01022). The research was funded by the Searle Scholars Program, the American Heart Association, and the National Institutes of Health (PO1-DK17433).

Original version received 27 April 1987 and accepted version received 6 July 1987.

REFERENCES

Ammann, D., F. Lanter, R. Steiner, P. Schulthess, Y. Shijo, and W. Simon. 1981. Neutral carrier based hydrogen ion selective microelectrode for extra- and intracellular studies. *Analytical Chemistry.* 53:2267–2269.

Aronson, P. S. 1981. Identifying secondary active transport in epithelia. *American Journal of Physiology.* 240:F1–F11.

Aronson, P. S. 1983. Mechanisms of active H^+ secretion in the proximal tubule. *American Journal of Physiology.* 245:F647–F659.

Aronson, P. S. 1985. Kinetic properties of the plasma membrane Na^+-H^+ exchanger. *Annual Review of Physiology.* 47:545–560.

Barac-Nieto, M., H. Murer, and R. Kinne. 1980. Lactate-sodium cotransport in rat renal brush border membranes. *American Journal of Physiology.* 239:F496–F506.

Barac-Nieto, M., H. Murer, and R. Kinne. 1982. Asymmetry in the transport of lactate by basolateral and brush border membranes of rat kidney cortex. *Pflügers Archiv.* 392:366–371.

Biagi, B., T. Kubota, M. Sohtell, and G. Giebisch. 1981. Intracellular potentials in rabbit proximal tubules perfused *in vitro. Journal of Membrane Biology.* 88:25–31.

Boron, W. F., and E. L. Boulpaep. 1983*a*. Intracellular pH regulation in the renal proximal tubule of the salamander. Na-H exchange. *Journal of General Physiology.* 81:29–52.

Boron, W. F., and E. L. Boulpaep. 1983*b*. Intracellular pH regulation in the renal proximal tubule of the salamander. Basolateral HCO_3^- transport. *Journal of General Physiology.* 81:53–91.

Brand, P. H., and R. S. Stansbury. 1980. Lactate absorption in *Thamnophis* proximal tubule: transport versus metabolism. *American Journal of Physiology.* 238:F218–F228.

Brand, P. H., and R. S. Stansbury. 1981. Lactate transport by *Thamnophis* proximal tubule: sodium dependence. *American Journal of Physiology.* 240:F388–F394.

Burg, M., J. Grantham, M. Abramow, and J. Orloff. 1966. Preparation and study of fragments of single rabbit nephrons. *American Journal of Physiology.* 210:1293–1298.

Burg, M. B., C. Patlak, N. Green, and D. Villey. 1976. Organic solutes in fluid absorption by renal proximal convoluted tubules. *American Journal of Physiology.* 231:627–637.

Cook, D. L., M. Ikeuchi, and W. Y. Fujimoto. 1984. Lowering of pH$_i$ inhibits Ca^{2+}-activated K^+ channels in pancreatic B-cells. *Nature.* 311:269–271.

Craig, F. N. 1946. Renal tubular reabsorption, metabolic utilization and isomeric fractionation of lactic acid in the dog. *American Journal of Physiology.* 146:146–159.

Davenport, H. W. 1958. The ABC of Acid-Base Chemistry. 4th ed. University of Chicago Press, Chicago, IL.

De Hemptinne, A., R. Marrannes, and B. Vanheel. 1983. Influence of organic acids on intracellular pH. *American Journal of Physiology.* 245:C178–C183.

Deuticke, B. 1982. Monocarboxylate transport in erythrocytes. *Journal of Membrane Biology.* 70:89–103.

Deuticke, B., E. Beyer, and B. Forst. 1982. Discrimination of three parallel pathways of lactate transport in the human erythrocyte membrane by inhibitors and kinetic properties. *Biochimica et Biophysica Acta.* 684:96–110.

Deuticke, B., I. Rickert, and E. Beyer. 1978. Stereoselective, SH-dependent transfer of lactate in mammalian erythrocytes. *Biochimica et Biophysica Acta.* 507:137–155.

Dubinsky, W. P., and E. Racker. 1978. The mechanism of lactate transport in human erythrocytes. *Journal of Membrane Biology.* 44:25–36.

Forster, J., P. S. Steels, and E. L. Boulpaep. 1980. Organic substrate effects on and heterogeneity of *Necturus* proximal tubule function. *Kidney International.* 17:479–490.

Forte, J. G., and T. E. Machen. 1986. Ion transport by gastric mucosa. *In* Physiology of Membrane Disorders. 2nd ed. T. E. Andreoli, J. F. Hoffman, D. D. Fanestil, and S. G. Schultz, editors. Plenum Publishing Corp., New York. 535–559.

Frömter, E. 1981. Electrical aspects of tubular transport of organic substances. *In* Renal Transport of Organic Substances. R. Greger, F. Lang, and S. Silbernagl, editors. Springer-Verlag, New York. 30–44.

Geibel, J. P., W. F. Boron, and G. Giebisch. 1987. Effects of acetate on HCO_3^- transport in S3 segment of the rabbit proximal tubule. *Kidney International.* 31:408. (Abstr.)

Guggino, S. E., G. J. Martin, and P. W. Aronson. 1983. Specificity and modes of the anion exchanger in dog renal microvillus membranes. *American Journal of Physiology.* 244:F612–F621.

Gullans, S. R., P. C. Brazy, and V. W. Dennis. 1984. Interactions between gluconeogenesis and sodium transport in rabbit proximal tubule. *American Journal of Physiology.* 246:F859–F869.

Halestrap, A. P., and R. M. Denton. 1974. Specific inhibition of pyruvate transport in rat liver mitochondria and human erythrocytes by α-cyano-4-hydroxycinnamate. *Biochemical Journal.* 138:313–316.

Hildmann, B., C. Storelli, W. Haase, M. Barac-Nieto, and H. Murer. 1980. Sodium ion/L-lactate co-transport in rabbit small-intestinal brush-border-membrane vesicles. *Biochemical Journal.* 186:147–156.

Hohmann, B., P. P. Frohner, R. Kinne, and K. Baumann. 1974. Proximal tubular lactate transport in rat kidney: a micropuncture study. *Kidney International.* 5:261–270.

Kostos, V., N. W. DiTullio, J. Fush, L. Cieslinski, and H. L. Saunders. 1975. The effect of 3-mercaptopicolinic acid on phosphoenolpyruvate carboxykinase (GTP) in the rat and guinea pig. *Archives of Biochemistry and Biophysics.* 171:459–465.

Martell, A. E., and R. Smith. 1977. Critical Stability Constants. Vol. 3: Other Organic Ligands. Academic Press, Inc., New York.

Mengual, R., G. Leblanc, and P. Sudaka. 1983. The mechanism of Na-L-lactate cotransport by brush-border membrane vesicles from horse kidney. *Journal of Biological Chemistry.* 258: 15071–15078.

Morgunov, N., and E. L. Boulpaep. 1987. Electrochemical analysis of renal Na-glucose cotransport in salamander proximal tubules. *American Journal of Physiology.* 252:F154–F169.

Nakhoul, N. L., and W. F. Boron. 1986. Effect of Na-dependent acetate transport on pH_i in rabbit proximal straight tubules. *Kidney International.* 29:373.

Nord, E. P., S. H. Wright, I. Kippen, and E. M. Wright. 1983. Specificity of the Na^+-dependent monocarboxylic acid transport pathway in rabbit renal brush border membranes. *Journal of Membrane Biology.* 72:213–221.

Roos, A., and W. F. Boron. 1981. Intracellular pH. *Physiological Reviews.* 61:296–434.

Sackin, H., and E. L. Boulpaep. 1981. The isolated, perfused salamander proximal tubule: methods, electrophysiology and transport. *American Journal of Physiology.* 241:F39–F52.

Sackin, H., and E. L. Boulpaep. 1983. Rheogenic transport in the renal proximal tubule. *Journal of General Physiology.* 82:819–851.

Samarzija, I., and E. Frömter. 1976. Renal transport of glutamate and aspartate. Evidence for Na-dependent uptake from the peritubular surface into proximal tubular cells. *Pflügers Archiv.* 365:R15.

Samarzija, I., V. Molnar, and E. Frömter. 1981. The stoichiometry of Na^+ coupled anion absorption across the brushborder membrane of rat renal proximal tubule. *Advances in Physiological Science.* 11:419–423.

Siebens, A. W., and W. F. Boron. 1985. Effects of lactate and SITS on depolarization-induced alkalinization in *Ambystoma* proximal tubules. *Federation Proceedings.* 44:1898. (Abstr.)

Siebens, A. W., and W. F. Boron. 1986*a*. Effect of electroneutral luminal and basolateral lactate transport on cell pH (pH_i) in isolated perfused *Ambysoma* proximal tubules. *Kidney International.* 25:376.

Siebens, A. W., and W. F. Boron. 1986*b*. Intracellular alkalinization induced by Na-lactate cotransport in salamander renal proximal tubules. *In* Ion Gradient Coupled Transport. F. Alvarado and C. H. van Os, editors. Elsevier/North-Holland, Amsterdam. 321–324.

Sokol, P. P., P. D. Holohan, and C. R. Ross. 1985. Electroneutral transport of organic cations in canine renal brush border membrane vesicles (BBMV). *Journal of Pharmacology and Experimental Therapeutics.* 233:694–699.

Spencer, T. L., and A. L. Lehninger. 1976. L-Lactate transport in Ehrlich ascites-tumour cells. *Biochemical Journal.* 154:405–414.

Steels, P. W., and E. L. Boulpaep. 1976. Effect of pH on ionic conductances of the proximal tubule epithelium of *Necturus* and the role of buffer permeability. *Federation Proceedings.* 35:465. (Abstr.)

Steinmetz, P. R. 1985. Epithelial hydrogen ion transport. *In* The Kidney: Physiology and Pathophysiology. D. W. Seldin and G. G. Giebisch, editors. Raven Press, New York. 1441–1458.

Storelli, C., A. Corcelli, G. Cassano, B. Hildmann, and C. Lippe. 1980. Polar distribution of sodium-dependent and sodium-independent transport system for L-lactate in the plasma membrane of rat enterocytes. *Pflügers Archiv.* 388:11–16.

Thomas, R. C. 1984. Experimental displacement of intracellular pH and the mechanism of its subsequent recovery. *Journal of Physiology.* 354:3P–22P.

Ullrich, K. J., G. Rumrich, and S. Kloss. 1982*a*. Reabsorption of monocarboxylic acids in the proximal tubule of the rat kidney. I. Transport kinetics of D-lactate, Na^+-dependence, pH-dependence and effect of inhibitors. *Pflügers Archiv.* 395:212–219.

Ullrich, K. J., G. Rumrich, and S. Kloss. 1982*b*. Reabsorption of monocarboxylic acids in the proximal tubule of the rat kidney. II. Specificity for aliphatic compounds. *Pflügers Archiv.* 395:220–226.

Ullrich, K. J., G. Rumrich, and S. Kloss. 1982*c*. Reabsorption of monocarboxylic acids in the proximal tubule of the rat kidney. III. Specificity for aromatic compounds. *Pflügers Archiv.* 395:227–231.

Wright, E. M. 1985. Transport of carboxylic acids by renal membrane vesicles. *Annual Review of Physiology.* 47:127–141.