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Cell pH and Luminal Acidification in *Necturus* Proximal Tubule

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Summary. Cellular potential and pH measurements (pH_i) were carried out in the perfused kidney of *Necturus* on proximal tubules with standard and recessed-tip glass microelectrodes under control conditions and after stimulation of tubular bicarbonate reabsorption. Luminal pH and net bicarbonate reabsorption were measured in parallel experiments with recessed-tip glass or antimony electrodes, both during stationary microperfusions as well as under conditions of isosmotic fluid transport. A mean cell pH of 7.15 was obtained in control conditions. When the luminal bicarbonate concentration was raised to 25 and 50 mM, pH_i rose to 7.44 and 7.56, respectively. These changes in pH_i were fully reversible. Under all conditions intracellular H^+ was below electrochemical equilibrium. Thus the maintenance of intracellular pH requires "active" H^+ extrusion across one or both of the cell membranes. The observed rise in pH_i and the peritubular depolarization after stimulation of bicarbonate reabsorption are consistent with enhanced luminal hydrogen ion secretion and augmentation of peritubular bicarbonate exit via an anion-conductive transport pathway.

Key words cell pH · *Necturus* proximal tubule · acidification · bicarbonate transport

Introduction

A large body of evidence supports the view that proximal tubular bicarbonate reabsorption as well as the titration of nonvolatile buffers are due to secretion of hydrogen ions across the luminal cell membrane (Malnic & Steinmetz, 1976; Malnic & Giebisch, 1979; Warnock & Rector, 1979, 1981). Supportive evidence is based on the presence of an "acid disequilibrium pH" after carbonic anhydrase inhibition, the sensitivity of tubular bicarbonate reabsorption to carbonic anhydrase inhibitors and the fact that key aspects of the kinetics of tubular acidification are quite similar for bicarbonate and nonbicarbonate buffers (Malnic & Steinmetz, 1976; Cassola, Giebisch & Malnic, 1977; Giebisch, Malnic, de Mello, & de Mello Aires, 1977; Malnic & Giebisch, 1979). An important additional feature of tubular hydrogen ion secretion in the proximal tubule is its strong dependence on the luminal sodium concentration (Ullrich, Rumrich & Baumann, 1975; Sacktor, 1977; Warnock & Rector, 1979, 1981; Kinsella & Aronson, 1980; Boron & Boulpaep, 1981; Chan & Giebisch, 1981).

Although a large number of studies have characterized the process of tubular acidification by measurements of *luminal* pH, bicarbonate and pCO_2 measurements (Malnic & Steinmetz, 1976; Malnic & Giebisch, 1979; Warnock & Rector, 1979, 1981), little information on *cell* pH is available. Clearly though knowledge of tubular cell pH is necessary to identify the nature of hydrogen ion translocation across the luminal and basolateral cell membranes and to assess the potential role of cell pH changes as a determinant of proximal tubular acidification.

The availability of recessed-tip glass microelectrodes having tip diameters of less than 1 μm (Thomas, 1974, 1978) has made it possible to measure stable cell pH values in the relatively large proximal tubule cells of *Necturus*. Similar measurements are also possible in the isolated perfused proximal tubule of *Ambystoma tigrinum* (Boron & Boulpaep, 1980*a*, *b*, 1981).

We report in the present study the first cellular pH measurements of proximal tubules of cells of *Necturus* under control conditions and following stimulation of tubular bicarbonate reabsorption by increasing the luminal buffer load. As determined in a series of parallel experiments in which *luminal* pH changes were monitored during stationary perfusion, proximal tubules of *Necturus* can effectively reduce the pH of alkaline bicarbonate solutions at a rate greatly exceeding that which could be accounted for by isosmotic fluid reabsorption. Based on the assessment of the H^+ electrochemical potential profile across the tubular epithelium, we conclude that the distribution of hydrogen ions across both the luminal and basolateral

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cell membrane is far from electrochemical equilibrium. In addition, stimulation of bicarbonate reabsorption is associated with a significant rise of cellular pH.

Materials and Methods

Experiments were performed at room temperature on adult *Necturus maculosus* of either sex obtained from Mogul-Ed Corporation (Oshkosh, Wisc.). They were maintained in flowing, charcoal-filtered tap water at 15 °C before use and anaesthetized by immersion in 0.07% tricaine methane-sulfonate (Finquel, Ayerst Lab. Inc., New York). The animals were prepared for double kidney perfusion by methods previously described in detail (Giebisch, Sullivan & Whittembury, 1973). Adequate perfusion was verified by injection into the aortic, and then into the portal cannula, of FD & C Green-colored Ringers's solution. The initial perfusion solution contained: NaCl 90 mM, KCl 2.5 mM, $NaHCO_3$ 10 mM, NaH_2PO_4 0.5 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1.0 mM, glucose 2.2 mM, polyvinyl pyrrolidone (PVP) 15 g/liter, and heparin 1,000 U/liter. The solutions were gassed with either 99% O_2, 1% CO_2 or with 1% CO_2 in air (experiments in which antimony microelectrodes were used), and the pH adjusted to 7.6 by addition of HCl or NaOH.

Two methods of luminal perfusion were used: (i) *Continuous* tubular perfusion via the glomerulus by means of a double-barreled micropipette (OD 10–20 μm), one barrel containing the above control Ringer's solution and the other a modified Ringer's solution in which part of the chloride was replaced by bicarbonate to give bicarbonate concentrations of 10, 25 or 50 mM, respectively. One of the solutions was colored by 0.05% FD C-Green. Tubular perfusions did not contain PVP or heparin. The two barrels were connected via polytheylene tubing to oil-filled syringes, and perfusion was achieved by manual pressure. (ii) Stationary microperfusion of single proximal tubules was carried out by placement of double-barreled micropipettes into appropriate early proximal tubules, one barrel containing Sudan-black colored castor oil and the other barrel containing Ringer's solution with bicarbonate concentrations of 10, 25 and 50 mM. In these experiments luminal pH was measured by means of antimony microelectrodes and luminal volume changes were assessed from measurements of diameter of the tubule lumen and the rate of change of the menisci of the split oil droplets (Malnic & de Mello-Aires, 1972).

Half-times of the rate of change of bicarbonate reabsorption were obtained from semilogarithmic plots of the approach of luminal bicarbonate concentrations to steady-state values as a function of time. Bicarbonate concentrations were calculated by the Henderson Hasselbalch equation assuming a luminal pCO_2 equal to that of the perfusion solutions. Details of the method have been described elsewhere (Malnic & de Mello-Aires, 1972).

Peritubular membrane potential differences were measured with Ling-Gerard microelectrodes drawn from 2 mm OD glass capillaries (Corning 7740 or 1720). They were filled with 3 M KCl by boiling. Tip potentials were less than 5 mV, and resistance values ranged between 20–50 MΩ. These electrodes were identical in composition, size, and shape to those used for the construction of the recessed-tip pH electrodes. The KCl-filled electrodes were connected, via a Ag/AgCl half-cell, to the input of a high impedance electrometer (model F-23, WP Instruments, Hamden, CT). The output of the electrometer was recorded on a strip-chart recorder (Gould #220). The external reference electrode was a Ag/AgCl half-cell connected to the peritoneal cavity by a 3 M KCl-agar bridge.

Ion-Selective Electrodes

pH glass microelectrodes. These were made as described by Thomas (1974, 1978). Briefly, Corning glass 7740 or 1720 microelectrodes, identical to those used for transepithelial and peritubular potential (*PD*) measurements, were used as outer insulating electrodes. The inner, pH-sensitive electrodes were drawn from Corning 0150 glass (1.0 mm OD). Their tips were sealed by local application of heat using a microforge. A pH microelectrode was sealed onto the end of a fine stainless steel tube and lowered into the insulating pipette. A glass-to-glass seal was made close to the tip of the pH-sensitive glass by local heating and the application of pressure (140–170 lbs/cm^2) via the steel tube. The electrodes were filled with a solution containing 0.1 M NaCl and 0.1 M Na citrate (pH 6) and heated to 90–95 °C for 1 hr. They were then stored with their tips immersed in chromic acid until used. The electrodes were connected via a Ag/AgCl half cell to the input of a high impedance electrometer (Model F23, WPI, Hamden, CT). The output of the electrometer was recorded on a strip-chart recorder (Gould #220). The reference electrode was similar to that used for *PD* measurements. The electrodes were calibrated in Ringer's titrated with HCl or NaOH to pH values ranging between 5.5 and 8.0. Electrodes were used for intracellular measurements if they gave a response of more than 55 mV per unit pH change and if the response time was less than 1.5 min. The mean slope of the electrodes used was 56.8 ± 0.3 mV/pH unit, and the correlation between the potential and pH was always greater than 0.99.

Antimony microelectrodes. Both single- and double-barreled antimony microelectrodes were prepared as described by Vieira and Malnic (1968). Preamplification and recording procedures were similar to those used for *PD* and glass microelectrodes. The reference electrode was filled with Ringer-Agar. Electrodes were calibrated in Ringer's solutions with pH adjusted between 7.6 and 8.5. Intratubular steady-state bicarbonate concentration, acidification half-times, and bicarbonate net reabsorption rates were calculated as described previously (Malnic & de Mello-Aires, 1972).

Results are expressed as means ± SE, and differences between groups were analyzed by Student's *t*-test.

Results

Control Cell pH (pH_i) of Proximal Tubule Cells

The experiments in this group were performed under free-flow conditions. Figure 1 *A* and *B* shows representative recordings of both reference and pH-sensitive electrodes from neighboring cells of a surface proximal tubule. The mean value of peritubular membrane potential (E_{pt}) in tubules in which pH_i was also measured was 57.9 mV (*see* Table 1). This value is in good agreement with previously published values for proximal tubule cells in the perfused *Necturus* kidney (Boulpaep, 1976*b*; Giebisch, 1961). In Fig. 1 *B* a typical recording of a cell impalement with a recessed-tip pH electrode is shown. These impalements were considered acceptable only if the pH electrode recorded a rapid potential change upon entering the cell followed by a stable potential during which the recorded potential varied by less than ±1 mV for a period of at least 1 min. This steady potential was then corrected using the mean value for E_{pt} determined in the same tubule and the pH_i determined from the calibration curve. From inspection of Table 1 it is apparent that the mean value for pH_i was 7.15. A somewhat higher cell pH (7.29) has been

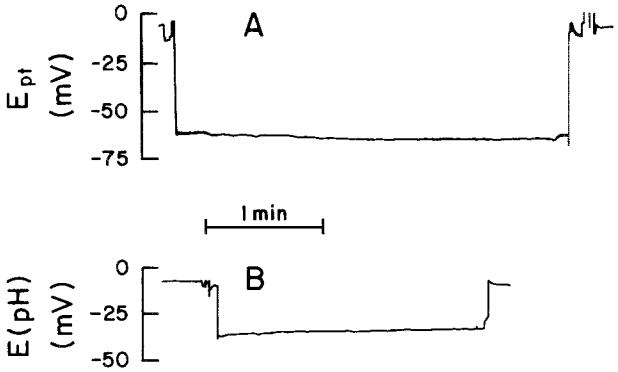

Fig. 1. Record of electrical potential difference (E_{pt}) across basolateral membrane of a proximal tubule cell (*A*) and record from recessed-tip pH electrode in a neighboring tubule cell of the same tubule (*B*)

Table 1. Intracellular pH and peritubular membrane potential of *Necturus* proximal tubule cells

Peritublar perfusate	Number of tubules	E_{pt} (mV)	pH_i
Control	20	-57.9 ± 0.8 (98)	7.15 ± 0.02 (46)
Control[a]	6	-60.2 ± 2.9 (18)	7.17 ± 0.05 (8)
NH_4Cl[b]	6	-36.6 ± 3.4 (18)[a]	7.44 ± 0.05 (8)[c]

[a] The values represent a separate series of studies in which the effects of NH_4Cl were determined on the same tubule.
[b] During this period 20 mM NH_4Cl was included in the peritubular perfusate without change in pH_o.
[c] $P<0.001$, as determined by a paried comparison.

reported in the proximal tubule of *Ambystoma* exposed to bicarbonate Ringer's (Boron & Boulpaep, 1980*a*, *b*, 1981). Indirect assessments of the cell pH of renal tubules have given estimates of cell pH similar to those measured directly (Struyvenberg, Morrison & Relman, 1968; Khuri et al., 1974; Bichara, Paillard, Leviel & Gardin, 1980).

To confirm the intracellular localization of the tip of the pH microelectrode, E_{pt} and pH_i were also measured during peritubular perfusion with ammonium chloride Ringer. It is well established that this maneuver leads to preferential NH_3 diffusion into the cell and subsequent hydrogen ion trapping with the result that the cell interior alkalinizes (Boron & deWeer, 1976). Results from relevant experiments on proximal tubule cells in *Necturus* are also summarized in Table 1. Replacement of 20 mM sodium chloride in the peritubular perfusion fluid by ammonium chloride (iso pH) caused both reversible depolarization of E_{pt} and cellular alkalinization.[1]

[1] Electrodes constructed from Corning 0150 glass have high selectivity for H^+ over NH_4^+. Estimates of H^+/NH_4^+ are of the order of 10^{11} (Dole, 1941; Isard, 1967).

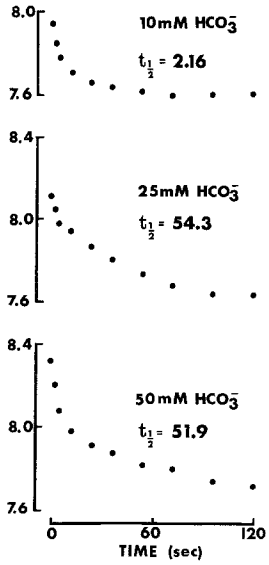

Fig. 2. Representative examples of pH changes in tubular lumen during stopped-flow microperfusion with Ringer's solutions containing different bicarbonate concentrations. The $t_{\frac{1}{2}}$ of the equilibration of bicarbonate concentrations is also given

Intraluminal pH and Acidification of Luminal Fluid

Free-flow data. Intraluminal pH was measured under free-flow conditions using both recessed-tip glass pH electrodes and double-barreled antimony electrodes. Luminal pH was determined with the glass electrodes by first measuring the transepithelial potential difference (E_{te}) with a conventional microelectrode (tip < 1 μm) and then inserting a recessed-tip electrode into the same tubular segment. Mean values of -5.0 ± 0.81 mV ($n=9$) for E_{te} and of 7.59 ± 0.02 ($n=9$) for intratubular pH were obtained. The double-barreled antimony microelectrodes had a somewhat larger tip diameter (6–10 μm). Using these electrodes yielded a somewhat smaller E_{te} of -2.9 ± 0.32 mV ($n=40$) and a mean intratubular pH of 7.61 ± 0.05 ($n=40$).

Stationary Microperfusion Data

Double-barreled antimony electrodes were used to measure tubular pH during stopped-flow microperfusion with solutions containing 10, 25 or 50 mM bicarbonate, equilibrated with air. The initial pH of each of these solutions was therefore above control tubular pH.

Figure 2 summarizes examples of the time course of luminal pH changes, indicating that each of these perfusion solutions was effectively acidified by the tubular epithelium. The time course of luminal acidification showed an initial rapid phase followed by a slow phase of approach to the steady-state pH value and is similar to observations made in rat proximal

Table 2. Steady-state pH and bicarbonate in *Necturus* proximal tubule

Luminal perfusate HCO_3^-	n	Steady-state pH	Steady-state HCO_3^- (mM)
10	26	7.56±0.03	6.4±0.3
25	23	7.68±0.05	8.9±0.8[a]
50	10	7.76±0.02[a]	9.7±0.3[a]

Comparison with 10 mM HCO_3^- perfusion
[a] $P<0.01$.

convoluted tubules (Malnic & de Mello Aires, 1972). It should be noted that the slow phase of the luminal pH change was only observed in those experiments in which an initial bicarbonate concentration of 25–50 mM was used.

In similar studies done in rat proximal convoluted tubules (Malnic & de Mello Aires, 1972), the initial rapid phase of luminal acidification was shown to be the result of CO_2 equilibration of the perfusion solution. Since the time-course of luminal acidification in *Necturus* proximal tubules was qualitatively the same, we have assumed a similar mechanism for this early phase. Accordingly the $t_{\frac{1}{2}}$ for CO_2 equilibration of the various perfusion solutions was; 2.16±0.29 sec with 25 mM HCO_3^- perfusate ($n=12$), and 5.31±0.43 sec with 50 mM HCO_3^- perfusate ($n=6$). The slow phase of luminal acidification seen with 25 and 50 mM HCO_3^- perfusates would then reflect HCO_3^- reabsorption by the tubule epithelium (Malnic & de Mello Aires, 1972).

Final, steady-state values of luminal bicarbonate concentration and pH values are summarized in Table 2, and bicarbonate reabsorptive half times as well as bicarbonate net fluxes are shown in Table 3. The steady-state luminal pH during 50 mM HCO_3^- perfusion was significantly higher than that measured with either 10 or 25 mM HCO_3^- perfusates. The reason(s) for this are not entirely apparent. It is possible that in those experiments a true steady-state had not yet been reached. Additionally, pH_i increased transiently with luminal perfusion of 50 mM HCO_3^- solutions (*see* below). If during the period of observation pH_i was still elevated, then the electrochemical driving force for H^+ across the luminal cell membrane would be reduced and a higher luminal steady-state pH could result.

Despite the observed difference in steady-state luminal pH, it is apparent that, similar to the behavior of the rat proximal convoluted tubule (Malnic & de Mello-Aires, 1972; Malnic & Steinmetz, 1976; Cassola et al., 1977; Giebisch et al., 1977; Malnic & Giebisch, 1979), bicarbonate reabsorption is stimulated with the increase in luminal bicarbonate concentration.

Table 3. Rates of bicarbonate reabsorption in *Necturus* proximal tubule

Luminal perfusate	n	HCO_3 $t_{\frac{1}{2}}$ (sec)	$J_{HCO_3}x$ (nmol/cm²·sec)
25	23	54.3±4.8	0.51±0.07
50	10	51.9±3.6	1.34±0.13

x Difference between 25 and 50 mM HCO_3^- perfusion: $P<0.01$. J_{HCO_3} was calculated according to the expression (Malnic & de Mello Aires, 1972)

$$J_{HCO_3}=\frac{\ln 2}{t_{\frac{1}{2}}}\cdot\frac{r}{2}\cdot(HCO_3^- - HCO_3^-)$$

where $t_{\frac{1}{2}}$ is the acidification half-time, r is the tubular radius and HCO_3^- and HCO_3^- are initial and steady-state bicarbonate concentrations.

Table 4. Comparison of rates of bicarbonate transport and net fluid movement

Perfusate (HCO_3^-)	Fluid reabs., $t_{\frac{1}{2}}$	J_v (cm³/cm²·sec)	HCO_3 reabs., $t_{\frac{1}{2}}$	J_{HCO_3} (mol/cm²·sec)
10 mM	2100 sec	8.26×10^{-7}	—	—
25 mM	2460 sec	7.05×10^{-7}	54 sec	0.51×10^{-9}
50 nM	2400 sec	7.23×10^{-7}	52 sec	1.34×10^{-9}

The results of a parallel series of experiments in which the half-times of volume reabsorption of solutions with different bicarbonate concentrations were measured are also shown in Table 4. It is apparent that the half time of volume reabsorption (>40 min) is very much greater than the half time value of bicarbonate reabsorption (~50 sec). Thus bicarbonate ions cannot be reabsorbed by mechanisms primarily dependent on luminal volume reabsorption.

pH_i Changes during Stimulated Acidification

Another series of experiments were carried out in which single proximal tubules were first perfused with solutions containing 10 mM bicarbonate Ringer's solution and the cells punctured with either conventional or recessed-tip microelectrodes. Following such control measurements, the luminal perfusion solution was abruptly changed to one containing either 25 or 50 mM bicarbonate. When cell *PD* and pH had reached new steady-state values, the lumen once again was perfused with the initial control Ringer's solution.

Typical recordings obtained with both conventional and pH microelectrodes are shown in Fig. 3. We note that the peritubular membrane potential was reversibly depolarized by the high luminal bicarbonate Ringer's. These potential changes were also detected by the pH electrode but the latter also showed slower potential changes reflecting intracellular alka-

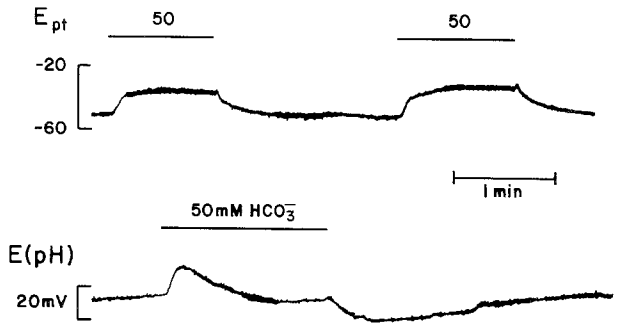

Fig. 3. Experimental records of changes in basolateral *PD* (E_{pt}) and cell pH (E(pH)) obtained in proximal tubular cells during luminal perfusion with 50 mM bicarbonate Ringer's solution

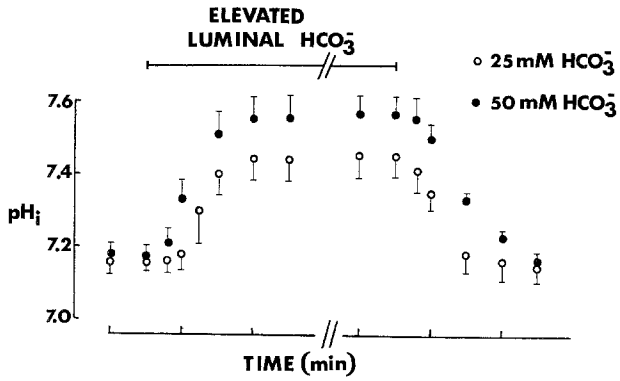

Fig. 4. Changes in intracellular pH of proximal tubular cells during luminal perfusion at elevated bicarbonate concentration. Means of 5 cells (25 mM HCO_3^-) and 8 cells (50 mM HCO_3^-. Vertical bars are one SE

linization. pH_i returned to control values upon perfusion of the lumen with control Ringer's.

The mean values of perfusion experiments with two levels of elevated bicarbonate concentrations are summarized in Fig. 4. Stimulation of acidification by 25 mM bicarbonate leads to a significant rise of pH_i from a control value of 7.16 ± 0.02 to 7.44 ± 0.06 ($n=5$). pH_i returned to control values within 2 min after reintroduction of the control Ringer's solution. The cellular pH changes with luminal perfusion with 50 mM bicarbonate were more pronounced. pH_i rose from a control value of 7.18 ± 0.03 ($n=8$) to a mean value of 7.56 ± 0.06 ($n=8$). These changes were also reversible upon reestablishment of the control luminal bicarbonate concentration.

Discussion

The present study characterizes the acidification characteristics of the proximal tubule of the *Necturus* kidney by relating luminal pH to cellular pH changes. It also allows an assessment of electrochemical potential gradients of hydrogen ions across the luminal and peritubular cell membranes.

The first relevant micropuncture studies performed on amphibian tubules, including *Necturus*, did not localize the proximal tubule as a site where significant urinary acidification took place (Giebisch, 1956; Montgomery & Pierce, 1937). However, it should be noted that due to significant volume reabsorption along the proximal tubule (Bott, 1962) at constant luminal bicarbonate concentrations, significant isosomotic bicarbonate reabsorption takes place along the proximal tubule of *Necturus*.

In the experiments reported here, the pH of the vascular perfusion solution was 7.6 and pCO_2 7.6 mm Hg. Accordingly, a steady-state intratubular bicarbonate concentration of 7.2 mM would be expected if transepithelial CO_2-equilibration were to occur. Even at higher initial luminal bicarbonate concentrations, net acidification ceased when the luminal pH approached the pH of the peritubular vascular perfusate. If the luminal pH were determined solely by equilibration of H^+ or HCO_3^- with the transepithelial voltage (~5 mV, lumen negative), a luminal pH not more than 0.1 pH unit below that of the perfusion fluid would be expected. Indeed, during perfusion with different bicarbonate concentrations we observed steady-state bicarbonate concentration, in the range of the peritubular bicarbonate concentrations. These results confirm previous observations that bicarbonate reabsorption across the proximal tubule of *Necturus*, is proportional to net fluid movement, but that this nephron segment is not able to generate significant transepithelial bicarbonate (pH) gradients (Montgomery & Pierce, 1937; Giebisch, 1956; Bott, 1962).

When solutions containing bicarbonate in concentrations exceeding those of the vascular perfusate are used in *Necturus* proximal tubules, acidification half-times can be subdivided into an initial, fast phase, with values of the order of 2 sec, and into a slower phase with half-times of the order of 50 sec. The latter slow phase was absent with perfusions of 10 mM bicarbonate. It has been shown previously in experiments of rat proximal convoluted tubule that the first rapid phase of pH change is dependent on CO_2 equilibration, whereas the second slow phase is due to the continuous fall in luminal bicarbonate until steady-state values were reached (Malnic & de Mello-Aires, 1972). It is apparent that similar events also determine the luminal pH changes in the proximal tubule of *Necturus*.

The fact that in the present experiments the initial transepithelial pH and bicarbonate gradients were dissipated but no pH (or bicarbonate) concentration differences established across the tubular epithelium raises the question whether passive bicarbonate diffusion out of the lumen could fully explain the observed

pH changes. We believe that several points argue against such an interpretation.

The alternative to passive bicarbonate movement being solely responsible for the luminal pH changes is that cellular hydrogen ion secretion participates in tubular bicarbonate reabsorption, a mechanism known to play a key role in the mammalian proximal tubule (Malnic & Steinmetz, 1976; Cassola et al., 1977; Giebisch et al., 1977; Malnic & Giebisch, 1979; Warnock & Rector, 1979, 1981; Bichara et al., 1980). In this regard, the nature of the electrochemical gradient of hydrogen ions across the luminal cell membrane in the present study (*see* below), and that reported for *Ambystoma* kidney (Boron & Boulpaep, 1980*a*, *b*, 1981) is consistent with a mechanism of luminal hydrogen ion secretion in proximal cells of amphibian kidneys and supports the view that bicarbonate ions are reabsorbed by hydrogen ion secretion. It is presently not resolved whether other mechanisms, such as bicarbonate diffusion from the lumen to the peritubular fluid through the paracellular shunt pathway or nondiffusional, i.e., carrier-mediated bicarbonate transport (Maren, 1967), could also contribute to the observed net reabsorption of bicarbonate.

Two additional comments are relevant. It is possible to analyze the contribution of a particular ion to the overall conductance of the epithelium:

$$G_T = \Sigma_{gi} = g_{Na} + g_{Cl} + g_{HCO_3} + \cdots$$

where gi are the partial ionic conductances of the respective ions. Since Na^+ and Cl^- are the major conductive ion species across the *Necturus* proximal tubule (Boulpaep, 1976*b*), g_{HCO_3} would be expected to be only a fraction of either g_{Na} or g_{Cl}. An apparent transepithelial bicarbonate permeability (P_{HCO_3}) can be calculated from the present data, and using the following expression (Hodgkin & Katz, 1949; Ullrich, 1973):

$$P_{HCO_3} = \frac{\ln 2}{t_{\frac{1}{2}}} \cdot \frac{r}{2} \cdot \frac{\exp(EF/RT) - 1}{EF/RT}$$

where E is the transepithelial electrical potential difference, F the Faraday constant, R the gas constant, T the absolute temperature, and r the tubular radius. From the $t_{\frac{1}{2}}$ of bicarbonate disappearence from the lumen of tubules perfused with either 25 or 50 mM HCO_3^- (50 sec), and using a transepithelial potential value of -5 mV, the apparent P_{HCO_3} would be 1.8×10^{-5} cm·sec^{-1}. This value is about twice that of chloride (0.8×10^{-5} cm·sec^{-1}), a highly permeant ion species in the *Necturus* proximal tubule (Kimura & Spring, 1978).

Alternatively, the rate of net bicarbonate reabsorption driven by the transepithelial potential difference can be calculated by the expression (Hodgkin & Katz, 1949):

$$J_{HCO_3} = P_{HCO_3} \cdot zF[HCO_3] \cdot E/RT$$

where $[HCO_3^-]$ is the luminal bicarbonate concentration and z, F, R and T have their usual meanings. If, at the extreme, it is assumed that $P_{HCO_3} = P_{Cl} = 0.8 \times 10^{-5}$ cm·sec^{-1}, then for a transepithelial potential of -5 mV and a luminal $[HCO_3^-] = 25$ mM, J_{HCO_3} would be 3.9×10^{-11} mole·cm^{-2}·sec^{-1}. This value is approximately one order of magnitude less than the measured value of J_{HCO_3} (*see* Table 3). Thus the observed rates of bicarbonate reabsorption cannot be attributed solely to passive electrodiffusion.

Finally, it is possible that bicarbonate reabsorption is coupled directly to reabsorption of tubular fluid (Kedem & Katchalsky, 1963; Hoshiko & Lindley, 1967). In view of the large difference in the $t_{\frac{1}{2}}$ values for volume reabsorption (2400 sec) as compared to those for bicarbonate reabsorption (50 sec), such a coupling of ion and fluid flows is unlikely.

Taken together, the above considerations argue strongly that additional mechanism(s) for transepithelial bicarbonate reabsorption exist in the *Necturus* proximal tubule.

The data obtained in this study permit calculation of the electrochemical potential differences of hydrogen ions across the membranes of proximal tubule cells in *Necturus* kidney. Figure 5 provides a summary of relevant data during control conditions and after luminal perfusion with a solution containing 50 mM bicarbonate. It is apparent that in control conditions the cell acts as a hydrogen ion sink, i.e., the transfer of hydrogen ions out of the cell across its luminal and peritubular cell membrane occurs against sizeable electrochemical gradients. Thus, in the absence of net hydrogen ion fluxes, as for example in the present stationary microperfusion studies, maintenance of a cell-pH of 7.15 requires active H^+ extrusion (or an equivalent process)[2] at one or both cell membranes. In view of the presence of a Na^+/H^+ antiport system in luminal brush border vesicles of proximal tubules (Kinsella & Aronson, 1980), it is likely that the primary site for cellular H^+ extrusion is at this membrane. However, other modes of H^+ extrusion and/or the presence of H^+ extrusion mechanisms at the basolateral membrane cannot be ruled out. In this regard, recent studies in *Necturus* (Guggino et al., 1981, and *unpublished observations*) and *Ambystoma* (Boron & Boulpaep, 1980*a*, *b*, 1981) proximal tubules have shown that the basolateral cell membrane contains pH-regulating mechanisms, the net effect being the cellular extrusion of H^+.

[2] The term active H^+ extrusion as used in this context does not imply a particular mechanism, and simply refers to a process by which the end result is a reduction of the intracellular $[H^+]$. Clearly this would include all primary active (e.g., H^+ pump) and secondary active (e.g., Na^+/H^+ exchange) mechanisms. For a detailed review of these mechanisms, *see* Roos and Boron (1981).

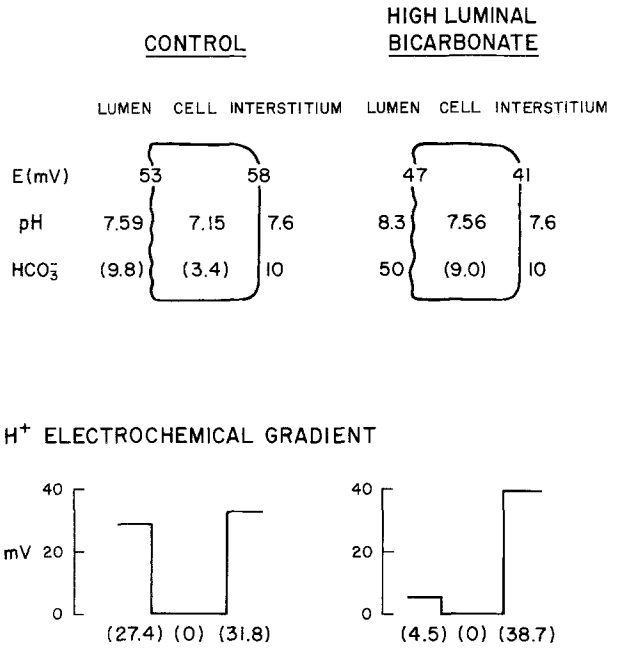

Fig. 5. Electrochemical potential gradients for H ions in control proximal tubule and during luminal perfusion with 50 mM HCO_3^-. The upper diagram shows mean *PD*, pH, and $[HCO_3^-]$ data, and the lower diagram gives the electrochemical gradient calculated from these data. Luminal bicarbonate concentrations in control conditions were calculated from steady-state pH values. Cell bicarbonate concentrations were calculated from pH_i and a pCO_2 corresponding to that of the perfusion fluid (1%)

When the lumen is perfused with a solution containing 50 mM bicarbonate, the electrochemical gradient for H^+ across the luminal cell membrane is markedly reduced whereas that across the peritubular cell membrane changes only little. This favors H^+ movement from cell to lumen, which is consistent with the observed increase of bicarbonate transport found after elevation of the luminal bicarbonate concentration. The cellular alkalinization measured during luminal perfusion with the 25 and 50 mM HCO_3^- solutions most likely reflects this increased rate of cellular H^+ extrusion. Alternatively, this cellular alkalinization could reflect the entry of HCO_3^- from the lumen into the cell. This, however, seems unlikely in view of the low permeability of the luminal cell membrane to HCO_3^- in both *Necturus* (Guggino et al., 1981) and *Ambystoma* (Boron & Boulpaep, 1981) proximal tubules. The observation that cell pH changes significantly after elevation of the luminal bicarbonate concentration makes it also unlikely that transfer of bicarbonate from the lumen to the interstitium could proceed only by diffusion along the intercellular shunt pathway. Such a mode of ion translocation would not be expected to involve changes in cellular pH.

We also noted significant cell depolarization following luminal perfusion with solutions having a high bicarbonate concentration. The mechanism underlying this potential change is not fully understood, but the following possibility should be considered. It is well established that the peritubular cell membrane of *Necturus* proximal tubule has, besides an electroneutral anion exchange mechanism (Edelman, Bouthier & Anagnostopoulos, 1981; Guggino et al., 1981; Shindo & Spring, 1981), a sizeable conductive pathway for electrodiffusion of bicarbonate ions (Burckhardt & Frömter, 1980; Matsumura, Guggino & Giebisch, 1981). Accordingly, it would be expected that the normal bicarbonate concentration gradient ($[HCO_3]_{extracellular} > [HCO_3^-]_{cell}$) contributes to the cell negative potential. Hence, reduction of this concentration gradient by an increase of the cellular bicarbonate concentration as reflected by the rise in cell pH, would be expected to depolarize the tubule cell. For instance, accelerated operation of luminal electroneutral sodium-hydrogen ion exchange (Ullrich et al., 1975; Sacktor, 1977; Boron & Boulpaep, 1980*a*, *b*, 1981; Kinsella & Aronson, 1980; Chan & Giebisch, 1981), a rise in cellular bicarbonate, and enhanced bicarbonate exit across a conductive peritubular transport pathway could account for the observed potential changes. Whether other factors contribute to the observed cell depolarization is presently unknown. In this regard, studies in mammalian proximal tubules have suggested that changes in extracellular pH (and therefore intracellular pH) may alter transcellular Na^+ and Cl^- transport, and hence transepithelial and membrane electrical properties (Berry, 1981). Whatever the precise origin of the peritubular depolarization, it assures that the electrochemical gradient acting upon bicarbonate ions as they exit the cell remains fairly constant.

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