Regulation of pH in Rat Papillary Tubule Cells in Primary Culture

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Abstract

To investigate the mechanisms responsible for urinary acidification in the terminal nephron, primary cultures of cells isolated from the renal papilla were grown as monolayers in a defined medium. Morphologically, cultured cells were epithelial in type, and similar to collecting duct principal cells. Cell pH measured fluorometrically in monolayers grown on glass slides showed recovery from acid loads in Na^+-free media. Recovery was inhibited by cyanide, oligomycin A, and *N*-ethylmaleimide. Cyanide and oligomycin inhibited recovery less in the presence than in the absence of glucose. When cells were first acid loaded in a Na^+-free medium and then exposed to external Na^+, pH recovery also took place. This recovery exhibited first-order dependence on Na^+ concentration and was inhibited by 5-(*N*-ethyl-*N*-isopropyl)amiloride. These studies demonstrate that in culture, collecting duct principal cells possess at least two mechanisms for acid extrusion: a proton ATPase and an Na^+-H^+ exchanger. The former may be responsible for some component of the urinary acidification observed in the papillary collecting duct in vivo; the role of the latter in acid-base transport remains uncertain.

Introduction

H^+ secretion is attributed to a proton ATPase in mammalian distal nephron (1–3). This transport mechanism is thought to reside in a specific cell type, the intercalated cell (4), which is similar to the mitochondria-rich cell of the turtle bladder (5, 6). Although a membrane-bound ATPase has also been purified from whole bovine medulla (7), the cellular localization of this transport mechanism is uncertain. Intercalated cells have not been identified in the papillary collecting duct in rat, rabbit, or humans (4), yet acidification of the tubular fluid seems to occur within the papilla (8–12).

The purpose of the current experiments was to examine the modes of H^+ secretion in cells isolated from rat renal papilla and grown in primary culture. The results of these studies demonstrate the presence of two H^+ transport systems, one having characteristics consistent with an H^+-ATPase and one having characteristics of an Na^+-H^+ exchanger.

Methods

Papillary collecting duct cells were isolated using a modification of methods reported by Grenier, Rollins, and Smith (13). Kidneys were harvested from 150–250-g Sprague-Dawley rats and papillae were dissected at their junctions with the medullary rays. The tissue was minced and suspended in 10 ml of Kreb's-Ringer phosphate buffer, pH 7.3, containing 2 mg/ml collagenase (CLS II; Worthington Biochemical Corp., Freehold, NJ). This mixture was incubated for 2 h at 37°C, gassed with 5% CO_2/95% O_2, and periodically agitated by aspiration into a 10-ml pipette. When the tissue was well dispersed, 2 vol of distilled water were added and the suspension allowed to stand for 5 min. The cells were then pelleted by centrifugation at 500 *g* for 5 min and washed twice in Kreb's-Ringer phosphate buffer. The cells were then suspended in 10 ml of a 1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle's media, containing 10% fetal calf serum, penicillin-streptomycin (1:100), and 10 mM Hepes. Five-million cells were then plated in 2 ml of the same medium in 35-mm plastic culture dishes (Costar, Cambridge, MA) containing a 9 × 18-mm glass coverslip. The cultures were maintained at 37°C and gassed with 5% CO_2/95% O_2 in a humidified incubator. After 24 h of incubation, the medium was changed to one that was serum free and contained 5 μg/ml transferrin, 5 μg/ml insulin, 50 nM hydrocortisone, 10 nM sodium selenite, and 5 pM triiodothyronine. Thereafter the medium was changed every 48 h. Cell fluorescence experiments were performed when the cells had achieved 70–90% confluence on the coverslips.

H^+ transport was assessed by observing changes in the fluorescence of intracellular 2,7-biscarboxyethyl-5(6)-carboxyfluorescein (BCECF).[1] The acetoxymethyl ester of this probe (BCECF-AM) was added to culture dishes in a final concentration of 10 μM and the cells incubated with it for 1 h. Thereafter the coverslips were removed from the dishes and washed with the buffer to be used for the various protocols. In addition to the salts specified in these protocols (see Results), this buffer always included 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgSO_4$, and 15 mM Hepes titrated with Tris base to the desired pH. 5.5 mM D-glucose was present except in the glucose-free buffers where it was replaced with mannitol. Each coverslip was then mounted in a holder constructed to maintain it within an acrylic cuvette at an angle of 68° to the incident light beam. Fluorescence intensity was monitored at 30°C in a Perkin-Elmer LS-5 fluorescence spectrophotometer at two excitation wavelengths, 450 and 504 nm, and at one emission wavelength, 550 nm.

Calibrations of cell pH were performed by exposing monolayers to 140 mM KCl buffered with Tris-Hepes at various pH values, adding 1 μg/ml nigericin, and observing the fluorescence ratios of the readings at 504- and 450-nm excitation wavelengths. The resulting calibration curves typically had r values > 0.99 and did not differ significantly among monolayers from the same culture group obtained on the same day. Thus, a single calibration was performed daily to calculate apparent cell pH values observed during the experimental maneuvers.

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1. *Abbreviations used in this paper:* BCECF, 2,7-biscarboxyethyl-5(6)-carboxyfluorescein; BCECF-AM, acetoxymethyl BCECF.

After each change of solution in the cuvette, the pH-independent fluorescence at 450 nm excitation was adjusted to a value of 20 to compensate for loss of dye from cells or loss of cells from the coverslip. Tracings were not accepted if this value changed by > 5% during recording of a pH transient. The tracings shown depict the results of representative experiments. Each maneuver was performed three times or more in at least two different cell culture groups (Tables I and II).

Quantification of the rate of Na^+-dependent pH recovery was assessed by determining the slope of the initial pH change after addition of Na^+. The S/V versus S relationship, where S = $[Na^+]$ in millimolars and V = rate of pH recovery in units per minute; was calculated and apparent K_m and V_{max} value was derived to produce the result for an individual monolayer.

BCECF-AM was obtained from Molecular Probes, Inc., Eugene, OR. Oligomycin, *N*-ethylmaleimide, and nigericin were obtained from Sigma Chemical Co., St. Louis, MO. The 5-(*N*-ethyl-*N*-isopropyl)amiloride was synthesized by a previously described procedure (14). Before use BCECF-AM and 5-(*N*-ethyl-*N*-isopropyl)amiloride were dissolved in dimethyl sulfoxide, oligomycin and nigericin were dissolved in ethanol, and *N*-ethylmaleimide was dissolved in water. Stock solution additions were always < 1% of the volume of the solutions in the cuvette, and vehicles alone at this volume were without effects on cell pH.

For electron microscopy, cell monolayers were grown on plastic coverslips and fixed in Karnovsky's solution. For transmission electron microscopy, they were postfixed in 1% osmium tetroxide and then embedded in Spur's low viscosity resin. Thin sections were stained with lead citrate and uranyl acetate and then examined with an EMU-4B electron microscope (RCA Corp., Lancaster, PA) operated at 50 kV. For scanning electron microscopy, fixed specimens underwent critical point drying with CO_2. They were then mounted on carbon planchets and carbon coated in a vacuum chamber (Denton Vacuum Inc., Cherry Hill, NJ). The monolayers were then examined with a scanning electron microscope (model 1200; Advanced Metals Research Corp., Bedford, MA).

Succinic dehydrogenase staining was performed on formalin-fixed monolayers grown on glass slides by the method of Pearse (15).

Results

Morphology of papillary cells in culture. Fig. 1 is a phase-contrast micrograph of papillary cells grown in primary culture. They demonstrate a homogeneous polygonal shape and are ~ 13 μm in diameter. Evidence of vectorial fluid transport is present in the form of cell blisters or hemicysts. Representative cultures examined by transmission electron microscopy did not show evidence of fibroblast contamination. A homogeneous epithelial cell type was revealed (Fig. 2) that demonstrated apical microvilli, intercellular junctions, basolaterally located nuclei, basolateral cell infoldings, and a small number of mitochondria. Fig. 3 is a scanning electron micrograph of the cultured cells that shows the apical surfaces covered with short microvilli and long, single, centrally placed cilia. This morphology is characteristic of collecting duct principal cells (4). Further evidence that the cultured cells are principal cells rather than intercalated cells is provided by the failure to demonstrate positive succinic dehydrogenase staining (Fig. 4) (16).

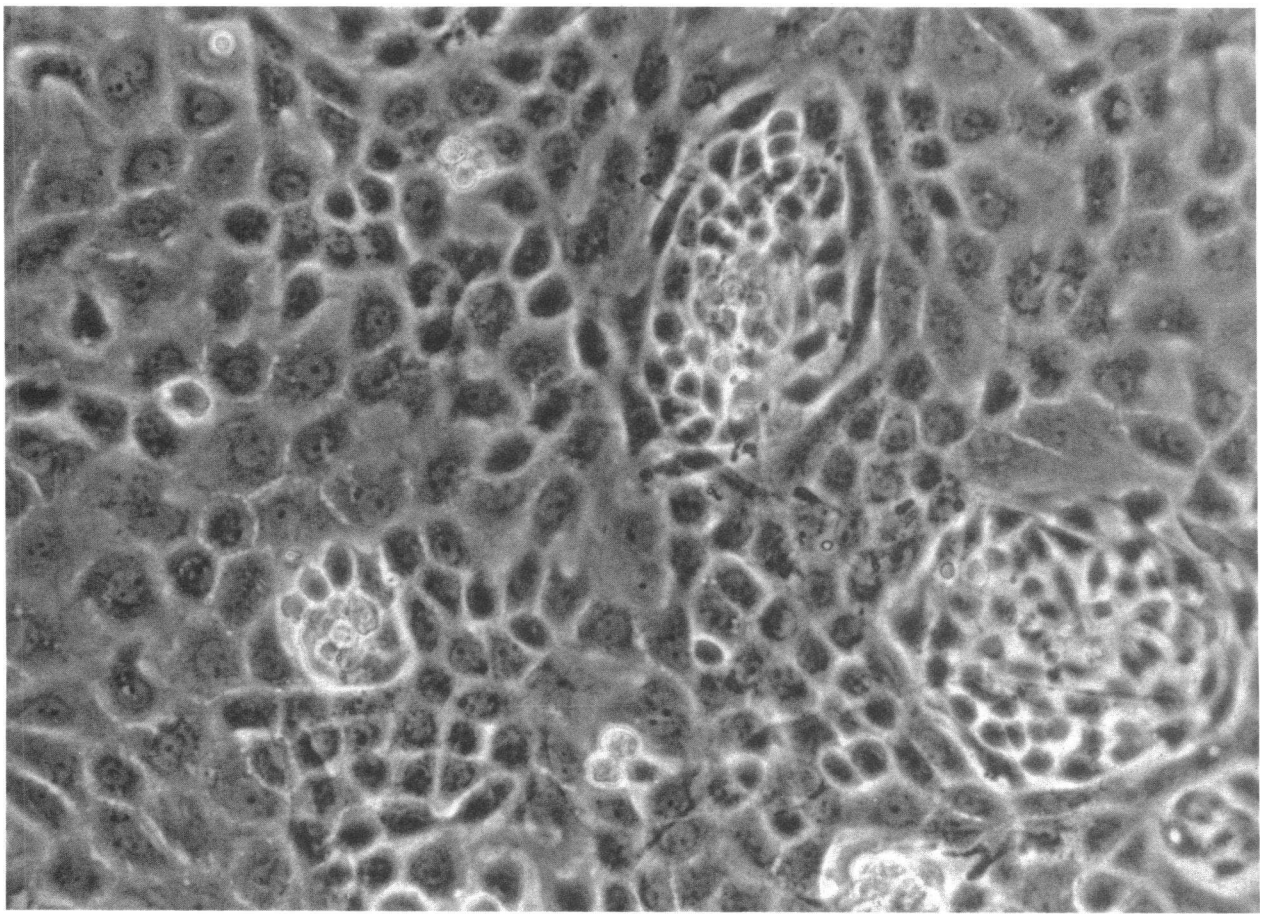

Figure 1. Phase-contrast photomicrograph of rat renal papillary cells in primary culture. × 320.

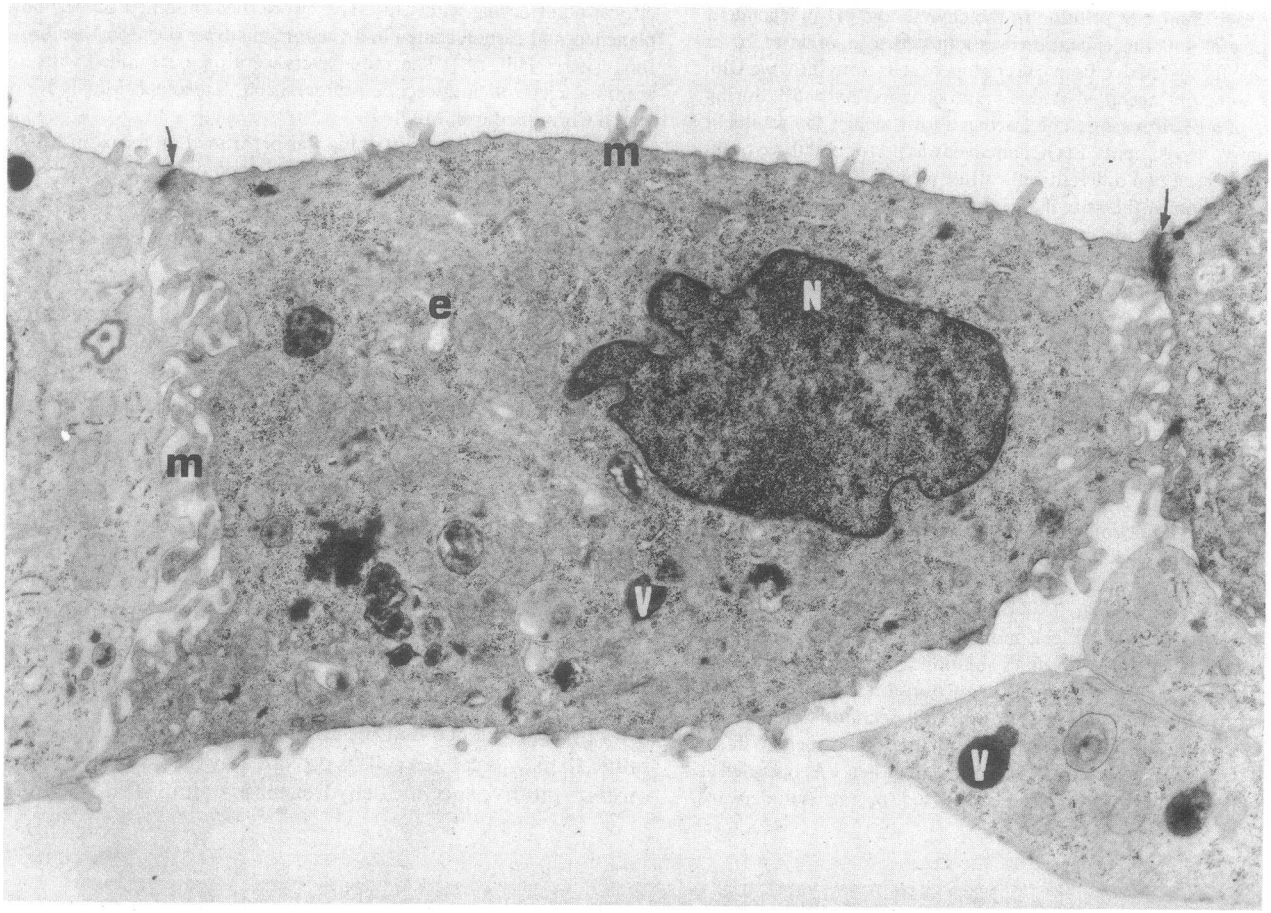

Figure 2. Transmission electronmicrograph of renal papillary cells in primary culture. Microvilli (*m*); nucleus (*N*); endoplasmic reticulum (*e*); autophagic vacuoles (*V*); and cellular junctions (*arrows*). × 12,800.

Na^+-independent pH regulation. The monolayers were loaded with acid by two protocols: (*i*) exposure to and removal from NH_4Cl, and (*ii*) exposure to propionate. The initial BCECF cell fluorescence in both protocols was determined in an extracellular medium containing 135 mM NaCl, buffered with 15 mM Hepes-Tris at pH 7.3. The left tracing of Fig. 5 *A* shows the change in cell pH that occurred when the original buffer was replaced with one containing 115 mM choline Cl and 20 mM NH_4Cl. Cell pH rose abruptly, presumably due to the rapid entry of NH_3. When the NH_4^+-containing buffer was changed to one containing choline alone, cell pH then decreased to a value below baseline because of the rapid loss of NH_3 and retention of the relatively impermeant NH_4^+. After this maneuver, cell pH increased towards baseline. The leftmost tracing of Fig. 5 *B* shows the result of replacing the initial Na^+-containing buffer with one containing 115 mM choline Cl and 20 mM propionate. Cell pH fell abruptly, presumably due to the entry of the relatively permeant propionic acid, and then recovered towards baseline.

Effect of metabolic inhibitors on Na^+-independent pH regulation. The right tracings of Fig. 5 show the effect of CN^- on Na^+-independent pH regulation. Incubation in a Na^+-containing, glucose-free buffer with 2 mM KCN for 20 min prevented the recovery of cell pH from the imposed acid loads. This is consistent with a requirement for metabolic energy of cell pH regulation (17). Since medullary and papillary tissue appear to have a high capacity for glycolysis (18), we examined whether pH recovery in the absence of Na^+ could be sustained by glucose alone. Fig. 6 shows studies utilizing the same protocols as in Fig. 5. As can be seen, considerable capacity for pH recovery was retained by the cells after incubation with KCN in the presence of 5.5 mM glucose.

The results of these experiments are summarized in the topmost sections of Table I (NH_4^+ loading) and Table II (propionate loading). Cyanide significantly inhibited pH recovery in both glucose-containing and glucose-free buffers. However, despite similar falls in pH after acid loading (ΔpH) and starting pH values (pH nadir), the slopes of pH recovery were significantly greater after KCN exposure in glucose-containing solutions compared with KCN exposure in glucose-free solutions.

Oligomycin, an inhibitor of the mitochondrial H^+-ATPase, prevents oxidative ATP generation (19). In the next series of experiments, the effect of this inhibitor on the Na^+-independent recovery of cell pH after acid loads was assessed. The left tracings of Fig. 7 indicate the control responses of the individual monolayers to the two acid-loading protocols. The right tracings show the results of the same acid-loading protocols after incubation of the monolayers in a glucose-free buffer with 5 μg/ml oligomycin. This treatment also abolished cell pH recovery.

In a manner similar to the cyanide experiments, monolayers exposed to oligomycin in a glucose-containing buffer

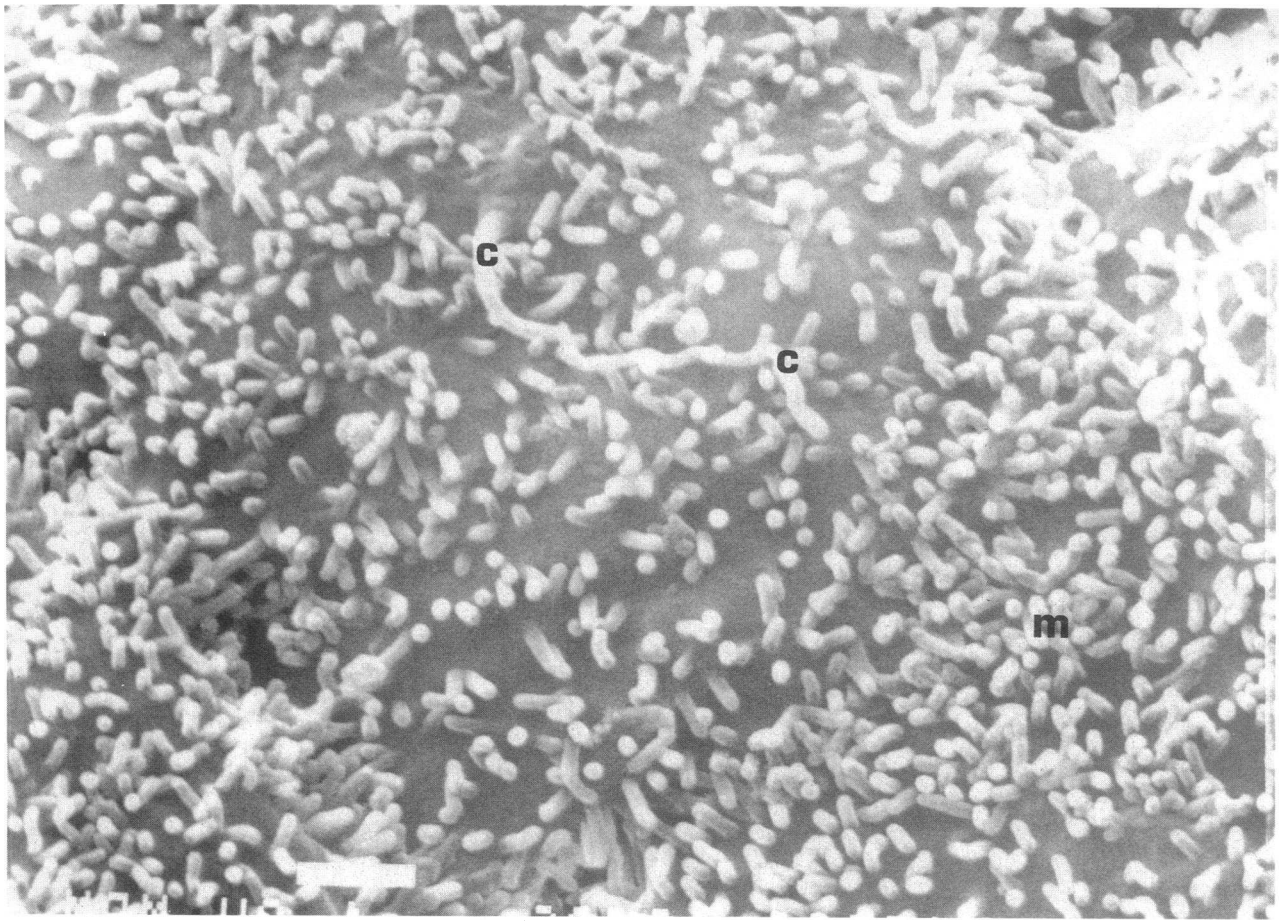

Figure 3. Scanning electron photomicrograph of renal papillary cells in culture. Short microvilli (*m*); and central cilium (*c*). × 15,000.

maintained a greater rate of pH recovery after acid loading than monolayers exposed to the inhibitor in glucose-free buffer. These results are summarized in the middles sections of Table I (NH_4^+ loading) and Table II (propionate loading). In glucose-containing solutions, oligomycin did not significantly inhibit pH recovery after NH_4^+ loading, but did significantly inhibit pH recovery after propionate. In the absence of glucose, however, the pH recovery rates after oligomycin exposure were significantly less than in glucose-containing buffer. These results confirm the requirement for metabolic energy of pH recovery under these experimental conditions.

Effect of a plasma membrane H^+-ATPase inhibitor on Na^+-independent pH regulation. *N*-ethylmaleimide inhibits proton transport and H^+-ATPase activity in a number of epithelia (7, 17, 20). The effect of this agent on cell pH regulation was examined in the next series of studies. The left tracings of Fig. 8 indicate the control responses of the individual monolayers to the two acid-loading protocols. The right tracings show the results of the same protocols after incubation of the monolayers in a buffer containing 10 μM *N*-ethylmaleimide for 20 min. As shown, this agent also abolished Na^+-independent pH recovery from acid loads.

The results of the experiments with *N*-ethylmaleimide are summarized in the lowest sections of Table I (NH_4^+ loading) and Table II (propionate loading). As can be seen, despite equivalent or greater ΔpH values and similar or lower pH nadir values, *N*-ethylmaleimide exposure (in the presence of glucose) abolished pH recovery. These results suggest that Na^+-independent pH recovery is due to the action of an H^+-ATPase.

Effect of Na^+ on pH recovery. The effect of Na^+ on cell pH recovery was investigated by incubating monolayers in a solution containing 140 mM choline Cl and buffered with Hepes-Tris at pH 7.1. H^+ loading was accomplished by adding 1 μg/ml nigericin, allowing cell pH to stabilize, and adding 10 mg/ml albumin to terminate the effect of the nigericin. Then small volumes of a 22.5% NaCl solution were added to achieve various Na^+ concentrations ranging from 3 to 60 mM. Only a very slow pH recovery (below 0.01 pH U/min) was observed without added Na^+ using this protocol; this was neglected in calculating rates of Na^+-dependent pH recovery. Addition of equivalent moles of a choline Cl solution also did not affect cell pH recovery. Initial rates of recovery in response to NaCl were used to generate a Hanes-Woolf relationship. The composite graph is shown in Fig. 9. Table III reports K_m and V_{max} values from 13 individual monolayers from five culture groups, each with an *r* value for the Hanes-Woolf plots > 0.9. Kinetic values were comparable by either method.

The effect of the amiloride-analogue 5-(*N*-ethyl-*N*-isopropyl)amiloride on Na^+-dependent pH recovery was investigated by exposing monolayers acid loaded with nigericin in Na^+-free media to different concentrations of 5-(*N*-ethyl-*N*-isopropyl) amiloride or to vehicle alone followed by 50 mM NaCl. The initial rates of pH recovery per minute were used to generate a

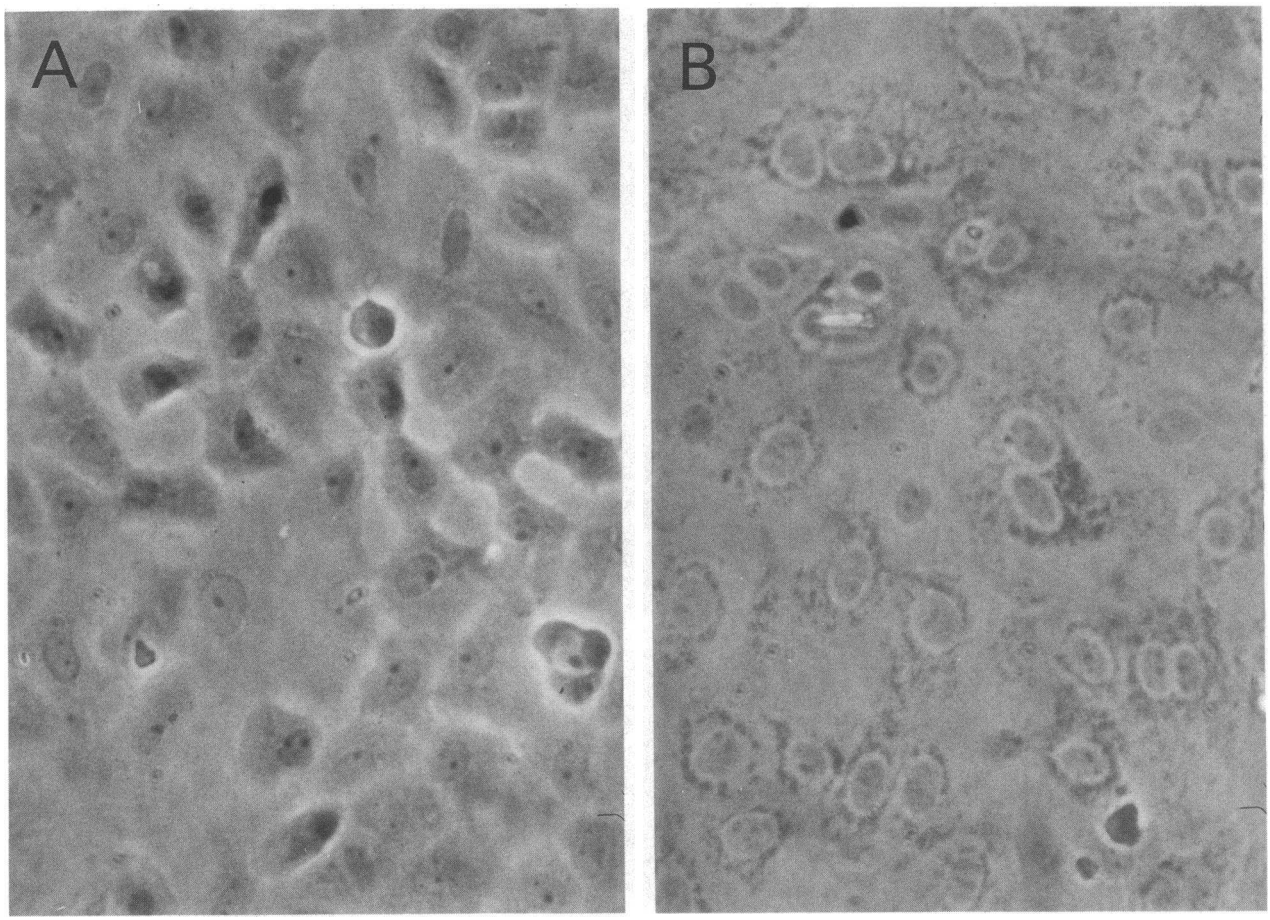

Figure 4. Succinic dehydrogenase staining of renal papillary cells in primary culture (*A*), and for comparison mouse proximal tubule cells in primary culture (*B*). Phase-contrast photomicrograph. × 384.

Dixon plot, as shown in Fig. 10. The inhibition constant (K_i) value derived from this relationship was 0.8 μM.

Discussion

Cytoplasmic pH regulation is a characteristic of most living cells, which possess several systems capable of transporting acid or base equivalents across the plasma membrane (21). These include cation-H^+ and anion-HCO_3^- (or OH^-) exchangers, anion-H^+ and cation-HCO_3^- (or OH^-) symporters, and electrogenic H^+ and HCO_3^- transporters. These systems have been characterized as simple diffusive mechanisms or facilitated (carrier mediated) transport, and have been shown to be passive, secondarily active, or directly coupled to supplies of metabolic energy. The pH regulating systems of renal tubule cells are also involved in the transepithelial movement of acid or base equivalents and, thus, urinary acidification.

BCECF has many characteristics that make it an ideal probe for intracellular pH (22, 23). BCECF-AM is lipophylic and rapidly enters the cell, where it is converted to the fluorescent BCECF by intracellular esterases. BCECF has a pK_a of $\sim$ 7.2, which makes it a sensitive probe for cytoplasmic pH in the physiologic range. It is confined to the cytoplasmic cellular compartment and has a sufficiently stable isosbestic point to permit correction for any leakage that may occur. Titration of solutions of BCECF have verified the fact that the fluorescence response of the dye to pH changes in instantaneous (results not shown), thus confirming its suitability for determination of cell pH transients.

The question of whether metabolic processes causing the shift of acid or base equivalents to or from compartments within the cell not accessible to BCECF could be responsible for the observed alterations in cell pH has not been examined in the current studies. Other investigators using maneuvers similar to those used in these studies to alter cell pH have demonstrated appropriate changes in proton extrusion as measured by titration of extracellular medium or extracellular pH changes (17, 24).

The cultured cells used in these studies are derived from the white papilla. The original cell suspension, therefore, should contain inner medullary collecting duct segments designated $IMCD_2$ and $IMCD_3$ (4) as well as loop of Henle thin limb cells, interstitial cells, and uroepithelial cells that line the renal pelvis. The efficacy of hypotonic lysis for removing these contaminants has not been verified. However, the cells as grown do not demonstrate morphology consistent with origin from the latter cell types. The preponderance of the cells are, in fact, epithelial in type. This is apparent from the intercellular junctions observed in transmission electron micrographs (25). It is clear, however, that these structures do not restrict access of peptide hormones to the apical membrane (13). Thus it is unlikely that the effects of maneuvers used in the current stud-

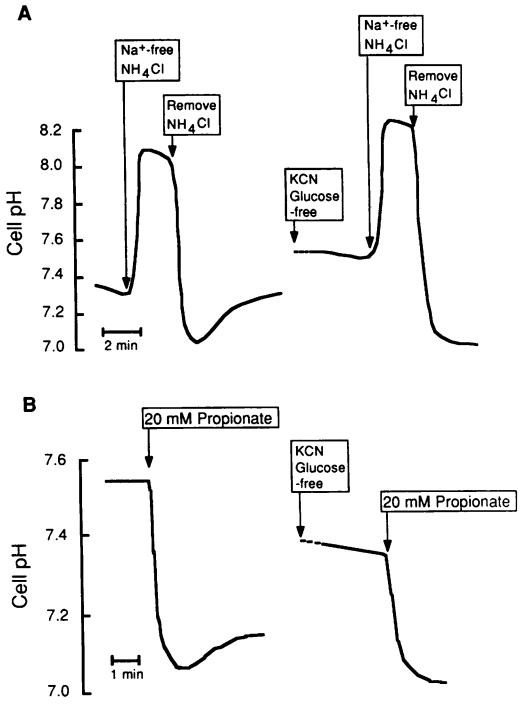

Figure 5. Effect of CN^- and glucose removal on Na^+-independent pH recovery. BCECF-loaded monolayers were initially exposed to an extracellular medium containing 135 mM NaCl and 10 mM Hepes-Tris at pH 7.3. At the indicated points the cells were acid loaded in the absence of Na^+ by exposure to and removal from an NH_4Cl-containing medium (*A*) or exposure to propionate (*B*). In both panels the tracings show pH recovery from Na^+-free acid loading before and after incubation of the monolayers for 20 min in a Na^+-containing but glucose-free medium with 2 mM KCN. Each panel depicts the results of a representative monolayer.

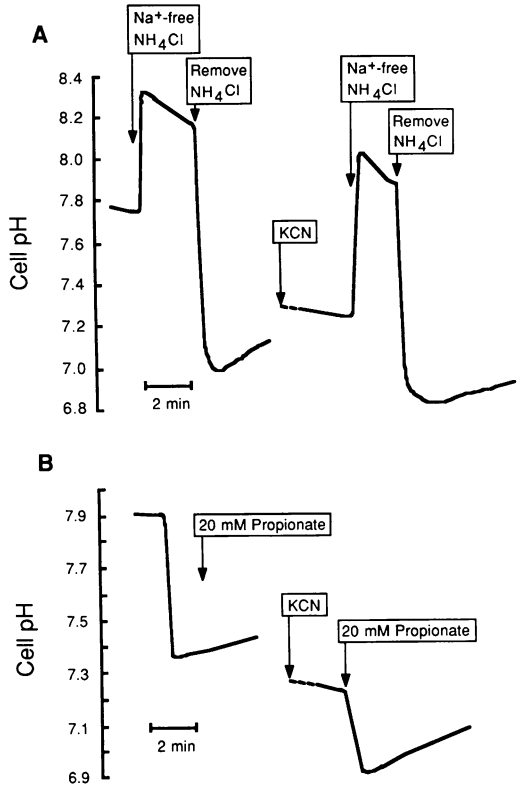

Figure 6. Effect of CN^- on Na^+-independent pH recovery in the presence of glucose. The experiments were performed in a identical manner to those shown in Fig. 4, except that all solutions contained 5.5 mM glucose.

ies to alter acid-base transport are confined to the apical membrane and, thus, can serve to localize these pH regulating systems to this membrane.

In addition to the fact that intercalated cells have not been observed in rat papilla, the source of the cultures under study, no cells conforming to their morphology were observed in scanning electron micrographs. Because of its greater content of mitochondria compared with principal cells, intercalated cells have been identified by succinic dehydrogenase staining (16). Proximal tubule cells in primary culture show positive succinic dehydrogenase staining because of their large numbers of mitochondria, similar to intercalated cells. The cultures reported on here lack such staining. Finally, observation of BCECF-loaded cultures with fluorescence microscopy does not clearly indicate cellular pH heterogeneity as would be expected from the presence of subpopulations of cells transporting acid or base (26). From these considerations it appears likely that the pH regulatory systems observed in the current studies are a property of the rat papillary collecting duct principal cell in primary culture.

The proton translocating ATPase has been identified in turtle bladders, a turtle bladder epithelial cell line, and in rat and bovine renal medulla (7, 20, 27, 28). It has not, to our knowledge, been localized to a particular cell type within these tissues, nor have the studies cited above ruled out its presence in inner papillary tissue. The Na^+-independent pH regulation described in the present studies have characteristics suggesting that it may be a result of the action of an H^+-ATPase. Interference with the supply of metabolic energy for transport by two different inhibitors of oxidative metabolism abolishes Na^+-independent pH regulation. Provision of glucose in the presence of the oxidative metabolism inhibitors CN^- and oligomycin preserved Na^+-independent pH regulation to some degree. This is consistent with maintenance of the supply of ATP through the glycolytic capacity of cells in the renal papilla (18). Finally, the effect of exposure of the cells to *N*-ethylmaleimide in the presence of glucose provides further support for the idea that this form of pH regulation is due to the H^+-ATPase. This inhibitor has been shown to be specific for H^+-ATPases derived from bovine renal medulla (including papilla?), endocytotic vesicles, Golgi complex, and microsomes of various morphologies (7, 28–32). These experiments, then, support the notion that rat papillary duct principal cells possess an H^+-ATPase.

Distal nephron acidification has not generally been thought to depend on Na^+. In turtle urinary bladder, a distal nephron model, electrogenic proton secretion is not critically dependent on Na^+ transport (33). Na^+ absorption may provide a favorable electrical profile for the H^+ pump across the luminal membrane (34, 35), but electrically silent Na^+-H^+ exchange has not been demonstrated in this tissue. Similar H^+ transport characteristics have been demonstrated in mammalian cortical collecting duct, again without apical Na^+-H^+ exchange (36). Outer medullary collecting duct cell suspensions

Table I. Cell pH Values and Transients in CPCD Cells Acid Loaded by Exposure to and Removal from NH_4Cl

	Initial pH	NH_4^+ pH	pH nadir	ΔpH	Slope
	U	*U*	*U*	*U*	*U/min*
Control	7.81±0.06	8.25±0.06	7.25±0.07	1.01±0.08	0.08±0.02
KCN +G	7.54±0.19	8.17±0.12	7.10±0.16	1.08±0.06	0.04±0.02
P vs. C (n = 6/2)	NS	NS	NS	NS	<0.025
Control	7.36±0.10	8.08±0.04	6.86±0.10	1.22±0.09	0.04±0.00
KCN −G	7.41±0.03	7.97±0.03	7.04±0.06	0.93±0.05	−0.02±0.01
P vs. C (n = 7/2)	NS	NS	NS	<0.02	<0.001
P, C vs. C	<0.005	<0.05	<0.02	NS	NS
P, +G vs. −G	NS	NS	NS	NS	<0.02
Control	7.46±0.08	7.66±0.16	7.27±0.10	0.38±0.19	0.02±0.01
Oligo +G	7.49±0.09	7.56±0.09	7.25±0.02	0.30±0.11	0.01±0.00
P vs. C (n = 3/2)	NS	NS	NS	NS	NS
Control	7.76±0.04	8.14±0.05	7.03±0.05	1.11±0.06	0.07±0.01
Oligo −G	7.48±0.05	7.95±0.08	7.19±0.06	0.77±0.12	−0.02±0.01
P vs. C (n = 8/3)	<0.005	<0.02	<0.02	<0.01	<0.001
P, C vs. C	<0.01	<0.005	<0.05	<0.001	<0.025
P, +G vs. −G	NS	<0.02	NS	NS	<0.05
Control	7.77±0.05	8.10±0.04	7.03±0.12	1.07±0.07	0.13±0.02
NEM +G	7.57±0.08	7.90±0.07	6.62±0.09	1.28±0.04	−0.02±0.01
P vs. C (n = 4/2)	<0.05	<0.01	<0.002	<0.05	<0.005

Values are means±SEM; n, number of slides per number of platings. Controls are values from maneuvers performed before incubation of individual slides with inhibitors. The maneuvers were then repeated after 20 min incubation with the indicated inhibitors, KCN, oligomycin(oligo), or *N*-ethylmaleimide (NEM) in the presence and absence of glucose (+G or −G). Comparisons within groups, inhibitor versus control (P vs. C), are by paired t test; comparisons between groups, between controls (P, C vs. C), or between inhibitors with and without glucose (P, +G vs. −G), are by unpaired t test.

Table II. Cell pH Values and Transients in CPCD Cells Acid Loaded by Exposure to Propionate

	Initial pH	pH nadir	ΔpH	Slope
	U	*U*	*U*	*U/min*
Control	7.72±0.10	7.37±0.02	0.35±0.10	0.02±0.01
KCN +G	7.47±0.14	7.20±0.11	0.27±0.07	0.02±0.01
P vs. C (n = 5/3)	NS	NS	NS	NS
Control	7.51±0.06	7.13±0.08	0.38±0.03	0.04±0.01
KCN −G	7.59±0.13	7.25±0.08	0.35±0.06	−0.03±0.01
P vs. C (n = 4/3)	NS	NS	NS	<0.05
P, C vs. C	NS	<0.02	NS	NS
P, +G vs. −G	NS	NS	NS	<0.002
Control	7.57±0.09	7.28±0.05	0.29±0.07	0.04±0.00
Oligo +G	7.44±0.03	7.13±0.06	0.31±0.05	0.01±0.00
P vs. C (n = 6/2)	NS	NS	NS	<0.001
Control	7.71±0.02	7.18±0.02	0.53±0.03	0.02±0.01
Oligo −G	7.50±0.03	7.04±0.02	0.46±0.02	−0.02±0.00
P vs. C (n = 5/2)	<0.01	<0.001	NS	<0.005
P, C vs. C	NS	NS	<0.02	NS
P, +G vs. −G	NS	NS	<0.05	<0.002
Control	7.72±0.04	7.15±0.06	0.57±0.05	0.02±0.01
NEM +G	7.48±0.06	6.93±0.05	0.55±0.03	−0.03±0.00
P vs. C (n = 4/2)	<0.02	<0.01	NS	<0.01

Values are means±SEM; n, number of slides per number of platings. Controls are values from maneuvers performed before incubation of individual slides with inhibitors. The maneuvers were then repeated after 20 min incubation with the indicated inhibitors, KCN, oligomycin(oligo), or *N*-ethylmaleimide (NEM) in the presence and absence of glucose (+G or −G). Comparisons within groups, inhibitor versus control (P vs. C), are by paired t test; comparisons between groups, between controls (P, C vs. C), or between inhibitors with and without glucose (P, +G vs. −G), are by unpaired t test.

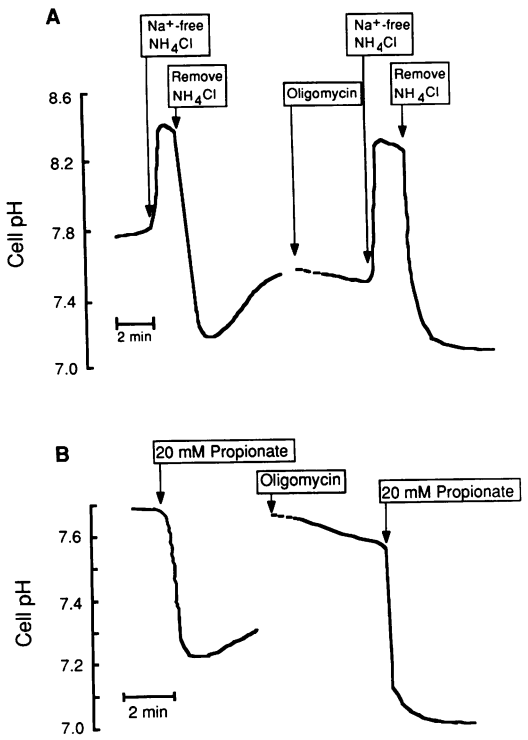

Figure 7. Effect of oligomycin on Na^+-independent pH recovery. The experiments were performed identically to those preceding except that Na^+-independent pH recovery was evaluated before and after incubation for 20 min with 5 μg/ml oligomycin in glucose-free buffer.

freshly isolated from rabbit kidneys also failed to demonstrate Na^+ dependence of pH regulation in studies performed utilizing techniques similar to those used in the current experiments (17). In contrast, La Belle has identified an amiloride-sensitive Na^+-H^+ exchanger in vesicles from rabbit medullary tissue (presumably including papilla) (37). Na^+ kinetics are not available for comparison, but the amiloride concentration at which 50% inhibition was observed ($K_{0.5}$) was 60 μM. The current studies have revealed a K_i value of 0.8 μM for 5-(*N*-ethyl-*N*-isopropyl)amiloride. This compound has been shown to be a 50 to 140 times more potent inhibitor for Na^+-H^+ exchange systems in a variety of tissues (38, 39). An effect of 5-(*N*-ethyl-*N*-isopropyl)amiloride itself to dissipate cell-to-extracellular pH gradients was not observed in the current studies.

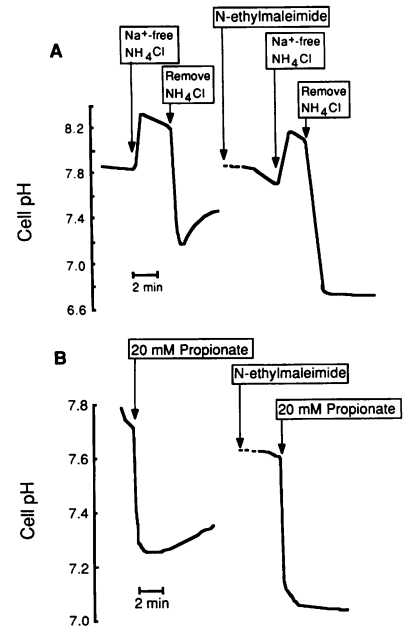

Figure 8. Effect of *N*-ethylmaleimide on Na^+-independent pH recovery. The experiments were performed identically to those preceding except that pH recovery was evaluated before and after incubation of the cells for 20 min with 10 μM *N*-ethylmaleimide.

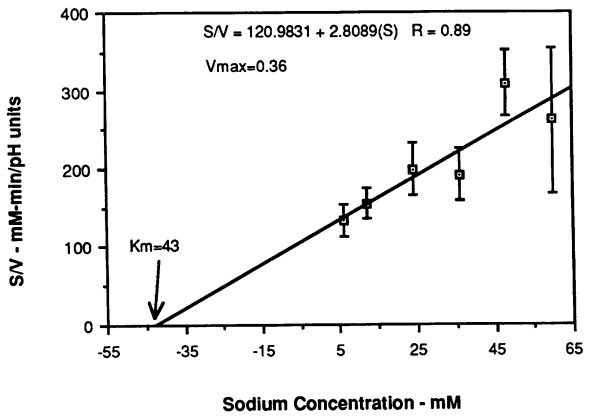

Figure 9. A composite Hanes-Woolf plot of Na^+-dependent pH recovery following an acid load. BCECF-loaded monolayers of papillary cells were acid loaded in media containing 140 mM choline Cl buffered with Hepes-Tris to pH 7.1 and nigericin (1 μg/ml). Albumin was used to terminate the effect of the ionophore, and small quantities of a concentrated NaCl solution were added to generate different external Na^+ concentrations. Mean±SEM of initial rates of pH change at each of six different Na^+ concentrations were used to construct the figure.

Na⁺-H⁺ exchange has, of course, been demonstrated in renal cortical vesicles, proximal tubules (perfused, in suspension, or in primary culture), and in several cultured renal cell

Table III. Kinetic Values Derived from Hanes-Woolf Plots of Individual Slides

Exp. No.	*n*	*r* value	K_m	V_{max}
1	3	0.997	9	0.13
2	5	0.975	34	0.17
3	4	0.905	62	0.22
4	4	0.952	38	0.53
5	4	0.938	27	0.38
6	5	0.967	32	0.43
7	8	0.918	115	1.52
8	3	1.000	20	0.30
9	4	0.979	34	0.43
10	4	0.980	69	0.70
11	4	0.950	43	0.38
12	4	0.967	53	0.34
13	4	0.961	65	0.38
		Means±SEM	46±8	0.45±0.10

Values for K_m (millimolars) and V_{max} (pH units per minute) are derived from linear regressions of plots of $[Na^+]$/pH change as a function of $[Na^+]$ for the indicated number of points (*n*) in 13 individual slides of 5 different platings.

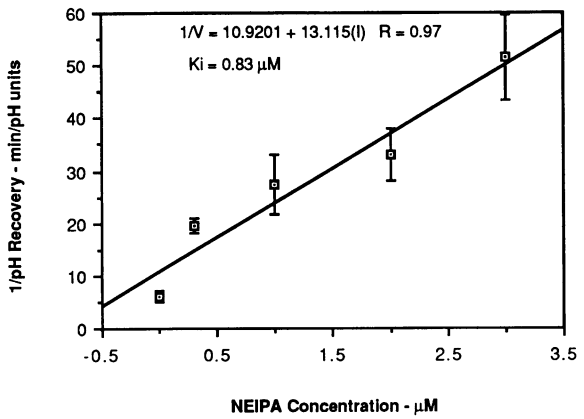

Figure 10. Dixon Plot of the effect of 5(*N*-ethyl-*N*-isopropyl)amiloride on Na^+-dependent pH recovery. The cells were acid loaded as in Fig. 8. 5(*N*-ethyl-*N*-isopropyl)amiloride was added followed by NaCl to produce an external Na^+ concentration of 50 mM. Each point is the mean±SEM of initial rates of pH change of four to six individual monolayers.

lines (40–48). Due to differences in technique, V_{max} values for pH are not amenable to direct comparison; K_m values for Na^+, however, are expressed in similar units, and appear to be between two and eight times higher in the primary papillary cultures than in other preparations. This value of ~ 46 mM is more than threefold higher than K_m values obtained in primary cultures of baby mouse proximal tubule cells by identical techniques (unpublished observations).

As indicated above, these studies cannot distinguish between apical and basolateral processes. It seems reasonable to consider that a papillary collecting duct H^+-ATPase would be apically situated, as in other acid-secreting renal segments or uroepithelial tissue, in juxtaposition to the compartment being acidified (8–11, 33). It is quite possible, however, that the Na^+-H^+ exchanger is present in the basolateral cell membrane where it serves to regulate cell pH, as has been described in rabbit cortical collecting duct (49).

In summary, evidence for two modes of pH regulation in papillary tubule cells in primary culture have been presented. These systems are consistent with the presence of both an H^+-ATPase and an Na^+-H^+ exchanger. The existence of these systems may provide the cellular mechanisms in principal cells for internal pH regulation as well as the urinary acidification observed in the renal papilla.

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