

Spontaneous Luminal Disequilibrium pH in S₃ Proximal Tubules

Role in Ammonia and Bicarbonate Transport

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Abstract

We determined whether a spontaneous luminal disequilibrium pH, pH_{dq} (pH measured – pH equilibrium), was present in isolated perfused rabbit S₂ and S₃ proximal tubules. Luminal pH was measured by perfusing with the fluorescent pH probe 1,4-DHPN, and the equilibrium pH was calculated from the measured collected total CO₂ and dissolved CO₂ concentrations. S₂ tubules failed to generate a spontaneous pH_{dq} . S₃ tubules generated a spontaneous acidic pH_{dq} of -0.46 ± 0.15 ($P < 0.05$), which was obliterated following the addition of carbonic anhydrase (0.1 mg/ml) to the perfusate. In S₃ tubules perfused and bathed in 4 mM total ammonia, luminal total ammonia rose from 4.08 ± 0.05 mM (perfusate) to 4.95 ± 0.20 mM (collected fluid) ($P < 0.02$). Carbonic anhydrase added to the perfusate prevented the rise in the collected total ammonia concentration. We conclude that the rabbit S₃ proximal tubule lacks functional luminal carbonic anhydrase. The acidic pH_{dq} in the S₃ segment enhances the diffusion of NH₃ into the lumen. In contrast, the S₂ segment has functional luminal carbonic anhydrase.

Introduction

Carbonic anhydrase plays an important role in bicarbonate absorption and acidification of the proximal tubule (1). Histochemical studies have revealed that not all segments of the nephron contain carbonic anhydrase and that within a nephron segment, some cells may stain positively for the enzyme while other cells appear to lack detectable enzyme (2–4). The presence of carbonic anhydrase correlates well with measured net bicarbonate reabsorptive rates in the segments that have been studied.

Two forms of carbonic anhydrase exist in the kidney, a soluble cytoplasmic enzyme and a membrane-bound form (2–16). Cytoplasmic carbonic anhydrase is thought to supply protons in the cell for transport across the apical membrane by catalyzing the formation of carbonic acid from CO₂ and water. Membrane-bound carbonic anhydrase present in the luminal membrane of the renal tubule increases the rate of dehydration of carbonic acid formed in the lumen from the titration of filtered bicarbonate by secreted protons (17). The uncatalyzed rate of carbonic acid dehydration is too slow to match the high rate of proton secretion in the proximal tubule. Consequently, in the absence of luminal carbonic anhydrase, proton secretion can result in carbonic acid accumulation and in an acidic disequilibrium pH in the lumen.

Using micropuncture in rat proximal convoluted tubules,

Rector et al. (17) found that no luminal pH disequilibrium was detectable despite a high rate of bicarbonate absorption. Intravenous infusion of acetazolamide, a carbonic anhydrase inhibitor, resulted in a large acidic disequilibrium pH in the lumen which was attributed to inhibition of the luminal membrane-bound enzyme. Recent micropuncture studies in the rat have confirmed that membrane bound carbonic anhydrase is in functional contact with the lumen of the proximal convoluted tubule (18, 19). The straight portion of the proximal tubule, however, cannot be studied by micropuncture because it is not accessible at the surface of the kidney. In this study, therefore, we utilized the isolated perfused tubule technique to determine whether the superficial proximal straight tubule (S₂) and the outer medullary proximal straight tubule (S₃) of the rabbit contain endogenous luminal carbonic anhydrase. The results demonstrate that the S₃ segment does not possess luminal enzyme and therefore generates a spontaneous acidic luminal disequilibrium pH as a consequence of proton secretion. The acidic disequilibrium pH is shown to enhance ammonia secretion by this segment.

Methods

Procedures. Tubules were dissected from pathogen-free New Zealand White rabbits (Small Animal Breeding Facility, National Institutes of Health, NIH). The rabbits were decapitated and one kidney was removed and sliced into coronal sections. The location of the microdissected tubules is depicted in Fig. 1. Superficial proximal straight tubules (S₂ segments) were dissected from the superficial medullary rays. The outer medullary proximal straight tubules (S₃ segments) were dissected by locating the beginning of a descending thin limb of Henle in the outer medulla and dissecting towards the cortex. The mean lengths of the dissected segments were (a) S₂: 1.07 ± 0.04 mm ($n = 4$); and (b) S₃: 1.35 ± 0.01 mm ($n = 20$). No attempt was made to distinguish between the S₃ segment of superficial and juxtamedullary nephrons. To measure luminal pH, the tubules were transferred to a specially designed perfusion chamber mounted on the stage of the microfluorometer, and were perfused in vitro using the pH-sensitive fluorescent dye 1,4-dihydroxyphthalonitrile (1,4-DHPN).¹ The chamber was blackened to prevent reflected excitation or emission light from impairing the fluorescent measurements.

The tubules were mounted on concentric glass pipettes as described (20) except that a guard pipette was not used on the perfusion end. All studies were performed with the tubule resting on the coverslip, which was necessary to minimize scattering of the excitation and emission light by the bathing solution. The bathing solution, which was preheated to 37°C in a water jacketed chamber and bubbled with 6.0% CO₂/94% O₂, flowed by gravity through Saran tubing into the perfusion chamber at 3–5 ml/min. Using this system, the bathing solution could be continuously exchanged with minimal movement of the tubule and without formation of bubbles in the perfusion chamber. Fine adjustment of the perfusion chamber temperature to $37 \pm 0.5^\circ\text{C}$ was made using a nichrome wire as a resistance heater. Bath pH was continuously monitored with a pH microelectrode (MI-410, Microelectrode Inc., Londonderry, NH).

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1. Abbreviations used in this paper: 1,4-DHPN, 1,4-dihydroxyphthalonitrile.

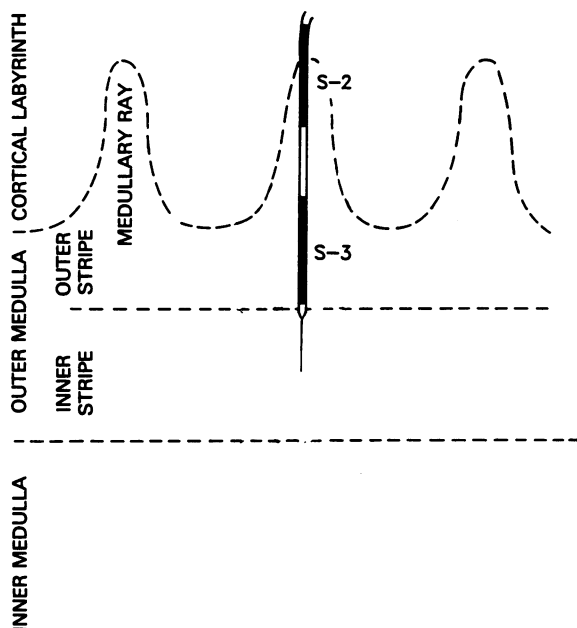


Figure 1. Location of microdissected tubules. S₂ segments were dissected from superficial medullary rays and S₃ segments were dissected from the outer stripe of the outer medulla.

The perfusate was continuously bubbled at 22°C with 5% CO₂/95% O₂ and flowed by gravity into the perfusion pipette. The tubules were perfused at a rate of 2–3 nl/min per mm.

Solutions. The composition of the perfusate and bathing solutions were as follows: Na⁺, 146 mM; K⁺, 5 mM; Ca²⁺, 2 mM; Mg²⁺, 1.2 mM; Cl⁻, 118 mM; HCO₃⁻, 25 mM; phosphate, 2.5 mM; lactate, 4 mM; sulfate, 1.2 mM; citrate, 1 mM; glucose, 5.5 mM; and alanine, 6 mM. Albumin was not added to the bath. In some experiments ammonium chloride (4 mM) was added to the perfusate and bathing solutions in replacement for 4 mM sodium chloride. The osmolality of all the solutions was ~290 mosmol/kg H₂O as measured by a vapor pressure osmometer (Wescor, Inc., Logan, UT). The composition of the perfusate and bathing solutions was identical except in those experiments where carbonic anhydrase B (0.1 mg/ml) was added to the perfusate. Carbonic anhydrase B was purchased from Sigma Chemical Co., St. Louis, MO.

Analytical techniques. Luminal pH was measured immediately distal to the perfusion pipette (initial 50 μm) and at the end of the tubules (final 100 μm) using the pH sensitive fluorescent probe 1,4-DHPN in the perfusate at a concentration of 410 μM (Fig. 2 A). Emission spectra were monitored from the luminal probe (excitation 370 to 407 nm) using a recently described microfluorometer (21) coupled to a standard perfusion apparatus (Fig. 2 B). The fluorescent probe demonstrates a spectral shift in its peak emission wavelength with change in pH (21). The ratio of the 512:455-nm fluorescence measured simultaneously corresponds to a specific pH (21). Since the two intensities are determined simultaneously, the measurement is independent of the probe concentration, fluctuation in the intensity of the excitation source, or changes

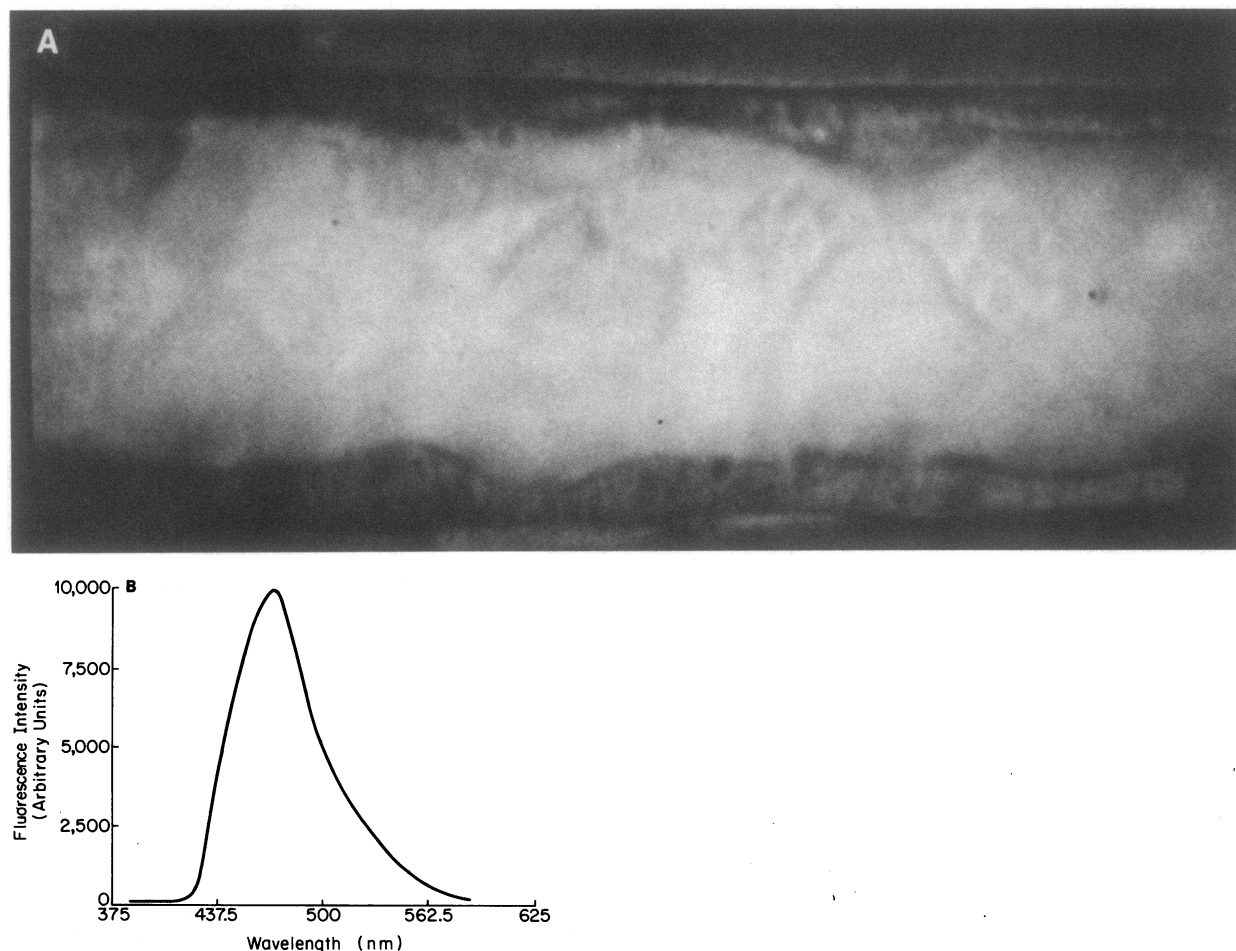


Figure 2. (A) Digitized image of a tubule perfused with the impermeant fluorescent pH probe, 1,4-DHPN (410 μM). (B) Emission spectrum of luminal 1,4-DHPN obtained from a 100-μm spot at the end of an S₃ proximal straight tubule (pH 6.68).

in the optical path length. Calibration of the probe was performed initially by perfusing Hepes-buffered standards at known pH values into the tubule at rapid perfusion rates and monitoring the 512:455 fluorescence ratio. This *in situ* calibration curve was found to be identical to the *in vitro* calibration curve indicating that the spectral characteristics of the probe were not altered by the physical properties and geometry of the tubule. A typical calibration curve is shown in Fig. 3. The intensity of the tubule autofluorescence was not different from zero (given the excitation wavelengths and number of scans, 10 per measurement, used in this study), precluding the need to subtract the autofluorescence spectrum from the spectrum of luminal 1,4-DHPN. In addition, fluorescence was not detectable after the perfusate containing the probe was replaced with a dye-free perfusate, indicating that there was no cellular uptake of the probe during the course of an experiment. All reported pH values are the mean result of three determinations. 1,4-DHPN was purchased from Molecular Probes, Inc. (Junction City, OR).

Total carbon dioxide (TCO_2) concentration in the perfusate, bath, and collected fluid was determined by microcalorimetry (22). The partial pressure of CO_2 (PCO_2) in the bath was calculated with the Henderson-Hasselbach equation using the measured bath TCO_2 and pH assuming a pK of 6.1 and a solubility coefficient for CO_2 of 0.0301. Given the slow perfusion rates used in this study, the end luminal PCO_2 was assumed to equal the bath PCO_2 .

In some studies the ammonia concentration in the perfusate bath and collected fluid was measured using a microfluorometric assay as previously described (23). In those experiments in which the collected ammonia concentration was measured, the pH of the luminal fluid could not be measured simultaneously because of interference with the ammonia assay caused by the fluorescence emission of the pH probe.

The end luminal disequilibrium pH was defined as the measured end luminal pH minus the end luminal equilibrium pH calculated using

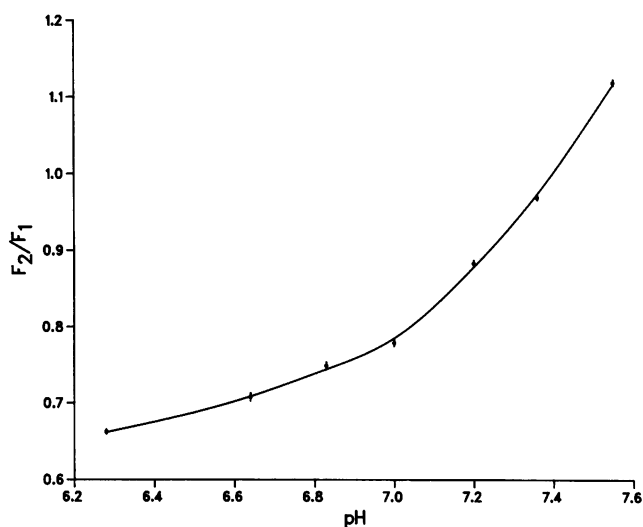


Figure 3. Calibration of 1,4-dihydroxyphthalonitrile (1,4-DHPN). 1,4-DHPN was calibrated using the microfluorometer in a solution containing Na^+ , 146 mM; K^+ , 5 mM; Ca^{2+} , 2 mM; Mg^{2+} , 1.2 mM; Cl^- , 118 mM; phosphate, 2.5 mM; HCO_3^- , 25 mM; lactate, 4 mM; sulfate, 1.2 mM; citrate, 1 mM; glucose, 5.5 mM; alanine, 6 mM; 1,4-DHPN, 410 μM . The fluorescence emission ratio of 512:455 nm was obtained at various pH values by altering the bicarbonate concentration at a constant PCO_2 (5%). Each point represents the mean \pm SEM of four determinations. The curve was not affected by altering the Na^+ , K^+ , Cl^- , Ca^{2+} , Mg^{2+} , PO_4^{3-} , buffer concentration or by the addition of 4 mM NH_4Cl or 0.1 mg/ml carbonic anhydrase. An identical calibration curve was obtained by perfusing the tubules rapidly with Hepes-buffered standards at known pH values indicating that the physical properties and geometry of the tubule did not alter the spectral properties of the dye.

the following equation: end luminal equilibrium $\text{pH} = 6.1 + \log [\text{TCO}_2 - 0.0301 \times \text{PCO}_2 / 0.0301 \times \text{PCO}_2]$, where TCO_2 = Total CO_2 (mM) of the collected fluid and PCO_2 = the end luminal PCO_2 (mmHg) which was assumed to equal the bath PCO_2 .

In some experiments fluid absorption was measured microfluorometrically using raffinose as a volume marker as previously described (24).

The transepithelial potential difference was measured using calomel cells connected to the perfusate and bath solution by NaCl bridges. Since the ionic composition of the perfusate and bath solutions was identical in all studies, liquid junction potentials were symmetrical.

Statistics. All values are reported as mean \pm standard error of the mean. Statistical significance was determined using either paired or unpaired *t* tests.

Results

Luminal pH in S_3 proximal tubules. The left side of Fig. 4 A depicts the luminal pH measured at the beginning (50 μm distal to the perfusion pipette tip), and at the end (final 100 μm) of five S_3 tubules. The mean collection rate was 2.55 ± 0.11 nl/min per mm. The measured luminal pH fell significantly from a mean of 7.43 ± 0.01 at the beginning of the tubules to 6.89 ± 0.17 at the end ($P < 0.05$). The right side of Fig. 4 A depicts the

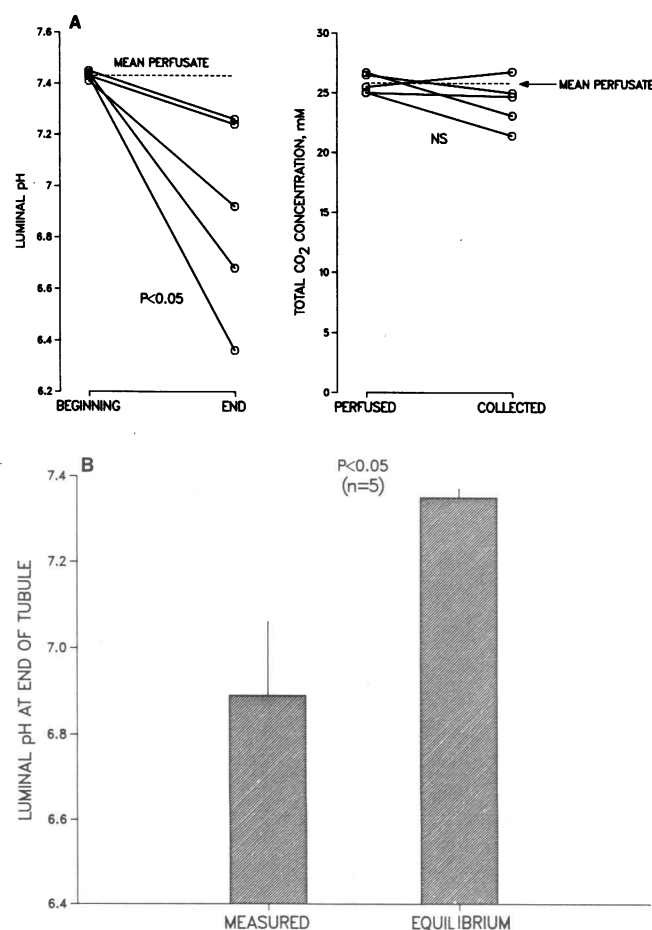


Figure 4. (A) Perfusate and end luminal pH with simultaneously measured perfusate and collected total CO_2 concentration in S_3 proximal tubules. Mean collection rate was 2.55 ± 0.11 nl/min per mm. (B) Measured end luminal pH and calculated end luminal equilibrium pH in the same S_3 proximal tubules.

perfusate and collected luminal total CO₂ concentration measured by microcalorimetry in the same tubules. The mean collected total CO₂ concentration of 24.2±0.9 mM was not significantly different from the perfusate concentration of 25.8±0.4 mM. The measured fluid absorption rate in a different set of tubules under the same conditions was 0.06±0.01 nl/min per mm ($P < 0.02$, $n = 3$). Fig. 4 B compares the mean measured end luminal pH and the mean end luminal equilibrium pH in the same experiments as shown in Fig. 4 A. The measured end luminal pH of 6.89±0.17 was significantly lower than the end luminal equilibrium pH of 7.35±0.02 ($P < 0.05$). The mean disequilibrium pH was -0.46 U. Thus, these results demonstrate the presence of a spontaneous acidic disequilibrium pH in the lumen of the S₃ segment.

A luminal disequilibrium pH should be dissipated if the lumen is perfused with a solution containing carbonic anhydrase. The results of such experiments are depicted in Fig. 5 and Table I. When S₃ tubules were perfused with 0.1 mg/ml carbonic anhydrase (mean collection rate 2.27±0.13 nl/min per mm), the measured mean end luminal pH increased significantly from 6.89±0.17 to 7.39±0.01 ($P < 0.05$). The mean collected total CO₂ concentration of 26.6±0.8 mM was not significantly different from the mean collected total CO₂ concentration measured in the absence of exogenous luminal carbonic anhydrase. The mean pH value determined in the presence of carbonic anhydrase was not significantly different from the mean equilibrium pH value. Thus, we conclude that the S₃ segment of the rabbit proximal tubule normally lacks endogenous luminal carbonic anhydrase.

Luminal pH in S₂ proximal tubules. The left side of Fig. 6 A shows the luminal pH measured at the beginning and at the end of four superficial S₂ proximal tubules (mean collection rate 2.56±0.18 nl/min per mm). The mean luminal pH fell from 7.43±0.01 to 7.10±0.05 at the end ($P < 0.01$). The right side of Fig. 6 A depicts the perfused and collected luminal total CO₂ concentration measured in the same tubules. In contrast to the S₃ segment, the mean collected total CO₂ concentration fell significantly from 26.4±0.2 mM to 12.2±1.4 mM ($P < 0.01$). Unlike the S₃ segment, there was no significant difference between the mean measured end luminal pH of 7.10±0.05 and the mean equilibrium value of 7.04±0.07 (Fig. 6 B). These results indicate

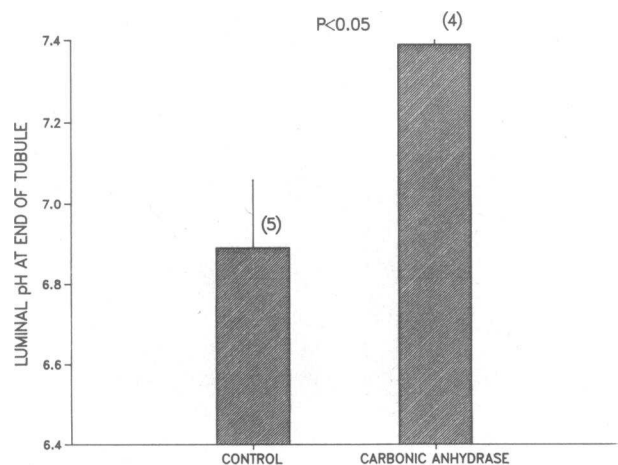


Figure 5. Effect of luminal carbonic anhydrase (0.1 mg/ml) on measured end luminal pH in the S₃ proximal tubules. Mean collection rate was 2.27±0.13 nl/min per mm ($n = 4$).

Table I. Effect of Exogenous Luminal Carbonic Anhydrase on Acidification in S₃ Proximal Straight Tubules

	Control	Carbonic anhydrase
Collection rate (nl·min ⁻¹ ·mm ⁻¹)	2.55±0.11	2.27±0.13
Potential difference (lumen-bath) (mV)	-1.2±0.1	-1.9±0.5
Perfusate total CO ₂ concentration (mM)	25.8±0.4	27.0±0.1
Collected total CO ₂ concentration (mM)	24.2±0.9	26.6±0.8
Luminal pH beginning	7.43±0.01	7.39±0.01
Luminal pH end	6.89±0.17	7.39±0.01*
End luminal PCO ₂ ‡ (mmHg)	42.7±0.4	41.8±0.8
Equilibrium pH§	7.35±0.02	7.41±0.01
Disequilibrium pH	-0.46±0.15	-0.02±0.01*

Values are means±SEM. Control $n = 5$, carbonic anhydrase $n = 4$. Mean tubule length 1.2±0.2 mm in control group and 1.2±0.1 mm in carbonic anhydrase group. Carbonic anhydrase, 0.1 mg/ml carbonic anhydrase B in lumen.

* $P < 0.05$ vs. control.

‡ Calculated from bath total CO₂ and bath pH.

§ Calculated from measured collected total CO₂ concentration and calculated end luminal PCO₂.

that the lumen of the rabbit superficial S₂ segment is normally in functional contact with membrane bound carbonic anhydrase which prevents formation of a spontaneous disequilibrium pH.

Ammonia transport in S₃ proximal tubules. Previous studies in the cortical collecting duct have shown that the presence of an acidic disequilibrium pH in the lumen enhances total ammonia secretion in that segment by increasing the transepithelial NH₃ concentration difference which drives NH₃ secretion (25). Further experiments were performed to test whether the luminal disequilibrium pH in the S₃ proximal tubule also enhances ammonia secretion. Five S₃ segments were perfused and bathed in symmetrical solutions containing 4 mM total ammonia in the absence and presence of exogenous carbonic anhydrase added to the perfusate. At a mean collection rate of 2.12±0.19 nl/min per mm, the total ammonia concentration increased significantly from a mean of 4.08±0.05 mM in the perfusate to 4.95±0.20 mM in the collected fluid ($P < 0.02$, $n = 5$) (Fig. 7). As in the previous experiments in the S₃ segment, the total CO₂ concentration in the collected fluid did not differ from the perfusate concentration (Fig. 7). When carbonic anhydrase (0.1 mg/ml) was added to the perfusate (mean collection rate 2.18±0.38 nl/min per mm), the collected total ammonia concentration did not differ significantly from the perfusate (Table II). These results indicate that the presence of an acidic luminal disequilibrium pH in the S₃ segment enhances total ammonia secretion. This presumably occurs because the acidic disequilibrium pH increases the diffusional entry of NH₃ into the lumen (see Discussion).

Discussion

Fluorescent measurement of luminal pH. In this study, new methodology was developed to measure luminal pH in the iso-

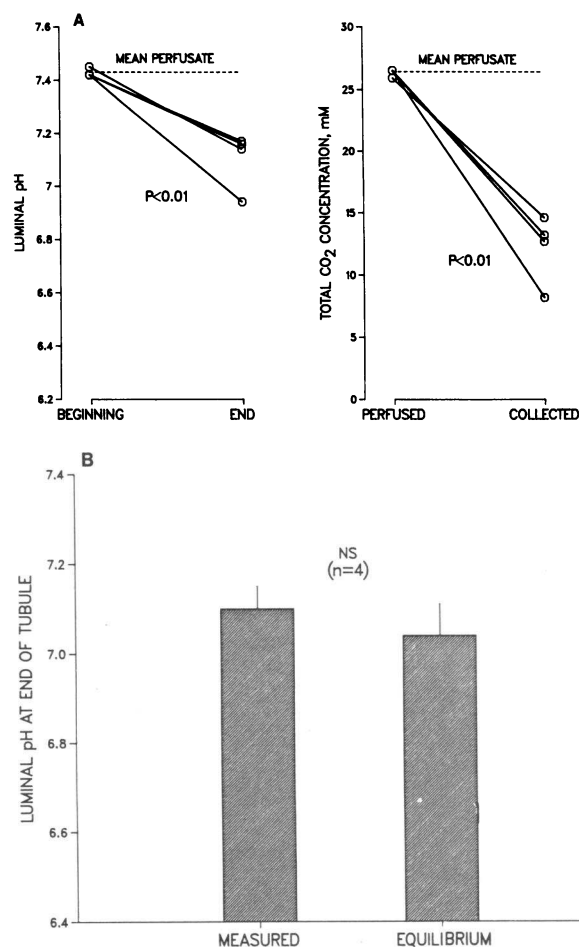


Figure 6. (A) Perfusate and end luminal pH with simultaneously measured perfusate and collected total CO₂ concentrations in S₂ proximal tubules. Mean collection rate was 2.56 ± 0.18 nl/min per mm ($n = 4$). (B) Measured end luminal pH and calculated end luminal equilibrium pH in S₂ proximal tubules.

lated perfused renal tubule preparation. In previous studies, luminal pH was monitored by placing a pH electrode into the collection pipette as the tubular fluid was collected under oil

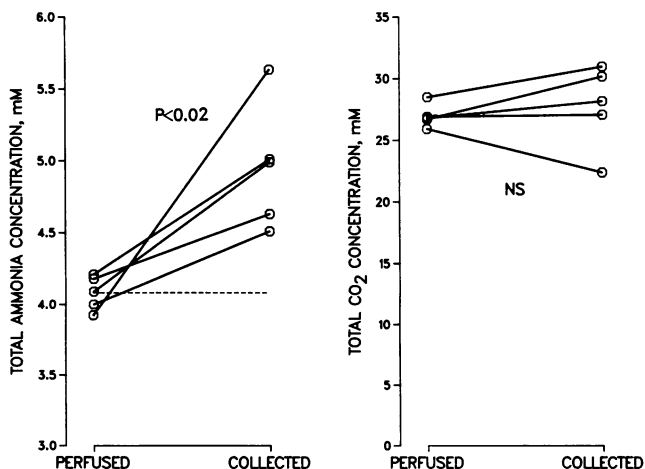


Figure 7. Total ammonia and total CO₂ concentrations in S₃ proximal tubules bathed and perfused with 4 mM NH₄Cl. Mean collection rate was 2.19 ± 0.19 nl/min per mm ($n = 5$).

Table II. Effect of Exogenous Luminal Carbonic Anhydrase on Collected Ammonia and Bicarbonate Concentrations in S₃ Proximal Straight Tubules

	Control	Carbonic anhydrase
Collection rate (nl · min ⁻¹ · mm ⁻¹)	2.12 ± 0.19	2.18 ± 0.38
Potential difference (lumen-bath) (mV)	-2.3 ± 0.2	-2.3 ± 0.3
Perfusate total CO ₂ concentration (mM)	27.0 ± 0.4	26.0 ± 0.4
Collected total CO ₂ concentration (mM)	27.8 ± 1.5	27.9 ± 0.5
Perfusate total ammonia concentration (mM)	4.08 ± 0.05	4.11 ± 0.05
Collected total ammonia concentration (mM)	4.95 ± 0.20	$4.06 \pm 0.07^*$

Values are mean \pm SEM. Control $n = 5$, carbonic anhydrase $n = 6$.

Mean tubule length 1.5 ± 0.2 mm in control group and 1.5 ± 0.1 mm in carbonic anhydrase group. Perfusate and bath were identical and contained 25 mM HCO₃⁻ and 4 mM total ammonia.

* $P < 0.01$ vs. control.

(26–28). Difficulties with the method included (a) loss of CO₂ into the oil, (b) measurement of the transepithelial potential difference at the collection end was made separately in time and needed to be subtracted from the voltage measured by the pH electrode, and (c) only equilibrium pH values could be measured since any disequilibrium pH present in the lumen of the tubule would be dissipated in the collection pipette.

These considerations prompted the development of a new technique for monitoring luminal pH in this study. By perfusing the tubules with the impermeant fluorescent pH probe, 1,4-DHPN, and monitoring the fluorescent emission spectra with a videomicrofluorometer, luminal pH could be measured while the luminal fluid was in contact with the tubule. This approach makes it possible, for the first time, to potentially measure both longitudinal and radial luminal pH gradients in the isolated perfused tubule and to measure end luminal pH without the difficulties encountered by the microelectrode technique.

Disequilibrium pH. Simultaneous determinations of end luminal pH and collected fluid total CO₂ concentrations revealed the presence of an acidic disequilibrium pH in the lumen of the S₃ segment of the proximal tubule. This observation indicates that functional luminal carbonic anhydrase is absent in this segment in contrast to the superficial proximal convoluted segment (17–19, 29) and the S₂ segment of the proximal tubule (present study). This conclusion was confirmed by the finding that the addition of carbonic anhydrase to the luminal fluid of the S₃ segment obliterated the disequilibrium pH.

When protons are secreted into the lumen of the proximal tubule and react with filtered bicarbonate, carbonic acid is formed. In the proximal convoluted tubule and the early proximal straight tubule, the carbonic acid formed is rapidly dehydrated to form dissolved CO₂ and water, a process catalyzed by endogenous luminal carbonic anhydrase. However, other segments of the nephron such as the distal convoluted tubule (18), the cortical collecting tubule (25), and the papillary collecting tubule (30) lack luminal carbonic anhydrase. In these segments,

in the absence of carbonic anhydrase, the rate of carbonic acid dehydration is low, allowing carbonic acid to accumulate even when protons are secreted at modest rates. Accumulation of carbonic acid without a concomitant elevation of the dissolved CO_2 concentration lowers the luminal pH below the equilibrium pH value predicted from the bicarbonate and dissolved CO_2 concentration, resulting in a so-called acid disequilibrium pH. The acidic disequilibrium pH in the S_3 segment of the proximal tubule was -0.46 indicating that the luminal carbonic acid concentration increased almost threefold (from 3.5 to $10.2 \mu\text{M}$).

Although the acidic disequilibrium pH was most likely due to proton secretion, other mechanisms should be considered. First, absorption of fluid by the tubule in the absence of functional membrane bound carbonic anhydrase could concentrate luminal carbonic acid to a greater extent than dissolved CO_2 given the high permeability of the proximal tubule to CO_2 (26, 31). Under the conditions of this study, however, the rate of fluid absorption in the S_3 segment was very low ($0.06 \text{ nl/min per mm}$) and could not have accounted for the large increase in the carbonic acid concentration observed. Second, it is possible for bicarbonate secretion to generate an acidic luminal disequilibrium pH by titrating nonbicarbonate buffers to generate carbonic acid at a rate greater than the uncatalyzed dehydration of carbonic acid to dissolved CO_2 and water. However, bicarbonate secretion has not been described in the proximal tubule and in addition the concentration of luminal nonbicarbonate buffers in the present study was low making this mechanism unlikely. Finally, although both fluid absorption and bicarbonate secretion can generate an acid disequilibrium pH, neither mechanism would account for the finding that the end luminal pH was more acidic than the perfusate pH. We therefore conclude that proton secretion in the absence of luminal carbonic anhydrase results in the generation of excess carbonic acid and a spontaneous acidic disequilibrium pH in the S_3 segment.

The disequilibrium pH was dissipated by luminal carbonic anhydrase (0.1 mg/ml). It has been suggested that carbonic anhydrase functions to increase the diffusion of CO_2 in unstirred layers adjacent to cell membranes (32, 33) and that in the absence of functional membrane bound carbonic anhydrase, luminal pH decreases as a result of an accumulation of CO_2 not carbonic acid (34–36). A previous study by Schwartz in the isolated rabbit proximal straight tubule noted in agreement with the above hypothesis, a decrease in apparent CO_2 permeability in the presence of luminal acetazolamide (26). However, this finding was not confirmed in the rat proximal convoluted tubule where acetazolamide failed to alter the apparent CO_2 permeability (31). The reason for the discrepancy is unclear. However, given the high permeability of the proximal tubule to CO_2 (26, 31) and the relatively slow perfusion rates used in this study, it is very unlikely that the end luminal PCO_2 was greater than the bathing solution PCO_2 under control conditions in the absence of luminal carbonic anhydrase. The most likely explanation for the elevation of luminal pH during perfusion with carbonic anhydrase is that the concentration of carbonic acid decreased secondary to an increase in its dehydration rate constant.

The precise transition site where the proximal straight tubule loses functional luminal carbonic anhydrase was not determined in the present study. The tubules were deliberately dissected from the most superficial and the most distal segments of the proximal straight tubule. The mean length of the S_3 segments was $1.35 \pm 0.01 \text{ mm}$ ($n = 20$) in length and therefore some tubules

extended into the medullary rays from the outer stripe of the outer medulla, which is $\sim 1 \text{ mm}$ in thickness. In a previous electron micrographic study of the rabbit proximal tubule, S_3 cells were found chiefly in the innermost regions of the cortex and the outer stripe of the outer medulla (37). A large luminal disequilibrium pH was consistently detected independent of which end of the tubule was attached to the collection pipette indicating that the part of the proximal straight tubule that lacks carbonic anhydrase is more extensive than just the outer medullary portion of the proximal straight tubule.

Previous histochemical studies have demonstrated membrane bound carbonic anhydrase in the S_2 segment in all species studied except the human where the enzyme appears to be weakly present or absent (2, 3, 38–40). In the S_3 segment of the mouse, human, and dog, histochemical studies have suggested that this segment completely lacks cytoplasmic and membrane bound enzyme (2, 38–40). In the rat S_3 segment, staining of the luminal membrane is either weakly present or absent with most staining being found in the basal infoldings and lateral cell membranes (3). In the rabbit S_3 segment, the brush border and cytoplasm stained positively with less intense staining of the basal and lateral membranes (2). Considering the results of the present study, the histochemical staining of the apical membrane of the rabbit evidently does not correlate with the presence of enzyme activity in the luminal compartment.

Ammonium transport. In additional studies of the S_3 segment involving the simultaneous measurement of bicarbonate and ammonia transport, we found a significant increase in the collected ammonia concentration relative to the perfusate despite the lack of a significant change in the collected bicarbonate concentration. In these studies, there was no significant decrease in the collected TCO_2 concentration relative to the perfusate in the presence or absence of exogenous luminal carbonic anhydrase. Whether the collected luminal TCO_2 concentration differs from the perfusate depends on two competing factors (a) proton secretion which decreases the collected TCO_2 concentration, and (b) fluid absorption, which increases the collected TCO_2 concentration. In the S_3 segment, these factors presumably balanced so that no significant change in the collected TCO_2 concentration was detected. The low rate of fluid absorption indicated that the rise in the collected ammonia concentration was due to ammonia secretion, not water absorption. The subsequent finding that ammonia secretion was diminished in the presence of exogenous luminal carbonic anhydrase indicated that the secretion of ammonia was dependent on the presence of spontaneous luminal disequilibrium pH.

Under symmetrical conditions (perfusate same as bath), the S_3 segment secreted ammonia. Addition of carbonic anhydrase to the luminal perfusate inhibited the ammonia secretion. Theoretically, ammonia could be secreted either as NH_3 or as NH_4^+ (24, 41, 42). Since the driving force for direct NH_4^+ secretion should not be affected by changes in luminal pH, the inhibition of ammonia secretion in the presence of luminal carbonic anhydrase supports the hypothesis that ammonia secretion by the S_3 segment occurs as a result of the diffusional entry of NH_3 into the lumen as has been demonstrated in the proximal convoluted tubule (43) and the S_2 segment of the proximal straight tubule (44). An acid disequilibrium pH will lower the luminal NH_3 concentration by shifting the $\text{NH}_3\text{--NH}_4^+$ buffer reaction towards NH_4^+ , creating a transepithelial NH_3 concentration difference, which will drive the diffusional entry of NH_3 into the lumen.

Ammonia is produced predominantly in proximal tubules and is transferred to the collecting duct by a sequence of events involving absorption of ammonia from the loops of Henle, accumulation of total ammonia in the medullary interstitium, and transepithelial secretion into the collecting ducts (41). A recent study by Good et al. (45) demonstrates that the NH_3 gradient that drives secretion of NH_3 into the collecting ducts is largely due to a high concentration of NH_3 in the medullary interstitium. The mechanism by which total ammonia accumulates in the renal medulla is not entirely understood. Based on the finding that isolated perfused thick ascending limbs from rats reabsorb NH_4^+ against an NH_4^+ concentration gradient, Good et al. (41, 45, 46) have recently proposed that this transport process powers a countercurrent multiplier for ammonia, which could account for the accumulation of ammonia in the medulla. In simplest terms, this multiplier, like that proposed by Kuhn and Ramel for NaCl (47) involves recycling of ammonia between the two limbs of the loop of Henle. That is, the total ammonia absorbed by the thick ascending limb must enter the descending limb for multiplication to occur. The portion of the descending limb adjacent to the thick ascending limbs in the outer stripe of the outer medulla and the medullary rays is the proximal straight tubule (thick descending limb [48]). Transepithelial ammonia secretion in the proximal straight tubule as demonstrated in this study (Fig. 7, Table II) and by Garvin et al. (44), should contribute to the countercurrent multiplication of ammonia and therefore to ammonia accumulation in the renal medulla.

The acid disequilibrium pH may enhance countercurrent multiplication of ammonia in vivo by increasing ammonia secretion in S_3 proximal straight tubules. Whether there actually is a luminal disequilibrium pH in vivo depends on whether proton secretion in the S_3 segment can lower the luminal pH below the pH of the tubule fluid entering it from the S_2 segment. The S_2 segment was observed to lower the luminal bicarbonate concentration to an average limiting value of $\sim 7\text{--}9$ mM corresponding to a limiting pH of $\sim 6.7\text{--}6.9$ (49). Evidence from micropuncture studies indicate that a similar limiting pH is already reached at the end of the rat superficial proximal convoluted tubule (50). It is thus likely that the pH of the tubular fluid exiting the S_2 segment of the proximal straight tubule normally is at the limiting value in vivo. Therefore, for the luminal pH to fall still lower in the S_3 segment requires that the limiting luminal pH be lower in the S_3 segment than the S_2 segment. Whether the S_3 segment lowers its luminal pH below the limiting luminal pH in the S_2 segment has not been measured. The minimal luminal pH generated by the S_3 segment depends on several factors including the mechanism of proton secretion (Na^+/H^+ exchange vs. H^+ pump), the tubule proton and bicarbonate permeability, and cell pH. In addition, diffusional entry of NH_3 tends to decrease the luminal acidic disequilibrium pH by reacting with secreted protons.

In summary, under the conditions of this study, the S_3 segment of the rabbit proximal tubule generated a spontaneous acidic disequilibrium pH of -0.46 , which was obliterated by perfusing the lumen with carbonic anhydrase. This result indicates that the S_3 segment lacks luminal membrane bound carbonic anhydrase and that the luminal fluid is acidified by proton secretion. The acidic luminal pH enhances the diffusional entry of NH_3 into the lumen. The bicarbonate absorption rate was low and was not enhanced in the presence of exogenous luminal carbonic anhydrase. In contrast, the superficial S_2 segment of

the rabbit proximal tubule did not generate a luminal disequilibrium pH despite marked bicarbonate absorption, indicating that the tubular fluid is in contact with endogenous membrane-bound carbonic anhydrase.

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