Increased Na/H Antiporter and $Na/3HCO_3$ Symporter Activities in Chronic Hyperfiltration

A Model of Cell Hypertrophy

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ABSTRACT The effect of chronic hyperfiltration, a model of cell hypertrophy, on H/HCO_3 transporters was examined in the in vivo microperfused rat proximal tubule. Hyperfiltration was induced by uninephrectomy with subsequent increased dietary protein. After 2 wk the hyperfiltration group had a higher glomerular filtration rate (2.21 ± 0.13 vs. 1.48 ± 0.12 ml/min), associated with increased kidney weight (1.71 ± 0.05 vs. 1.23 ± 0.04 g). HCO_3 absorptive rate measured in tubules perfused with an ultrafiltrate-like solution (25 mM HCO_3) was higher in the hyperfiltration group (183 ± 17 vs. 109 ± 16 pmol/mm per min). The activities of the apical membrane Na/H antiporter and basolateral membrane $Na/3HCO_3$ symporter were assayed using the measurement of cell pH [(2′7′)-bis(carboxyethyl)-(5,6)-carboxyfluorescein] in the doubly microperfused tubule in the absence of contact with native fluids. After 2 wk of hyperfiltration Na/H antiporter activity, assayed as the effect of luminal Na removal on cell pH, was increased 114%. Basolateral membrane $Na/3HCO_3$ symporter activity, assayed as the effect of a decrease in peritubular [HCO_3] (25 to 5 mM) or in peritubular [Na] (147 to 25 mM) in the absence of luminal and peritubular chloride, was increased 77 and 113%, respectively, in the hyperfiltration group. Steady-state cell pH, measured with physiologic, ultrafiltrate-like luminal and peritubular perfusates, was significantly higher in the hyperfiltration group (7.27 ± 0.02 vs. 7.14 ± 0.03). In similar studies, performed 24 h after uninephrectomy and protein feeding, kidney weight was increased 10%, Na/H antiporter activity 39%, and $Na/3HCO_3$ symporter activity 46%. At this time cell pH was not different between the two groups. The results demonstrate that chronic hyperfiltration is associated with parallel increases in Na/H antiporter and $Na/3HCO_3$ symporter activities. If a decrease in cell pH is the signal that triggers these adaptations, it occurs early, and the adaptations can be maintained in the absence of sustained cell acidification.

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J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/91/02/0195/23 $2.00
Volume 97 February 1991 195–217

INTRODUCTION

Cell growth may be associated with DNA synthesis and cell division (hyperplasia), or may occur in the absence of these processes (hypertrophy). Hyperplasia has been demonstrated to be associated with activation of the Na/H antiporter, although the role of antiporter activation remains unclear (Grinstein and Rothstein, 1986; Moolenar, 1986). Recently, Ganz et al. (1989) found that mitogenic stimulation of mesangial cells increased the activities of Na-dependent and -independent Cl/HCO_3 exchangers, in addition to the Na/H antiporter. Thus, many H/HCO_3 transporters are activated in hyperplasia. The cell mechanisms mediating hypertrophy are not understood, but appear to be different than those responsible for hyperplasia (Beer et al., 1987; Norman et al., 1988).

Chronic hyperfiltration (chronically elevated single nephron glomerular filtration rate) is associated with proximal tubule cell hypertrophy, with little cell division (Johnson and Roman, 1966; Hayslett et al., 1968; Fine et al., 1978; Hayslett, 1979; Trizna et al., 1981; Tabei et al., 1983; Brenner, 1985; Fine, 1986; Johnston et al., 1987). Chronic hyperfiltration is also associated with increased apical membrane Na/H antiporter activity (Cohn et al., 1982; Harris et al., 1984, 1986; Nord et al., 1985). While it is possible that Na/H antiporter stimulation is merely related to the hypertrophic growth response, the increased activity may also be a component of an increase in the capacity of the proximal tubule to secrete protons in this condition. Because glomerular hyperfiltration leads to increased filtered loads of bicarbonate, maintenance of normal acid–base balance requires increased proximal tubule proton secretion and bicarbonate reabsorption.

Transepithelial proton secretion in the proximal tubule is mediated by an apical membrane Na/H antiporter (65%), an apical membrane H translocating ATPase (35%), and a basolateral membrane $Na/3HCO_3$ symporter (100% of basolateral transport) (Alpern, 1990). In a number of chronic conditions, increased rates of proximal tubule proton secretion are attributable to parallel increases in apical and basolateral membrane transporter activities. Proximal tubule apical membrane Na/H antiporter and basolateral membrane $Na/3HCO_3$ symporter activities are increased in chronic metabolic acidosis (Cohn et al., 1983; Kinsella et al., 1984; Tsai et al., 1984; Harris et al., 1986; Jacobsen et al., 1986; Akiba et al., 1987; Preisig and Alpern, 1988) and chronic potassium deficiency (Seifter and Harris, 1984; Soleimani et al., 1990), conditions associated with increased rates of bicarbonate absorption in the proximal tubule (Chan et al., 1982; Kunau et al., 1985). These increased transporter activities represent intrinsic adaptations in the transport proteins in that they are present in the absence of contact with the inducing environment. In that proximal tubule cell pH (pH_i) is decreased in both of these conditions (Adam et al., 1986), it may be that the decreased pH_i is the signal that initiates and maintains the adaptations.

In these studies we examined the effect of chronic hyperfiltration on proximal tubule acidification. The results demonstrate that chronic hyperfiltration is associated with marked hypertrophy of the proximal tubule and an increased capacity for transepithelial proton secretion. Na/H antiporter and $Na/3HCO_3$ symporter activities are increased in parallel at 24 h and 12–18 d of hyperfiltration. This parallel

stimulation of the two transporters is similar to that observed in chronic metabolic acidosis and chronic potassium deficiency. However, unlike chronic metabolic acidosis and chronic potassium deficiency, a decreased proximal tubule pH_i could not be demonstrated in chronic hyperfiltration. It is not presently clear whether this generalized increase in H/HCO_3 transporter activities is common to all hypertrophic responses, or is unique to cells whose major function is transepithelial bicarbonate transport.

METHODS

Animal Groups

Experiments were performed using male Sprague-Dawley rats (Sasco Breeding Laboratories, Omaha, NE) weighing 210–325 g. Control rats were sham-nephrectomized and fed a 24% protein diet, and hyperfiltering rats were uninephrectomized and fed a 40% protein diet. Both diets were made by NBCo Biochemicals, a division of ICN Biomedicals, Inc. (Cleveland, OH). This protocol was designed based on the results of Harris et al. (1984), who demonstrated that when the effects of uninephrectomy and varying dietary protein on Na/H antiporter activity were assessed, Na/H antiporter activity could be expressed as the same linear function of glomerular filtration rate (GFR), irrespective of how GFR was altered.[1]

Animals were housed in individual cages so they could be individually fed 20 g of their respective diets daily. All animals were allowed water ad lib. Animals in both groups ingested similar amounts of diet. Studies were performed at 24 h and 2 wk (12–18 d). At 24 h control rats lost 13 ± 2 g (n = 11) and hyperfiltering rats lost 13 ± 2 g (n = 9) (NS). At 2 wk control rats gained 61 ± 3 g (n = 33) and hyperfiltering rats gained 60 ± 3 g (n = 33) (NS).

Measurement of Single Kidney GFR

Rats were anesthetized with an intraperitoneal injection of Inactin (100 mg/kg body weight with additional doses as needed) and placed on a heated table that maintained body temperature at 36.5–37.5°C. The right femoral artery was catheterized for monitoring blood pressure and obtaining blood samples, the left jugular vein was catheterized for continuous infusion, and the left ureter was cannulated with polyethylene tubing (PE10) through a midline abdominal incision. After placement of these lines animals were given a 0.5-cm^3 bolus of Ringers' bicarbonate containing exhaustively dialyzed [^{3}H]methoxy-inulin (70 μCi/ml) (New England Nuclear, Boston, MA), followed by a constant infusion of the same solution at 1.5 cm^3/h. Ringers' bicarbonate contained (in mM): 105 NaCl, 25 $NaHCO_3$, 4 Na_2HPO_4, 5 KCl, 1 $MgSO_4$, and 1.8 $CaCl_2$. Timed urine collections were made under oil in preweighed vials and the collected volume was measured. Blood samples were collected between urine collections.

In Vivo Microperfusion

Rats were prepared as previously described (Preisig and Alpern, 1988) and were continuously infused at 1.5 cm^3/h with Ringers' bicarbonate (see above). The left kidney was exposed by a flank incision and immobilized in a Lucite cup. The ureter was cannulated (PE50) to ensure free drainage of urine. Proximal tubule transit time was measured with an intravenous injection

[1] The results of Harris et al. (1984) described above suggest that the relevant factor that altered Na/H antiporter activity was GFR rather than dietary protein or uninephrectomy per se. Nevertheless, it is possible that the effects of these maneuvers on antiporter activity are independent of the consequent effect on GFR.

of 0.02 ml of 10% lissamine green dye, and only those kidneys with transit times of < 12 s were accepted for study.

Transepithelial volume and bicarbonate transport studies. Surface loops of proximal convoluted tubules were microperfused with a thermally insulated microperfusion pump (Wolfgang Hampel, Berlin, Germany) as previously described (Preisig et al., 1987). The perfusion pipette, which was placed in a proximal loop, contained 68 μCi/ml of exhaustively dialyzed [^{3}H]methoxy-inulin. An oil block was placed proximal to the perfusion pipette, and a hole was left proximal to the block for glomerular ultrafiltrate to escape. A collection pipette was placed in a late proximal loop, an oil block was inserted distally, and a timed collection was made. Collected samples were stored under HEPES-equilibrated paraffin oil (Preisig et al., 1987). After the collection the perfused segment was filled with microfil (Canton Bio-Medical Products, Boulder, CO). On a subsequent day the kidney was incubated in 6 N HCl for 60 min, allowing dissection of the microfil casts and measurement of the length of the perfused segment.

Basolateral membrane studies. Capillary and luminal pipettes were placed using a Leitz dissecting microscope (Leitz Wetzler, Rockleigh, NJ). Peritubular capillaries were perfused as previously described (Alpern, 1985) with a 12–14-μm tip pipette designed to allow rapid changes between two perfusion fluids. After placement of the capillary pipette the lumen of a proximal convoluted tubule was perfused at 40 nl/min using a thermally insulated microperfusion pump with a 6–8-μm tip pipette as previously described (Alpern, 1985). This pipette (loading pipette) contained the acetoxymethyl derivative of (2′7′)-bis(carboxyethyl)-(5,6)-carboxyfluorescein (BCECF-AM; 50–100 μM; Molecular Probes, Junction City, OR). The compound BCECF-AM is nonfluorescent, and thus its presence in the luminal fluid does not influence the measurement of cell pH. After the cells had been loaded for 5–7 min the dissecting microscope was moved out of position and a Leitz epifluorescence microscope (MPV compact system; Leitz Wetzler) was moved into position.

Apical membrane studies. The peritubular capillary and loading pipettes were as described above. After loading the cells with BCECF for 6 min, the loading pipette was removed. A second pipette (6–8-μm tip) was placed in the lumen, and the lumen was perfused in a retrograde fashion as previously described (Alpern and Chambers, 1986). The luminal perfusion setup also allows for rapid changes between two solutions. After all pipettes were placed, the epifluorescent microscope was moved into position.

In our system, the half-time of a fluid change is ~200 ms. In these studies the initial rate of change in pH_i was linear for 2–3 s. Tubules were perfused for 2–4 h after completion of surgery. There was no apparent time-dependent change in measured values over the course of the experiment.

The perfusates are listed in Table I. All solutions were bubbled with 7% CO_2/93% O_2 at 37°C. The [calcium gluconate] in solutions 5–8 were chosen to give ionized [Ca^{2+}] = 1.2–1.5 mM (Alpern, 1987). 4-Acetamido-4′-isothiocyanostilbene-2,2′ disulfonic acid (SITS) and all solution chemicals were purchased from Sigma Chemical Co., St. Louis, MO.

Analysis and Calculations

GFR. Aliquots of plasma and urine were placed into scintillation fluid for liquid scintillation counting (model LS3801; Beckman Instruments, Inc., Fulton, CA). Single kidney GFR (milliliters per minute) was calculated from the equation

$$\text{GFR} = (U_{in} \cdot V)/P_{in} \quad (1)$$

where U_{in} and P_{in} are the urine and plasma counts of tritium, respectively, and V is the urine flow rate (milliliters per minute). In each animal three to six measurements of GFR were obtained from the timed urine collections and averaged to obtain a single value for the animal.

Proximal tubular volume and bicarbonate absorption. In studies measuring the rates of transepithelial volume and bicarbonate absorption, the total collected volume was determined using calibrated constant-bore pipettes. From this volume an aliquot was removed for measurement of total CO_2 (tCO_2). The remaining volume was placed into the scintillation cocktail for liquid scintillation counting.

The perfusion rate (V_p, nanoliters per minute) was calculated from the equation

$$V_p = (I_c/I_p)(V_c) \quad (2)$$

where I_c and I_p are the collected and perfused inulin counts, respectively, and V_c is the collection rate (nanoliters per minute).

TABLE I

Perfusion Solutions

	1	2	3	4	5	6	7*	8*
NaCl	120	140	127	—	—	—	—	—
$NaHCO_3$	25	5	25	—	25	25	—	—
KCl	5	5	3	3	—	—	—	—
$MgSO_4$	1	1	1	1	1	1	1	1
Na_2HPO_4	1	1	—	—	—	—	—	—
K_2HPO_4	—	—	1	1	1	1	—	—
$CaCl_2$	1.8	1.8	1.8	1.8	—	—	—	—
Glucose	5	5	—	—	5	5	—	—
Alanine	5	5	—	—	5	5	—	—
Urea	5	5	5	5	5	5	—	—
Choline Cl	—	—	—	127	—	—	—	—
Choline HCO_3	—	—	—	25	—	—	25	10
NH_4HCO_3	—	—	—	—	—	—	—	15
NMDG‡ gluconate	—	—	—	—	—	122	—	—
Na gluconate	—	—	—	—	122	—	—	—
K gluconate	—	—	—	—	3	3	5	5
Ca gluconate	—	—	—	—	9.3	9.3	3.6	3.6

All units are millimolar; all solutions were bubbled with 7% CO_2/93% O_2 at 37°C.
*Solutions titrated to pH 7.32 with *N*-methyl-D-glucamine hydroxide and brought to final osmolality of 295–300 mosmol with sucrose.
‡*N*-Methyl-D-glucamine.

Volume flux per tubule length (J_v, nanoliters per millimeter per minute) was calculated as

$$J_v = (V_p - V_c)/L \quad (3)$$

where L is the perfused length (millimeters).

The [tCO_2] in the perfusated and collected fluids was measured using microcalorimetry. The rate of bicarbonate absorption (J_{HCO_3}, picomoles per millimeter per minute) was calculated as

$$J_{HCO_3} = [(C_pV_p) - (C_cV_c)]/L \quad (4)$$

where C_p and C_c are the HCO_3 concentrations (measured as total CO_2) of the perfused and collected fluids, respectively.

Measurement of pH_i. As previously described (Alpern, 1985), fluorescence was measured alternately at 500- and 450-nm excitation (emission, 530 nm) using an epifluorescence microscope with interference filters (Corion Corp., Holliston, MA). All results were corrected

for background. The fluorescence excitation ratio (F_{500}/F_{450}) was calculated as the mean of two 500-nm excitation measurements divided by the 450-nm excitation measurement obtained between them. The results of our intracellular dye calibration (see Results) were used to convert fluorescence excitation ratios to an apparent pH_i.

The initial rate of change in pH_i was calculated as previously described (Alpern, 1985). The initial rate of change of 500-nm fluorescence [$d(F_{500})/dt$] during a fluid change was divided by the 450-nm excitation fluorescence (F_{450}) corrected for background at the time of the fluid change (interpolated from the measurements before and after the fluid change). We have previously demonstrated that fluorescence at 450-nm excitation is not measurably affected by pH_i and thus can be considered constant (Alpern, 1985). This rate of change in the fluorescence ratio was converted to a rate of change in pH_i (dpH_i/dt, pH units/minute) by dividing by the slope of the in vivo calibration of BCECF [$d(F_{500}/F_{450})/dpH_i$].

Steady-state changes in pH_i were calculated as the mean of the initial and recovery steady-state pH_i's minus the steady-state pH_i after the experimental maneuver.

Calibration of the pH-sensitive dye. Intracellular BCECF was calibrated in vivo in tubules from both control and hyperfiltering rats using the nigericin technique as previously described (Alpern, 1985). Tubules were perfused with well-buffered solutions containing 25 mM HEPES, 60 mM K_2HPO_4, 10 μg/ml nigericin, and appropriate amounts of $NaHCO_3$ to give pH's ranging from 6.6 to 7.6. Before adding the $NaHCO_3$, the HEPES and K_2HPO_4 solutions were pH titrated to pH's 6.6, 7.0, 7.4, and 7.6 at 37°C with NaOH. All solutions were brought to a final osmolality of 295–300 mosmol with raffinose. Cells were loaded with BCECF before exposure to nigericin, and then perfused with one of the above solutions in the capillary. Over ~5 min the fluorescence excitation ratio approached a stable value that was used as the result.

Intracellular buffer capacity. The intracellular buffer capacity was determined in tubules from both control and hyperfiltering rats using the technique of rapid NH_3/NH_4^+ addition as previously described (Roos and Boron, 1981; Preisig and Alpern, 1988). The NH_3 rapidly enters the cell and combines with intracellular protons to form NH_4^+, leading to a rapid cell alkalinization. After fluorescence with 500-nm excitation a rapid initial increase in fluorescence intensity (cell alkalinization) occurred, followed by a slow decrease in fluorescence intensity (cell pH defense and NH_4^+ diffusion). The initial deflection with 500-nm excitation was divided by the 450-nm value (interpolated from neighboring 450-nm measurements) to calculate the magnitude of change in the F_{500}/F_{450} ratio. In some tubules the decrease in fluorescence intensity began near the peak of the initial deflection, leading to an underestimate of the magnitude of the initial deflection. This was dealt with by extrapolating the 500-nm fluorescence curve back to the time of the fluid change, as previously described by Roos and Boron (1981). The buffer capacity (β, millimolar/pH unit) is given by the formula (Roos and Boron, 1981)

$$\beta = \frac{[NH_4^+]_i}{\Delta pH_i} \tag{5}$$

where $[NH_4^+]_i$ is the intracellular $[NH_4^+]$ after addition of NH_3/NH_4^+. The initial pH_i was calculated from the fluorescence excitation ratio just before the addition of NH_3/NH_4^+, and the final pH_i was calculated from the fluorescence ratio at the peak of the spike. The difference between these two values was used as ΔpH_i. The intracellular concentration of NH_4^+ was calculated as

$$[NH_4^+]_i = [NH_4^+]_o \cdot 10^{(pH_o - pH_i)} \tag{6}$$

where $[NH_4^+]_o$ is the $[NH_4^+]$ of the perfusate and pH_o is the pH of the perfusate. pH_i was calculated from the fluorescence excitation ratio just after addition of NH_3/NH_4^+ (at the peak of the spike).

Cell height and volume. The measurement of cell height was done on slices of the left kidney that had been harvested without attempt to maintain the lumen patent, fixed in formalin, and stained with periodic acid schiff (PAS). All measurements were by R. J. Alpern, without knowledge of the animal group. In each kidney measurements of the inner and outer tubule diameters were taken of five surface proximal tubules cut transversely. Cell height was calculated as the difference between the inner and outer diameters divided by 2, expressed in micrometers. Epithelial volume per millimeter tubule length was calculated by treating the tubule as two concentric cylinders.

H-equivalent fluxes. The H-equivalent fluxes in response to changes in either luminal or capillary perfusate composition (J_H^{eq}, picomoles per millimeter per minute) were calculated from the equation

$$J_H^{eq} = dpH_i/dt \cdot \beta \cdot V/mm \quad (7)$$

where dpH_i/dt is the initial rate of change in pH_i (pH units/minute), β is the buffer capacity (mM/pH unit), and V/mm is the cell volume per millimeter tubule length (liters per millimeter).[2]

Statistics

The slopes and intercepts of the calibration curves were determined by linear regression analysis. All results are expressed as mean ± SE. Statistical significance was assessed by the Student's t test for unpaired data.

RESULTS

The purpose of these studies was to use the condition of chronic glomerular hyperfiltration as a model of cell hypertrophy to examine the effects on proximal tubule bicarbonate absorption, and on the intrinsic properties of the transporters responsible for transcellular bicarbonate absorption, the apical membrane Na/H antiporter, and basolateral membrane $Na/3HCO_3$ symporter. Rats with chronic hyperfiltration, induced by uninephrectomy and 40% protein feeding, were compared with sham-operated rats maintained on a normal (24%) protein diet.

2-wk Hyperfiltration

Arterial blood gases, plasma values, kidney weights, and transport rates are shown in Table II. There were no differences in arterial blood gases or plasma [Na], [K], or [Cl], confirming that this protocol of chronic hyperfiltration was not associated with a systemic acid–base disorder or potassium deficiency, conditions previously shown to induce stimulatory adaptations in the apical membrane Na/H antiporter (Cohn et al., 1983; Kinsella et al., 1984; Seifter and Harris, 1984; Tsai et al., 1984; Harris et al., 1986; Jacobsen et al., 1986; Akiba et al., 1987; Talor et al., 1987; Preisig and Alpern, 1988; Krapf, 1989; Ruiz et al., 1989; Soleimani et al., 1990), basolateral membrane $Na/3HCO_3$ symporter (Akiba et al., 1987; Preisig and Alpern, 1988; Krapf, 1989;

[2] To calculate V/mm (cell volume per millimeter tubular length), the epithelial volume rather than cell water was used, and therefore cell volume has been overestimated to the extent that extracellular and dry weight volumes contribute to epithelial volume. However, if we assume that the cell water increase in hypertrophy is proportional to the increase in epithelial volume, the results will be the same, although the absolute values may be slightly overestimated.

Ruiz et al., 1989; Soleimani et al., 1990), and rates of bicarbonate absorption (Chan et al., 1982; Cogan, 1984; Kunau et al., 1985). Serum creatinine levels were higher in the hyperfiltration group, reflecting the loss of 50% of kidney mass, and left kidney weight was increased in the hyperfiltration group, reflecting the hypertrophy that occurs in response to chronic hyperfiltration (Johnson and Roman, 1966; Hayslett et al., 1968; Fine et al., 1978; Hayslett, 1979; Trizna et al., 1981; Tabei et al., 1983; Brenner, 1985; Fine, 1986; Johnston et al., 1987).

GFR. Left kidney GFR was measured in animals from both groups. As shown in Table II, in the hyperfiltration group single kidney GFR was 2.21 ± 0.13 ml/min

TABLE II

2-wk Control and Hyperfiltering Groups: Plasma Values, Arterial Blood Gases, Kidney Weight, GFR, and Transport Rates

	Control group	Hyperfiltration group
Arterial blood gases		
pH	7.38 ± 0.01 (n = 32)	7.37 ± 0.01 (n = 33)
pCO_2, *mmHg*	44.2 ± 0.8 (n = 32)	44.8 ± 0.6 (n = 33)
$[HCO_3]$, *mM*	26.4 ± 0.3 (n = 32)	25.9 ± 0.3 (n = 33)
Plasma values*		
Creatinine, *mg%*	0.47 ± 0.03 (n = 9)	0.62 ± 0.04‡ (n = 10)
[Na], *meq/liter*	144 ± 1 (n = 9)	144 ± 1 (n = 10)
[K], *meq/liter*	3.3 ± 0.1 (n = 9)	3.2 ± 0.1 (n = 10)
[Cl], *meq/liter*	109 ± 1 (n = 9)	108 ± 1 (n = 10)
Hct, %	44.7 ± 0.5 (n = 26)	44.1 ± 0.4 (n = 27)
Plasma protein, *g%*	5.8 ± 0.1 (n = 27)	5.6 ± 0.1 (n = 27)
Kidney weight, *g*	1.23 ± 0.04 (n = 33)	1.71 ± 0.05§ (n = 33)
Single kidney GFR, *ml/min*	1.48 ± 0.12 (n = 4)	2.21 ± 0.13‖ (n = 4)
Transport rates		
Volume absorption, *nl/mm · min*	2.47 ± 0.26 (n = 9)	4.13 ± 0.62‡ (n = 5)
Bicarbonate absorption, *pmol/mm · min*	109 ± 16 (n = 9)	183 ± 17‡ (n = 5)

*Plasma Na, K, and Cl concentrations are expressed as milliequivalents per liter plasma water, and plasma creatinine concentration is expressed as milligrams per deciliter plasma.

‡$P < 0.02$ vs. control.

§$P < 0.00001$ vs. control.

‖$P < 0.01$ vs. control.

(n = 4), which was significantly higher than in the control group (1.48 ± 0.12 ml/min [n = 4], $P < 0.01$), demonstrating that the surgical and dietary protocol induced chronic hyperfiltration. This 49% stimulation of GFR is similar in magnitude to that observed by Harris et al. (1984) of 46% for the same surgical and dietary protocols.

Rates of volume and bicarbonate absorption. In vivo microperfusion was used to compare the rates of volume and bicarbonate absorption in the two groups. Tubule lumens were perfused with an ultrafiltrate-like solution (solution 1, Table I) containing 25 mM HCO_3 at 15 nl/min. The rates of volume and bicarbonate absorption are presented in Table II. In the hyperfiltration group J_v and J_{HCO_3} were 4.13 ± 0.62

nl/mm · min and 183 ± 17 pmol/mm · min, respectively (n = 5), which were significantly higher than observed in the control group (2.47 ± 0.26 nl/mm · min and 109 ± 16 pmol/mm · min, respectively; $P < 0.02$ in both cases) (n = 9). To determine if the increased rate of bicarbonate absorption was associated with intrinsic adaptations in the transport mechanisms responsible for transcellular bicarbonate absorption, we next assayed the activities of the apical membrane Na/H antiporter and basolateral membrane Na/3HCO_3 symporter in the two groups.

Calibration of the pH-sensitive dye. To ensure that the surgical and dietary regimen did not affect the pH sensitivity of BCECF, the dye was calibrated in vivo in tubules from the two groups of animals. The data are shown in Fig. 1. Chronic hyperfiltration had no effect on the in vivo calibration of BCECF, as neither the slopes nor intercepts were significantly different (control: slope = 1.52 ± 0.09 and intercept = −8.92 ± 0.62; hyperfiltration: slope = 1.39 ± 0.10 and intercept = −8.05 ± 0.70). Therefore, the data were combined to define the calibration curve used in these studies (slope = 1.46 ± 0.07 and intercept = −8.52 ± 0.47). For this calibration curve the standard error for cell pH's calculated from single fluorescent ratio measurements is 0.11 pH units over the pH_i range of 6.6–7.6.

Apical membrane Na/H antiporter activity. In these studies antiporter activity was assayed by rapidly removing and re-adding Na to the luminal perfusate; choline was the replacement cation (solution 3 vs. 4, Table I). Peritubular capillaries were perfused continuously with a solution containing 5 mM HCO_3 (solution 2, Table I) with 1 mM SITS added.[3] We have previously shown with this protocol that the effect of changes in luminal [Na] on pH_i is due to changes in the activity of the amiloride-sensitive Na/H antiporter (Alpern and Chambers, 1986). Addition of SITS to the peritubular perfusate allows changes in the activity of the apical membrane Na/H antiporter to have the greatest effect on pH_i, and acidification of the peritubular perfusate prevents a SITS-induced cell alkalinization (Alpern and Chambers, 1986).

The absolute values of the mean initial rates of change in pH_i (dpH_i/dt) for all tubules are summarized in Fig. 2. In tubules from control rats, when luminal [Na] was changed from 152 to 0 mM (solution 3 vs. 4, Table I), dpH_i/dt was 3.12 ± 0.47 pH units/min (n = 8), and steady-state pH_i decreased from 7.27 ± 0.07 to 6.94 ± 0.06. Upon readdition of 152 mM Na to the luminal perfusate, pH_i increased at an initial

[3] We have previously shown (Alpern and Chambers, 1986) that when the basolateral membrane bicarbonate transporters were operational, the pH_i response to luminal Na removal was biphasic, consisting of an initial cell acidification attributable to the apical membrane Na/H antiporter and a late cell alkalinization due to the effects of lowering cell [Na] on the basolateral membrane Na/3HCO_3 symporter. Because the basolateral membrane bicarbonate transporters dominate control of pH_i, the early cell acidification attributable to the Na/H antiporter was small (0.04 pH units), extremely transient, and inconsistent. Inhibition of the basolateral membrane Na/3HCO_3 symporter with peritubular SITS resulted in luminal Na removal causing a greater cell acidification (0.25 pH units), which was not followed by a late alkalinization, was reproducible, and was inhibited by luminal amiloride. Therefore, in these studies the activity of the apical membrane Na/H antiporter is assayed in the presence of peritubular SITS. However, because SITS inhibits basolateral membrane base efflux it causes a cell alkalinization, which would secondarily inhibit the Na/H antiporter (Aronson et al., 1982) and possibly make it difficult to assay. To avoid this cell alkalinization, the capillary perfusate is acidified.

rate of 3.00 ± 0.35 pH units/min to a pH_i of 7.33 ± 0.07 (n = 8). When the same luminal perfusate [Na] changes were made in tubules from the hyperfiltration group, dpH_i/dt was faster (4.73 ± 0.41 [n = 8] and 4.69 ± 0.47 pH units/min [n = 8], $P < 0.03$ and $P < 0.02$, respectively), demonstrating a stimulatory adaptation in the apical membrane Na/H antiporter. With these fluid changes in the hyperfiltration group, pH_i decreased from 7.27 ± 0.04 to 6.90 ± 0.03 and returned to 7.33 ± 0.04.

In response to the luminal fluid [Na] changes in the above study, steady-state pH_i decreased by 0.32 ± 0.04 pH units in tubules from control animals and by 0.43 ± 0.04 pH units in tubules from the hyperfiltration group. Although these values were not significantly different, the larger change in the tubules from the hyperfiltration group is supportive of the observed differences in dpH_i/dt described above.

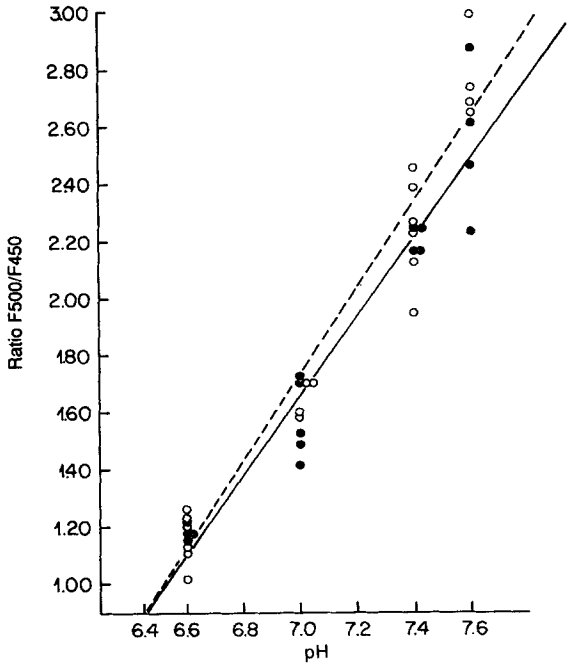

FIGURE 1. In vivo calibration of BCECF in control and hyperfiltering rats. F_{500}/F_{450} ratio is plotted as a function of pH. Open circles and dashed line are results from control animals; closed circles and solid line are results from hyperfiltering rats. The calibration was done using the nigericin technique (see Methods). The slopes are 1.52 and 1.39 (NS) and the y intercepts are −8.92 and −8.05 (NS) in tubules in control and hyperfiltering animals, respectively.

Basolateral membrane Na/3HCO$_3$ symporter. To compare the activities of the basolateral membrane Na/3HCO_3 cotransporter in the two groups, the effect of changing peritubular fluid [HCO_3] and pH on pH_i was examined. For these studies the lumen and capillaries were initially perfused with an ultrafiltrate-like solution containing 25 mM HCO_3 (pH 7.32) (solution 1, Table I). In tubules from control animals, when peritubular [HCO_3] and pH were rapidly decreased from 25 (pH 7.32) to 5 mM (pH 6.62) (solution 2, Table I) and then returned to 25 mM (pH 7.32), dpH_i/dt was 5.79 ± 0.52 (n = 12) and 4.90 ± 0.33 pH units/min (n = 12), respectively (Fig. 3). With these fluid changes pH_i decreased from 7.14 ± 0.03 to 6.78 ± 0.03, and returned to 7.14 ± 0.03. When the same fluid changes were made in tubules from hyperfiltering animals, dpH_i/dt was significantly faster (7.27 ± 0.34

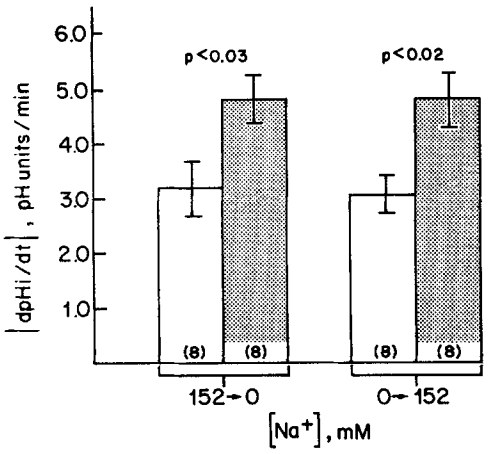

FIGURE 2. Initial rate of change in pH_i in response to a luminal fluid sodium change. The absolute value of the initial rate of change in pH_i ($|dpH_i/dt|$), is plotted on the y axis. Open bars represent results from tubules in 2-wk control animals; stippled bars are results from tubules in 2-wk hyperfiltering animals. The left set of bars are the data when luminal fluid [Na] was changed from 152 to 0 mM, and the right set of bars are the data when luminal fluid [Na] was returned from 0 to 152 mM.

[n = 11] and 6.73 ± 0.38 pH units/min [n = 11; Fig. 3], $P < 0.03$ and $P < 0.002$, respectively). In the tubules from the hyperfiltration group pH_i decreased from 7.27 ± 0.02 to 6.84 ± 0.02, and returned to 7.27 ± 0.02. The initial pH_i was significantly higher in the hyperfiltration group (7.14 ± 0.03 [control] and 7.27 ± 0.02 [hyperfiltration], $P < 0.002$), as was the pH_i in the recovery period ($P < 0.002$).

In these studies steady-state pH_i changed by 0.36 ± 0.02 pH units in tubules from control animals, and by 0.44 ± 0.02 pH units in tubules from the hyperfiltration group ($P < 0.002$). These studies demonstrate that an adaptation has occurred in a bicarbonate transport mechanism on the basolateral membrane in the hyperfiltration group.

To further demonstrate that the bicarbonate transport mechanism that adapted was the $Na/3HCO_3$ symporter, studies were done in which peritubular [Na] was rapidly changed from 147 to 25 and returned to 147 mM in the absence of luminal

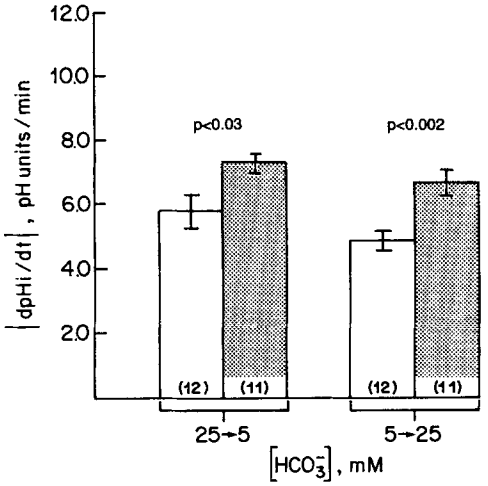

FIGURE 3. Initial rate of change in pH_i in response to a peritubular fluid bicarbonate change. Y axis as described for Fig. 2. Open bars represent results from tubules in 2-wk control animals; stippled bars are results from tubules in 2-wk hyperfiltering animals. The left set of bars are the data when peritubular fluid [HCO_3] was changed from 25 to 5 mM, and the right set of bars are the data when peritubular fluid [HCO_3] was returned from 5 to 25 mM.

and peritubular chloride (solutions 5 and 6, Table I). Luminal and peritubular chloride were removed to eliminate contributions from the basolateral membrane Na-dependent Cl/HCO_3 exchanger (Preisig and Alpern, 1989). In tubules from control rats dpH_i/dt was 6.61 ± 0.46 pH units/min (n = 9) when peritubular [Na] was decreased, and 5.49 ± 0.51 pH units/min (n = 9) when [Na] was returned to control values (Fig. 4). With these fluid changes pH_i decreased from 7.23 ± 0.04 to 6.89 ± 0.03 and returned to 7.23 ± 0.03. In tubules from the hyperfiltration group dpH_i/dt was significantly faster when peritubular [Na] was decreased (9.97 ± 1.00 pH units/min, $P < 0.007$; n = 7) (Fig. 4). When [Na] was returned to 147 mM, dpH_i/dt was faster (7.17 ± 0.89 pH units/min, n = 7) but did not reach statistical significance (P = 0.10) (Fig. 4). In the hyperfiltration group pH_i decreased from 7.28 ± 0.04 to 6.88 ± 0.03, and returned to 7.32 ± 0.04.

In these studies steady-state pH_i decreased 0.34 ± 0.03 pH units (n = 9) in tubules from control animals and 0.42 ± 0.01 pH units (n = 7) in tubules from the

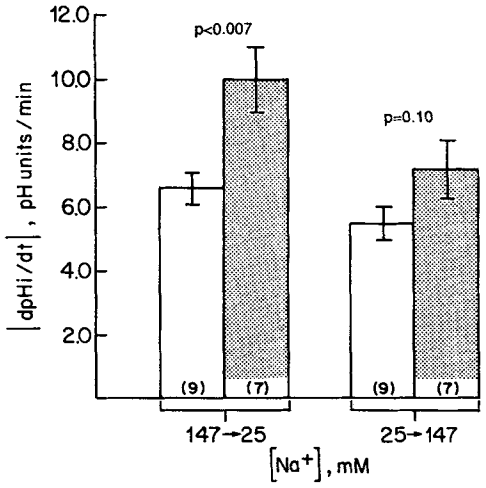

FIGURE 4. Initial rate of change in pH_i in response to a peritubular fluid sodium change in the absence of luminal and peritubular chloride. Y axis as described in Fig. 2. Open bars represent data from tubules in 2-wk control animals; stippled bars are data from tubules in 2-wk hyperfiltering animals. The left set of bars are the data when peritubular fluid [Na] was changed from 147 to 25 mM, and the right set of bars are the data when peritubular fluid [Na] was changed from 25 to 147 mM.

hyperfiltration group. These values are significantly different ($P < 0.03$). Taken together with the studies in which peritubular perfusate $[HCO_3]$/pH was changed, these studies demonstrate a stimulatory adaptation in the basolateral membrane $Na/3HCO_3$ symporter in the hyperfiltration group.

Buffer capacity. The assays for Na/H and $Na/3HCO_3$ transporter activities measured an initial rate of change in pH_i in response to a change in the driving force for a transported species. We have interpreted differences in dpH_i/dt between the two groups to reflect differences in H-equivalent movement across one of the membranes. However, this interpretation of the dpH_i/dt measurements requires knowledge of the buffer capacity and cell volume per millimeter tubule length.

Buffer capacity was measured using the technique of NH_3/NH_4^+ addition (see Methods). There was no Na or Cl in either of the perfusates to eliminate any influence of Na- or Cl-coupled transporters in the measurement of buffer capacity (solutions 7 and 8, Table I). In tubules from control animals NH_3/NH_4^+ addition

caused cell pH to increase by 0.13 ± 0.02 pH units, and buffer capacity was 62.0 ± 10.3 mM/pH unit (n = 6). In tubules from the hyperfiltration group, cell pH increased by 0.14 ± 0.02 pH units and buffer capacity was 77.2 ± 13.7 mM/pH unit (n = 6, NS vs. control group). This higher, though not significantly different, value in the hyperfiltration group is in the wrong direction to explain the differences in the dpH_i/dt measurements.

Cell volume. As shown by Eq. 7, only a decrease in cell volume per millimeter tubule length could explain the differences in the dpH_i/dt measurements. Since chronic hyperfiltration is associated with cellular hypertrophy, we expected that cell volume per millimeter tubule length would be increased, if anything, in the hyperfiltering group, and therefore once again would be in the wrong direction to explain the observed differences in dpH_i/dt between the two groups. To confirm this, we measured the inner and outer diameters of the proximal tubule on histologic sections, and estimated cell volume per millimeter tubule length as described in Methods.

Five control and four hyperfiltering kidneys were studied. Cell height was 14.1 ± 0.6 μm in tubules from control animals (n = 25) and 18.0 ± 0.8 μm in tubules from hyperfiltering animals (n = 20), $P < 0.0002$. Calculated cell volume was significantly larger in tubules from hyperfiltering animals compared with tubules from control animals (18.9 ± 1.1 vs. 13.4 ± 0.6 × 10^{-10} liters/mm, respectively, $P < 0.0001$). Our observed effect of chronic hyperfiltration on cell height and volume are in good agreement with those obtained in both rats and rabbits after a reduction in renal mass and/or changes in dietary protein intake (Hayslett et al., 1968; Trizna et al., 1981; Tabei et al., 1983; Johnston et al., 1987).

As can be seen from Eq. 7, since cell volume per millimeter tubule length was significantly larger in tubules from hyperfiltering animals, the dpH_i/dt measurements do not accurately reflect the increase in transporter activity that occurred in the hyperfiltering animals. To correct for differences in cell volume per millimeter tubule length, we calculated a rate of proton-equivalent flux (J_H^{eq}, picomoles per millimeter per minute) using Eq. 7, which is a more accurate estimate of transporter activity. The results are presented in Table III. As can be seen, Na/H antiporter and $Na/3HCO_3$ symporter activities were increased 77–121%. Since cell volume per millimeter tubule length increased only 41%, these results demonstrate that chronic hyperfilteration is associated with an increased effective density of transporters, and that the increased transporter activity is not due solely to an increase in cell mass.

24-h Hyperfiltration

In the above 2-wk studies, when the lumen and capillaries were perfused with similar ultrafiltrate-like solutions, steady-state pH_i was higher in the hyperfiltering group. These results suggest that a decreased pH_i, as occurs in chronic metabolic acidosis and chronic potassium deficiency, is not the signal for initiation and maintenance of the adaptations in hyperfiltration. However, it is possible that pH_i is decreased at an earlier time point in chronic hyperfiltration, and is responsible for initiation of the adaptations. It was also possible that either the Na/H antiporter or the $Na/3HCO_3$ transporter was activated first, and that this led to activation of the other transporter at a later time.

To test these hypotheses, steady-state pH_i, cell size, and Na/H and Na/3HCO_3 transporter activities were examined 24 h after uninephrectomy and 40% protein feeding, and compared with a 24-h post-sham nephrectomy group fed a 24% protein diet. Arterial blood gases, plasma values, and kidney weights for these two groups are presented in Table IV. Again, there were no differences in arterial blood gases or plasma [K], confirming that this protocol of hyperfiltration was not associated with a systemic acid–base disorder or potassium deficiency. Serum creatinine was slightly though not statistically higher in the uninephrectomized group, reflecting the recent loss of 50% of kidney mass. Left kidney weight was increased in the hyperfiltration group, suggesting that the hypertrophic response to uninephrectomy and protein feeding was underway within 24 h. A similar response of kidney weight at 24 h was observed by Harris et al. (1984).

TABLE III

H-Equivalent Fluxes

Protocol	J_H^{ss} Control	J_H^{ss} Hyperfiltration	% Increase	$P <$
	pmol/mm · min			
2-wk hyperfiltration				
Na/H antiporter				
[Na] 152 → 0 mM	293	626	114	0.001
0 → 152 mM	281	620	121	0.001
Na/3HCO_3 symporter				
[HCO_3] 25 → 5 mM	543	962	77	0.0001
5 → 25 mM	460	890	93	0.0001
[Na] 147 → 25 mM	620	1,319	113	0.002
25 → 147 mM	515	949	84	0.004
24-h hyperfiltration				
Na/H antiporter				
[Na] 152 → 0 mM	421	584	39	0.02
0 → 152 mM	358	486	36	0.01
Na/3HCO_3 symporter				
[HCO_3] 25 → 5 mM	742	1,081	46	0.01
5 → 25 mM	771	917	19	0.03

Apical membrane Na/H antiporter activity. As described above, Na/H antiporter activity was assayed by rapidly removing and re-adding Na to the luminal perfusate. Peritubular capillaries were perfused with a solution containing 5 mM HCO_3 (solution 2, Table I) with 0.5 mM SITS added. The lumen was initially perfused with a solution containing 152 mM Na (solution 3, Table I), which was rapidly changed to one containing 0 mM Na (solution 4, Table I).

The absolute values of the mean initial rates of change in pH_i (dpH_i/dt) for all tubules are summarized in Fig. 5. In tubules from control rats, when luminal [Na] was changed from 152 to 0 mM, dpH_i/dt was 3.81 ± 0.40 pH units/min (n = 13) and steady-state pH_i decreased from 7.10 ± 0.06 to 6.80 ± 0.06. Upon readdition of 152 mM Na to the luminal perfusate pH_i increased at an initial rate of 3.24 ± 0.24 pH_i units/min to a pH_i of 7.09 ± 0.08 (n = 13). When the same luminal perfusate [Na]

TABLE IV

24-h Control and Hyperfiltering Groups: Plasma Values, Arterial Blood Gases, and Kidney Weight

	Control group	Hyperfiltration group
Arterial blood gases		
pH	7.37 ± 0.01 (n = 11)	7.36 ± 0.01 (n = 8)
pCO_2, *mmHg*	51.6 ± 2.9 (n = 11)	51.3 ± 2.2 (n = 8)
$[HCO_3]$, *mM*	29.7 ± 0.8 (n = 11)	29.3 ± 0.7 (n = 8)
Plasma values*		
Creatinine, *mg%*	0.45 ± 0.05 (n = 4)	0.57 ± 0.03 (n = 3)
[Na], *meq/liter*	146 ± 1 (n = 4)	145 ± 1 (n = 3)
[K], *meq/liter*	3.8 ± 0.2 (n = 4)	3.7 ± 0.1 (n = 3)
[Cl], *meq/liter*	106 ± 1 (n = 4)	106 ± 1 (n = 3)
Hct, %	43.3 ± 1.0 (n = 11)	44.0 ± 1.7 (n = 7)
Plasma protein, *g%*	5.7 ± 0.1 (n = 11)	5.8 ± 0.1 (n = 7)
Kidney weight, *g*	1.11 ± 0.04 (n = 10)	1.22 ± 0.03‡ (n = 9)

*Plasma Na, K, and Cl concentrations are expressed as milliequivalents per liter plasma water, and plasma creatinine concentration is expressed as milligrams per deciliter plasma.
‡P = 0.054 vs. control.

changes were made in tubules from the hyperfiltration group, dpH_i/dt was faster (5.28 ± 0.40 [n = 11] and 4.39 ± 0.35 pH units/min [n = 11], $P < 0.02$ and $P < 0.01$, respectively). With these fluid changes in the hyperfiltering group, pH_i decreased from 6.97 ± 0.06 to 6.61 ± 0.06 and returned to 6.98 ± 0.07.

In response to the luminal fluid [Na] changes in the above studies, steady-state pH_i decreased by 0.31 ± 0.02 pH units in tubules from the control animals, and by 0.36 ± 0.02 pH units in tubules from the hyperfiltering group. Again, although these values are not statistically different, the larger change in the tubules from the hyperfiltration group is supportive of the observed differences in dpH_i/dt.

Basolateral membrane $Na/3HCO_3$ symporter. To compare the activities of the basolateral membrane $Na/3HCO_3$ cotransporter in the two groups, the effect of changing peritubular fluid $[HCO_3]$ and pH on pH_i was examined. In these studies the

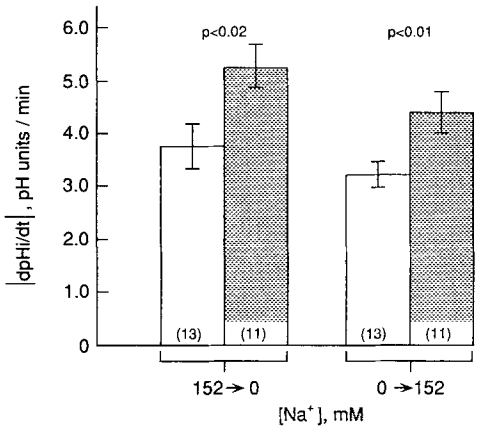

FIGURE 5. Initial rate of change in pH_i in response to a luminal fluid sodium change. Y axis as described in Fig. 2. Open bars represent results from tubules in 24-h control animals; stippled bars are results from tubules in 24-h hyperfiltering animals. The left set of bars are the data when luminal fluid [Na] was changed from 152 to 0 mM, and the right set of bars are the data when luminal fluid [Na] was returned from 0 to 152 mM.

lumen and capillaries were initially perfused with a solution containing 25 mM HCO_3 (pH 7.32; solution 1, Table I). In tubules from control animals, when peritubular [HCO_3] and pH were rapidly decreased from 25 (pH 7.32) to 5 mM (pH 6.62; solution 2, Table I) and then returned to 25 mM (pH 7.32), dpH_i/dt was 6.71 ± 0.63 (n = 10) and 6.97 ± 0.41 (n = 10), respectively (Fig. 6). With these fluid changes pH_i decreased from 7.07 ± 0.03 to 6.63 ± 0.03 and returned to 7.05 ± 0.02. When the same fluid changes were made in tubules from the hyperfiltering group, dpH_i/dt was significantly faster (9.77 ± 0.86 [n = 10] and 8.29 ± 0.40 [n = 10], Fig. 6; $P < 0.01$ and $P < 0.04$, respectively). In these tubules pH_i decreased from 7.03 ± 0.03 to 6.58 ± 0.02 and returned to 7.04 ± 0.03.

The initial pH_i tended to be lower in the hyperfiltering group (7.07 ± 0.03 [control] and 7.03 ± 0.03 [hyperfiltration]), although this was not statistically different (P = 0.36). In these studies steady-state pH_i changed by 0.44 ± 0.03 pH units in tubules from control animals and by 0.45 ± 0.01 pH units in tubules from the hyperfiltering group (NS).

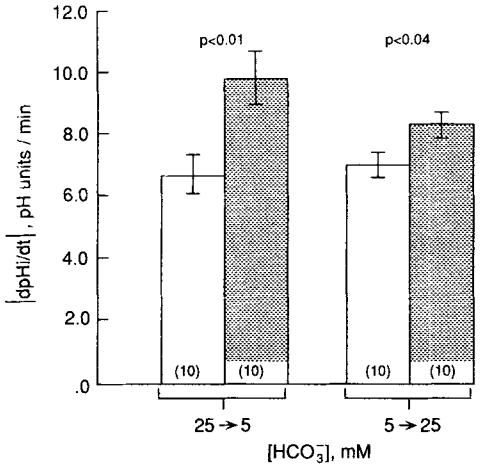

FIGURE 6. Initial rate of change in pH_i in response to a peritubular fluid bicarbonate change. Y axis as described in Fig. 2. Open bars represent results from tubules in 24-h control animals; stippled bars are results from tubules in 24-h hyperfiltering animals. The left set of bars are the data when peritubular fluid [HCO_3] was changed from 25 to 5 mM, and the right set of bars are the data when peritubular fluid [HCO_3] was changed from 5 to 25 mM.

Cell volume. Measurements were taken of the inner and outer diameters of surface proximal tubules in four control and four hyperfiltering kidneys as described in Methods. Cell height was 11.6 ± 0.5 μm in tubules from control animals (n = 20) and 11.5 ± 0.3 μm in tubules from hyperfiltering animals (n = 20), NS. Calculated cell volume per millimeter tubule length was 15.6 ± 0.8 × 10^{-10} liters/mm in tubules from control animals and 16.0 ± 0.6 × 10^{-10} liters/mm in tubules from hyperfiltering animals, NS.

To compare the percent stimulation of transporter activity at 24 h and 2 wk, we calculated a J_H^{eq} flux for the 24-h studies. These data are presented in Table III. As can be seen, Na/H antiporter and Na/3HCO_3 symporter activities were increased 19–46% after 24 h of hyperfiltration. In the absence of an increase in cell volume per millimeter tubule length, this once again represents an increase in the effective density of transporters. The 35% average increase in transporter activity after 24 h of

hyperfiltration represents approximately one-third of the average increase in transporter activity present after 2 wk of hyperfiltration.

DISCUSSION

Increases in GFR lead to an increased filtered load of bicarbonate which necessitates increased rates of proximal tubular bicarbonate absorption to maintain acid–base balance. Chronic hyperfiltration has been associated with proximal tubular hypertrophy (Johnson and Roman, 1966; Hayslett et al., 1968; Hayslett, 1979; Fine et al., 1978; Trizna et al., 1981; Tabei et al., 1983; Brenner, 1985; Fine, 1986; Johnson et al., 1987), and increased rates of volume and sodium absorption (Weber et al., 1975; Bank et al., 1978; Fine et al., 1978; Trizna et al., 1981; Tabei et al., 1983; Wong et al., 1984; Maddox et al., 1986; Johnston et al., 1987). Furthermore, rates of bicarbonate absorption have been shown to be increased in free-flow micropuncture studies (Bank et al., 1978; Wong et al., 1984; Maddox et al., 1986). Increased apical membrane Na/H antiporter activity has been demonstrated in chronic hyperfiltration associated with uninephrectomy, protein feeding, renal failure (5/6 nephrectomy), and diabetes mellitus (Cohn et al., 1982; Harris et al., 1984, 1986; Nord et al., 1985).

The present studies demonstrate that chronic hyperfiltration of 2 wk duration is associated with increased activities of the Na/H antiporter and Na/3HCO_3 symporter, an increased capacity for HCO_3 absorption, and cell hypertrophy in the proximal tubule. Because tubules in control and hyperfiltering animals were perfused with the same solutions and at the same perfusion rates, the different rates of bicarbonate absorption[4] and transporter activities are not attributable to the higher luminal flow rate or mean luminal bicarbonate concentration that would be associated with an increase in GFR. These studies do not address whether the increase in transporter activities is due to an increase in transporter number or an increased turnover rate per transporter.

It is interesting to compare the degree of increase in transporter activity with the magnitude of increase in membrane surface area. Indeed, Trinza et al. (1981) found that rates of volume absorption increased in parallel with cylindrical luminal surface area in proximal tubules dissected from uninephrectomized rabbits. To examine this question in our studies, transporter activities and volume and bicarbonate fluxes were expressed per square centimeter cylindrical luminal surface area (Table V). Luminal diameter values were used from Hayslett et al. (1968), who fixed the tissue under free-flow conditions in control and uninephrectomized rats. As can be seen, when expressed per square centimeter surface area both Na/H antiporter and Na/3HCO_3 symporter activities are increased significantly. Rates of volume and bicarbonate absorption increased by 43% in tubules from hyperfiltering animals. These latter

[4] In the studies measuring rates of volume and bicarbonate absorption, tubules from both control and hyperfiltering rats were initially perfused with similar solutions at the same rate. However, the faster rates of volume and bicarbonate absorption in the hyperfiltration group would lower the mean luminal flow rate and bicarbonate concentration compared with the control group. Studies on the flow dependence of volume and solute absorption suggest that the lower mean luminal flow rate and bicarbonate concentration would lead to relatively slower rates of transport. Thus, in our studies we may have underestimated the magnitude of the stimulation in volume and bicarbonate absorptive capacity in the hyperfiltration group.

changes, however, were of borderline statistical significance ($P < 0.07$ and 0.08, respectively). Taken together these results suggest a degree of transporter stimulation out of proportion to the increase in surface area. Such a comparison, however, must be viewed as speculative as the appropriate comparison would be to the true brush border and basolateral membrane surface areas determined by morphometric studies.

The conclusion that increases in transporter activities are greater than the degree of hypertrophy is supported by the fact that transporter activities increase to a greater extent than cell volume. In Table III transporter activity is expressed as the rate of H-equivalent movement for each experimental protocol. As can be seen, after

TABLE V

H-Equivalent Fluxes and Rates of Volume and Bicarbonate Absorption after 2 Wk of Hyperfiltration

	J_H^{eq}							
	Control		Hyperfiltration		% Increase		$P <$	
	pmol/mm·min	*×10³ pmol/cm²·min*	*pmol/mm·min*	*×10³ pmol/cm²·min*	*pmol/mm·min*	*pmol/cm²·min*	*pmol/mm·min*	*pmol/cm²·min*
Na/H antiporter								
[Na] 152 → 0	293	3.62	626	6.59	114	82	0.001	0.01
0 → 152	281	3.47	620	6.53	121	88	0.001	0.01
$Na/3HCO_3$ symporter								
[HCO_3] 25 → 5	543	6.70	962	10.13	77	51	0.0001	0.001
2 → 25	460	5.68	890	9.37	93	65	0.0001	0.0001
[Na] 147 → 25	620	7.65	1,319	13.88	113	81	0.002	0.01
25 → 147	515	6.36	949	9.99	84	57	0.004	0.02

	Volume and bicarbonate absorption			
	Volume absorption		Bicarbonate absorption	
	nl/mm·min	*×10³ nl/cm²·min*	*pmol/mm·min*	*×10³ pmol/cm²·min*
Control	2.47	3.05	109	1.35
Hyperfiltration	4.13	4.35	183	1.93
% Increase	67	43	68	43
$P <$	0.02	0.07	0.02	0.08

2 wk of hyperfiltration J_H^{eq} increased 77–121%, while cell volume only increased 41% (see Results), and after 24 h J_H^{eq} increased 19–46% with no measureable change in cell volume. Thus, transporter activities increased to a greater extent than did cell size, suggesting that cell hypertrophy is not the sole explanation for the enhanced transporter activities. In fact, if transporter activities increased in proportion to cell volume, there would have been no change in dpH_i/dt (see Eq. 7).

A similar pattern of responses, e.g., increased Na/H antiporter and $Na/3HCO_3$ symporter activities, cell hypertrophy, and enhanced rates of water and solute transport, is also seen in chronic metabolic acidosis and chronic potassium deficiency (Chan et al., 1982; Cohn et al., 1983; Kinsella et al., 1984; Seifter and Harris, 1984;

Tsai et al., 1984; Kunau et al., 1985; Fine, 1986; Jacobsen et al., 1986; Akiba et al., 1987; Preisig and Alpern, 1988; Soleimani et al., 1990). In these latter conditions cell acidification occurs (Adam et al., 1986) and has been considered a possible signal for the adaptations.

In the studies where peritubular fluid $[HCO_3]$/pH was changed, the only studies where tubules were perfused with physiological solutions, baseline and recovery pH_i's were higher in the 2-wk hyperfiltration group. When tubules were perfused in the absence of luminal and peritubular Cl there was also a tendency for pH_i to be higher in the hyperfiltration group, but this did not achieve statistical significance ($P = 0.33$ and 0.08 for the control and recovery periods, respectively). In the study where capillaries were perfused with SITS, cell pH's were similar in the two groups, but we consider pH_i in the presence of capillary SITS to be difficult to interpret. The association of increased pH_i with adaptations in the Na/H antiporter and $Na/3HCO_3$ symporter indicates that a decrease in pH_i is not a prerequisite, at least, for the maintenance of the adaptations.[5]

To examine if there was a decreased pH_i at an earlier time point during the adaptation, we performed similar studies at 24 h. At this time there was a tendency for pH_i to decrease, although this did not achieve statistical significance. However, the absence of a statistically significant difference in baseline pH_i should not be interpreted to mean that cell acidification was not present or involved in the initiation and/or maintenance of the adaptative response. It is entirely possible that the degree of cell acidification observed, although insufficient to achieve statistical significance, was of sufficient magnitude to initiate the adaptations.

After 24 h of hyperfiltration the activities of both transporters and kidney weight were increased, although measured cell height and volume were not different between the two groups. It was possible that hyperfiltration directly activated one transporter, and that this caused changes in cell composition which secondarily activated the other transporter. However, our results suggest that this is not the case, as transporter activities are increased in parallel at 24 h (Figs. 5 and 6, Table III). In addition, approximately one-third of the change in transporter activities that is present after 2 wk of hyperfiltration has occurred by 24 h. This time course is similar to that reported by Harris et al. (1986).

The signal for the adaptation in chronic hyperfiltration is presently unresolved. As discussed above, a decreased cell pH may be an early cell signal, but even if this is true, the cause of the decreased cell pH is not presently clear. One theory (work

[5] One concern in the studies after 2 wk of hyperfiltration is that the faster dpH_i/dt's in the hyperfiltration group may be due to the more alkaline pH_i, rather than an adaptation occurring in the $Na/3HCO_3$ symporter, possibly through an allosteric effect of pH_i on the $Na/3HCO_3$ symporter. However, there are two pieces of data that would suggest that this is not the case, and that an adaptation has occurred in the $Na/3HCO_3$ symporter. First, in the studies in which peritubular $[HCO_3]$ was changed from 25 to 5 mM, baseline pH_i varied from 7.05 to 7.35 in the control group. Over this pH_i range of 0.30 pH units there was no correlation between dpH_i/dt and pH_i ($P = 0.62$). Thus, it is unlikely that the difference in steady-state pH_i between the two groups (0.13 pH units) could explain the difference in the dpH_i/dt measurements. Second, in the same studies, when peritubular $[HCO_3]$ was returned from 5 to 25 mM, dpH_i/dt was faster in the hyperfiltration group, yet steady-state pH_i before the fluid change was not statistically different (6.78 ± 0.03 [control] vs. 6.84 ± 0.02 [hyperfiltration]).

hypothesis) proposes that increases in luminal flow rate stimulate proximal tubular $NaHCO_3$ and NaCl absorption, which then leads to hypertrophy and adaptive increases in transporter activities. Increases in luminal flow rate have been demonstrated to increase proximal tubular transport by modifying the luminal substrate concentration profile, and by poorly understood direct effects on the epithelium (Barfuss and Schafer, 1979; Green et al., 1981; Chan et al., 1982; Alpern et al., 1983). It is presently unclear how increased transport would then lead to increased transporter activity and hypertrophy.

A second possible signal for the adaptation is growth factors. Increases in GFR could lead to release of growth factors that cause cell hypertrophy. It is now well recognized in many tissues that growth factor actions include activation of the Na/H antiporter (Grinstein and Rothstein, 1986; Moolenar, 1986). However, in these tissues the activated Na/H antiporter does not mediate transepithelial transport, and may be a different Na/H antiporter than that present on the proximal tubular apical membrane (Haggerty et al., 1988). Fine et al. (1985) have shown that insulin and PGE1 cause hypertrophy of proximal tubular cells in primary culture and stimulate the Na/H antiporter. However, these studies (Fine et al., 1985) did not examine whether the activated antiporter was on the apical membrane. Our studies and those of Harris and co-workers (1984, 1986), Nord et al. (1985), and Cohn et al. (1982) clearly demonstrate increased apical membrane Na/H antiporter activity in chronic hyperfiltration.

Increased $Na/3HCO_3$ symporter activity was somewhat unexpected in these studies, and initially was felt to be more consistent with the work hypothesis than an effect of growth factors. Growth factors had traditionally been thought to activate only the Na/H antiporter. However, recently Ganz et al. (1989) found that arginine vasopressin, a growth factor in mesangial cells, stimulated the Na/H antiporter and the Na-dependent and -independent Cl/HCO_3 exchangers in these cells. Mesangial cells do not possess a $Na/3HCO_3$ symporter. Thus, it is possible that growth factors, released in response to chronic hyperfiltration, activate the Na/H antiporter and the $Na/3HCO_3$ symporter. It is also possible that similar growth factors mediate the response to chronic metabolic acidosis or chronic potassium deficiency.

In summary, chronic hyperfiltration is a model for cell hypertrophy. The present studies demonstrate that the increase in apical membrane Na/H antiporter activity in this condition is associated with a parallel increase in basolateral membrane $Na/3HCO_3$ symporter activity. These adaptations, similar to those in chronic metabolic acidosis and chronic potassium deficiency, contribute to an increased capacity for transcellular proton secretion. The increased transporter activities may be unique to this epithelium whose major function is H/HCO_3 transport, or may represent the response of all cells to increase the activity of multiple H/HCO_3 transporters during hypertrophy.

This work was supported by a National Institutes of Diabetes, Digestive, and Kidney Disease grant (RO1-DK39298), two Biomedical Research Support grants (2-SO7-RR05426-26 and 2-SO7-RR05426-27), a Grant-In-Aid from the American Heart Association, Texas Affiliate (G-121), a grant from the National Kidney Foundation of Texas, and by funds from the Ruby B. Hexter estate.

Original version received 19 December 1989 and accepted version received 18 June 1990.

REFERENCES

Adam, W. R., A. P. Koretsky, and M. W. Weiner. 1986. ^{31}P-NMR in vivo measurement of renal intracellular pH: effects of acidosis and K^+ depletion in rats. *American Journal of Physiology.* 251 (*Renal Fluid and Electrolyte Physiology* 20):F904–F910.

Akiba, T., V. K. Rocco, and D. G. Warnock. 1987. Parallel adaptation of the rabbit renal cortical sodium/proton antiporter and sodium/bicarbonate cotransporter in metabolic acidosis and alkalosis. *The Journal of Clinical Investigation.* 80:308–315.

Alpern, R. J. 1985. Mechanism of basolateral membrane $H^+/OH^-/HCO_3^-$ transport in the rat proximal convoluted tubule. A sodium-coupled electrogenic process. *The Journal of General Physiology.* 86:613–636.

Alpern, R. J. 1987. Apical membrane chloride/base exchange in the rat proximal convoluted tubule. *The Journal of Clinical Investigation.* 79:1026–1030.

Alpern, R. J. 1990. Cell mechanisms of proximal tubule acidification. *Physiological Reviews.* 70:79–114.

Alpern, R. J., and M. Chambers. 1986. Cell pH in the rat proximal convoluted tubule. Regulation by luminal and peritubular pH and sodium concentration. *The Journal of Clinical Investigation.* 78:502–510.

Alpern, R. J., M. G. Cogan, and F. C. Rector, Jr. 1983. Flow dependence of proximal tubular bicarbonate absorption. *American Journal of Physiology.* 245 (*Renal Fluid and Electrolyte Physiology* 14):F478–F484.

Aronson, P. S., J. Nee, and M. A. Suhm. 1982. Modifier role of internal H^+ in activating the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Nature.* 299:161–163.

Bank, N., W. Su, and H. S. Aynedjian. 1978. A micropuncture study of HCO_3 reabsorption by the hypertrophied proximal tubule. *The Yale Journal of Biology and Medicine.* 51:275–282.

Barfuss, D. W., and J. A. Schafer. 1979. Flow dependence of nonelectrolyte absorption in the nephron. *American Journal of Physiology.* 236 (*Renal Fluid and Electrolyte Physiology* 5):F163–F174.

Beer, D. G., K. A. Zweifel, D. P. Simpson, and H. C. Pitot. 1987. Specific gene expression during compensatory renal hypertrophy in the rat. *Journal of Cellular Physiology.* 131:29–35.

Brenner, B. M. 1985. Nephron adaptation to renal injury or ablation. *American Journal of Physiology.* 249 (*Renal Fluid and Electrolyte Physiology* 18):F324–F337.

Chan, Y. L., B. Biagi, and G. Giebisch. 1982. Control mechanisms of bicarbonate transport across the rat proximal convoluted tubule. *American Journal of Physiology.* 242 (*Renal Fluid and Electrolyte Physiology* 11):F532–F543.

Cogan, M. G. 1984. Chronic hypercapnia stimulates proximal bicarbonate reabsorption in the rat. *The Journal of Clinical Investigation.* 74:1942–1947.

Cohn, D. E., K. A. Hruska, S. Klahr, and M. R. Hammerman. 1982. Increased Na^+/H^+ exchange in brush border vesicles from dogs with renal failure. *American Journal of Physiology.* 243 (*Renal Fluid and Electrolyte Physiology* 12):F293–F299.

Cohn, D. E., S. Klahr, and M. R. Hammerman. 1983. Metabolic acidosis and parathyroidectomy increase Na^+-H^+ exchange in brush border vesicles. *American Journal of Physiology.* 245 (*Renal Fluid and Electrolyte Physiology* 14):F217–F222.

Fine, L. G. 1986. The biology of renal hypertrophy. *Kidney International.* 29:619–634.

Fine, L. G., B. Badie-Dezfooly, A. G. Lowe, A. Hamzeh, J. Wells, and S. Salehmoghaddam. 1985. Stimulation of Na^+/H^+ antiport is an early event in hypertrophy of renal proximal tubular cells. *Proceedings of the National Academy of Sciences, USA.* 82:1736–1740.

Fine, L. G., W. Trizna, J. J. Bourgoignie, and N. S. Bricker. 1978. Functional profile of the isolated uremic nephron. Role of compensatory hypertrophy in the control of fluid reabsorption by the proximal straight tubule. *The Journal of Clinical Investigation.* 1508–1518.

Ganz, M. B., G. Boyarsky, R. B. Sterzel, and W. F. Boron. 1989. Arginine vasopressin enhances pHi regulation in the presence of HCO_3^- by stimulating three acid-base transport systems. *Nature.* 337:648–651.

Green, R., R. J. Moriarty, and G. Giebisch. 1981. Ionic requirements of proximal tubular fluid reabsorption: flow dependence of fluid transport. *Kidney International.* 20:580–587.

Grinstein, S., and A. Rothstein. 1986. Mechanisms of regulation of the Na^+/H^+ exchanger. *The Journal of Membrane Biology.* 90:1–12.

Haggerty, J. G., N. Agarwal, R. F. Reilly, E. A. Adelberg, and C. W. Slayman. 1988. Pharmacologically different Na/H antiporters on the apical and basolateral surfaces of cultured porcine kidney cells (LLC-PK1). *Proceedings of the National Academy of Sciences, USA.* 85:6797–6801.

Harris, R. C., B. M. Brenner, and J. L. Seifter. 1986. Sodium-hydrogen exchange and glucose transport in renal microvillus membrane vesicles from rats with diabetes mellitus. *The Journal of Clinical Investigation.* 77:724–733.

Harris, R. C., J. L. Seifter, and B. M. Brenner. 1984. Adaptation of Na^+/H^+ exchange in renal microvillus membrane vesicles. Role of dietary protein and uninephrectomy. *The Journal of Clinical Investigation.* 74:1979–1987.

Hayslett, J. P. 1979. Functional adaptation to reduction in renal mass. *Physiological Reviews.* 59:137–164.

Hayslett, J. P., M. Kashgarian, and F. H. Epstein. 1968. Functional correlates of compensatory renal hypertrophy. *The Journal of Clinical Investigation.* 47:774–782.

Jacobsen, C., U. Kragh-Hansen, and M. I. Sheikh. 1986. Na^+-H^+ exchange in luminal-membrane vesicles from rabbit proximal convoluted and straight tubules in response to metabolic acidosis. *Biochemistry Journal.* 239:411–416.

Johnson, H. A., and J. M. V. Roman. 1966. Compensatory renal enlargement: hypertrophy versus hyperplasia. *The American Journal of Pathology.* 49:1–13.

Johnston, J. R., B. M. Brenner, and S. C. Hebert. 1987. Uninephrectomy and dietary protein affect fluid absorption in rabbit proximal straight tubules. *American Journal of Physiology.* 253 (*Renal Fluid and Electrolyte Physiology* 22):F222–F233.

Kinsella, J., T. Cujdik, and B. Sacktor. 1984. Na^+-H^+ exchange activity in renal brush border membrane vesicles in response to metabolic acidosis: the role of glucocorticoids. *Proceedings of the National Academy of Sciences, USA.* 81:630–634.

Krapf, R. 1989. Mechanisms of adaptation to chronic respiratory acidosis in the rabbit proximal tubule. *The Journal of Clinical Investigation.* 83:890–896.

Kunau, R. T., Jr., J. I. Hart, and K. A. Walker. 1985. Effect of metabolic acidosis on proximal tubular total CO_2 absorption. *American Journal of Physiology.* 249 (*Renal Fluid and Electrolyte Physiology* 18):F62–F68.

Maddox, D. A., J. F. Horn, F. C. Famiano, and F. J. Gennari. 1986. Load dependence of proximal tubular fluid and bicarbonate reabsorption in the remnant kidney of the Munich-Wistar rat. *The Journal of Clinical Investigation.* 77:1639–1649.

Moolenar, W. H. 1986. Effects of growth factors on intracellular pH regulation. *Annual Review of Physiology.* 48:363–376.

Nord, E. P., A. Hafezi, J. D. Kaunitz, W. Trizna, and L. G. Fine. 1985. pH gradient-dependent increased Na^+-H^+ antiport capacity of the rabbit remnant kidney. *American Journal of Physiology.* 249 (*Renal Fluid and Electrolyte Physiology* 18):F90–F98.

Norman, J. T., R. E. Bohman, G. Fischmann, J. W. Bowen, A. McDonough, D. Slamon, and L. G. Fine. 1988. Patterns of mRNA expression during early cell growth differ in kidney epithelial cells destined to undergo compensatory hypertrophy versus regenerative hyperplasia. *Proceedings of the National Academy of Sciences, USA.* 85:6768–6772.

Preisig, P. A., and R. J. Alpern. 1988. Chronic metabolic acidosis causes an adaptation in the apical membrane Na/H antiporter and basolateral membrane $Na(HCO_3)_3$ symporter in the rat proximal convoluted tubule. *The Journal of Clinical Investigation.* 82:1445–1453.

Preisig, P. A., and R. J. Alpern. 1989. Basolateral membrane H-OH-HCO_3 transport in the proximal tubule. *American Journal of Physiology.* 256 (*Renal Fluid and Electrolyte Physiology* 25):F751–F765.

Preisig, P. A., H. E. Ives, E. J. Cragoe, Jr., R. J. Alpern, and F. C. Rector, Jr. 1987. Role of the Na^+-H^+ antiporter in rat proximal tubule bicarbonate absorption. *The Journal of Clinical Investigation.* 80:970–978.

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

Ruiz, O. S., J. A. L. Arruda, and Z. Talor. 1989. Na-HCO_3 cotransport and Na-H antiporter in chronic respiratory acidosis and alkalosis. *American Journal of Physiology.* 256 (*Renal Fluid and Electrolyte Physiology* 25):F414–F420.

Seifter, J. L., and R. C. Harris. 1984. Chronic K depletion increases Na-H exchange in rat renal cortical brush border membrane vesicles (BBMV). *Kidney International.* 25:282. (Abstr.)

Soleimani, M., J. A. Bergman, M. A. Hosford, and T. D. McKinney. 1990. Potassium depletion increases luminal Na^+/H^+ exchange and basolateral Na^+:$CO_3^=$: HCO_3^- cotransport in rat renal cortex. *The Journal of Clinical Investigation.* 86:1076–1083.

Tabei, K., D. J. Levenson, and B. M. Brenner. 1983. Early enhancement of fluid transport in rabbit proximal straight tubules after loss of contralateral renal excretory function. *The Journal of Clinical Investigation.* 72:871–881.

Talor, Z., W.-C. Yang, J. Shuffield, E. Sack, and J. A. L. Arruda. 1987. Chronic hypercapnia enhances V_{max} on Na-H antiporter of renal brush-border membranes. *American Journal of Physiology.* 253 (*Renal Fluid and Electrolyte Physiology* 22):F394–F400.

Trizna, W., N. Yanagawa, Y. Bar-Khayim, B. Houston, and L. G. Fine. 1981. Functional profile of the isolated uremic nephron. *The Journal of Clinical Investigation.* 68:760–767.

Tsai, C.-J., H. E. Ives, R. J. Alpern, V. J. Yee, D. G. Warnock, and F. C. Rector, Jr. 1984. Increased V_{max} for Na^+/H^+ antiporter activity in proximal tubule brush border vesicles from rabbits with metabolic acidosis. *American Journal of Physiology.* 247 (*Renal Fluid and Electrolyte Physiology* 16):F339–F343.

Weber, J., K. Y. Lin, and N. S. Bricker. 1975. Effect of sodium intake on single nephron glomerular filtration rate and sodium reabsorption in experimental uremia. *Kidney International.* 8:14–20.

Wong, N. L., G. A. Quamme, and J. H. Dirks. 1984. Tubular handling of bicarbonate in dogs with experimental renal failure. *Kidney International.* 25:912–918.