Potassium Depletion Increases Luminal Na^+/H^+ Exchange and Basolateral $Na^+:CO_3^=:HCO_3^-$ Cotransport in Rat Renal Cortex

Manoocher Soleimani, Julia A. Bergman, Melanie A. Hosford, and T. Dwight McKinney
Nephrology Section, Department of Medicine, Indiana University Medical Center, and Veteran's Administration Medical Center, Indianapolis, Indiana 46202-5116

Abstract

Most HCO_3^- reabsorption in proximal tubules occurs via electroneutral Na^+/H^+ exchange in brush border membranes (BBMS) and electrogenic $Na^+:CO_3^=:HCO_3^-$ cotransport in basolateral membranes (BLMS). Since potassium depletion (KD) increases HCO_3^- reabsorption in proximal tubules, we evaluated these transport systems using BBM and BLM vesicles, respectively, from control (C) and KD rats. Feeding rats a potassium deficient diet for 3–4 wk resulted in lower plasma $[K^+]$ (2.94 mEq/liter, KD vs. 4.47 C), and higher arterial pH (7.51 KD vs. 7.39 C). KD rats gained less weight than C but had higher renal cortical weight. Influx of 1 mM $^{22}Na^+$ at 5 s (pH_o 7.5, pH_i 6.0, 10% CO_2, 90% N_2) into BLM vesicles was 44% higher in the KD group compared to C with no difference in equilibrium uptake. The increment in Na^+ influx in the KD group was DIDS sensitive, suggesting that $Na^+:CO_3^=:HCO_3^-$ cotransport accounted for the observed differences. Kinetic analysis of Na^+ influx showed a K_m of 8.2 mM in KD vs. 7.6 mM in C and V_{max} of 278 nmol/min/mg protein in KD vs. 177 nmol/min/mg protein in C. Influx of 1 mM $^{22}Na^+$ at 5 s (pH_o 7.5, pH_i 6.0) into BBM vesicles was 34% higher in the KD group compared to C with no difference in equilibrium uptake. The increment in Na^+ influx in the KD group was amiloride sensitive, suggesting that Na^+/H^+ exchange was responsible for the observed differences. Kinetic analysis of Na^+ influx showed a K_m of 6.2 mM in KD vs. 7.1 mM in C and V_{max} of 209 nmol/min/mg protein in KD vs. 144 nmol/min/mg protein in C. Uptakes of Na^+-dependent [^{3}H]glucose into BBM and [^{14}C]succinate into BLM vesicles were not different in KD and C groups, suggesting that the Na^+/H^+ exchanger and $Na^+:CO_3^=:HCO_3^-$ cotransporter activities were specifically altered in KD. We conclude that adaptive increases in basolateral $Na^+:CO_3^=:HCO_3^-$ cotransport and luminal Na^+/H^+ exchange are likely responsible for increased HCO_3^- reabsorption in proximal tubules of KD animals. (*J. Clin. Invest.* 1990. 86:1076–1083.) Key words: hypokalemia • metabolic alkalosis • renal growth • acid-base balance

Portions of these studies were presented at the Annual meeting of the American Society for Clinical Investigation, Washington, DC, May, 1989, and published in abstract form (*Clin. Res.* 37:585*a*, 1989).

Address correspondence to Dr. Soleimani, Nephrology Section, Department of Medicine, Fesler Hall 108, 1120 South Drive, Indiana School of Medicine, Indiana, IN 46202-5116.

Received for publication 3 October 1989 and in revised form 5 June 1990.

J. Clin. Invest.

0021-9738/90/10/1076/08 $2.00
Volume 86, October 1990, 1076–1083

Introduction

Potassium depletion has been associated with an increased ability of the renal tubules to reabsorb HCO_3^- (1–4). This adaptive change in HCO_3^- reabsorption has been invoked as a possible mechanism involved in the pathogenesis of systemic metabolic alkalosis in disorders associated with potassium depletion (5). Selective potassium deficiency has been shown to cause metabolic alkalosis in humans (6, 7) and in most studies in rats (1–4, 8, 9). In experiments in dogs (10–12) and rabbits (13), however, potassium deficiency resulted in metabolic acidosis. The majority of studies evaluating the effect of potassium depletion on renal acidification processes have been conducted in rats. In the rat, segmental analysis of the nephron indicates that potassium depletion is associated with increased HCO_3^- reabsorption by the proximal tubules (1–4).

Most HCO_3^- reabsorption in the kidney results from the collective effects of the Na^+/H^+ exchange system in the brush border membrane (14) and the $Na^+:CO_3^=:HCO_3^-$ cotransport system in the basolateral membrane (15–23) of the proximal tubule acting in series. In the present studies we have used brush border membrane (BBM)[1] and basolateral membrane (BLM) vesicles isolated from the renal cortex of potassium-depleted and control rats to study the characteristics of luminal Na^+/H^+ exchange and basolateral $Na^+:CO_3^=:HCO_3^-$ cotransport. The results suggest that potassium depletion induces a parallel adaptation in these transport processes. The increase in the activity of luminal Na^+/H^+ exchange and basolateral $Na^+:CO_3^=:HCO_3^-$ cotransport is, evidently, due to an increase in the V_{max} of the transporters.

Methods

Animal model. Potassium depletion was induced by placing male Sprague Dawley rats (175–200 g) on a potassium deficient diet similar to that employed by others (24) for 3–4 wk. The potassium deficient diet was prepared by replacing K^+ salts with Na^+. The composition of control and potassium deficient diets is given in Table I. Rats were housed two per cage. The total amount of food provided to animals in each control and experimental cage was 15 g/d. Both control and experimental animals readily consumed their respective diets. Animals had free access to water until they were killed.

Membrane vesicle preparation. Animals were killed by intraperitoneal injection of 12.5 mg of sodium pentobarbital. Arterial blood for blood gas determinations and other chemical analysis was obtained from the abdominal aorta at the time of sacrifice. BBM vesicles were isolated from the renal cortices by a Ca^{++} aggregation method (25) as employed previously (26). BLM vesicles were isolated from the renal

1. *Abbreviations used in this paper:* BBM, brush border membrane; BLM, basolateral membrane; C, control; DIDS, disodium 4,4′ diisothiocyanostilbene-2,2′-disulfonate; KD, potassium depletion.

Table I. Constituents of Control and Potassium Deficient Diets

	Control	K-Deficient
Casein	360 g	360 g
Corn starch	200 g	200 g
Sucrose	320 g	320 g
Corn oil	35 ml	35 ml
Peanut oil	35 ml	35 ml
Vitamins*	10 g	10 g
$CaHPO_4$	10 g	10 g
$MgSO_4$	6 g	6 g
NaCl	6 g	6 g
K_2HPO_4	8.3 g	0
Na_2HPO_4	0	6.8 g
KCL	9.5 g	0

* Vitamin diet fortification mixture, ICN Pharmaceuticals, Inc., Cleveland, OH.

cortices by a minor modification of differential and Percoll gradient centrifugation described previously (21) using Sorvall models RC5C superspeed and OTD 55B ultracentrifuges (DuPont Co., Wilmington, DE). Both BBM and BLM vesicles were frozen and stored at −70°C and used within 2 wk of preparation. The purity of the vesicle preparations was estimated by measuring the specific activities of alkaline phosphatase and Na^+, K^+-ATPase in the initial homogenate and final membrane suspension. The specific activity of alkaline phosphatase in BBM vesicles and initial homogenates was 171.3±14.2 and 19.3±2.4 μmols P-nitrophenol/mg protein/min in the control (n = 3) and 198.1±18.9 and 21.6±2.0 μmols P-nitrophenol/mg protein/min in the K-depleted group (n = 3) ($P > 0.05$). The specific activity of Na^+, K^+-ATPase in BLM vesicles and initial homogenates was 4.30±0.33 and 0.38±0.03 nmol Pi/min/mg protein, respectively, in the control, and 3.99±0.36 and 0.39±0.05 nmol Pi/min/mg protein, respectively, in the K-depleted group ($P > 0.05$). Additionally, HCO_3^--dependent $^{22}Na^+$ uptake in BBM and amiloride sensitive $^{22}Na^+$ uptake in BLM vesicles were measured to further evaluate the contamination of BBM with BLM and vice versa. In BBM vesicles, HCO_3^--dependent $^{22}Na^+$ uptake was 5.7%±5.8 of total uptake in control membranes and 5.3%±7.6 in potassium depletion (KD) membranes ($P > 0.05$). Conversely, amiloride sensitive $^{22}Na^+$ uptake in BLM vesicles from control animals was 3.7%±6.7 of total uptake compared to 6.1%±5.3 in the KD group ($P > 0.05$). Thus, these results indicate relatively little cross contamination of BBM with BLM and vice versa. The BBM vesicle size, calculated from external Na^+ and equilibrium values for $^{22}Na^+$ uptake, was not significantly different in K-depleted and control groups (1.40±0.21 μl/mg protein in C vs. 1.81±0.25 KD, $P > 0.05$). The BLM vesicle size was 1.77±0.29 μl/mg protein in control compared to 1.97±0.35 in K-depleted animals ($P > 0.05$).

Transport measurements. Intravesicular content of $^{22}Na^+$, [^{3}H]glucose, and [^{14}C]succinate were assayed in triplicate at room temperature by a rapid filtration method, as previously described (27). The ice-cold medium used to dilute and wash the vesicles consisted of 170 mM K gluconate, 10 mM Hepes/TMA hydroxide, pH 7.5. Each filter (0.45 μm, DAWP; Millipore Continental Water Systems, Bedford, MA) was placed in 5 ml of scintillation fluid (Ready-Solv HP; Beckman Instruments Inc., Fullerton, CA) and radioactivity was determined by scintillation spectroscopy. The membrane suspension and all experimental media were continuously gassed with 100% N_2 or 10% CO_2/90% N_2. Every solution was titrated to the indicated pH with TMA hydroxide. The final composition of the experimental media and other details of the protocols are given in the figure legends. All experiments were performed using vesicles treated with valinomycin (0.5 mg/ml) and preequilibrated in media of appropriate composition to ensure that $[K^+]_o = [K^+]_i$ during uptake measurements. The adequacy of voltage clamping by this method has been verified previously (23, 28). For kinetic studies, uptake was measured at 4 s, since we and others have shown that under conditions similar to those used in the present studies $^{22}Na^+$ uptake via Na^+/H^+ exchange in BBM vesicles (29) and via $Na^+:CO_3^=:HCO_3^-$ cotransport in BLM vesicles is linear up to this time (30). Preliminary results indicated that uptakes in BBM and BLM vesicles from potassium depleted and control animals were also linear at these times (data not shown).

The permeability of vesicles to hydrogen ions was evaluated as described previously (31, 32). Briefly, an outward H^+ gradient was imposed across both the BBM and BLM vesicles (pH_i 6.0, pH_o 7.5) of control and K-depleted animals. 10 μl of BBM or BLM vesicles were then added to 2 ml of a sodium-free medium (pH 7.5) that contained 6 μM acridine orange. The fluorescence of acridine orange was determined at room temperature using a spectrofluorimeter (LS-3B; Perkin-Elmer Corp., Norwalk, CT) with excitation and emission wavelengths of 492 and 530. The rate of change of acridine orange fluorescence after the initial quenching was measured and used to determine the proton permeability of the vesicles. The relative rate of change of acridine orange fluorescence in BBM vesicles from KD animals to that of control group was 1.23±0.27-fold for three experiments ($P > 0.05$). Similarly, BLM vesicles of K-depleted animals did not show any significant difference in proton permeability compared to control (relative rate of change of acridine orange fluorescence in KD group to that of control animals was 1.15±0.14-fold for three experiments, $P > 0.05$).

The data are presented as the mean±SEM. Statistical analysis was determined using t test for paired or unpaired observations. A value of $P < 0.05$ was considered statistically significant.

Materials. ^{22}NaCl (sp act, 906 mCi/mg), [^{14}C]succinate (sp act, 56 mCi/mmol) and [^{3}H]glucose (sp act, 14.4 mCi/mmol) were obtained from New England Nuclear (Boston, MA). Percoll, valinomycin, acridine orange, amiloride/HCL, and disodium 4,4′ diisothiocyanostilbene-2,2′-disulfonate (DIDS) were obtained from Sigma Chemical Co. (St. Louis, MO). Valinomycin was dissolved in 95% ethanol and added to the membrane vesicles in a 1:100 dilution. Other chemicals were obtained from Sigma Chemical Co.

Results

Body and kidney weights in control and potassium depleted animals are given in Table II. At the time that they were killed, potassium-depleted animals weighed significantly less than controls ($P < 0.01$). Compared to their initial weights, potassium-depleted rats gained only 13%, whereas control animals gained 47% ($P < 0.04$). However, when compared to controls, kidney weights in the potassium-depleted animals were 56% higher (3.21±.088 vs. 2.05±0.048/two kidneys, $P < 0.001$). The kidney per body weight ratio in the potassium-depleted animals was twice that of control animals (0.014±0.0004 vs. 0.007±0.0001, $P < 0.001$).

Plasma $[K^+]$ and arterial blood gas values in control animals and ones fed the potassium deficient diet are given in Table III. Plasma $[K^+]$ was significantly lower in the potassium deficient rats (2.94±0.073 mEq/liter vs. 4.47±0.095 mEq/liter in controls, $P < 0.001$). Arterial pH was significantly higher in potassium depleted rats (7.51±0.008 vs. 7.39±0.007 in controls, $P < 0.001$). Potassium-depleted animals had an arterial pCO_2 of 43.1 mm Hg (control, 39.2 mm Hg) and a calculated plasma HCO_3^- concentration of 31.2 mM (control, 23.7 mM). These results confirm that rats fed a potassium deficient diet develop both hypokalemia and metabolic alkalosis. This is in accordance with previous studies evaluating the effect of dietary-induced potassium depletion on systemic acid base balance (1–4, 8, 9).

Table II. Body and Kidney Weights in Control and Potassium-Depleted Animals

	Body weight				
	Initial	Final	Weight gain (%)	Kidney weight	Kidney/body weight
	g	*g*		*g*	
Control	187.6±2.1	274.8±6.1	46.5±2.3	2.05±0.048	0.007±0.0001
K-depleted	189.9±1.9	215.8±3.9* ($P < 0.01$ [$n = 32$])	13.1±1.1* ($P < 0.04$ [$n = 32$])	3.21±0.088* ($P < 0.001$ [$n = 28$])	0.014±0.0004* ($P < 0.001$ [$n = 28$])

To examine for possible effects of potassium depletion on the luminal Na^+/H^+ exchanger, the influx of Na^+ into BBM vesicles was measured in the presence of an inwardly directed pH gradient. The time course of pH-dependent $^{22}Na^+$ uptake in vesicles isolated from control and potassium depleted rats is shown in Fig. 1. An inward pH gradient resulted in the transient uphill accumulation of Na^+ (overshoot) in both control and potassium-depleted groups. However, at early time points, the potassium depleted group showed increased Na^+ influx compared to controls with no significant difference in the equilibrium values. These results suggest that potassium depletion activates the Na^+/H^+ exchange system. To determine if the increment in Na^+ influx was indeed because of increased activity of Na^+/H^+ exchange system, the initial rate (5 s) of $^{22}Na^+$ influx was measured in the presence of an inward pH gradient with or without 1 mM amiloride in the external solution (Fig. 2). $^{22}Na^+$ influx was 34% higher in vesicles from potassium-depleted animals compared to control (1.56±0.16 vs. 1.15±0.14 nmol/mg protein, $P < 0.05$). The increment in the $^{22}Na^+$ influx in the potassium depleted group was completely abolished in the presence of 1 mM amiloride, suggesting that the observed difference between the two groups resulted from increased activity of the Na^+/H^+ exchanger in the potassium-depleted group. To examine the mechanism of increased activity of the Na^+/H^+ exchanger in BBM vesicles from potassium-depleted rats, we evaluated the kinetics of this exchanger by measuring initial rates (4 s) of $^{22}Na^+$ influx as a function of the $[Na^+]$ in vesicles isolated from control and potassium-depleted animals. Hanes-Woolf plots of the results (Fig. 3) demonstrate an increase in the V_{max} for Na^+ with no significant change in K_m in the potassium-depleted group, consistent with either an increase in the number and/or turnover rate of the Na^+/H^+ transporter.

We next evaluated the activity of the $Na^+:CO_3^=:HCO_3^-$ cotransporter in BLM vesicles isolated from control and potassium-depleted rats. In the experiment illustrated in Fig. 4, the time course of Na^+ influx into these vesicles was assayed in the presence of an inward pH and HCO_3^- gradient. An inward HCO_3^- gradient was associated with an accumulation of Na^+ above equilibrium (overshoot) both in control and potassium-depleted groups. However, at early time points, the potassium-depleted group showed increased Na^+ influx compared to control with no significant differences in the equilibrium values. Since in these experiments, an inward HCO_3^- gradient was imposed in the presence of inward pH gradient, the possibility of a pH effect as the cause of the increment in $^{22}Na^+$ influx in potassium-depleted group cannot be excluded. Such an effect could be possible specifically if there was significant contamination of the BLM vesicles with BBM in this group. Although there were no differences in enrichment ratios for BLM in the two groups of vesicles, to further address this possibility, we measured the initial rate of HCO_3^--dependent Na^+ influx in the presence and absence of 1 mM DIDS, an inhibitor of $Na^+:CO_3^=:HCO_3^-$ cotransport that does not have any effect on the Na^+/H^+ exchange system. The results are shown in Fig. 5. $^{22}Na^+$ influx in vesicles from potassium depleted rats was 44% greater than that in control vesicles (2.56±0.31 vs. 1.77±0.26 nmol/mg protein, $P < 0.04$). Furthermore, the increment in $^{22}Na^+$ influx in the potassium-depleted group was completely abolished when DIDS was added to the external solution, suggesting that the observed difference between the potassium depleted and control groups was mediated via $Na^+:CO_3^=:HCO_3^-$ cotransport. To examine the mechanism of increased activity of $Na^+:CO_3^=:HCO_3^-$ cotransport in BLM vesicles from potassium-depleted rats, we evaluated the kinetics of this transport process. The initial rate (4 s) of $^{22}Na^+$ influx in vesicles isolated from control and potassium-depleted animals was measured as a function of the $[Na^+]$. Hanes-Woolf plots of the results are shown in Fig. 6, and demonstrate that the V_{max} for Na^+ was increased in the potassium-depleted group with no significant change in K_m. These results are consistent with either an increase in the number or turnover rate of the transporter.

To determine if potassium depletion affects the luminal Na^+/H^+ exchange and basolateral $Na^+:CO_3^=:HCO_3^-$ cotransport processes specifically or causes more generalized effects, we compared the initial rates (5 s) of Na^+-dependent [^{3}H]glucose (33) and [^{14}C]succinate (34) uptake in BBM and BLM vesicles, respectively, isolated from both control and potassium depleted rats (Table IV). The relative rate of Na^+-dependent [^{3}H]glucose uptake was not different in control and potassium-depleted groups. Similarly, the relative rate of Na^+ de-

Table III. Plasma [K^+] and Arterial Blood Gases in Control and Potassium-depleted Animals

	[K^+]	pH	pCO_2	pO_2
Control	4.47±0.095	7.39±0.007	39.2±0.5	97.2±2.7
K-depleted	2.94±0.073* ($P < 0.001$ [$n = 14$])	7.51±0.008* ($P < 0.001$ [$n = 13$])	43.1±0.7* ($P < 0.01$ [$n = 13$])	93.8±1.9

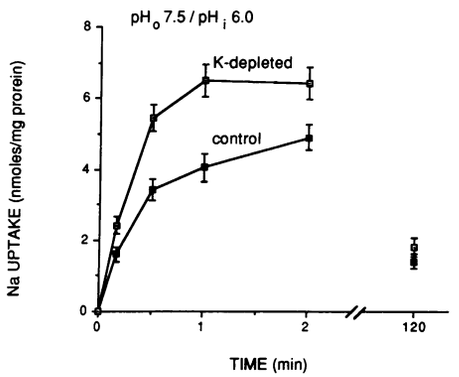

Figure 1. Effect of pH gradient on Na^+ influx. BBM vesicles from potassium-depleted and control animals were preequilibrated for 120 min at 20°C in a medium consisting of 52 mM TMA gluconate, 108 mM potassium gluconate, 52 mM Mes, 42 TMA hydroxide, pH 6.0. Uptake of 1 mM $^{22}Na^+$ into the vesicles was assayed in the presence of a medium consisting of 52 mM TMA gluconate, 108 potassium gluconate, 31 mM mannitol, 10 mM Mes, 31 mM TMA hydroxide, pH 7.5. Values shown for uptake represent mean±SE for experiments performed in triplicate on three different membrane preparations.

pendent [^{14}C]succinate uptake was not different between the two groups.

Discussion

Induction of potassium deficiency in rats (and humans) results in the development of metabolic alkalosis (1–4, 6–9). This is manifested by an elevated plasma HCO_3^- concentration and is associated with the (paradoxical) excretion of an acid urine (1, 35, 36). It has been suggested that potassium deficiency increases renal tubular H^+ secretion and subsequently, bicarbonate reabsorption (1–7). Both metabolic alkalosis and paradoxical aciduria could result from these processes (1–4, 35, 36). Several studies employing free-flow micropuncture or in

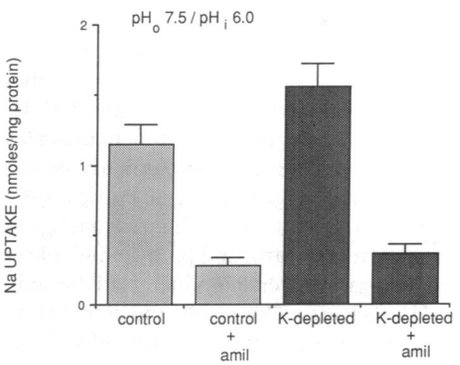

Figure 2. Effect of amiloride on pH dependent Na^+ influx. The 5-s uptake of $^{22}Na^+$ into BBM vesicles preequilibrated in pH 6.0 medium was assayed in the presence of pH 7.5 medium as described in Fig. 1. Amiloride (1 mM) was added as the hydrochloride salt to the external solution. Valinomycin was added at the start of preequilibration period. Values shown for uptake represent mean±SE for experiments performed in triplicate on four different membrane preparations.

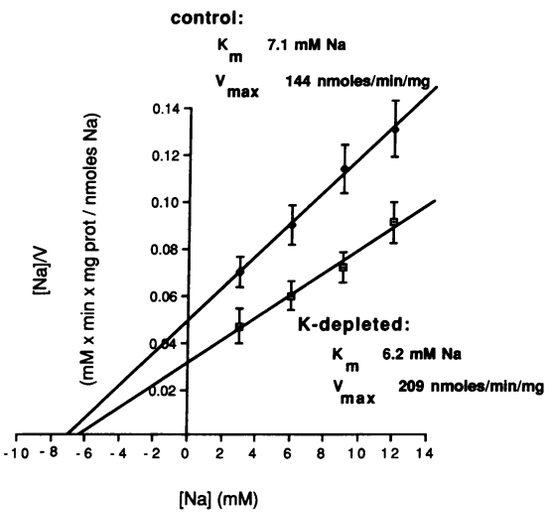

Figure 3. Kinetics of pH dependent Na^+ influx into BBM vesicles (BBMV). BBMV from potassium-depleted and control animals were preequilibrated for 120 min at 20°C in a medium consisting of 67 mM TMA gluconate, 92 mM potassium gluconate, 52 mM Mes, 42 TMA hydroxide, pH 6.0. The 4-s uptake of $^{22}Na^+$ into vesicles was assayed in the presence of a medium consisting of 67 mM TMA gluconate, 92 potassium gluconate, 31 mM mannitol, 10 mM Mes, 31 mM TMA hydroxide, pH 7.5. The Na^+ concentration was varied by replacing TMA gluconate with sodium gluconate in the uptake medium. Valinomycin was added at the start of preequilibration period. Values shown for uptake represent mean±SE for experiments performed in triplicate on three different membrane preparations.

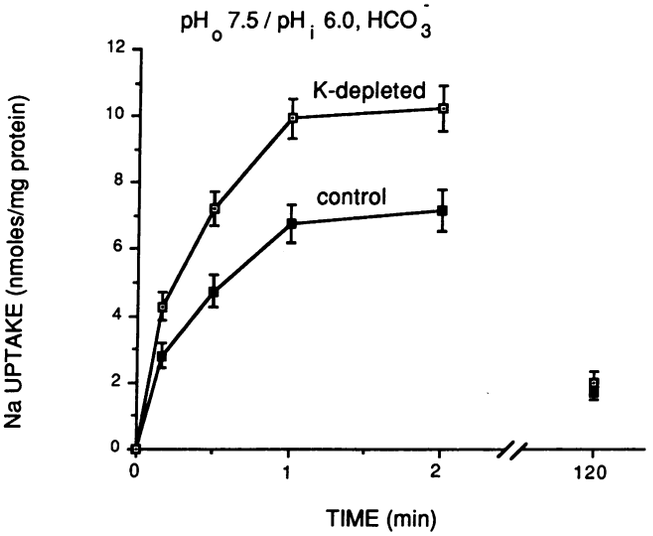

Figure 4. Effect of HCO_3^- gradient on Na^+ influx into BLM vesicles (BLMV). BLMV from potassium-depleted and control animals were preequilibrated for 120 min at 20°C in a medium consisting of 52 mM TMA gluconate, 108 mM potassium gluconate, 52 mM Mes, 42 TMA hydroxide, pH 6.0, gassed with 10% CO_2. Uptake of 1 mM $^{22}Na^+$ into vesicles was assayed in the presence of a medium consisting of 52 mM TMA gluconate, 51 potassium gluconate, 57 mM potassium bicarbonate, 31 mM mannitol, 10 mM Mes, 31 mM TMA hydroxide, pH 7.5, gassed with 10% CO_2. Values shown for uptake represent mean±SE for experiments performed in triplicate on three different membrane preparations.

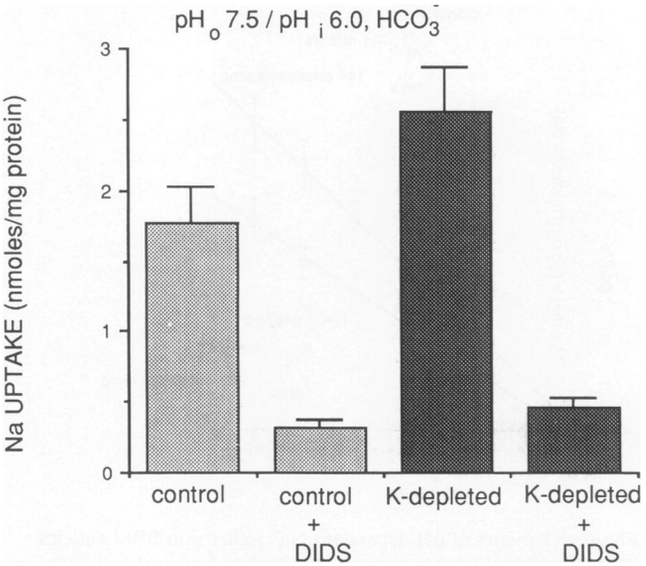

Figure 5. Effect of DIDS on HCO_3^--dependent Na influx in BLMV. The 5 s uptake of 2 mM $^{22}Na^+$ into the BLM vesicles preequilibrated in pH 6.0 medium was assayed in the presence of 57 mM HCO_3^-, 10% CO_2 at pH 7.5 as described in Fig. 4. DIDS (1 mM) was added as the disodium salt to the external solution. Valinomycin was added at the start of the preequilibration period. Values shown for uptake represent mean±SE for experiments performed in triplicate on four different membrane preparations.

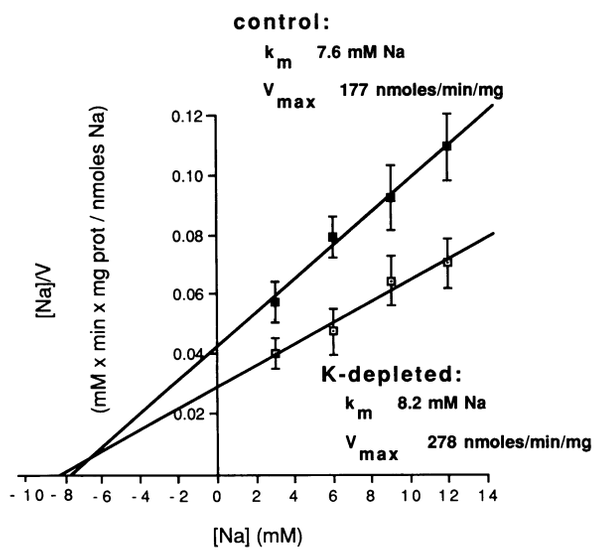

Figure 6. Kinetics of HCO_3^--dependent Na^+ influx into BLMV. BLMV from potassium-depleted and control animals were preequilibrated for 120 min at 20°C in a medium consisting of 67 mM TMA gluconate, 92 mM potassium gluconate, 52 mM Mes, 42 TMA hydroxide, pH 6.0 and gassed with 10% CO_2. The 4-s uptake of $^{22}Na^+$ into vesicles was assayed in the presence of a medium consisting of 67 mM TMA gluconate, 35 potassium gluconate, 57 mM potassium bicarbonate, 31 mM mannitol, 10 mM Mes, 31 mM TMA hydroxide, pH 7.5. The Na^+ concentration was varied by replacing TMA gluconate with sodium gluconate in the uptake medium. Valinomycin was added at the start of preequilibration period. Values shown for uptake represent mean±SE for experiments performed in triplicate on three different membrane preparations.

Table IV. Na^+-dependent [^{3}H]Glucose and [^{14}C]Succinate Uptakes into BBMV and BLMV from Potassium-Depleted and Control Animals

Influx	Percentage of control
Na-dependent [^{3}H]glucose cotransport (BBMV)	107±8.2
Na-dependent [^{14}C]succinate cotransport (BLMV)	113±8.4

BBM and BLM vesicles from potassium-depleted and control animals were preequilibrated for 120 min at 20°C in a medium consisting of 52 mM TMA gluconate, 108 mM potassium gluconate, 52 mM TMA gluconate, 42 mM Hepes, 21 TMA hydroxide, pH 7.5. The 5-s uptake of 50 μM [^{3}H]glucose into brush border membrane vesicles was assayed in the presence of a medium consisting of 52 mM TMA gluconate, 108 potassium gluconate, 52 mM sodium gluconate, 42 mM Hepes, 21 mM TMA hydroxide, pH 7.5. The 5-s uptake of 5 μM [^{14}C]succinate into basolateral membrane vesicles was assayed in the presence of a similar medium. Values shown for uptake represent mean±SE for experiments performed in triplicate on three different membrane preparations.

vivo microperfusion techniques in rat kidneys have demonstrated that the rate of proximal tubular HCO_3^- reabsorption is elevated in potassium deficiency (1–4). Using continuous microperfusion techniques in proximal tubules enhanced HCO_3^- reabsorption has been observed when tubules and peritubular capillaries of potassium deficient rats were exposed to HCO_3^- concentrations typically observed in hypokalemia (3). However, in some other reports an increase in the absolute amount of HCO_3^- reabsorbed by the proximal tubules of potassium deficient rats was not shown (9, 37).

The majority of HCO_3^- filtered across the glomerulus is reabsorbed in the proximal tubule by a process of (secondarily) active proton secretion. The principal pathway for movement of protons from cell to tubular fluid is via luminal membrane Na^+/H^+ exchange (14). HCO_3^- transport across the BLM of the proximal tubule is mediated predominantly via electrogenic Na^+:HCO_3^- cotransport (15–23) with an apparent stoichiometry of three equivalents of base per Na^+ ion (18, 23). Recent studies indicate that the three base equivalents are comprised of one $CO_3^=$ ion and one HCO_3^- ion cotransported with one Na^+ ion (30). It has been suggested that potassium deficiency increases the rate of H^+ secretion via activation of the Na^+/H^+ exchanger in the proximal tubular BBM resulting in increased HCO_3^- reabsorption (38, 39). Results of preliminary experiments evaluating Na^+/H^+ exchange in rat renal cortical BBM vesicles by Seifter et al. (39) are consistent with this conclusion. In BBM vesicles isolated from control and potassium-depleted rats, they showed that potassium depletion increased the activity of luminal Na^+/H^+ exchange. Our results indicate that potassium depletion increases the activity of pH dependent $^{22}Na^+$ influx in renal cortical BBM vesicles. The increase in $^{22}Na^+$ influx was amiloride sensitive, suggesting that Na^+/H^+ exchange was responsible for the increase. The time course of the pH dependent $^{22}Na^+$ influx showed persistently higher levels of $^{22}Na^+$ influx at early time points but no difference in equilibrium values in vesicles from potassium-depleted animals. Moreover, our results suggest that increased activity of the Na^+/H^+ exchanger is due to an increase in the V_{max} with no

significant change in the K_m of the exchange process. These findings are consistent with either an increase in the number or turnover rate of the Na^+/H^+ exchange transport proteins. The cellular mechanism(s) by which potassium depletion increases Na^+/H^+ exchange, and, therefore, H^+ secretion into the proximal tubular fluid remains unknown. It has been suggested that increased H^+ secretion by proximal tubules in potassium-depleted rats results from intracellular acidosis. A reduced intracellular pH in proximal tubule cells has been reported in this setting (40, 41). The mechanism(s) responsible for generation of this intracellular acidosis is unknown as discussed below. This acidic pH could increase the rate of the Na^+/H^+ exchanger via at least two mechanisms. First, intracellular acidosis increases substrate (H^+) availability for the exchanger at the inner surface of the luminal membrane. Second, an increase in the intracellular proton concentration can stimulate the exchanger by binding to the modifier site located at the inner surface of the Na^+/H^+ exchange transport protein (42).

We also studied the effect of potassium depletion on pathways involved in the exit of HCO_3^- across the BLM of proximal tubule cells. The increase in HCO_3^- dependent $^{22}Na^+$ influx we observed in BLM vesicles prepared from potassium-depleted animals was DIDS sensitive suggesting that increased activity of the $Na^+:CO_3^=:HCO_3^-$ cotransporter was responsible for the results. The time course of HCO_3^--dependent $^{22}Na^+$ influx showed persistently higher levels of $^{22}Na^+$ influx at early time points, but not at equilibrium, in vesicles from potassium-depleted animals. Kinetic studies evaluating the nature of these adaptive changes showed that there was an increase in V_{max} with no change in K_m. These results are consistent with either an increase in the number or turnover rate of the $Na^+:CO_3^=:HCO_3^-$ cotransporters. Increased activity of this cotransporter, in association with increased activity of luminal Na^+/H^+ exchange, could lead to increased reabsorption of HCO_3^- and, thus, an increase in plasma [HCO_3^-] in the potassium-depleted state.

The molecular mechanism(s) by which potassium depletion induces adaptive changes in luminal Na^+/H^+ exchange and basolateral $Na^+:CO_3^=:HCO_3^-$ cotransport is not certain. By unknown mechanisms potassium depletion in rats causes generalized renal growth (43). Whether there is an association between the renal growth and the observed acid–base changes or between the renal growth and the adaptive changes in luminal Na^+/H^+ exchange and basolateral $Na^+:CO_3^=:HCO_3^-$ cotransport is not clear. One speculation is that potassium depletion could function as a growth factor. Cell growth in a renal epithelial cell line (BSC-1) was stimulated by a low medium concentration of potassium (44). Growth factors have been shown to increase the activity of pH regulating mechanisms (45, 46). Therefore, it is possible that potassium depletion, by virtue of its growth promoting characteristics, may activate processes involved in regulation of cell pH; e.g., Na^+/H^+ exchange and $Na^+:CO_3^=:HCO_3^-$ cotransport. Interestingly, potassium depletion does not induce metabolic alkalosis (47) or accelerated renal growth in rabbits (McKinney, T. D., and K. K. Davidson, unpublished observations).

Another possibility with regard to the effect of potassium depletion on adaptive changes in luminal Na^+/H^+ exchange and basolateral $Na^+:CO_3^=:HCO_3^-$ cotransport is that potassium depletion might increase the electrochemical driving forces for one or both of these transport processes. It is noteworthy in this regard that increased activity of one of these transport processes will affect the other transporter if cell pH is to be maintained in a narrow range. A primary increase in activity of the Na^+/H^+ exchanger will increase cell pH. Under the effect of cytosolic carbonic anhydrase this increased pH will lead to generation of more HCO_3^-, thus providing additional substrate for the $Na^+:CO_3^=:HCO_3^-$ cotransporter. On the other hand, a primary increase in $Na^+:CO_3^=:HCO_3^-$ cotransporter activity will decrease intracellular pH due to HCO_3^- extrusion across the basolateral membrane. Subsequently, this would provide more substrate for the Na^+/H^+ exchanger in the luminal membrane. Since proximal tubule cell pH is lower in potassium depleted rats (40, 41), it would appear that the luminal Na^+/H^+ exchanger is not the primary process affected since an alkaline cell pH would be the expected result of a primary increase in activity of this exchanger. The mechanism(s) responsible for the intracellular acidosis in proximal tubule cells of potassium depleted rats is not certain. It has been postulated that this may result from H^+ redistribution (48). However, another plausible explanation is that the intracellular pH falls because of increased base extrusion across the basolateral membrane due to increased activity of the basolateral $Na^+:CO_3^=:HCO_3^-$ cotransporter. The cellular mechanism by which potassium depletion might activate this cotransporter remains speculative. $Na^+:CO_3^=:HCO_3^-$ cotransport is an electrogenic process and is, therefore, affected by changes in membrane potential. Alpern (17) has shown in rat proximal tubule cells that increasing peritubular potassium from 5 to 50 mEq/liter, a maneuver that has been shown to cause depolarization of the basolateral membrane, induces cell alkalinization. In the presence of barium, the same maneuver failed to change the cell pH. In the presence of HCO_3^- and a relative electropositive vesicle interior (produced by the combination of the potassium ionophore valinomycin an inwardly directed potassium gradient) causes transient uptake of Na^+ above equilibrium values in basolateral membrane vesicles isolated from rabbit renal cortex (21). These results suggest that the exit of HCO_3^- across the BLM is sensitive to changes in membrane potential. With a stoichiometry of three equivalents of HCO_3^- ions/Na^+ ion, membrane depolarization should decrease, whereas hyperpolarization should increase the rate of base extrusion via $Na^+:CO_3^=:HCO_3^-$ cotransport. Using a double barrel microelectrode, Cemerikic et al. (49) have shown that potassium deficiency induces hyperpolarization of the basolateral membrane of proximal tubule cells. Therefore, it is possible that changes in membrane potential induced by potassium depletion could be responsible for the increased activity of the $Na^+:CO_3^=:HCO_3^-$ cotransporter observed in our studies. According to this scheme, potassium depletion induces hyperpolarization of the basolateral membrane that, in turn, increases the electrochemical driving force for base exit across the BLM via electrogenic $Na^+:CO_3^=:HCO_3^-$ cotransport. This leads to acidification of the cell providing more substrate for the Na^+/H^+ exchanger in the luminal membrane whose activity subsequently increases. The intracellular acidosis in proximal tubule cells observed in potassium depletion would also be compatible with the above scheme. It is noteworthy that the increases in the luminal Na^+/H^+ exchanger and basolateral $Na:CO_3^=:HCO_3^-$ cotransporter induced by potassium depletion

may be underestimated because of the generation of a metabolic alkalosis that has been shown to decrease the activities of these two transport systems (50).

In summary, potassium depletion causes a parallel adaptive increase in the luminal Na^+/H^+ exchanger and basolateral $Na:CO_3^=:HCO_3^-$ cotransporter in rat kidney proximal tubule. These adaptive changes are probably responsible in the generation and maintenance of metabolic alkalosis in potassium depletion.

Acknowledgements

The authors acknowledge the excellent secretarial assistance of Marcia A. Harmon.

These studies were supported in part by National Institutes of Health Program, project grant Am-17387, and by the Veteran's Administration.

References

1. Rector, F. C., Jr., H. A. Bloomer, and D. W. Seldin. 1964. Effect of potassium deficiency on the reabsorption of bicarbonate in the proximal tubule of the rat kidney. *J. Clin. Invest.* 43:1976–1982.

2. Kunau, R. T., Jr., A. Frick, F. C. Rector, Jr., and D. W. Seldin. 1968. Micropuncture study of the proximal tubular factors responsible for the maintenance of alkalosis during potassium deficiency in the rat. *Clin. Sci. (Lond.).* 34:223–231.

3. Chan, Y. L., B. Biagi, and G. Giebisch. 1982. Control mechanisms of bicarbonate transport across the rat proximal convoluted tubule. *Am. J. Physiol.* 242:F532–543.

4. Capasso, G., R. Kinne, G. Malnic, and G. Giebisch. 1987. Renal bicarbonate reabsorption in the rat. I. Effect of hypokalemia and carbonic anhydrase. *J. Clin. Invest.* 80:409–414.

5. Seldin, D. W., and F. C. Rector, Jr. 1972. The generation and maintenance of metabolic alkalosis. *Kidney Int.* 1:306–321.

6. Hernandez, R. E., M. Schambelan, M. G. Cogan, J. Colman, R. C. Morris, Jr., and A. Sebastian. 1987. Dietary NaCl determines severity of potassium depletion-induced metabolic alkalosis. *Kidney Int.* 31:1356–1367.

7. Jones, J. W., A. Sebastian, H. N. Hutler, M. Schambelan, J. M. Sutton, and E. D. Biglieri. 1982. Systemic and renal acid-base effects of chronic dietary potassium depletion in humans. *Kidney Int.* 21:402–410.

8. Luke, R. B., and H. Levitin. 1967. Impaired renal conservation of chloride and the acid-base changes associated with potassium depletion in the rat. *Clin. Sci. (Lond.).* 32:511–526.

9. Levine, D. Z., T. Walker, and L. A. Nash. 1973. Effect of KCl on proximal tubular function in normal and potassium depleted rats. *Kidney Int.* 4:318–325.

10. Burnell, J. M., E. M. Teubner, and D. P. Simpson. 1974. Metabolic acidosis accompanying potassium deprivation. *Am. J. Physiol.* 227:329–333.

11. Garella, S., B. Chang, and S. I. Kahn. 1979. Alterations of hydrogen ion homeostasis in pure potassium depletion: studies in rats and dogs during the recovery phase. *J. Lab. Clin. Med.* 93:321–331.

12. Hulter, H. A., A. Sebastian, J. F. Sigala, J. H. Licht, R. D. Glynn, M. Schambelan, and E. G. Biglieri. 1980. Pathogenesis of renal hyperchloremic acidosis resulting from dietary potassium restriction in the dog: role of aldosterone. *Am. J. Physiol.* 238:F79–F91.

13. McKinney, T. D., and K. K. Davidson. 1987. Effect of potassium depletion and protein intake in vivo on renal tubular bicarbonate transport in vitro. *Am. J. Physiol.* 252:F509–F516.

14. Aronson, P. S. 1983. Mechanisms of active H^+ secretion in the proximal tubule. *Am. J. Physiol.* 245:F647–F659.

15. Boron, W. F., and E. L. Boulpaep. 1983. Intracellular pH regulation in the renal proximal tubule of the salamander. Basolateral HCO_3^- transport. *J. Gen. Physiol.* 81:53–94.

16. Biagi, B. A., and M. Sohtell. 1986. Electrophysiology of basolateral bicarbonate transport in the rabbit proximal tubule. *Am. J. Physiol.* 250:F267–F272.

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

18. Yoshitomi, K., B. C. Burckhardt, and E. Fromter. 1985. Rheogenic sodium-bicarbonate cotransport in the peritubular cell membrane of rat renal proximal tubule. *Pfluegers Arch. Eur. J. Physiol.* 405:360–366.

19. Sasaki, S., T. Shiigai, and T. Takeuchi. 1985. Intracellular pH in the isolated perfused rabbit proximal straight tubule. *Am. J. Physiol.* 249:F417–F423.

20. Alpern, R. J., and M. Chambers. 1986. Cell pH in the rat proximal convoluted tubule. Regulation by luminal and peritubular pH and sodium concentration. *J. Clin. Invest.* 78:502–510.

21. Grassl, S. M., and P. S. Aronson. 1986. Na^+/HCO_3^- co-transport in basolateral membrane vesicles isolated from rabbit renal cortex. *J. Biol. Chem.* 261:8778–8783.

22. Akiba, T., R. J. Alpern, J. Eveloff, J. Calamina, and D. G. Warnock. 1986. Electrogenic sodium/bicarbonate cotransport in rabbit renal cortical basolateral membrane vesicles. *J. Clin. Invest.* 78:1472–1478.

23. Soleimani, M., S. M. Grassl, and P. S. Aronson. 1987. Stoichiometry of Na^+-HCO_3^- cotransport in basolateral membrane vesicles isolated from rabbit renal cortex. *J. Clin. Invest.* 79:1276–1280.

24. Whinnery, M. A., and R. T. Kunau, Jr. 1979. Effect of potassium deficiency on papillary plasma flow in the rat. *Am. J. Physiol.* 237:F226–F231.

25. Evers, C., W. Hasse, H. Murer, and R. Kinne. 1978. Properties of brush border membrane vesicles isolated from rat kidney cortex by calcium precipitation. *Membr. Biochem.* 1:202–219.

26. McKinney, T. D., and M. E. Kunnemann. 1985. Procainamide transport in rabbit renal cortical brush border membrane vesicles. *Am. J. Physiol.* 249:F532–F541.

27. Soleimani, M., and P. S. Aronson. 1989. Effects of acetazolamide on Na/HCO_3 cotransport in basolateral membrane vesicles isolated from rabbit renal cortex. *J. Clin. Invest.* 83:945–951.

28. Kinsella, J. L., and P. S. Aronson. 1980. Properties of the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Am. J. Physiol.* 238:F461–F469.

29. Mahnensmith, R. L., and P. S. Aronson. 1985. Interrelationship among quinidine, amiloride, and lithium as inhibitors of the renal Na^+/H^+ exchanger. *J. Biol. Chem.* 260:12586–12592.

30. Soleimani, M., and P. S. Aronson. 1989. Ionic mechanism of Na-HCO_3 cotransport in rabbit renal basolateral membrane vesicles. *J. Biol. Chem.* 264:18302–18308.

31. Reenstra, W. W., D. G. Warnock, V. J. Yee, and J. G. Forte. 1981. Proton gradients in renal cortex brush border membrane vesicles. *J. Biol. Chem.* 256:11663–11666.

32. Ives, E. I. 1985. Proton/hydroxyl permeability of proximal tubule brush border vesicles. *Am. J. Physiol.* 248:F78–86.

33. Aronson, P. S., and B. Sacktor. 1974. Transport of D-glucose by brush border membranes isolated from the renal cortex. *Biochim. Biophys. Acta.* 356:231–243.

34. Wright, S. H., and T. M. Wunz. 1987. Succinate and citrate transport in renal basolateral and brush border membranes. *Am. J. Physiol.* 253:F432–439.

35. Broch, O. J. 1950. Low potassium alkalosis with acid urine in ulcerative alkalosis. *Scand. J. Clin. Lab. Invest.* 2:113–117.

36. Van Slyke, K. K., and E. I. Evans. 1947. The paradox of aciduria in the presence of alkalosis caused by hypokalemia. *Ann. Surg.* 126:545–550.

37. Cogan, M. G., and F. Y. Liu. 1983. Metabolic alkalosis in the rat: evidence that reduced glomerular filtration rather than enhanced tubular bicarbonate reabsorption is responsible for maintaining the alkalotic state. *J. Clin. Invest.* 71:1141–1160.

38. Rector, F. C. 1973. Acidification of the urine. *Handb. Physiol.* 00:431–454.

39. Seifter, J. L., and R. C. Harris. 1984. Chronic K depletion increases Na-H exchange in rat renal cortical brush border membrane vesicles. *Kidney Int.* 25:282a.

40. 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. *Am. J. Physiol.* 20:F904–910.

41. Jones, B., and D. P. Simpson. Influence of alterations in acid-base conditions on intracellular pH of intact renal cortex. 1983. *Renal Physiol.* 6:28–35.

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

43. Schrader, G. A., C. O. Prickett, and W. D. Salmon. 1935. Symptomatology and pathology of potassium and magnesium deficiencies in the rat. *J. Nutr.* 14:85–110.

44. Walsh-Reitz, M. M., and F. G. Toback. 1983. Kidney epithelial cell growth is stimulated by lowering extracellular potassium concentration. *Am. J. Physiol.* 244:C429–432.

45. Moolenaar, W. H., R. Y. Tsien, P. T. van der Saag, S. W. de Laat. 1982. Na^+/H^+ exchange and cytoplasmic pH in the action of growth factors in human fibroblasts. 1983. *Nature (Lond.).* 384:645–648.

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

47. McKinney T. D., and K. K. Davidson. 1987. Effect of potassium depletion and protein intake in vivo on renal tubular bicarbonate transport in vitro. *Am. J. Physiol.* 252:F509–F516.

48. Cooke, R. E., W. E. Segar, B. D. Cheek, F. E. Coville, and D. C. Darrow. 1952. The extrarenal correction of alkalosis associated with potassium deficiency. *J. Clin. Invest.* 31:798–805.

49. Cemerikic, D., C. S. Wilcox, and G. Giebisch. 1982. Intracellular potential and K^+ activity in rat kidney proximal tubular cells in acidosis and K^+ depletion. *J. Membr. Biol.* 69:159–165.

50. Akiba, T., V. K. Vito, and D. G. Warnock. 1987. Parallel adaptation of the rabbit renal cortical sodium/proton antiporter and sodium/bicarbonate cotransporter in metabolic acidosis and alkalosis. *J. Clin. Invest.* 80:308–315.