Dietary acid increases blood and renal cortical acid content in rats

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Wesson, Donald E. Dietary acid increases blood and renal cortical acid content in rats. *Am. J. Physiol.* 274 (*Renal Physiol.* 43): F97–F103, 1998.—We examined whether dietary acid that increases net acid excretion (NAE) without measurably decreasing plasma pH or total CO_2 (tCO_2) causes net acid retention. Control rats drinking distilled H_2O were compared with those drinking 40 mM $(NH_4)_2SO_4$, 40 mM Na_2SO_4, or drinking Na_2SO_4 and given aldosterone (Na_2SO_4 + Aldo) to increase NAE without dietary acid. Systemic plasma tCO_2 increased in Na_2SO_4 + Aldo animals, but systemic and stellate vessel plasma tCO_2 and pH were not different from control among remaining groups. NAE increased in $(NH_4)_2SO_4$ and Na_2SO_4 + Aldo but not in Na_2SO_4 animals. Blood base excess (BBE) decreased compared with its respective baseline in $(NH_4)_2SO_4$ (-0.44 ± 0.06 vs. 0.66 ± 0.04 μmol/ml; $P < 0.01$, paired *t*-test), increased in Na_2SO_4 + Aldo (0.79 ± 0.05 vs. 0.61 ± 0.03 μmol/ml; $P < 0.04$, paired *t*-test), but was unchanged in Na_2SO_4 animals. Renal cortical H^+ content assessed by microdialysis of the renal cortex in situ increased in $(NH_4)_2SO_4$, decreased in Na_2SO_4 + Aldo, but was unchanged in Na_2SO_4 animals. The data show that dietary acid sufficient to increase NAE without decreasing plasma tCO_2 or pH nevertheless decreases BBE and increases renal cortical acid content, consistent with net acid retention.

acidification; base excess; buffer; diet; kidney interstitium; microdialysis

DIETARY ACID INCREASES renal acid excretion (4, 5, 16), but the physiological alteration induced by this dietary maneuver that mediates a sustained increase in renal acidification is not clear. This laboratory has shown that a dietary acid protocol that does not measurably decrease plasma pH or total CO_2 (tCO_2) nevertheless increases distal tubule acidification and renal acid excretion (16). These data might suggest that physiological responses to dietary acid fully rids the animal of ingested acid and completely normalizes acid-base status. This scenario would suggest that parameters other than altered acid-base status mediate a sustained increase in renal acidification induced by chronic dietary acid. Alternatively, because chronically administered acid is buffered mostly in tissues rather than in plasma (9), acid retention induced by this dietary protocol might be inadequately reflected by quantitative alterations in plasma acid-base parameters. Tissue acid content might more accurately reflect acid retention induced by this dietary change.

The present studies tested the hypothesis that dietary acid sufficient to increase renal acid excretion without measurably altering plasma acid-base parameters nevertheless causes acid retention. Blood base excess (BBE) was quantitated in rats with standard titration techniques, and renal parenchymal acid content was assessed using microdialysis of the renal cortex. The data show that dietary acid decreases BBE and increases renal cortical acid content, consistent with net acid retention.

MATERIALS AND METHODS

Male and female Munich-Wistar rats (Harlan Sprague Dawley, Houston, TX), 247–311 g, eating a minimum electrolyte diet (ICN Nutritional Biochemicals, Cleveland, OH) were used. Control animals drank distilled H_2O with this diet. The highest $(NH_4)_2SO_4$ concentration that rats would consistently drink was 40 mM. Seven days of this drinking solution with this diet increased distal tubule acidification and urine acid excretion (16) and was the acid-ingesting protocol used. Two additional control groups were studied. First, animals drinking 40 mM Na_2SO_4 were studied to control for sulfate ingestion. Second, animals drinking Na_2SO_4 additionally received daily intramuscular injections of 7 μg/100 g body wt aldosterone monoacetate (Sigma Chemical, St. Louis, MO) suspended in 0.5 ml corn oil to increase renal acid excretion (13) without augmented dietary acid. The remaining three groups received daily intramuscular injections of 0.5 ml corn oil without aldosterone. Preliminary studies showed that rats of similar weight ingested 16.7 ± 0.6 g/day of diet when drinking distilled H_2O, 16.9 ± 0.7 g/day when drinking the Na_2SO_4 solution, and 16.7 ± 0.8 g/day for the $(NH_4)_2SO_4$ solution. To ensure that each group ingested the same amount, animals received exactly 16 g/day. The diet contained 20% protein and the following electrolytes (in μeq/g of diet): 21.7 Na^+, 43.5 K^+, and 13.8 Cl^-. All animals underwent placement and securing of a heparinized polyethylene tube (PE-50) in the right carotid artery for chronic vascular access under ketamine hydrochloride (100 mg/kg body wt; Parke-Davis, Morris Plains, NJ) anesthesia and allowed a 7-day recovery. The arterial line was flushed daily with 10% heparin in 5% dextrose in H_2O and capped with a metal plug. The exterior end of the arterial line was placed in a stainless steel spring to prevent damage and sutured to a skin site on the animal's back from which its hair had been sheared. Each animal of the four groups [control, $(NH_4)_2SO_4$, Na_2SO_4, and Na_2SO_4 + aldosterone] underwent three consecutive periods of drinking solutions while in metabolic cages. During the baseline period (*week 1*), all animals ingested distilled H_2O. For the experimental period (*week 2*), animals drank the experimental solution for that group (control animals continued to drink distilled H_2O) and received the corn oil injections with or without aldosterone as described. During the recovery period (*week 3*), all animals drank distilled H_2O.

Whole blood titration. On *day 7* of each period, 1.35 ml of heparinized arterial blood was drawn anerobically from animals resting comfortably in a restraining device and replaced with an equivalent blood volume from an identically treated paired animal. Arterial blood gases were measured with a blood gas analyzer (Radiometer, Copenhagen, Denmark), and plasma tCO_2 was measured by flow-through ultrafluorometry (12), as done in this laboratory (16) with 0.35 ml of the blood.

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The remaining 1 ml was briefly bubbled with 100% O_2, placed under H_2O- and 5% CO_2-equilibrated mineral oil, then titrated (Radiometer PHM82 meter, TTT80 titrator, and ABU80 autoburette) to pH 7.4 at 37°C with 0.1 N HCl when initial pH was >7.4 and with 0.1 N NaOH when ≤7.4. The technique normalizes blood pH to a constant P_{CO_2} with added acid or alkali, uncovering any metabolic differences in blood acid-base content. This "blood base excess" was designated with a positive sign when acid was added to achieve pH 7.4 and with a negative one when alkali was added. An aliquot spun in a capillary tube measured hematocrit and plasma protein by refractometry of the supernatant because these parameters influence BBE measurement (10).

Urine net acid excretion. Daily net acid excretion (NAE) was determined from urine excretion of HCO_3, NH_4^+, and titratable acid (TA) on *day 7* of each period. Urine was obtained anerobically from animals by percutaneous bladder puncture while under ketamine anesthesia as described (14). Urine pH was immediately determined on a blood gas analyzer (Radiometer), and NH_4^+ was measured with an ion-specific electrode and a flow-through cell (Orion Research, Cambridge, MA). Urine TA was measured using standard titrametric techniques. After anesthesia recovery, animals were returned to metabolic cages to ingest drinking solutions. Cumulative urine NAE was determined for each 7-day period in six additional animals per group, from which no blood was taken. For *week 1*, bladder urine was obtained as described on only *day 7*, and urine NAE for that period was calculated by multiplying the value for that day times seven. Daily urine NAE determinations were done for the subsequent two periods.

In vivo microperfusion of distal tubules. Animals were prepared for micropuncture of accessible distal tubules as described (18). Distal tubules were perfused at the early distal flow rate measured in situ (6 nl/min) (15), calibrated in vitro, and verified in vivo (18). An injected latex cast determined perfused tubule length after subsequent acid digestion of the kidney (18). Diet, but not drinking solution, was withheld the evening before study, yielding higher baseline HCO_3 reabsorption (6) and permitting differences in HCO_3 reabsorption to be more clearly seen. The perfusing solution contained the following (in mM): 41 Na^+, 4 K^+, 40 Cl^-, 5 HCO_3, and 200 raffinose. Perfusate HCO_3 concentration ($[HCO_3]$) and Cl^- concentration ($[Cl^-]$) were chosen to approximate these anion concentrations in early distal tubule fluid of control animals (15). Raffinose minimized fluid transport and thereby permitted a more focused study of HCO_3 transport (18). Infused and collected perfusate was analyzed for tCO_2 using flow-through fluorometry (see below).

Measurement of stellate vessel tCO_2 and pH. tCO_2 was measured in stellate vessel plasma using flow-through fluorometry (12) as done previously in this laboratory (16). Stellate vessel pH was measured in situ as described (2) using a glass-membrane pH microelectrode constructed as described (8). Briefly, the microelectrode was pulled from Corning 1720 glass (Electric Glass and Supply, Modesto, CA) redrawn to 1 mm outer diameter and ground to a tip diameter of 8 μm. The tip was covered with glass (World Precision Instruments, Sarasota, FL) composed of the following by weight (%): 60 SiO_2, 4 UO_2, 8 MgO, and 28 Na_2O. The electrode was filled with 1 M magnesium acetate, and a silver-silver chloride electrode was inserted in the open end and sealed in place with molten dental wax. The potential difference developed across the pH membrane was measured by connecting the silver portion of the silver-silver chloride electrode to the high-impedance input of an electrometer connected to an earth ground. The electrode tip was immersed in test solutions (standard buffers to pH 4.0, 6.84, 7.0, 7.384, and 10.0) at 37°C. The low input of the electrometer was connected to the test solution through a reference calomel in series with a variable-bias control box. Potential output was recorded on a multipen recorder. Slope of millivolts vs. pH relationship was 56.8 mV/pH for reference standards with pH 7.0 and 4.0 intercepts = 148 and 317 mV, respectively. Simultaneous pH measurements were done with the glass microelectrode and a glass macroelectrode (Corning ion analyzer, model 255) in blood under H_2O- and 6.7% CO_2-equilibrated mineral oil (this covers the exposed kidney during micropuncture) from control rats to which HCl, NaOH, or saline was added in vitro to create a physiological pH range. Table 1 shows the two methods yielded similar pH measurements.

Microdialysis of renal cortex. Relative renal cortical H^+ content was determined among the four groups by quantitating H^+ added to infused dialysate during in situ microdialysis of the renal cortex (11) as done previously in this laboratory (17). Changes in dialysate P_{CO_2} and tCO_2 were also measured to determine the altered H^+ concentration ($[H^+]$) component that mediated any changes in dialysate H^+ content. A microdialysis apparatus was constructed from a 5-mm-long piece of hollow fiber dialysis tubing (mol mass cutoff, 5,000 kDa; Hospal, Meyzieu, France) with 0.1 mm inner diameter as described (11). Each end of the dialysis tubing was connected to a 25-cm-long polyethylene tube (0.12 mm inner diameter, 0.65 mm outer diameter; Bioanalytical Systems, Indianapolis, IN) and sealed in place with cyanoacrylic glue (11). The left kidney was exposed through a flank incision in rats anesthetized with ketamine (100 mg/kg). The renal capsule was penetrated with a 31-gauge needle tunneled in the outer renal cortex ~1 mm from the renal surface for ~0.5 mm before exiting by penetrating the renal capsule again. The tip of the needle was inserted into one end of the dialysis probe, and the needle was pulled together with the dialysis tube until the dialysis fiber was situated within the renal cortex. The inflow and outflow tubes of the dialysis probe were tunneled subcutaneously through a bevel-tipped tube and exteriorized near the interscapular region. Subcutaneous tissue was closed with 3–0 prolene suture, and skin was closed with clips. A heparinized polyethylene tube (PE-50) was placed and secured in the right carotid artery for vascular access. The arterial line was flushed daily with 10% heparin in 5% dextrose in water and then capped with a metal plug. Exterior ends of the dialysis tubes and arterial line were sutured to skin on the animal's back sheared of hair. The exteriorized portions of the tubes were placed in a stainless steel spring to prevent damage.

Three procedures were done in an effort to identify the renal cortical fluid compartment with which the dialysis apparatus interfaced. First, 5% dextrose in H_2O was infused, and $[Na^+]$ and $[K^+]$ were measured in collected dialysate to determine whether the apparatus interfaced with intracellular fluid, consistent with ongoing cellular destruction. Col-

Table 1. *Simultaneous microelectrode and macroelectrode pH measurements in blood from control rats*

	Samples				
	1	*2*	*3*	*4*	*5*
Microelectrode	7.174	7.256	7.363	7.458	7.538
Macroelectrode	7.189	7.274	7.381	7.474	7.551

HCl or NaOH was added in vitro to some samples to create a physiological pH range.

lected dialysate [Na^+] and [K^+] were 127 ± 3.2 and 3.1 ± 0.3 meq/l, respectively, not consistent with intracellular fluid. To determine whether electrolyte constituents of systemic plasma could enter dialysate, we measured the entry of systemically infused $^{22}NaCl$ into dialysate through the microdialysis apparatus placed in the kidney as described. In vitro $^{22}NaCl$ recovery, evaluated by immersing dialysis membranes of five identically constructed probes into a solution of $^{22}NaCl$, was 99%. In four animals with the microdialysis apparatus given a 0.5 mCi intravenous bolus of $^{22}NaCl$ followed by 0.5 mCi/h, $^{22}NaCl$ concentration in collected dialysate was 96% of that in plasma. These data support ready communication between plasma and an extracellular fluid compartment in the renal cortex that interfaces with the microdialysis apparatus. Third, we quantitated possible contamination of the interfacing fluid compartment in the renal cortex with tubule contents. This was done by comparing [^{3}H]inulin concentration in late proximal tubules and collected dialysate. In vitro [^{3}H]inulin recovery, evaluated by immersing dialysis membranes of five identically constructed probes into a [^{3}H]inulin solution, was 89%. Five animals with the microdialysis apparatus in place were infused intravenously with 1 mCi of [^{3}H]inulin. The [^{3}H]inulin concentration in collected dialysate was 4.7% of that in late proximal tubules, consistent with minimal communication with tubule contents. Altogether, the data suggest that the renal cortical fluid compartment interfacing with the microdialysis apparatus is extracellular, is minimally contaminated with tubule contents and communicates with plasma electrolytes. Candidates include renal interstitial fluid as reported by others using similar techniques (11), but the present data do not exclude contributions from renal vascular and/or lymph fluid. Histological studies of tissue surrounding the inserted dialysis tubing of four animals 14 days after insertion of the microdialysis apparatus show only minimal amounts of fibrous tissue adjacent to the dialysis tube and a small number of predominantly mononuclear white blood cells.

Microdialysis of the renal cortex was done in comfortably restrained, conscious animals on *day 7* of each period. The inflow tube was connected to a gas-tight syringe filled with Ringer HCO_3 solution modified as described below and equilibrated with 6.7% CO_2. The CO_2% was chosen to approximate P_{CO_2} in rat renal cortex (2), recognizing that actual renal cortical P_{CO_2} levels remains controversial (3). The dialysate was infused at 3 μl/min (Harvard Apparatus), a flow rate determined to be optimal by others (11). The infusion pump was calibrated by measuring the volume of a timed solution infusion onto a siliconized glass slide under H_2O-equilibrated mineral oil. Preliminary studies produced a dialysate that when perfused as described in control animals yielded no net change in H^+ content (i.e., there was no difference in H^+ content between collected and infused dialysate). We reasoned that such a solution would gain H^+ when dialyzed against renal cortex with higher-than-control H^+ content and would lose H^+ if it were lower. This goal was achieved (see RESULTS) using Ringer HCO_3 with [HCO_3] = 26 meq/l and CO_2 equilibration as described. Three 20-min collection periods were done in five each of $(NH_4)_2SO_4$, Na_2SO_4, Na_2SO_4 + aldosterone, and control animals. Anerobically obtained, collected, and infused dialysate were analyzed for pH (Micro flow-through pH monitor; Lazar Research, Los Angeles, CA), P_{CO_2} (Micro flow-through CO_2 probe, Lazar Research), and tCO_2 by flow-through fluorometry.

Analytical methods. Collected and infused dialysate as well as arterial plasma were immediately analyzed for tCO_2 using flow-through ultrafluorometry as described (16). All samples were measured on the experimental day by comparing fluorescence of a 7- to 8-nl sample aliquot (corrected for a distilled H_2O blank run with each sample group) to a standard curve. A standard curve was constructed for each sample run using an identical volume of the following $NaHCO_3$ standards: 0, 2.5, 5, 10, 25, and 50 mM.

Calculations. Daily NAE was [NH_4^+] + (TA − [HCO_3]) multiplied times urine volume for that day. Net dialysate H^+ addition was calculated by multiplying the [H^+] difference between collected and infused dialysate (from the measured pH) times total volume of collected dialysate (3 μl/min × 20 min = ~60 μl). A positive value for net H^+ addition indicated greater H^+ content in collected compared with infused dialysate (i.e., H^+ gain). Net H^+ addition for each of the three collection periods were meaned to obtain a single value for that animal. This value was then meaned for each animal for a group value.

Statistical analysis. Results are means ± SE. Statistical significance was determined using analysis of variance when comparing more than two means. The Bonferroni method was used for *t*-test comparison of means ($P < 0.05$) for multiple different comparisons of the same parameter among the three groups. Student's *t*-test for paired observations was used where appropriate.

RESULTS

Animal growth with ingestion of drinking solutions. Body weights were not different among control, $(NH_4)_2SO_4$, Na_2SO_4, and Na_2SO_4 + aldosterone animals beginning *week 1* (266 ± 8, 268 ± 6, 269 ± 9 and 273 ± 9 g, respectively) or ending *week 3* (311 ± 10, 307 ± 9, 313 ± 11, and 314 ± 12 g, respectively). Ingested solution volume was similar among $(NH_4)_2SO_4$, Na_2SO_4, and Na_2SO_4 + aldosterone animals [38.7 ± 2.8, 40.9 ± 2.9, and 45.2 ± 3.4 ml/day, respectively; P = not significant (NS)] but that for each experimental group was higher than control (21.3 ± 1.8 ml/day, respectively, $P < 0.01$ for each experimental group).

Blood and urine changes. As depicted in Table 2, daily NAE during the characteristic intervention (*week 2*) was higher than that for the initial H_2O-ingesting period (*week 1*) in $(NH_4)_2SO_4$ and Na_2SO_4 + aldosterone animals but decreased in both groups at the end of the second H_2O-ingesting period (*week 3*). Plasma tCO_2 was higher than at *week 2* compared with *week 1* in the Na_2SO_4 + aldosterone animals but was not different from the *week 1* value in the remaining groups. Blood hematocrit and plasma protein were not different when comparing experimental periods within groups or comparing respective periods among groups. Cumulative 7-day NAE shown in Fig. 1 was higher at *week 2* than at *week 1* in $(NH_4)_2SO_4$ (39.8 ± 2.4 vs. 18.5 ± 1.5 meq/7 days, $P < 0.001$) and Na_2SO_4 + aldosterone (50.1 ± 3.9 vs. 18.9 ± 1.7 meq/7 days, $P < 0.001$) animals. By contrast, the *week 3* value for these two groups [$(NH_4)_2SO_4$ = 25.9 ± 2.2 meq/7 days; Na_2SO_4 + aldosterone = 32.3 ± 3.1 meq/7 days] was lower than the respective values at *week 2* ($P < 0.04$ for both groups) but higher than that at *week 1* ($P < 0.03$ for both groups).

Distal tubule bicarbonate reabsorption. Figure 2 shows that distal tubule net HCO_3 reabsorption was higher than control (11.7 ± 1.2 pmol·mm^{-1}·min^{-1}) in $(NH_4)_2SO_4$ (22.1 ± 1.7 pmol·mm^{-1}·min^{-1}, $P < 0.005$)

Table 2. *Blood and plasma parameters and urine net acid excertion when drinking H_2O at baseline (week 1), during ingestion of experimental drinking solution (week 2), and after returning to H_2O (week 3)*

	Blood				Plasma Protein, g/dl	Urine NAE, meq/day
	pH	P_{CO_2}, mmHg	tCO_2, mM	Hematocrit, %		
			Control, n = 6			
Week 1, H_2O	7.42 ± 0.03	39.5 ± 1.4	26.0 ± 1.3	47.9 ± 0.8	4.3 ± 0.2	2.6 ± 0.3
Week 2, H_2O	7.41 ± 0.04	40.3 ± 1.7	25.7 ± 1.4	47.8 ± 0.7	4.2 ± 0.1	2.8 ± 0.3
Week 3, H_2O	7.41 ± 0.03	40.9 ± 1.6	26.1 ± 1.5	48.2 ± 0.8	4.2 ± 0.1	2.8 ± 0.3
			$(NH_4)_2SO_4$, n = 5			
Week 1, H_2O	7.41 ± 0.03	40.4 ± 1.5	25.8 ± 1.7	48.4 ± 0.7	4.2 ± 0.1	2.7 ± 0.3
Week 2, $(NH_4)_2SO_4$	7.39 ± 0.04	39.2 ± 1.3	24.5 ± 1.4	47.5 ± 0.6	4.2 ± 0.2	5.7 ± 0.6*
Week 3, H_2O	7.40 ± 0.04	41.0 ± 1.4	25.7 ± 1.5	47.9 ± 0.9	4.1 ± 0.1	2.9 ± 0.2†
			Na_2SO_4, n = 5			
Week 1, H_2O	7.40 ± 0.05	39.6 ± 1.4	25.2 ± 1.4	48.3 ± 0.6	4.1 ± 0.1	2.5 ± 0.3
Week 2, Na_2SO_4	7.41 ± 0.03	41.3 ± 1.6	26.5 ± 1.5	47.6 ± 0.8	4.1 ± 0.2	3.1 ± 0.3
Week 3, H_2O	7.39 ± 0.03	41.9 ± 1.4	26.0 ± 1.4	47.4 ± 0.7	4.2 ± 0.1	2.8 ± 0.3
			Na_2SO_4 + aldosterone, n = 5			
Week 1, H_2O	7.39 ± 0.03	41.4 ± 1.5	25.3 ± 1.5	47.8 ± 0.7	4.1 ± 0.1	2.6 ± 0.3
Week 2, Na_2SO_4 + aldosterone	7.46 ± 0.04	43.5 ± 1.5	30.6 ± 1.7*	48.8 ± 0.8	4.4 ± 0.2	8.1 ± 0.7*
Week 3, H_2O	7.42 ± 0.04	41.3 ± 1.4	27.0 ± 1.5	48.0 ± 0.9	4.2 ± 0.1	3.9 ± 0.4†

Values are means ± SE; *n*, no. of animals. Chemical designations refer to drinking solution for the group at *week 2*. NAE, net acid excretion; tCO_2, total CO_2. *$P < 0.05$ vs. respective value at *week 1*, paired *t*-test. †$P < 0.05$ vs. respective value at *week 2*.

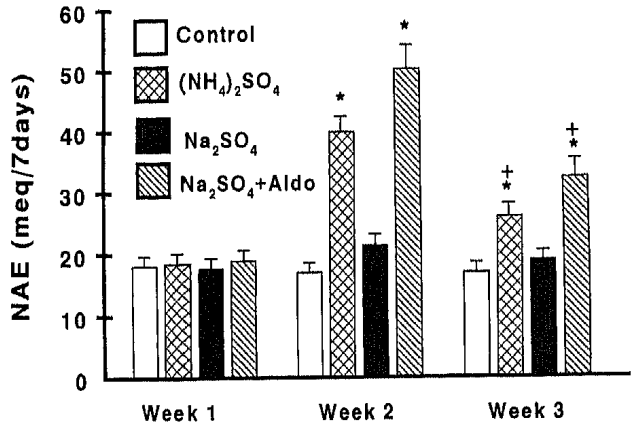

Fig. 1. Urine net acid excretion (NAE) in control, $(NH_4)_2SO_4$-ingesting, Na_2SO_4-ingesting, and Na_2SO_4-ingesting animals additionally given intramuscular aldosterone (Aldo). All animals received distilled H_2O during *week 1*, characteristic intervention described during *week 2*, and distilled H_2O again during *week 3*. *$P < 0.05$ vs. respective *week 1* value. +$P < 0.05$ vs. respective *week 2* value.

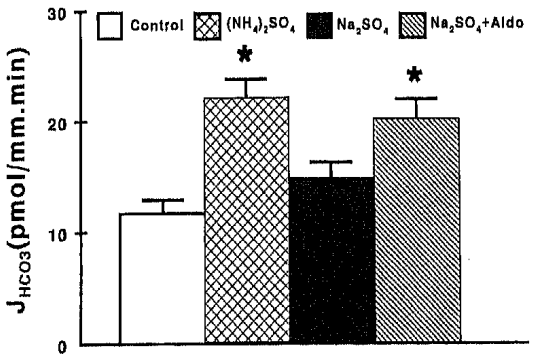

Fig. 2. Net HCO_3 reabsorption (J_{HCO_3}) in distal tubules determined by in vivo microperfusion 1 wk after undergoing the characteristic intervention in control, $(NH_4)_2SO_4$-ingesting, Na_2SO_4-ingesting, and Na_2SO_4-ingesting animals additionally given aldosterone (Aldo). *$P < 0.05$ vs. control.

and Na_2SO_4 + aldosterone (20.2 ± 1.8 pmol·mm^{-1}·min^{-1}, $P < 0.02$) animals, but that for the Na_2SO_4 (14.9 ± 1.4 pmol·mm^{-1}·min^{-1}) animals was not different from control.

Stellate vessel tCO_2 and pH. Stellate vessel tCO_2 and pH were not different among groups 1 wk after ingesting their characteristic drinking solutions, as shown in Table 3.

Blood base excess. Compared with respective *week 1* values, Fig. 3 shows that BBE at *week 2* was lower in $(NH_4)_2SO_4$ (−0.44 ± 0.06 vs. 0.66 ± 0.04 μmol/ml, $P < 0.001$) but was higher in Na_2SO_4 + aldosterone (0.79 ± 0.05 vs. 0.61 ± 0.04 μmol/ml, $P < 0.03$) animals. BBE in control and Na_2SO_4 animals was not different among the three periods. At the end of *week 3*, BBE in the $(NH_4)_2SO_4$ animals (0.48 ± 0.03 μmol/ml) was higher than that for this group at *week 2* ($P < 0.01$) but was not different from the respective *week 2* value in the remaining groups.

Renal cortical acid content. Microdialysis collected dialysate volume was not different from that of an identically timed infusion onto a glass slide under H_2O-equilibrated mineral oil (see MATERIALS AND METHODS) for control (60.4 ± 3.0 vs. 59.7 ± 2.5 μl, respectively; P = NS), $(NH_4)_2SO_4$ (59.3 ± 2.3 vs. 60.2 ± 1.9 μl, respectively; P = NS), Na_2SO_4 (59.5 ± 1.8 vs. 60.8 ± 2.2 μl, respectively; P = NS), and Na_2SO_4 + aldosterone

Table 3. *Stellate vessel tCO_2 and pH*

	Control, n = 6	$(NH_4)_2SO_4$, n = 6	Na_2SO_4, n = 6	Na_2SO_4 + Aldosterone, n = 5
tCO_2, mM	33.0 ± 1.4	30.1 ± 1.3	33.7 ± 1.3	35.9 ± 1.4
pH	7.25 ± 0.01	7.22 ± 0.01	7.26 ± 0.01	7.29 ± 0.01

Values are means ± SE; *n*, no. of animals. Chemical designations refer to content of drinking solution.

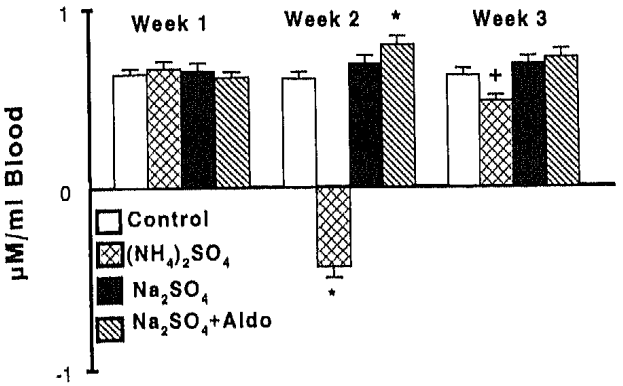

Fig. 3. Blood base excess in control, $(NH_4)_2SO_4$-ingesting, Na_2SO_4-ingesting, and Na_2SO_4-ingesting animals additionally given aldosterone (Aldo). All animals received distilled H_2O during *week 1*, characteristic intervention described during *week 2*, and distilled H_2O again during *week 3*. $^*P < 0.05$ vs. respective *week 1* value. $^+P < 0.05$ vs. respective *week 2* value.

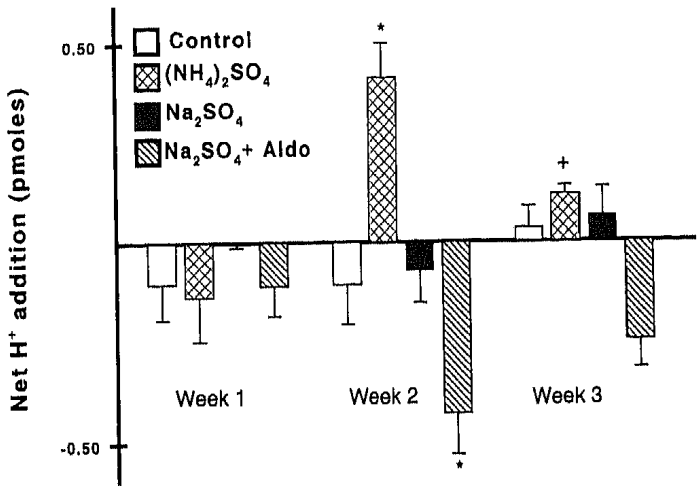

Fig. 4. Net H^+ addition to collected dialysate after in situ microdialysis against renal cortex of control, $(NH_4)_2SO_4$-ingesting, Na_2SO_4-ingesting, and Na_2SO_4-ingesting animals additionally given aldosterone (Aldo). All animals received distilled H_2O during *week 1*, characteristic intervention described during *week 2*, and distilled H_2O again during *week 3*. $^*P < 0.05$ vs. respective *week 1* value. $^+P < 0.05$ vs. respective *week 2* value.

(61.2 ± 2.0 vs. 60.4 ± 1.9 μl, respectively; P = NS) animals. Table 4 shows no pH, P_{CO_2}, or tCO_2 differences between collected and infused dialysate of control animals. Furthermore, Fig. 4 shows that net H^+ addition to dialysate of control animals was not different from zero for all three periods (mean of all controls = −0.057 ± 0.078 pmol). By contrast, Table 4 shows that collected compared with perfused dialysate pH and tCO_2 was lower during *week 2* in $(NH_4)_2SO_4$ animals and higher in Na_2SO_4 + aldosterone animals for this same period. These differences were no longer present in either of these two groups at *week 3*. Collected compared with infused dialysate acid-base parameters were not different for Na_2SO_4 animals in any period. Figure 4 shows that *week 2* compared with *week 1* net H^+ addition to dialysate was higher in $(NH_4)_2SO_4$ animals (0.413 ± 0.088 vs. −0.136 ± 0.110 pmol, $P < 0.002$, paired t-test) and lower in the Na_2SO_4 + aldosterone animals (−0.430 ± 0.102 vs. −0.109 ± 0.075 pmol, $P < 0.02$, paired t-test). Net H^+ addition at *week 3* compared with *week 2* was lower in $(NH_4)_2SO_4$ animals, but that in Na_2SO_4 + aldosterone animals was not different. Net dialysate H^+ addition in Na_2SO_4 animals was not different among the three periods.

DISCUSSION

Dietary acid increases distal tubule acidification and urine NAE and can do so without measurable alterations in plasma pH and tCO_2 (16). Furthermore, humans given diets that induce a 10-fold increase in urine NAE have barely detectable changes in plasma pH and $[HCO_3]$ (4). Together, these studies might suggest that physiological responses to a persistent increase in dietary acid intake not only balance steady-state acid excretion with intake to prevent progressive acid retention but also dynamically correct net acid retention. The latter achievement would return acid-base status to that prior to increased dietary acid despite ongoing acid intake. Because augmented urine NAE is sustained in this setting, this explanation for unchanged plasma acid-base parameters suggests that factors other than altered acid-base status mediate the sustained increase in urine NAE. Alternatively, if altered acid-base parameters are necessary mediators of increased renal acidification as suggested (7), then

Table 4. *Dialysate acid-base parameters*

	Control, $n = 5$			$(NH_4)_2SO_4$, $n = 5$			Na_2SO_4, $n = 5$			Na_2SO_4 + aldosterone, $n = 5$		
	pH	P_{CO_2}	tCO_2	pH	P_{CO_2}	tCO_2	pH	P_{CO_2}	tCO_2	pH	P_{CO_2}	tCO_2
Week 1												
Infused	7.30 ± 0.03	56.7 ± 1.7	27.5 ± 1.3	7.30 ± 0.02	57.2 ± 1.7	27.6 ± 1.4	7.29 ± 0.02	57.0 ± 1.7	27.2 ± 1.5	7.30 ± 0.02	55.8 ± 1.6	27.0 ± 1.4
Collected	7.31 ± 0.03	55.0 ± 1.5	27.8 ± 1.3	7.30 ± 0.02	55.4 ± 1.7	27.3 ± 1.4	7.30 ± 0.02	55.9 ± 1.6	26.9 ± 1.5	7.31 ± 0.02	54.8 ± 15	27.7 ± 1.5
Week 2												
Infused	7.29 ± 0.03	55.9 ± 1.7	27.0 ± 1.2	7.30 ± 0.03	56.6 ± 1.7	27.3 ± 1.4	7.30 ± 0.02	56.7 ± 1.7	27.5 ± 1.5	7.30 ± 0.02	56.8 ± 1.6	27.4 ± 1.5
Collected	7.30 ± 0.03	54.8 ± 1.6	27.2 ± 1.3	7.25 ± 0.02*	55.4 ± 1.5	22.9 ± 1.3*	7.32 ± 0.02	54.8 ± 1.6	27.9 ± 1.5	7.36 ± 0.02*	56.1 ± 1.5	30.9 ± 1.6*
Week 3												
Infused	7.31 ± 0.03	54.1 ± 1.6	27.2 ± 1.3	7.29 ± 0.02	56.9 ± 1.6	27.0 ± 1.4	7.30 ± 0.02	55.9 ± 1.6	26.9 ± 1.4	7.29 ± 0.02	57.1 ± 1.6	26.8 ± 1.4
Collected	7.30 ± 0.03	53.5 ± 1.5	26.7 ± 1.4	7.28 ± 0.02	55.6 ± 1.7	26.1 ± 1.3	7.30 ± 0.02	55.0 ± 1.5	26.5 ± 1.5	7.32 ± 0.02	56.0 ± 1.6	28.4 ± 1.5

Values are means ± SE; n, no. of animals. Chemical designations refer to content of drinking solution. $^*P < 0.05$ vs. infused value, paired t-test. P_{CO_2} is in units of mmHg; tCO_2 is in units of mM.

undetectable decreases in static systemic or stellate plasma $[H^+]/[HCO_3]$ or other indicators of an acid challenge are sufficient to induce an increase in urine NAE. The present studies determined whether a dietary protocol shown to increase urine NAE without measurable decreases in plasma pH or tCO_2 nevertheless increases tissue acid content. The data show this dietary protocol, which increases urine NAE without measurably decreasing plasma pH or tCO_2, nevertheless decreases BBE and increases renal cortical acid content.

The present studies also show that BBE and renal cortical acid content return to levels not different from control after augmented acid intake stops, consistent with excretion of retained acid. During the H_2O-drinking period following acid ingestion (*week 3*), urine NAE was less than during acid ingestion (*week 2*) but was higher than that for the initial H_2O period (*week 1*). This higher-than-baseline NAE during *week 3* likely contributed to correcting net acid retention at the end of *week 2*. Higher NAE continued a short time into *week 3* after acid intake had stopped, suggesting that ongoing acid intake per se was not necessary to sustain increased NAE. Daily urine NAE fell progressively during this final period to a value not different from control by *day 7* (see Table 2), a time when BBE and renal cortical acid content were comparable to control. Together, these data are consistent with a role for retained acid in mediating increased urine NAE.

Many studies show that increased urine NAE helps prevent progressive acid retention in response to dietary acid. The present studies show that physiological responses to dietary acid, including increased NAE, indeed prevented a progressive decline in plasma $[H^+]/tCO_2$ but did not normalize BBE or renal cortical acid content until acid intake stopped. Thus renal and other responses were not sufficient to fully rid the animal of retained acid until acid intake stopped. The present studies suggest that a sustained increase in dietary acid might have adverse metabolic consequences (1), even when this acid intake does not cause a major decrease in plasma $[H^+]$ or tCO_2. Less $(NH_4)_2SO_4$ or other means of increasing dietary acid might yield different results. Nevertheless, the present studies show that dietary acid that increases NAE without measurably altering plasma $[H^+]/tCO_2$ still causes acid retention. Other investigators reached similar conclusions using computational techniques (rather than direct techniques as in the present studies) to "normalize" blood pH and $[HCO_3]$ to a constant P_{CO_2} to demonstrate an inverse relationship between blood $[H^+]/[HCO_3]$ and NAE in humans ingesting diets with wide-ranging acid-base contents (4). Interestingly, the net BBE decrease in $(NH_4)_2SO_4$ animals of the present studies (0.66 to −0.44 μmol/ml or 1.1 μmol/ml; Fig. 3) is similar to the quantitative (but not statistically significant) fall in plasma tCO_2 (25.8 to 24.5 meq/l or 1.3 μmol/ml). Physiological mechanisms might have the sensitivity to detect and respond to such small changes in plasma tCO_2 or $[H^+]$. Alternatively, the resulting acid retention might be more accurately reflected (and possibly recognized physiologically) by phenomena other than decreased plasma $[H^+]/tCO_2$. Indicators of acid retention that might induce augmented NAE include titration level and/or quantity of tissue buffers, intracellular $[H^+]$, or $[H^+]$ in an extracellular compartment other than plasma (see below). Whether changes in these or possibly other indicators are sufficient to induce renal mechanisms of increased NAE without changes in plasma acid-base parameters awaits further study.

Microdialysis has been used to measure renal interstitial levels of biological substances (11, 17) but was adapted to assess renal cortical H^+ content in the present studies. The dialysate interfaces with a renal extracellular compartment that is minimally contaminated by tubule contents (see MATERIALS AND METHODS). Nevertheless, contamination of the fluid compartment that interfaces with the microdialysis apparatus by tubule contents might influence net H^+ addition to dialysate. If so, then greater net H^+ addition to dialysate of $(NH_4)_2SO_4$ animals with stimulated distal tubule acidification (Fig. 2) might be due to leakage of acidified tubule fluid into the compartment interfacing with the microdialysis apparatus. This explanation is not supported by Fig. 4, which shows lower dialysate H^+ addition in Na_2SO_4 + aldosterone animals that also had stimulated tubule acidification. These data suggest that tubule acidification had little influence on dialysate H^+ addition and that dietary acid increased H^+ content of the renal cortex.

Whether the extracellular compartment interfacing with dialysate was indeed renal interstitial fluid (RIF), as determined by others (11), plasma, or both was not definitively determined in the present studies. The increment in renal cortical H^+ content induced by dietary acid might contribute to increased renal acidification and likely entered the kidney from plasma. Indeed, electrolytes can enter the compartment that interfaces with the microdialysis apparatus from plasma (see MATERIALS AND METHODS). When H^+ addition is compared with plasma and interstitial fluid, lower protein content of the latter is consistent with lower buffering capacity and a steeper $[H^+]$ rise in RIF. A compartment whose $[H^+]$ changes briskly in response to added H^+ theoretically would be an ideal one to be exposed to renal $[H^+]$ sensing mechanisms, thereby enabling initiation of the cascade leading to augmented renal acidification before acid retention becomes severe. RIF simultaneously interfaces with blood (the likely source of H^+) and renal tubules (which excrete the excess H^+), offering a strategic location for a compartment to serve such a purpose. Testing this hypothesis requires a mechanism of H^+ transport into RIF and mechanisms of sensing its entry and await future studies.

Infused dialysate fluid was designed so that there was no net H^+ addition when interfaced with the renal cortex in situ (see MATERIALS AND METHODS). The fact that the pH of this fluid was lower than pH of systemic arterial plasma suggests that renal cortical extracellular $[H^+]$ is greater than that of systemic arterial

plasma, assuming ready diffusability of P_{CO_2} and $H^+/[HCO_3]$ across the microdialysis membrane. That the renal cortex is acid compared with systemic arterial plasma is supported by lower stellate vessel compared with systemic plasma pH in all groups of the present studies and in previous studies by other investigators (2). The comparatively acid environment of the rat renal cortex might promote renal tubule H^+ secretion in these animals which ordinarily excrete an acid urine. A further increase in the acid content of the renal cortex and/or other tissues might mediate the augmented tubule acidification induced by dietary acid.

In summary, the present studies show lower BBE and higher renal cortical H^+ content, consistent with net acid retention, in acid-ingesting animals with increased urine NAE but with measurably unchanged plasma acid-base parameters. The studies additionally show that these indicators of net acid retention reverted to levels not different from control when acid ingestion stopped. The data show that dietary acid causes net acid retention that is poorly reflected by altered plasma acid-base parameters and suggest that acid retention as determined by increased tissue acid content and/or other phenomena are sufficient mediators of the mechanisms of increased renal acid excretion.

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