The Effect of Uncomplicated Potassium
Depletion on Urine Acidification

RICHARD L. TANNEN

*From the Department of Metabolism, Walter Reed Army Institute of Research,
Washington, D. C. 20012*

A B S T R A C T Studies were performed on normal hu-
man subjects to determine the effects of potassium deple-
tion on urine acidification. Depletion was induced by
ingestion of a low potassium diet either alone or in
combination with a potassium-binding resin, and the
response of each subject to an acute ammonium chloride
load in the potassium-depleted state was compared to
his normal response. Urine pH was significantly higher
during potassium deficiency if sufficient potassium de-
pletion had been induced. No differences in blood acid-
base parameters, urinary flow rate, or urinary fixed
buffer excretion rate were found to account for this
change; however, the increase in urine pH was accom-
panied by a concomitant increase in net acid and am-
monium excretion. It is proposed that these changes
during potassium depletion reflect an increase in am-
monia diffusion into the urine, presumably as a result
of increased renal ammonia production. In addition, it
is speculated that changes in ammonia metabolism may
be a physiologic control mechanism for potassium con-
servation.

INTRODUCTION

Although the interrelationship between renal potassium
and hydrogen ion secretion and excretion have been
intensively studied (1), the effect of uncomplicated
potassium deficiency on urine acidification has received
little attention. In the few studies in humans of this
latter relationship, chronic potassium deficiency resulted
in an elevation of urine pH (2–4). It has been proposed
that this is the result of an inability to establish a nor-
mal blood to tubular fluid hydrogen ion gradient, i.e.,
an acquired distal renal tubular acidosis (2). Because

it appeared that a number of important variables had
not been considered in the design of these investiga-
tions, a reevaluation of the problem was undertaken.

The results of the present study demonstrate an un-
equivocal effect of potassium depletion on urine acidifi-
cation. They indicate that the elevation in urine pH is
the result of a primary increase in ammonia diffusion
into the urine rather than the result of an acquired
renal tubular acidosis. In addition, these findings sug-
gest possible new insight into the mechanism of the
renal control of potassium excretion.

METHODS

Urine acidification was evaluated by means of a modified
short ammonium chloride test (5) in nine men (ages 20–
26 yr) with normal renal function; at least once under
normal conditions, and at one or more degrees of potas-
sium depletion. All subjects were volunteers who gave
informed consent.

Five of the subjects had no history of significant ante-
cedent illness. Patient Da had idiopathic hypercalciuria with-
out evidence of nephrolithiasis. Three patients (D, B, and
Mi) had malaria with apparent cure 1–2 months before
study. Two of them (B and Mi) had a transient rise in
blood urea nitrogen (BUN) to approximately 35 mg/100
ml and transient mild proteinuria during the acute phase of
malaria. At the time of study, every patient had a normal
blood pressure, urinalysis, creatinine clearance, serum cal-
cium, phosphorus, uric acid, total protein, and globulin
concentrations; aminoaciduria was absent, and the urine
culture was negative.

A low electrolyte food powder [1] supplemented with sodium
chloride and served as a liquid formula was ingested daily
preceding both the normal and the potassium-depleted, am-
monium chloride studies. The food powder which contains
calcium caseinate, corn oil, and sucrose provides 21.9 g of
protein, 16.5 g of fat, 59.0 g of carbohydrate, 0.1 mmole
of sodium, 0.2 mmole of potassium, 0.25 mmole of magne-
sium, 10.0 mmoles of calcium, and 183 mg of phosphorus
per 100 g of food powder (6). The diet was given in quan-
tities to provide daily the following: 35 calories, 3 ml of
water, and 2 mmoles of sodium per kg of body weight.

Before the normal studies, the diet was ingested for 3 days and supplemented daily with potassium chloride providing 0.75 mmoles of potassium per kg of body weight. One patient (R) also underwent a second normal study preceded by 5 days of formula diet.

Varying degrees of potassium depletion were achieved in the following fashion: (a) 3 days of formula diet with potassium chloride being withdrawn on day 3 (one patient). (b) 3 days of formula diet without potassium chloride (two patients). (c) 5 days of formula diet without potassium chloride (six patients). (d) 5 days of formula diet without potassium chloride and with sodium-cycle sulfonic polystyrene cation exchange resin (Kayexalate)[2] ingestion (80 g/day) on days 1 and 2 (four patients).

The formula diet in the absence of potassium supplementation provided a daily intake of potassium of less than 1 mmole. During the potassium depletion periods, stool and urine were collected in order to quantitate the degree of potassium depletion.

The formula diet is deficient in magnesium (6); however, after 5 days of ingestion, a magnesium deficit of less than 2% of total body magnesium would be expected (7, 8). In every Kayexalate study, in two 5-day potassium depletion studies (R and Mc), and in three normal studies (N, S, and R-2), a daily magnesium chloride supplement of 4 mmoles was ingested. No difference was evident between the studies done with or without the magnesium supplement.

An interval of from 7 to 38 days occurred between acute ammonium chloride studies performed on an individual subject. The normal study antedated the potassium depletion studies in all patients with the exception of subject R, whose second normal study followed the potassium depletion studies.

The ammonium chloride study was perfomed in a similar fashion during the normal and potassium-depleted state. On the day of study the subjects were awakened at 6:30 a.m. and from then until the completion of the test remained in the upright position ingesting only water. A water load of 300–400 ml was given from 6:30 to 7:30 a.m., and approximately 100 ml/hr thereafter. After two 1-hr urine collections, ammonium chloride in gelatin capsules (2 mmoles/kg of body weight) was taken from 9:30 to 10:30 a.m. and eight subsequent hourly urines were collected. Urine was voided directly into flasks containing mineral oil and thymol. It was aspirated immediately into a syringe and stoppered, and pH and total CO_2 determinations were performed promptly. All other urine determinations were performed on frozen aliquots.

Venous blood for pH and total CO_2 determinations was collected before ingestion of ammonium chloride and 1, 2, 4, and 6 hr thereafter. These samples were drawn without stasis into heparinized plastic syringes, and the analyses were promptly performed. Blood electrolyte and creatinine determinations were performed on serum samples drawn before and 6 hr after ammonium chloride ingestion.

Chemical determinations. Blood and urine pH were determined anaerobically at 37°C with a Corning Model 12 blood pH system. Plasma and urine total CO_2 were determined manometrically with a Natelson microgasometer. (Scientific Industries, Inc., Springfield, Mass.). Chloride was measured with an Aminco Cotlove chloride titrator (American Instrument Co., Inc., Silver Spring, Md.). Stool potassium content was determined on nitric acid–perchloric acid digests. The following determinations were performed with a Technicon Autoanalyzer (Technicon

Corporation, Ardsley, N. Y.) using suitable modifications of the referenced techniques: sodium and potassium by flame photometry; phosphate (9); ammonium (10); and creatinine (11).

Total urinary organic acids and the contribution of organic acids to titratable acid were determined in the following fashion. Dephosphated urine specimens were titrated from pH 2.7 to 8.0 to determine total organic acids as described by Van Slyke and Palmer (12), and from urine to blood pH to determine the contribution of organic acids to titratable acid. Then both these values were corrected for the creatinine content of the specimen, assuming a pK_a' creatinine of 4.92 for the correction of titratable acid.

Calculation. Urine ionic strength (μ) was calculated as one-half the sum of the product of the molality of each measured ion and the square of its valence; and the product of the calculated unmeasured anion and the square of an assumed valence of one. Blood bicarbonate concentration and P_{CO_2} were calculated from the Henderson-Hasselbalch equation assuming a pK_a' of 6.10 and solubility coefficient of 0.0301; and urine values were calculated similarly using a pK_a' of (6.33 $- 0.5\sqrt{\mu}$) (13) and a solubility coefficient of 0.0309. Titratable acid was calculated from urine pH, blood pH, and urinary phosphate, and creatinine content, using a pK_a' phosphate of $(7.181 - 1.57 \sqrt{\mu})/(1 + 1.49 \sqrt{\mu}) + (\beta)\mu$ as documented experimentally by Schwartz, Bank, and Cutler (14), and a pK_a' creatinine of 4.92. Urine-free ammonia (NH_3) was calculated from the equation:

$$NH_3 = \text{total ammonia/antilog } (pK_a' - pH) + 1$$

using a pK_a' of $(8.89 + 0.5176 \sqrt{\mu})/(1 + 1.159 \sqrt{\mu})$ (15). Net acid was calculated as the sum of ammonium plus titratable acid minus bicarbonate. The data were calculated in part with an IBM 1401 computer using Fortran II code language.

RESULTS

Although control urine collections were made before the ingestion of ammonium chloride in all normal and potassium-depleted studies, only 8 of 10 studies from the 5-day potassium depletion protocols (both with and without Kayexalate) had mean control rates of urine flow of 0.5 ml/min or greater. In these eight studies control net acid excretion averaged 45.8 μmoles/min in the normal state and 44.2 μmoles/min during potassium depletion. The mean difference of 1.6 ± 8.7SD μmoles/min was not significant. Because of the variability in both rate of urine flow and phosphate excretion, these control observations were unsuitable for interpretation of the response to potassium depletion of urine pH and ammonium excretion.

The qualitative response to the ingestion of ammonium chloride by normal individuals in these studies has been previously described,[3] and the response in potassium-depleted subjects was similar. Because a relative plateau in urine pH and net acid excretion occurs from the 2nd hr (U_2) after the ingestion of ammonium chloride to the end of the study (U_8), these periods

[2] Winthrop Laboratories, New York.

[3] Tannen, R. L. 1969. The relationship between urine pH and acid excretion; the influence of urine flow rate. *J. Lab. Clin. Med.* 74: 757.
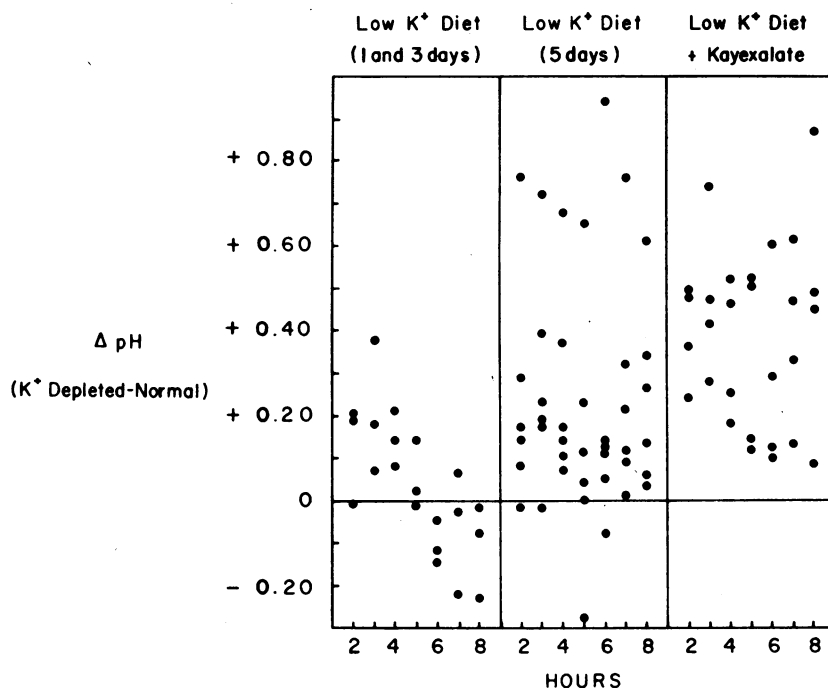
FIGURE 1 The effect of potassium depletion on urine pH. Each point in this and all subsequent figures represents a comparison between the response during a comparable time period of a paired potassium-depleted and normal study. Only the periods from 2 to 8 hr after the ingestion of ammonium chloride are plotted.

have been utilized in comparing the results of the normal and potassium-depletion studies. The mean values of each parameter during this time period (U_2-U_8) from each subject's potassium-depleted and normal study were compared and analyzed statistically utilizing a paired t test. In addition, every value obtained during a subject's potassium-depleted study was compared to the value obtained during the same time period of his normal study.

Urine pH. In Fig. 1, a comparison of the response of urine pH to the ingestion of ammonium chloride during the potassium-depleted and normal state is shown. In Table I, the mean urine pH (U_2-U_8)[4] during potassium-depleted and normal studies is recorded.

No difference in urine pH after the ingestion of ammonium chloride was noted between potassium-depleted and normal studies when potassium was absent from the diet for 1 or 3 days; nor between the two normal studies performed on subject R (Fig. 1).

---

[4] The mean pH reported were calculated by directly taking the arithmetic mean. All mean pH data were also calculated by converting individual pH values to hydrogen ion concentration, taking this arithmetic mean, and then converting back to pH. In no instance was the interpretation of the data affected by the method of calculating mean pH.

A statistically significant[5] increase in urine pH was noted in five of six studies when potassium was absent from the diet for 5 days; however, the magnitude of the change varied considerably from an increase in mean urine pH of 0.07 to 0.73 U (Fig. 1). The increase in mean urine pH of the entire group was from 4.97 to 5.20.

All four patients who received Kayexalate, in addition to ingesting the low potassium diet for 5 days, had a significant rise in urine pH; the increase in mean urine pH ranging from 0.15 to 0.52 U (Fig. 1). Mean urine pH for this entire group increased significantly ($P <$ 0.02) from 4.84 to 5.22 U.

Four subjects underwent two studies at different degrees of potassium depletion. Subject Da was studied after 1 and 3 days of potassium withdrawal; subject D after 3 and 5 days of potassium withdrawal; and subjects R and Mc after 5 days of potassium withdrawal alone, and also with Kayexalate ingestion. Da's response did not differ from normal at either level of potassium depletion. In the other three subjects urine pH was highest during the study in which they were the most severely depleted of potassium.

---

[5] The term significant will be used only to indicate events calculated to be statistically significant at the 0.05 level.

TABLE I
Mean Urinary Acid-

Subjects[*]	pH		Urine flow		Net acid		NH$_4$+	
	Norm.§	K+dep.‖	Norm.	K+dep.	Norm.	K+dep.	Norm.	K+dep.
			ml/min		μmoles/min		μmoles/min	
Δ pH less than 0.25								
Mi	4.68	4.79	1.8	2.2	54.8	51.7	35.3	35.5
A	4.94	5.12	1.4	1.9	81.7	81.9	50.2	56.7
R¶	5.03	5.02	2.5	1.9	72.1	76.6	57.7	57.8
Mc	4.86	4.93	1.7	1.0	98.3	85.9	67.6	58.2
Mc (Kaye)	4.86	5.01	1.7	1.4	98.3	93.2	67.6	65.8
Mean	4.87	4.97	1.8	1.7	81.0	77.9	55.7	54.8
P**	<0.05		NS		NS		NS	
Δ pH greater than 0.25								
D	5.10	5.35	2.4	2.3	92.3	100.2	72.9	81.3
B	5.23	5.96	0.9	2.2	55.5	57.2	32.8	46.1
R¶ (Kaye)	5.02	5.47	2.5	2.7	72.1	78.9	57.7	65.4
N (Kaye)	4.73	5.12	2.2	1.9	71.5	74.1	52.3	56.5
S (Kaye)	4.76	5.28	2.4	2.4	79.1	89.9	53.6	67.4
Mean	4.96	5.43	2.1	2.3	74.1	80.1	53.9	63.3
P**	<0.01		NS		<0.05		<0.01	

* If Kayexalate, as well as 5 days of a low potassium diet was ingested, it is indicated in parentheses.
‡ TA, titratable acid.
§ Norm., normal study.
‖ K+dep., potassium-depleted study.
¶ Norm. values are mean of two normal studies.
** P values refer to the comparison between normal and potassium-depleted studies.

The largest, and hence most readily detectable. changes in factors relating to the increase in urine pH would be expected in those studies in which urine pH increased the greatest amount. Therefore, in an attempt to define the factors responsible for the increase in urine pH, those five studies (from protocols employing either the 5 day low potassium diet alone or the low potassium diet and Kayexalate) in which mean urine pH increased by 0.25 U or more were arbitrarily analyzed as a group (Δ pH greater than 0.25 group). In addition, the remaining five studies (from protocols employing either the 5 day low potassium diet alone or the low potassium diet and Kayexalate) were also analyzed as a group (Δ pH less than 0.25 group). The two groups were compared to examine which factors were important in determining whether urine pH would increase during potassium depletion, and also to determine whether the changes noted in certain acid-base parameters were directly related to changes in urine pH.

Potassium balance and serum concentration. In Table II the potassium deficit occurred before the ammonium chloride study, and serum potassium concentrations before and during the ammonium chloride study are given for both the Δ pH greater than 0.25 and Δ pH less than 0.25 groups.

Neither the absolute amount of the potassium deficit (−201 vs. −196 mmoles) nor the degree of potassium depletion factored by body weight (−28.3 vs. −23.6 mmoles/10 kg) were significantly different between the Δ pH greater than 0.25 and the Δ pH less than 0.25 groups.

In the Δ pH greater than 0.25 group, control serum potassium concentration averaged 4.1 mM in the normal studies and 3.6 mM in the potassium depletion studies; 6 hr after the ingestion of ammonium chloride it averaged 4.4 mM and 4.0 mM, this latter difference is not significant. No difference in serum potassium concentration between normal and potassium-depleted studies was noted at either time in the Δ pH less than 0.25 group.

Acid-base. In Table I mean urinary acid-base parameters and in Table III blood acid-base parameters for the Δ pH greater than 0.25 and Δ pH less than 0.25 groups are given. In Figs. 2–5 differences in urinary

TA‡		HCO$_3^-$		Phosphate		Creatinine		Organic acids	
Norm.	K⁺dep.	Norm.	K⁺dep.	Norm.	K⁺dep.	Norm.	K⁺dep.	Norm.	K⁺dep.
μmoles/min		*μmoles/min*		*μmoles/min*		*μmoles/min*		*μmoles/min*	
19.6	16.3	0.1	0.1	17.2	13.4	11.1	10.6	21.0	27.5
31.7	25.4	0.2	0.3	34.4	26.3	12.8	15.1	49.0	23.1
15.0	19.1	0.7	0.4	14.3	19.5	10.1	11.1	6.7	15.6
31.0	27.8	0.2	0.1	31.6	29.7	13.9	12.6	11.0	15.6
31.0	27.6	0.2	0.2	31.6	29.3	13.9	13.2	11.0	14.4
25.7	23.2	0.3	0.2	25.8	23.6	12.4	12.5	19.7	19.2
	NS		NS		NS		NS		NS
19.6	19.4	0.3	0.5	20.3	23.0	11.8	12.4	22.1	18.9
22.9	13.3	0.2	2.2	26.9	19.7	10.4	9.7	12.9	10.7
15.0	15.3	0.7	1.8	14.3	18.2	10.1	10.1	6.7	8.7
19.3	17.9	0.2	0.3	17.8	19.3	10.3	10.3	22.5	8.6
25.8	23.4	0.2	1.0	25.0	27.1	11.2	10.9	9.1	7.1
20.5	17.9	0.3	1.2	20.9	21.5	10.8	10.7	14.7	10.8
	NS		NS		NS		NS		NS

acid-base parameters between the potassium-depleted and normal studies of the Δ pH greater than 0.25 group are shown.

Δ pH greater than 0.25 group. Mean urine pH after ammonium chloride ingestion increased from 4.96 during normal studies to 5.43 during potassium depletion. This increase in urine pH was not associated with any change in blood acid-base parameters during potassium depletion; blood pH, Pco₂, and plasma bicarbonate concentration were similar both before and after ammonium chloride ingestion in the normal and potassium-depleted studies (Table III).

An increase in urine flow rate will result in an increase in the urine pH of an acid urine (16, 17 and footnote 3). After the ingestion of ammonium chloride, urine flow rates were similar averaging 2.3 ml/min during potassium-depleted and 2.1 ml/min during normal studies (Table I). As shown in Fig. 2, urine pH was higher in every instance during potassium depletion regardless of the flow rate relationship between potassim-depleted and normal studies. No significant difference in phosphate, creatinine, or organic acid excretion

was noted between normal and potassium-depleted studies (Fig. 3, Table I).

Urinary net acid excretion after the ingestion of ammonium chloride was moderately but significantly greater in the potassium-depleted than in the normal studies, averaging 80.1 vs. 74.1 μmoles/min (Fig. 4, Table I). This was accompanied by a significantly higher rate of ammonium excretion averaging 63.3 μmoles/min during potassium depletion and 53.9 μmoles/min during the normal state (Fig. 4, Table I). Titratable acid excretion averaged 20.5 μmoles/min during normal studies and 17.9 μmoles/min during potassium depletion; bicarbonate excretion averaged 0.3 and 1.2 μmoles/min respectively; and the calculated titratable acid minus bicarbonate excretion decreased in every study (mean, 3.5 μmoles/min); however, none of these changes was statistically significant.

Titratable acid excretion, as calculated, included only the contribution of phosphate and creatinine, and not that of organic acids. The magnitude of the contribution of organic acids to titratable acid was estimated in some specimens from both normal and potassium-

TABLE II
Degree of Potassium Depletion

Subjects*	K+ balance		Serum K+			
			Control		Post NH₄Cl	
			Norm.	K⁺dep.	Norm.	K⁺dep.
	mmoles	*mmoles/10kg*	*mM*		*mM*	
Δ pH less than 0.25						
Mi	−198	−28.5	4.9	4.2	4.2	4.4
A	−205	−25.3	4.7	4.9	4.7	4.5
R	−170	−25.1	4.2	3.7	4.4	4.0
Mc	−168	−16.2	3.7	3.5	3.9	4.0
Mc(Kaye)	−240	−23.1	3.7	4.0	3.9	4.0
Mean	−196	−23.6	4.2	4.1	4.2	4.2
P‡			NS		NS	
Δ pH greater than 0.25						
D	−281	−38.2	4.0	3.5	3.8	3.9
B	−143	−22.3	4.0	3.7	4.7	3.8
R (Kaye)	−186	−27.4	4.2	3.5	4.4	3.9
N (Kaye)	−173	−25.0	4.4	4.0	4.4	4.4
S (Kaye)	−221	−28.8	4.0	3.1	4.6	3.8
Mean	−201	−28.3	4.1	3.6	4.4	4.0
P‡			<0.01		NS	

* Kayexalate studies indicated by (Kaye).
‡ *P* values refer to the comparison between normal and potassium-depleted studies.

depleted studies. 31 specimens from normal studies had a ratio of organic acid titratable acidity to total urinary organic acids of 0.14 ±0.01SE, and nine specimens from potassium-depleted studies a ratio of 0.16 ±0.02SE. Therefore, it is unlikely that the exclusion of organic acids from the calculation of titratable acid significantly influenced the interpretation of changes in either titratable acid or net acid excretion.

Following the ingestion of ammonium chloride, urinary-free ammonia concentration averaged 3.2 μM during normal studies, and 12.0 μM during potassium depletion. A decrease in urine flow rate can increase urine-free ammonia concentration; however, this increase was observed consistently regardless of the relationship in urine flow rate between normal and potassium-depleted studies (Fig. 5). Urinary Pco₂ did not differ significantly between the normal and potassium-depleted studies averaging 57 and 66 mm Hg.

Δ *pH less than 0.25 group.* In this group following the ingestion of ammonium chloride, mean urine pH was 4.87 during the normal studies and 4.97 during the potassium-depleted studies. Blood acid-base parameters were not significantly different during the potassium-depleted and normal studies (Table III). Urine flow

rate was similar, 1.7 and 1.8 ml/min respectively, and no significant difference in phosphate, creatinine, or organic acid excretion was noted (Table I).

Net acid excretion averaged 81.0 μmoles/min during normal studies and 77.9 μmoles/min during potassium depletion and ammonium excretion was 55.7 and 54.8 μmoles/min. No significant changes in titratable acid or bicarbonate excretion were observed during potassium depletion (Table I).

Urine free NH₃ concentration averaged 2.5 μM during normal studies and 3.7 μM during potassium depletion, and urine Pco₂ was 60 and 58 mm Hg.

Comparison of the Δ *pH greater than 0.25 and* Δ *pH less than 0.25 groups.* The differences between potassium-depleted and normal studies of the Δ pH greater than 0.25 group were compared with the differences found in the Δ pH less than 0.25 group utilizing a nonpaired *t* test.

As might be anticipated, based on the criteria for forming the two groups, the increase in urine pH was significantly larger in the Δ pH greater than 0.25 group than in the Δ pH less than 0.25 group. The responses of blood acid-base parameters; urine flow rate; and phos-

TABLE III
Blood Acid-Base Parameters

Subjects*	HCO₃⁻				pH				Pco₂			
	Control		Post NH₄Cl‡		Control		Post NH₄Cl		Control		Post NH₄Cl	
	Norm.	K⁺dep.	Norm.	K⁺dep.	Norm.	K⁺dep.	Norm.	K⁺dep.	Norm.	K⁺dep.	Norm.	K⁺dep.
	mM								*mm Hg*			
Δ pH less than 0.25												
Mi	28.1	27.7	22.3	23.0	7.28	7.31	7.27	7.28	61.8	56.7	50.3	50.4
A	29.3	26.9	24.6	22.3	7.37	7.34	7.30	7.33	52.3	51.3	51.5	43.3
R	28.2	26.4	22.5	23.7	7.31	7.33	7.27	7.29	57.7	51.7	50.4	50.3
Mc	25.9	28.0	20.8	22.0	7.33	7.33	7.29	7.27	50.6	54.7	44.0	48.9
Mc (Kaye)	25.9	25.3	20.8	22.6	7.33	7.33	7.29	7.32	50.6	49.5	44.0	45.2
Mean	27.5	26.9	22.2	22.7	7.32	7.33	7.28	7.30	54.6	52.8	48.0	47.6
Δ pH greater than 0.25												
D	30.2	27.8	24.8	22.5	7.38	7.24	7.29	7.23	52.7	66.9	53.0	54.8
B	25.6	24.9	19.7	20.8	7.35	7.37	7.29	7.33	47.8	44.4	41.6	40.2
R (Kaye)	28.2	28.0	22.5	23.1	7.31	7.33	7.27	7.29	57.7	54.7	50.4	49.3
N (Kaye)	26.6	26.4	22.0	19.8	7.34	7.30	7.28	7.26	50.8	55.4	47.6	44.7
S (Kaye)	30.1	30.4	22.7	24.6	7.36	7.37	7.33	7.32	55.0	54.2	44.2	48.6
Mean	28.1	27.5	22.3	22.2	7.35	7.32	7.29	7.29	52.8	55.1	47.4	47.5

* Kayexalate studies indicated by (Kaye).
‡ Mean value of bloods drawn 1, 2, 4, and 6 hr after ingesting NH₄Cl.

phate, creatinine, and organic acid excretion did not differ.

The increases in both net acid and ammonium excretion during potassium depletion in the Δ pH greater than 0.25 group were significantly different from the response of the Δ pH less than 0.25 group; indicating that these changes are directly related to the increase in urine pH. No significant differences between the two groups in the response of titratable acid or bicarbonate excretion were observed.

Electrolytes. In Table IV and Fig. 6, the urinary electrolyte response to the ammonium chloride load during the normal and potassium-depleted state is given for both the Δ pH less than 0.25 and the Δ pH greater than 0.25 groups.

The urinary electrolyte response to the ammonium chloride load was similar in the Δ pH greater than 0.25 and Δ pH less than 0.25 groups. Sodium excretion was greater in both groups during potassium depletion and chloride excretion did not change significantly; therefore the calculated (Cl⁻—Na⁺) excretion decreased significantly in both groups (Table IV, Fig. 6). Serum sodium, chloride, and creatinine concentrations were not significantly different during normal and potassium-depleted studies.

Potassium and potassium plus net acid excretion (K⁺+H⁺) decreased significantly in both groups during potassium depletion (Table IV). The decrease in (K⁺+H⁺) excretion was comparable in magnitude to and highly correlated with ($r = 0.81$, $P < 0.01$) the decrease in (Cl⁻—Na⁺) excretion as seen in Fig. 7, and a similar relationship exists for potassium excretion and (Cl⁻—Na⁺) excretion. The mean fall in (Cl⁻—Na⁺) was 55 μmoles/min, and in (K⁺+H⁺) was 58 μmoles/min in the Δ pH less than 0.25 group; while it was 42 and 41 μmoles/min respectively in the Δ pH greater than 0.25 group.

The ratio of urinary potassium excretion to urinary potassium plus net acid excretion (K⁺/K⁺+H⁺) decreased significantly from 0.55 to 0.38 during potassium depletion in the Δ pH less than 0.25 group and from 0.50 to 0.25 in the Δ pH greater than 0.25 group. This ratio is significantly lower during potassium depletion in the Δ pH greater than 0.25 group than in the Δ pH less than 0.25 group.

DISCUSSION

Clarke, Evans, Macintyre, and Milne demonstrated that urine pH was increased during potassium deficiency in three normal human subjects receiving ammonium chloride (2). In addition, net acid and ammonium ex-
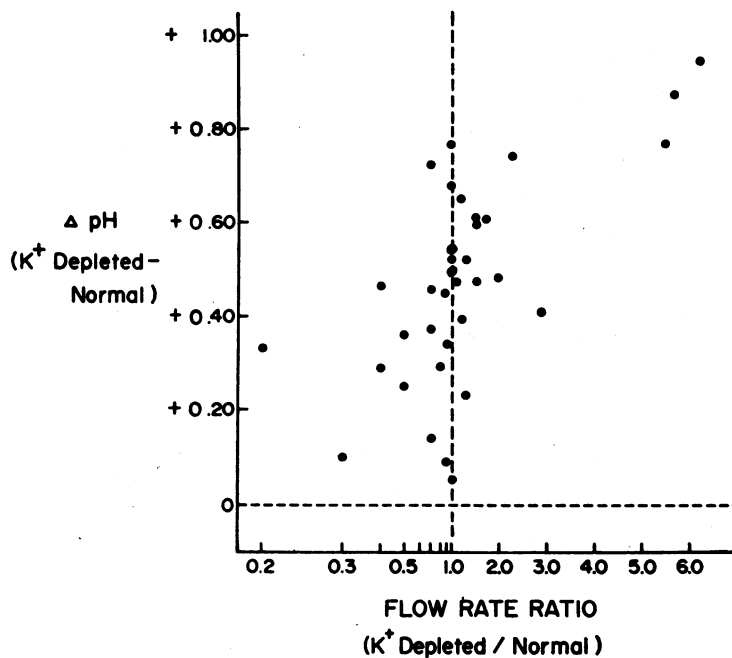
FIGURE 2 The relationship between changes in urine pH and changes in urinary flow rate. These points include only the Δ pH greater than 0.25 group. Urine pH was higher during potassium depletion regardless of whether urine flow rate increased or decreased.
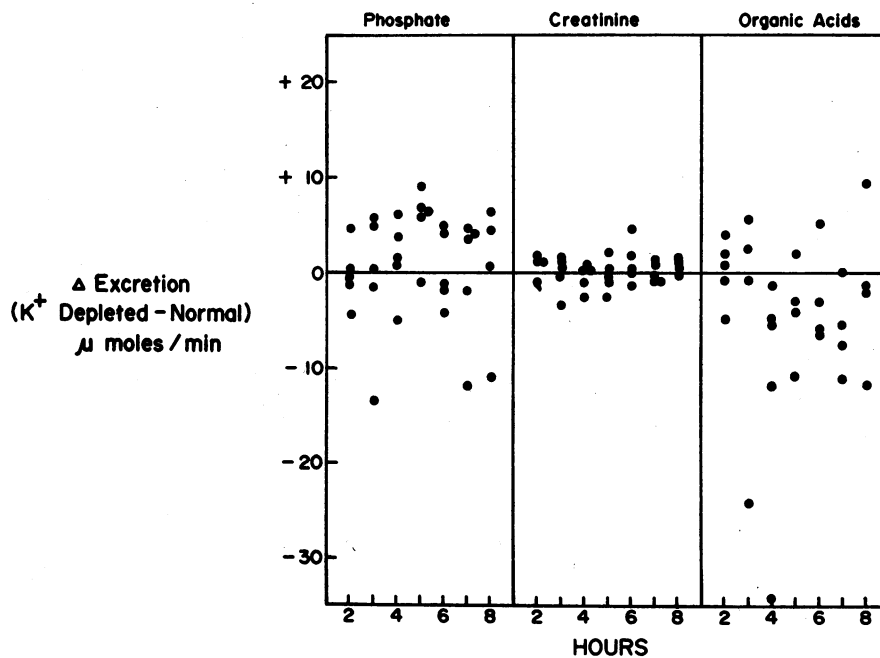

FIGURE 3 The response of urinary fixed buffer excretion to potassium depletion. These points include only the Δ pH greater than 0.25 group. No significant changes in phosphate, creatinine, or organic acid excretion occurred.

cretion were slightly decreased; however, a comparable diet was not ingested during the normal and potassium depletion studies. The authors concluded from these observations that potassium deficiency results in an acquired renal tubular acidosis, i.e., an inability to establish a normal blood to tubular fluid hydrogen ion gradient. This increase in urine pH during potassium deficiency was confirmed subsequently by two other groups (3, 4); however, in both of these studies inadequate data were reported to ascertain the mechanism responsible for the change.

In the present study the response of a potassium-deficient subject to an acute ammonium chloride load was investigated with special emphasis placed on controlling all other variables which may influence urine acidification. A minimum of 7 days separated the acute ammonium chloride studies; the same formula diet with the exception of potassium chloride content was ingested for 3 days preceding both the normal and potassium depletion studies; food was not ingested during the acute ammonium chloride study; subjects were maintained in the upright position during the ammonium chloride study (18); the same dose of ammonium chloride was ingested by the same subject at the same time of day; the urine flow rate was controlled, and specimens collected during comparable time periods were subjected to paired analysis.

Since human volunteers were being studied, an attempt was made to induce the least amount of potassium depletion necessary to observe an effect on urinary acidification. No difference in urine pH following the ingestion of ammonium chloride was detected between normal and potassium-depleted studies after either 1 or 3 days of a low potassium diet. After 5 days of a low potassium diet the effect was variable. Some subjects had an unequivocally higher urine pH, while others showed minimal increases or no change. When, in addition to 5 days of a low potassium diet, Kayexalate (80 g/day) was ingested on the first 2 days, urine pH following an ammonium chloride load was increased in all subjects during potassium depletion. These observations are consistent with previous studies which suggested that urine pH can be increased as a result of potassium deficiency (2–4).

In order to define the mechanism responsible for this increase in urine pH, the concomitant response of the other variables which might influence urinary acidification were analyzed. It would be expected that significant changes in these related variables would be detected most easily in those studies which showed the greatest increase in urine pH. For this reason all subjects demonstrating an increase in mean urine pH of 0.25 U or greater were arbitrarily put into a group referred to as the Δ pH greater than 0.25 group, and analyzed separately. In addition, this group was compared to the group of patients showing either a modest or no rise in urine pH, referred to as the Δ pH less than 0.25 group.
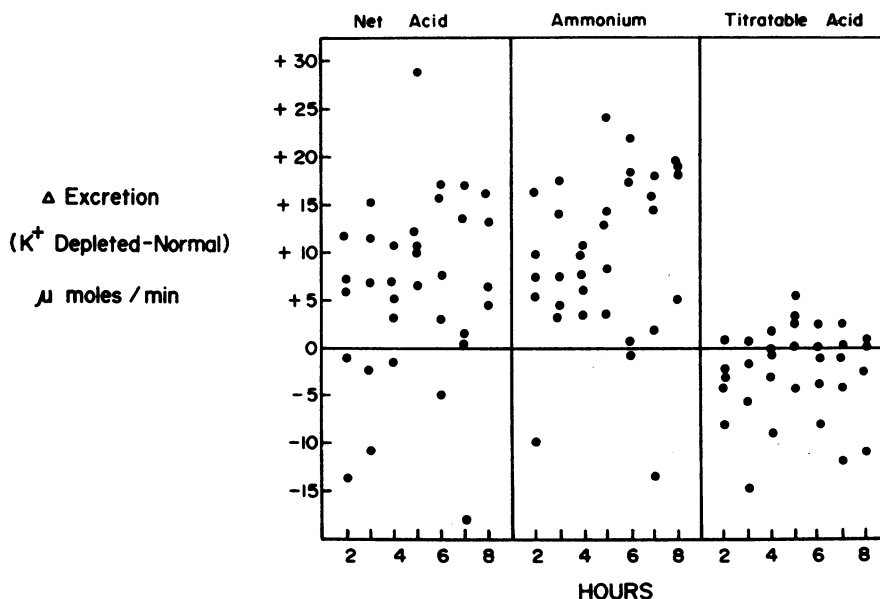

FIGURE 4 The response of urinary acid-base parameters to potassium depletion. These points include only the Δ pH greater than 0.25 group. Net acid and ammonium excretion were both significantly greater during potassium depletion, but the small decrease in titratable acid excretion was not significant.
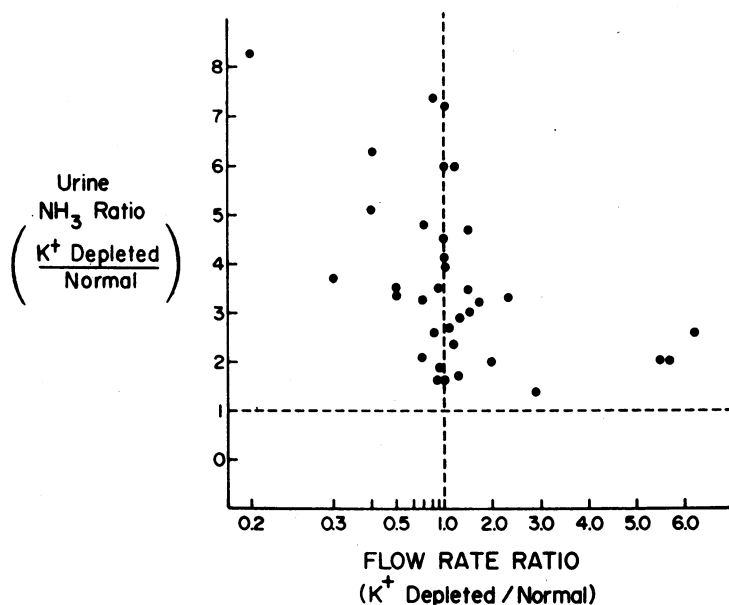
FIGURE 5 The relationship between changes in urinary free ammonia concentration (NH₃) and changes in urinary flow rate. These points include only the Δ pH greater than 0.25 group. The increase in free ammonia occurred regardless of whether urine flow rate increased or decreased.
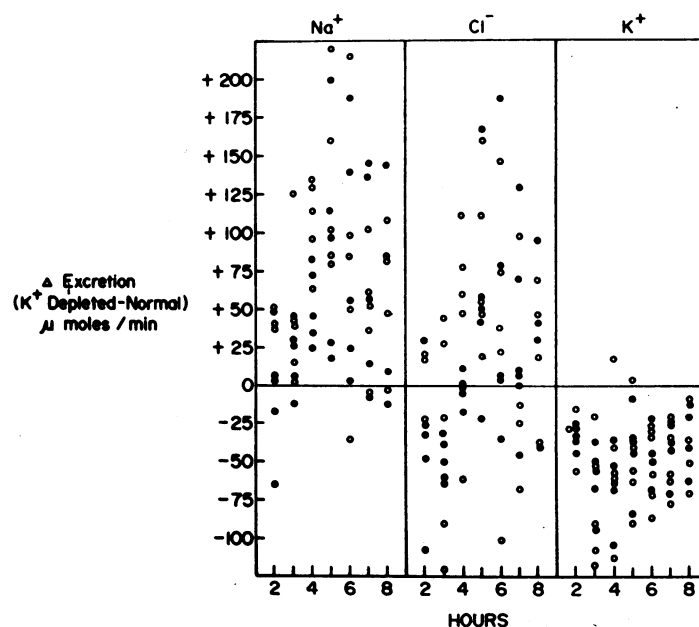

FIGURE 6 The response of urinary electrolytes to potassium depletion. The closed circles represent the Δ pH greater than 0.25 group and the open circles the Δ pH less than 0.25 group. Sodium excretion increased significantly, potassium excretion decreased significantly, and chloride excretion did not change significantly during potassium depletion.

822   R. L. Tannen

The degree of potassium depletion was not significantly greater in the Δ pH greater than 0.25 group than in the Δ pH less than 0.25 group (28 vs. 24 mmoles/10 kg) and considerable overlap existed between the two groups. Nevertheless, some observations suggested that the increase in urine pH might bear some relationship to the degree of potassium depletion. Although the degree of potassium depletion was not significantly larger in the Δ pH greater than 0.25 than Δ pH less than 0.25 group, a significant decrease in serum potassium concentration was observed only in the Δ pH greater than 0.25 group. An increase in urine pH was observed consistently in the Kayexalate protocol which provided the greatest potassium-depleting stress, was more variable in the 5 day low potassium diet protocol and did not occur in the 1 and 3 day protocols. In addition, three subjects studied at more than one level of potassium depletion had the highest urine pH when they were the most depleted of potassium. Although these observations suggest that urine pH is more likely to be increased with larger degrees of potassium depletion, it is clear that the relationship between changes in urine pH and potassium depletion varies considerably between individual subjects. The amount of potassium depletion associated with a rise in urine pH in this study was comparable to that observed in other studies (2–4), and is also of the same magnitude necesary to induce changes in renal concentrating ability in the human (19). Whether further degrees of potassium deficiency would result in greater changes in urine pH is unanswered.

The pH of an acid urine increases as urine flow rate is increased (16, 17)[8] and potassium depletion is known to result in a defect in water reabsorption (19, 20). This possible explanation for the rise in urine pH observed during potassium deficiency had not been considered in any of the previous investigations, however, it was specifically excluded in this study. As noted in Table I mean urine flow rate was not significantly different between potassium-depleted and normal studies, and, as seen in Fig. 2, urine pH increased during potassium depletion irrespective of urine flow rate. Since a specific attempt was made to control urine flow rate in this study, the possibility cannot be excluded that this mechanism contributes to the increase in urine pH seen in clinical settings associated with potassium depletion, as for example, in patients with hyperaldosteronism (21).

Urine pH was not increased because of a change in blood pH, P_{CO_2}, or bicarbonate. Nor did the pH increase

TABLE IV

Mean Urinary Electrolyte Parameters ($U_2–U_8$)

Subjects*	Na^+		Cl^-		$(Cl^- - Na^+)$		K^+		$(K^+ + H^+)$‡		$K^+/(K^+ + H^+)$	
	Norm.	K+dep.	Norm.	K+dep.	Norm.	K+dep.	Norm.	K+dep.	Norm.	K+dep.	Norm.	K+dep.
	μmoles/min		*μmoles/min*		*μmoles/min*		*μmoles/min*		*μmoles/min*			
Δ pH less than 0.25												
Mi	96	171	177	228	81	57	85	51	139	102	0.60	0.49
A	108	191	212	235	103	45	112	54	194	136	0.58	0.40
R	98	155	176	206	78	51	62	41	134	118	0.46	0.35
Mc	108	141	228	193	121	51	128	57	226	143	0.56	0.39
Mc (Kaye)	108	220	228	244	121	25	128	38	226	131	0.56	0.29
Mean	104	176	204	221	101	46	103	48	184	126	0.55	0.38
P§	<0.01		NS		<0.02		<0.02		<0.02		<0.01	
Δ pH greater than 0.25												
D	154	230	251	292	98	62	76	39	169	139	0.45	0.28
B	70	84	113	114	44	30	66	37	121	94	0.54	0.39
R (Kaye)	98	130	176	155	78	25	62	20	134	99	0.46	0.20
N (Kaye)	93	101	170	133	77	32	78	27	150	101	0.52	0.27
S (Kaye)	100	201	192	231	92	30	91	17	170	107	0.53	0.15
Mean	103	149	180	185	78	36	75	28	149	108	0.50	0.25
P§	NS		NS		<0.01		<0.01		<0.01		<0.01	

* Kayexalate studies indicated by (Kaye).
‡ ($K^+ + H^+$) indicates potassium plus net acid.
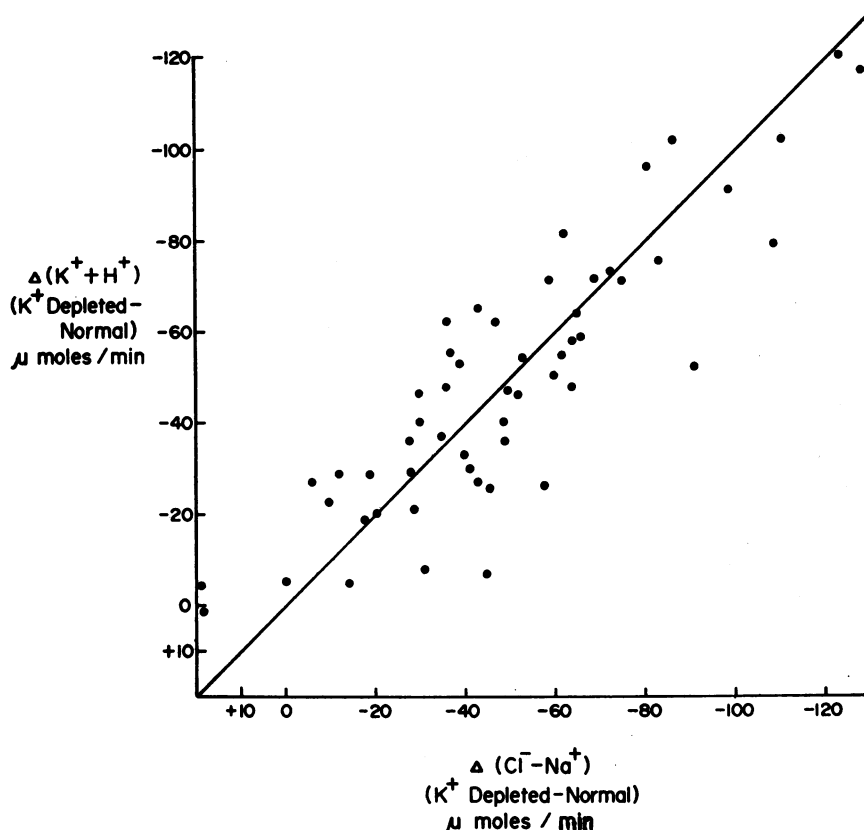§ P values refer to the comparison between normal and potassium-depleted studies.

FIGURE 7 The relationship between changes in potassium plus net acid excretion ($K^+ + H^+$) to changes in chloride minus sodium excretion ($Cl^- - Na^+$). These points include both the Δ pH greater than 0.25 and Δ pH less than 0.25 groups. The 45° identity line is drawn to indicate the relationship expected if the changes in both parameters are of equal magnitude. The decrease in ($K^+ + H^+$) excretion and the decrease in ($Cl^- - Na^+$) excretion which occurred during potassium depletion were correlated significantly and were approximately equal.

result from an increase in fixed buffering capacity of the urine; as seen in Fig. 3, no increase in phosphate, creatinine, or organic acid excretion was observed during potassium depletion.

Net acid excretion was found to be increased rather than decreased in those subjects demonstrating the greatest increase in urine pH. The increase was accounted for entirely by an increase in ammonium excretion of greater magnitude than the rise in net acid.[8] Neither net acid excretion nor ammonium excretion changed significantly in the Δ pH less than 0.25 group, indicating that the changes in both of these parameters are related in some fashion to the rise in urine pH. Significant changes were not found in either titratable

acid or bicarbonate excretion and although calculated titratable acid minus bicarbonate excretion decreased in every study, this change was also not statistically significant. In the urine pH range of these studies, it was expected that any changes in these parameters would be too small to validate statistically.[7]

It has been suggested that the increase in urine pH during potassium deficiency is the result of an inability to establish a normal blood to urine hydrogen ion gra-

---

[8] If the increases in net acid and ammonium excretion were the result of an increase in ammonium chloride absorption from the gastrointestinal tract during potassium deficiency, they should be associated with a decrease rather than an increase in urine pH.

[7] In the urine pH range of these studies virtually all the urinary phosphate is in the acidified form and therefore changes in phosphate excretion will have a much greater influence on titratable acid excretion than will changes in urine pH. Although phosphate excretion did not change significantly during potassium depletion in the Δ pH greater than 0.25 group, its variability in individual studies would serve to mask possible small changes in titratable acid excretion in the group as a whole resulting from the increase in urine pH. Also in this pH range changes in bicarbonate excretion would be minimal.

824   R. L. Tannen

dient (2). A priori, it might be expected that a defect in hydrogen ion transport would result in diminished, certainly not enhanced, excretion of an acute acid load. Indeed in classical distal renal tubular acidosis, the response to an acute ammonium chloride load is a combination of a higher urine pH and lower rate of net acid excretion than observed in normal subjects (5). However, the patient with classical renal tubular acidosis has a number of factors including a diminished glomerular filtration rate and chronic metabolic acidosis, as well as potassium deficiency, which might modify the response to an acid load. On the other hand, individuals with so-called "incomplete renal tubular acidosis," who have a normal glomerular filtration rate and no acidosis or hypokalemia, provide a relatively pure model of a hydrogen ion gradient defect. Relatively few of these patients have been reported in the literature. The three patients initially described by Wrong and Davies included one with subnormal and one with supernormal net acid excretion in response to an acute acid load (5). Two of the three patients reported by Buckalew, McCurdy, Ludwig, Chaykin, and Elkinton had relatively low rates of net acid excretion (22) and all six of the patients reported by Györy and Edwards had low rates of net acid excretion (23). One patient with incomplete renal tubular acidosis evaluated by us had an unequivocal diminution in net acid and ammonium excretion when compared to 18 normal subjects. Therefore it appears that net acid excretion in response to an acid load is diminished, if changed at all, when a defect in establishing a normal hydrogen ion gradient exists.

In addition, although it has frequently been pointed out that ammonium excretion is high in relationship to urine pH in individuals with renal tubular acidosis (5, 23), rates of ammonium excretion higher than normal are not noted usually in patients with either classic or incomplete renal tubular acidosis. On the contrary, the absolute rate of ammonium excretion most often has been unchanged or diminished (5, 22, 23). Since the response of subjects with potassium deficiency to an acute acid load is characterized by an increase in net acid and an even greater increase in ammonium excretion it would seem unlikely that this is the result of an acquired renal tubular acidosis.

A more plausible explanation for these experimental results is that potassium depletion causes a primary increase in ammonia diffusion into the urine. Theoretically if the excretion rate of net acid and fixed buffer, as well as the rate of urine flow, remain constant, an increase in ammonia diffusion into the urine would result in an increase in free ammonia concentration, an increase in the ratio of free ammonia to ammonium concentration, and a concomitant increase in urine pH

and rearrangement of other urinary buffer pair concentration ratios. The urine concentration and excretion rate of titratable acid would tend to decrease and those of bicarbonate[8] and ammonium to increase; but the magnitude of these changes would depend on the initial pH of the urine, the concentration of fixed buffer in the urine and the quantity of ammonia added. In actual fact, however, net acid excretion might increase rather than remain constant because the rise in urine pH would enhance the gradient for hydrogen ion secretion. If so, any added hydrogen ion would be excreted in the form of ammonium. The results of this study are consistent with such a sequence of events. Urine pH and free ammonia concentration increased concomitantly with an increase in ammonium and net acid excretion. As would be anticipated in the urine pH range of this study, the effects on titratable acid and bicarbonate excretion were minimal.

Other evidence exists to suggest that an increase in renal ammonia production accompanies potassium depletion. Potassium-depleted rats have been found to have increased activity of renal glutaminase (24) and glutamine transaminase-ω-amidase (25) enzymes and concomitantly an increase in urinary ammonium excretion. In addition, renal cortical slices from potassium-depleted rats have an increased rate of glucose production (26). These observations are all consistent with an increased rate of ammonia production. Conflict exists regarding the glutaminase activity in the kidneys of potassium-depleted dogs (27, 28); however, Gabuzda, Hall, Baertl, and Sancetta have shown by direct techniques in vivo that potassium depletion can increase renal ammonia production in normal dogs and also in humans with cirrhosis (28, 29). No direct measurements of renal ammonia production in normal humans have been reported to date.

Although this relationship between ammonia production and potassium depletion has been noted, its possible physiologic ramifications have not been elucidated. Some of the findings in this study suggest that an increase in renal ammonia production could possibly be a control mechanism for potassium conservation.

In this study potassium depletion was found to enhance the natriuretic response to an ammonium chloride load and other investigators have noted comparable findings (2, 3). On the other hand many investigators have described sodium retention during potassium depletion, and recently this has been ascribed to an increase in proximal tubular sodium reabsorption (30). Our findings during potassium deficiency probably differ from those

---

[8] Bicarbonate concentration will increase only if a sufficiently large decrease in urine P_{CO_2} does not occur. In this study urine P_{CO_2} was not significantly different during the normal and potassium-depleted state.

of others because of the administration of ammonium chloride which modifies renal sodium handling and also possibly because our studies were acute, while those in which sodium retention have been noted were chronic. This slightly greater natriuretic response to an ammonium chloride load during potassium depletion was accompanied by a consistent decrease in calculated $(Cl^- - Na^+)$ excretion which was quantitatively similar to and significantly correlated with a concomitant decrease in total potassium and net acid excretion $(K^+ + H^+)$, as seen in Fig. 7. This pattern of electrolyte excretion most likely represents a decrease in distal sodium-cation exchange and probably is a result, at least in part, of the decrease in aldosterone secretion which accompanies potassium depletion (31–33). This apparently is one manner in which urinary potassium excretion is altered in response to potassium deficiency.

In addition to the decrease in sodium-cation exchange, a striking decrease in the per cent of potassium in the total cation exchanged, i.e. the ratio of $K^+/(K^+ + H^+)$, was observed; and the per cent of potassium appears to be lowest in those studies showing the greatest increase in urine pH. In the Δ pH greater than 0.25 group this decrease in percentage of potassium comprising total cation exchange (49 ±8% SE) is far greater than the percentage decrease in serum potassium (14 ±3% SE), or estimated decrease in total body potassium (6 ±0.4% SE).[9] It presumably is also far greater than the decrease in intracellular and more specifically renal tubular potassium concentration (35). Therefore some mechanism other than a change in potassium concentration in some body compartment appears to be necessary to explain fully the decrease in $K^+/(K^+ + H^+)$ ratio. A possible explanation would be that aldosterone effects the renal tubule by selectively altering only sodium-potassium exchange; however, most evidence suggests that it always affects hydrogen ion exchange concomitantly as well (36–39). It is tempting to speculate that this unexplained decrease in potassium excretion might be the result of an increase in renal ammonia production accompanying potassium depletion. The more favorable gradient for hydrogen ion secretion resulting from increased ammonia production could enhance distal sodium-hydrogen ion exchange at the expense of sodium-potassium exchange. Further studies in an attempt to substantiate this thesis would be of interest.

ACKNOWLEDGMENTS

analysis; and Colonel Paul Teschan for his support and encouragement.

REFERENCES

1. Berliner, R. W. 1959–60. Renal mechanisms for potassium excretion. *Harvey Lect.* **55:** 141.
2. Clarke, E., B. M. Evans, I. Macintyre, and M. D. Milne. 1955. Acidosis in experimental electrolyte depletion. *Clin. Sci. (London).* **14:** 421.
3. Rubini, M. E., W. B. Blythe, E. G. Herndon, and W. H. Meroney. 1957. Influence of potassium deficiency on response to an acidifying salt. *Clin. Res.* **5:** 193.
4. Schloeder, F. X., and B. J. Stinebaugh. 1966. Defect of urinary acidification during fasting. *Metab. (Clin. Exp.)* **15:** 17.
5. Wrong, O., and H. E. F. Davies. 1959. The excretion of acid in renal disease. *Quart. J. Med.* **28:** 259.
6. Mitchell, J., and H. J. Gitelman. 1968. A low electrolyte liquid diet suitable for metabolic studies. *Amer. J. Clin. Nutr.* **21:** 1321.
7. Fitzgerald, M. G., and P. Fourman. 1956. An experimental study of magnesium deficiency in man. *Clin. Sci.* **15:** 635.
8. Dunn, M. J., and M. Walser. 1966. Magnesium depletion in normal man. *Metab. (Clin. Exp.)* **15:** 884.
9. Fiske, C. H., and T. J. Subbarow. 1925. The colorometric determination of phosphorus. *J. Biol. Chem.* **66:** 375.
10. Logsdon, E. E. 1960. A method for the determination of ammonia in biological materials on the autoanalyzer. *Ann. N. Y. Acad. Sci.* **87:** 801.
11. Chasson, A. L., M. J. Grady, and M. A. Stanley. 1961. Determination of creatinine by means of automatic chemical analysis. *Amer. J. Clin. Path.* **35:** 83.
12. Van Slyke, D. D., and W. W. Palmer. 1920. Studies of acidosis. XVI. The titration of organic acids in urine. *J. Biol. Chem.* **41:** 567.
13. Hastings, A. B., and J. Sendroy. 1925. The effect of variation in ionic strength on the apparent first and second dissociation constants of carbonic acid. *J. Biol. Chem.* **65:** 445.
14. Schwartz, W. B., N. Bank, and R. W. P. Cutler. 1959. The influence of urinary ionic strength on phosphate pK_2' and the determination of titratable acid. *J. Clin. Invest.* **38:** 347.
15. Bank, N., and W. B. Schwartz. 1960. Influence of certain urinary solutes on acidic dissociation constant of ammonium at 37°C. *J. Appl. Physiol.* **15:** 125.
16. Nutbourne, D. M., and H. E. de Wardener. 1961. The effect of a water diuresis on the urinary excretion of hydrogen ions in man. *Clin. Sci. (London).* **20:** 63.
17. Woeber, K. A., E. L. Reid, I. Kiem, and A. G. Hills. 1963. Diffusion of gases out of the distal nephron segment in man. I. NH_3. *J. Clin. Invest.* **42:** 1689.
18. Steinmetz, P. R., and N. Bank. 1963. Effects of acute increases in the excretion of solute and water on renal acid excretion in man. *J. Clin. Invest.* **42:** 1142.
19. Rubini, M. E. 1961. Water excretion in potassium-deficient man. *J. Clin. Invest.* **40:** 2215.
20. Relman, A. S., and W. B. Schwartz. 1956. The nephropathy of potassium depletion. A clinical and pathological entity. *N. Engl. J. Med.* **255:** 195.
21. Luetscher, J. A. 1964. Primary aldosteronism. Observations in six cases and review of diagnostic procedures. *Medicine.* **43:** 437.

---

[9] Calculated assuming a normal body potassium content of 49 mEq/kg (34).

22. Buckalew, V. M., Jr., D. K. McCurdy, G. D. Ludwig, L. B. Chaykin, and J. R. Elkinton. 1968. Incomplete renal tubular acidosis. Physiologic studies in three patients with a defect in lowering urine pH. *Amer. J. Med.* **45**: 32.

23. Györy, A. Z., and K. D. G. Edwards. 1968. Renal tubular acidosis. A family with an autosomal dominant genetic defect in renal hydrogen ion transport, with proximal tubular and collecting duct dysfunction and increased metabolism of citrate and ammonia. *Amer. J. Med.* **45**: 43.

24. Iacobellis, M., E. Muntwyler, and G. E. Griffin. 1954. Enzyme concentration changes in the kidneys of protein- and/or potassium-deficient rats. *Amer. J. Physiol.* **178**: 477.

25. Goldstein, L. 1964. Relation of renal glutamine trans-aminase-ω-amidase activity to ammonia excretion in the rat. *Nature (London).* **201**: 1229.

26. Goodman, A. D., R. E. Fuisz, and G. F. Cahill. 1966. Renal gluconeogenesis in acidosis, alkalosis, and potassium deficiency: its possible role in regulation of renal ammonia production. *J. Clin. Invest.* **45**: 612.

27. Iacobellis, M., E. Muntwyler, and G. E. Griffin. 1955. Kidney glutaminase and carbonic anhydrase activity and tissue electrolyte composition in potassium-deficient dogs. *Amer. J. Physiol.* **183**: 395.

28. Gabuzda, G. J., and P. W. Hall, III. 1966. Relation of potassium depletion to renal ammonium metabolism and hepatic coma. *Medicine.* **45**: 481.

29. Baertl, J. M., S. M. Sancetta, and G. J. Gabuzda. 1963. Relation of acute potassium depletion to renal ammonium metabolism in patients with cirrhosis. *J. Clin. Invest.* **42**: 696.

30. Lennon, E. J., and J. Lemann, Jr. 1968. The effect of a potassium-deficient diet on the pattern of recovery from experimental metabolic acidosis. *Clin. Sci. (London).* **34**: 365.

31. Johnson, B. B., A. H. Lieberman, and P. J. Mulrow. 1957. Aldosterone excretion in normal subjects depleted of sodium and potassium. *J. Clin. Invest.* **36**: 757.

32. Gann, D. S., C. S. Delea, J. R. Gill, Jr., J. P. Thomas, and F. C. Bartter. 1964. Control of aldosterone secretion by change of body potassium in normal man. *Amer. J. Physiol.* **207**: 104.

34. Cannon, P. J., R. P. Ames, and J. H. Laragh. 1966. Relation between potassium balance and aldosterone secretion in normal subjects and in patients with hypertensive or renal tubular disease. *J. Clin. Invest.* **45**: 865.

34. Edelman, I. S., and J. Leibman. 1959. Anatomy of body water and electrolytes. *Amer. J. Med.* **27**: 256.

35. Welt, L. G., W. Hollander, Jr., and W. B. Blythe. 1960. The consequences of potassium depletion. *J. Chronic Dis.* **11**: 213.

36. August, J. T., and D. H. Nelson. 1959. The dual action of aldosterone on renal sodium reabsorption in normal subjects. *Clin. Res.* **7**: 274.

37. Mills, J. N., S. Thomas, and K. S. Williamson. 1961. The effects of intravenous aldosterone and hydrocortisone on the urinary electrolytes of the recumbent human subject. *J. Physiol. (London).* **156**: 415.

38. Sonnenblick, E. H., P. J. Cannon, and J. H. Laragh. 1961. The nature of the action of intravenous aldosterone: evidence for a role of the hormone in urinary dilution. *J. Clin. Invest.* **40**: 903.

39. Liddle, G. W. 1958. Aldosterone antagonists. *Arch. Intern. Med.* **102**: 998.