

# Effect of Volume Expansion on Renal Citrate and Ammonia Metabolism in KCl-Deficient Rats

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**ABSTRACT** When rats with desoxycorticosterone acetate (DOCA)-induced potassium chloride deficiency are given sodium chloride there is simultaneously a partial correction of metabolic alkalosis and a marked reduction in urinary citrate excretion and renal citrate content. To examine DOCA's role in this phenomenon and to determine how sodium chloride alters renal metabolism, rats were made KCl deficient using furosemide and a KCl-deficient diet. Renal citrate and ammonia metabolism were then studied after chronic oral sodium chloride administration or acute volume expansion with isotonic mannitol. Although both maneuvers partially corrected metabolic alkalosis, sodium chloride raised serum chloride concentration while mannitol significantly decreased it. Urinary citrate excretion decreased to 10% of control in rats given NaCl and to 50% of control in rats infused with mannitol. The filtered load of citrate was constant or increased indicating increased tubular citrate reabsorption. Renal cortical citrate content also decreased approximately 50%. Renal cortical slices from KCl-deficient rats incubated in low or normal chloride media produced equal amounts of  $^{14}\text{CO}_2$  from [1,5- $^{14}\text{C}$ ]citrate. In addition, urinary ammonia excretion increased by over 300% in both groups. This occurred in the mannitol group despite increased urinary pH and flow rate indicating a rise in renal ammonia production.

It seems that neither DOCA nor an increase in serum chloride concentration explains the experimental results. Rather, it appears that volume expansion is responsible for increased renal tubular citrate reabsorption and renal ammonia production. As these renal metabolic

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responses ordinarily occur in response to acidosis, the data are consistent with the hypothesis that volume expansion reduces renal cell pH in KCl-deficient rats.

## INTRODUCTION

Recently, Adler, Zett, and Anderson (1) demonstrated that when desoxycorticosterone acetate (DOCA)<sup>1</sup>-induced chronic potassium chloride-deficient rats are given sodium chloride, but no potassium salts, renal cortical citrate content and urinary citrate excretion profoundly decrease. Renal citrate metabolism was examined because it has been shown that urinary citrate excretion and renal citrate content decrease in acidosis and increase in alkalosis (2, 3) presumably secondary to increased renal citrate oxidation in acidosis and a decrease in alkalosis (4). However, Adler and his colleagues noted that despite the enormous reductions in urinary and renal citrate seen after sodium chloride administration, metabolic alkalosis was still present (1). Inasmuch as changes in citrate metabolism in potassium-deficient diaphragm muscle have been shown to be directly related to alterations in intracellular rather than extracellular pH (5), it was postulated that sodium chloride administration in the potassium chloride-deficient rats may have caused a renal tubular cell acidosis. Unfortunately, direct measurement of renal tubular cell pH is presently not possible in vivo (6), so this was not able to be confirmed directly.

To test the hypothesis that renal tubular cell pH is lowered by sodium chloride ingestion in potassium chloride deficiency and to determine the mechanism through which sodium chloride induces changes in renal citrate metabolism, further experiments were performed. To eliminate the possible role of exogenously

<sup>1</sup>Abbreviation used in this paper: DOCA, desoxycorticosterone acetate.

administered DOCA, rats were made potassium chloride deficient by intraperitoneal furosemide administration. The roles played by extracellular chloride concentration and volume expansion in altering renal cell pH were studied by examining their effects on renal citrate and ammonia metabolism. The results indicate that the response of renal citrate metabolism to sodium chloride ingestion in the furosemide-induced potassium chloride-deficient rats was identical to the response of renal citrate metabolism to sodium chloride ingestion in rats made deficient with DOCA. In addition to the changes in renal citrate metabolism, sodium chloride or mannitol induced volume expansion increased ammonia excretion. These renal metabolic alterations appear to be due to an expansion of intravascular volume rather than a change in plasma chloride concentration. The data are consistent with the hypothesis that expansion of intravascular volume in potassium chloride-deficient rats leads to a decrease in renal tubular cell pH.

## METHODS

**Potassium chloride depletion protocol.** Male Sprague-Dawley rats weighing 350–375 g were used in all experiments. Each animal was placed on a zero potassium, zero chloride diet (obtained from Nutritional Biochemical Corporation, Cleveland, Ohio). Animals were allowed water containing 75 meq/liter of sodium bicarbonate ad libitum during the entire study. Rats were maintained on the potassium chloride-deficient diet for a 15-day period. On days 8–12 each rat received a daily intraperitoneal dose of 2 mg of furosemide.

**Repletion protocol.** At the end of the 15-day period all food was removed, and the rats were divided into four equal groups: a KCl-repleted group tube fed 10 ml of a 20% dextrose solution containing 200 meq/liter KCl twice daily; a K-depleted group tube fed 10 ml of 20% dextrose solution containing no electrolytes twice daily; an NaCl-repleted group tube fed 10 ml of a 20% dextrose solution containing 200 meq/liter sodium chloride twice daily; and a KHCO<sub>3</sub>-repleted group tube fed 10 ml of a 20% dextrose solution containing 200 meq/liter KHCO<sub>3</sub> twice daily. Solutions were administered for 3 days during which the sodium bicarbonate drinking water was continued ad libitum. Rats were weighed each morning during this 3-day repletion. After 3 days of tube feeding rats were sacrificed the next day as outlined below.

**Citrate and ammonia excretion experiments in repleted rats.** After receiving the sixth tube feeding each rat was placed in an individual metabolic cage and given only the sodium bicarbonate solution ad libitum. Urine was collected for the next 16 h. At the end of this time rats were removed from the cages which were then rinsed with distilled water to insure complete urine collection. Each urine collection was filtered and analyzed for creatinine, chloride, citrate, and ammonia.

**Tissue electrolyte and citrate experiments in repleted rats.** Animals were anesthetized by the intraperitoneal injection of inactin, 100 mg/kg of body weight. Each rat was anesthetized separately to avoid any delay before killing the rat by exsanguination from the abdominal aorta into a heparinized syringe. Portions of renal cortical tissue, liver, and gluteal muscle were removed and rapidly frozen in liquid

nitrogen for citrate analysis. Other portions of these tissues were placed in tared tubes for later determination of water and potassium content. All the frozen tissues were weighed and homogenized in the frozen state. The homogenate was centrifuged and the supernate saved for citrate analysis.

**<sup>14</sup>CO<sub>2</sub> production from [1,5-<sup>14</sup>C]citrate experiments.** As previously described (2) renal cortical slices were placed into specially constructed 25-ml Erlenmeyer flasks having a center well. Approximately 100 mg of renal cortex was used in each flask. Each flask contained 4 ml of a carbon dioxide-oxygen saturated Krebs-Ringer bicarbonate solution containing 1.0 meq/liter of potassium. The substrate was 1 mM acetate and 1 mM citrate. In addition, each flask contained 0.5 μCi of [1,5-<sup>14</sup>C]citrate (obtained from New England Nuclear, Boston, Mass.). The solution in one group of flasks contained 70 meq of sodium chloride, while the solution in the other group contained 110 meq of sodium chloride. Isoosmolarity was achieved by the addition of mannitol to the first flask. Flasks were incubated for 90 min in a metabolic shaker at 37°C, and <sup>14</sup>CO<sub>2</sub> was collected and counted as previously described (2). Results are expressed as disintegrations per minute per flask per 200 mg. Calculations of this value have also been previously described (2). Production of <sup>14</sup>CO<sub>2</sub> from labeled citrate in the remainder of the paper will be termed citrate decarboxylation.

**Mannitol expansion experiments.** Potassium chloride-depleted rats were employed in all experiments. After the 15-day depletion protocol the potassium chloride-depleted rats were anesthetized by the intraperitoneal injection of inactin, 100 mg/kg of body weight. A tracheostomy was performed in each animal to allow for removal of retained secretions. An indwelling jugular catheter was inserted for infusion, and a femoral artery line was placed for blood sampling. The infusion line was connected to a Harvard constant infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.). A bladder catheter was inserted suprapubically. After a 30-min stabilization period, animals were divided into two groups. The dextrose-infused group received 8.3 ml/h of a 5% dextrose solution over a 210-min period. The mannitol-infused group was given 8.3 ml/h of the 5% dextrose solution for 90 min to act as a control period. These animals were then infused with a 280 mM mannitol solution given at 37 ml/min for 150 min. In the dextrose group blood was obtained after the stabilization period and at the conclusion of the experiment. In the mannitol-infused group blood was obtained at the end of the stabilization period, at the end of the control period, and at the conclusion of the experimental period. Urine collected under oil in the dextrose-infused group was obtained as a single sample over the entire 210 min. In the mannitol-infused rats a 90-min control period urine was obtained, and 30 min after mannitol infusion was started a 60-min and two 30-min urines were collected under oil. Urines in both groups were analyzed for pH, creatinine, citrate, and ammonia. Each group was infused with radioisotopes immediately after the stabilization period. Dextrose-infused rats received 2 μCi each of Na <sup>22</sup>Cl and [<sup>14</sup>C]DMO and 10 μCi of tritiated water. Double this quantity was given to the mannitol-infused group to compensate for increased urinary losses. Isotopes were injected early to allow sufficient tissue equilibration time to take place. At the conclusion of the experiment animals in each group were killed by exsanguination from the abdominal aorta into a heparinized syringe. Portions of renal cortical tissue were removed and immediately frozen in liquid nitrogen, weighed, homogenized in a frozen state, and saved for citrate analysis as previously

TABLE I  
*Arterial Acid-Base Status and Arterial and Tissue Potassium Levels in Potassium Chloride-Repleted, Potassium-Depleted, Sodium Chloride-Repleted, and Potassium Bicarbonate-Repleted Rats\**

	Blood				Tissue potassium	
	pH	HCO <sub>3</sub>	PCO <sub>2</sub>	K	Muscle	Kidney
		meq/liter	mm Hg	meq/liter	meq/kg dry weight	
KCl repleted, <i>n</i> = 5	7.42‡ ±0.01	20.8‡ ±1.7	32‡ ±3	3.9‡ ±0.2	471‡ ±9	339 ±14
K depleted, <i>n</i> = 7	7.55§ ±0.02	36.4§ ±1.8	41§ ±1	2.3§ ±0.2	357§ ±9	319 ±9
NaCl repleted, <i>n</i> = 7	7.48 ±0.03	26.0‡§ ±1.2	35‡ ±2	2.6§ ±0.1	365§ ±10	318 ±5
KHCO <sub>3</sub> repleted, <i>n</i> = 6	7.55§ ±0.01	32.7§ ±1.0	38§ ±1	2.4§ ±0.2	348§ ±12	328 ±6

\* Values represent the mean ± SE of the mean.

‡ Differ from K deplete *P* < 0.01, using Student *t* test.

§ Differ from KCl replete *P* < 0.01, using Student *t* test.

described (2). A portion of renal cortex, plus liver, gluteal muscle, and cardiac muscle tissue were placed in tared tubes for later determination of water and potassium content. A portion of gluteal muscle, cardiac muscle, and liver tissue was placed in weighed homogenizing tubes for determination of intracellular pH, as previously described (7).

*Analytic methods.* Blood pH and PCO<sub>2</sub> were measured on a Radiometer BM3 MK2 blood micro system at 37°C. Urine pH was measured using a Radiometer PMS27 pH meter. Bicarbonate concentration in the blood was calculated from the Henderson-Hasselbalch equation employing a pK of 6.1. Sodium and potassium were measured on an IL flame photometer while chloride, creatinine, and blood urea nitrogen (BUN) were determined by autoanalyzer. Tissue water and electrolytes were determined by drying tissue to constant weight in a 70°C oven for 16 h, as previously described (2). Citrate was determined enzymatically (8), and ammonia was determined by microdiffusion (9). Triple isotope determination of tissue pH employed the methods described by Schloerb and Grantham (10). Radioactivity was measured in a three channel Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.) using an AES standardization and standard methods for determining efficiencies.

## RESULTS

*Effect of sodium chloride repletion on renal citrate metabolism and urinary ammonia excretion in KCl-depleted rats.* Extracellular acid-base status and serum and tissue potassium levels in the four groups of rats studied are shown in Table I. With the exception of the potassium chloride-repleted rats, metabolic alkalosis was present in each group. Compared to potassium-depleted and potassium bicarbonate-repleted animals, however, the severity of the metabolic alkalosis was less in the sodium chloride-repleted rats, *P* < 0.05. Thus, sodium chloride partially corrected the alkalosis,

a result previously shown by other investigators in DOCA-induced potassium-deficient animals (1, 11). The furosemide and dietary KCl restriction regimen induced a state of severe potassium depletion. Muscle potassium concentration was reduced 25% in each of the last three groups compared to values found both in the potassium chloride-repleted animals and normal values obtained in this and other laboratories (*P* < 0.001). Renal potassium concentration was also reduced but the degree of reduction, as expected, was less than in the muscle (1). It is important to note that despite administration of potassium bicarbonate serum and muscle potassium depletion was as severe as that found in sodium chloride and potassium-depleted animals who received no potassium. Administration of potassium bicarbonate, unlike potassium chloride, was unable to restore extracellular acid-base conditions, serum potassium concentration, or tissue potassium concentration to normal. The efficacy of sodium chloride administration in repleting chloride stores is shown both by the rise in serum chloride concentration from 80 to 94 meq/liter and by the large amounts of chloride in the urine of the NaCl-repleted rats (Table II).

In addition to partially correcting the extracellular metabolic alkalosis, administration of sodium chloride also altered renal metabolism. As shown in Fig. 1 and 2 urinary citrate excretion fell while urinary ammonia excretion rose in the sodium chloride-repleted rats. Citrate excretion in the sodium chloride-repleted group, compared to each of the three other groups, differed significantly (*P* < 0.001) as did ammonia excretion (*P* < 0.001 vs. K depleted and KHCO<sub>3</sub> and *P* < 0.02 compared to KCl-repleted animals). The alteration in

**TABLE II**  
*Arterial Blood Concentrations of Urea, Creatinine and Citrate, Urinary Chloride Excretion, and Renal Cortical Citrate Content in the Four Groups of Rats\**

	Blood			Urinary chloride excretion <i>meq/liter</i>	Renal cortical citrate content <i>μmol/g wet weight</i>
	Urea nitrogen <i>g/100 ml</i>	Creatinine <i>g/100 ml</i>	Citrate <i>μmol/ml</i>		
KCl replete, <i>n</i> = 5	11.2‡ ±0.6	0.48 ±0.04	0.059 ±0.005	121‡ ±25	0.346 ±0.060
K deplete, <i>n</i> = 7	20.7§ ±2.4	0.50 ±0.04	0.57 ±0.006	3.4§ ±0.5	0.316 ±0.046
NaCl replete, <i>n</i> = 7	12.0‡ ±1.2	0.46 ±0.02	0.060 ±0.008	147§ ±28	0.158‡§ ±0.025
KHCO <sub>3</sub> replete, <i>n</i> = 6	14.2 ±2.0	0.52 ±0.02	0.090‡§ ±0.008	4.2‡ ±0.8	0.819‡§ ±0.075

\* Values represent the mean ± SE of the mean.  
 ‡ Differ from deplete *P* < 0.01, using Student *t* test.  
 § Differ from KCl replete *P* < 0.01, using Student *t* test.

citrate excretion in NaCl-repleted rats apparently was not secondary to changes in the filtered load of citrate. Table II shows that serum creatinine levels were not significantly different between groups, and only in the potassium-depleted group was BUN elevated (*P* < 0.01), indicating equal glomerular filtration rates in the several groups. Blood citrate levels were elevated only in the potassium bicarbonate rats so filtered citrate in each of the first three groups studied probably was the same. Increased citrate excretion in the KHCO<sub>3</sub>

group is easily accounted for by the elevated filtered load. Evidence that the observed change in renal citrate metabolism in the NaCl-repleted rats was not due to filtered load is presented in the last column of Table II. The data show that in addition to the reduced citrate excretion, renal cortical citrate content also decreased significantly in the sodium chloride-repleted animals (*P* < 0.001 vs. each of the three other groups). Sodium chloride given to KCl-deficient rats, therefore, induces changes in both renal citrate and ammonia metabolism.

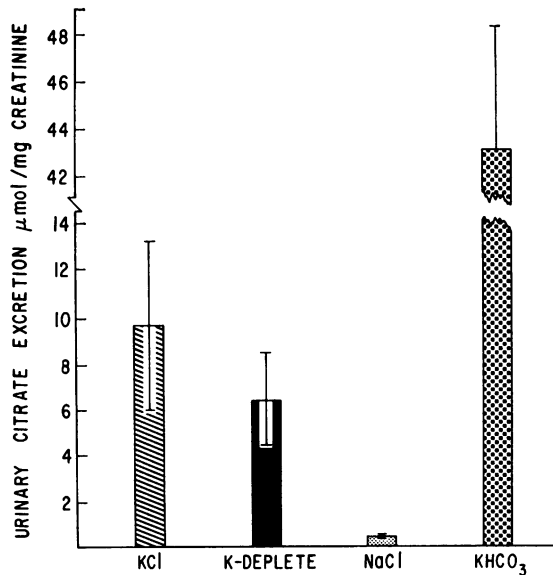


FIGURE 1 Urinary citrate excretion in the four groups of repleted rats. The brackets represent ±1 SE of the mean.

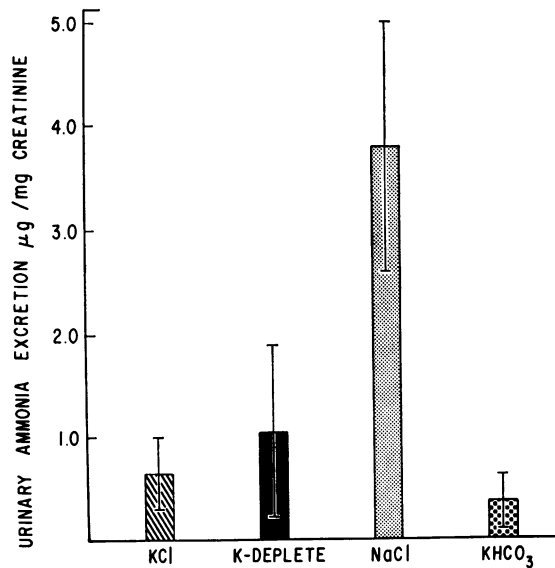


FIGURE 2 Urinary ammonia excretion in the four groups of repleted rats. The brackets represent ±1 SE of the mean.

TABLE III  
Arterial Blood Values in KCl-Depleted Rats Infused with either Dextrose or Mannitol Solutions\*

	pH	PCO <sub>2</sub>	HCO <sub>3</sub>	Na	Cl	K	Creatinine	Citrate
		mm Hg	meq/liter		meq/liter		g/100 ml	μmol/ml
Dextrose infused, n = 12								
Initial	7.50	46	36.3	—	—	—	—	—
	±0.02	±3	±1.8					
Final	7.49	46	35.1	134	79	2.4	0.56	0.073
	±0.02	±3	±1.6	±2	±2	±0.1	±0.06	±0.007
Mannitol infused, n = 13								
Initial	7.47	52	37.3	140	—	2.4	—	—
	±0.02	±3	±1.0	±4		±0.1		
Final	7.46	45‡	31.8‡§	113‡§	<70§	2.6	0.57	0.080
	±0.01	±2	±1.3	±3		±0.2	±0.06	±0.004

\* Values represent the mean ± SE of the mean.

‡ Value differs significantly from initial value ( $P < 0.05$ ).

§ Difference from final dextrose infused value statistically significant ( $P < 0.01$ ).

In an attempt to delineate the mechanism involved, further experiments were performed.

<sup>14</sup>CO<sub>2</sub> production from [1,5-<sup>14</sup>C]citrate *in vitro*. The results of the previous experiments might be due to alterations in the concentration of serum chloride bathing the kidney. It has been shown that urinary citrate excretion is dependent on the ability of renal tubules to reabsorb filtered citrate (12), and this resorptive capacity is probably a function of the renal tubular cells ability to oxidize citrate accumulated intracellularly (4, 13). Measurement of citrate decarboxylation by renal cortical tissue at different external chloride concentrations should, therefore, partially describe chloride's effect upon renal citrate metabolism. Renal cortical slices were obtained from potassium chloride-deficient rats whose extracellular acid-base status and serum and tissue potassium concentrations were statistically identical to those of the potassium-depleted rats shown in Table I. The slices were incubated in low (70 meq) and "normal" (110 meq) chloride solutions and <sup>14</sup>CO<sub>2</sub> production was measured. The results are shown in Fig. 3. As <sup>14</sup>CO<sub>2</sub> production is equal in the two groups it appears that chloride concentration does not directly affect renal citrate metabolism. To further examine the effect of chloride concentration on renal metabolism and to determine whether changes in extracellular volume might account for the observed results additional experiments were performed.

*Expansion of intravascular volume by mannitol in potassium-depleted rats: effect on renal citrate and ammonia metabolism.* Mannitol infusions were given to potassium chloride-depleted animals. The effect of this infusion was to expand intravascular volume and lower serum chloride concentration. Another group of animals was given low volume dextrose infusion over

a similar time period to serve as a control. Serum and tissue potassium concentrations in the animals before and after infusion were similar to that of the animals shown in Table I. Muscle potassium concentration after infusion in the dextrose and mannitol groups was 304 ± 30 and 312 ± 36 meq/kg of dry weight, respectively, compared to a muscle potassium concentration in non-potassium chloride-depleted animals of 465 meq/kg dry weight ( $P < 0.001$ ). Renal potassium content in the dextrose and mannitol rats was 267 ± 26 and 252 ± 32

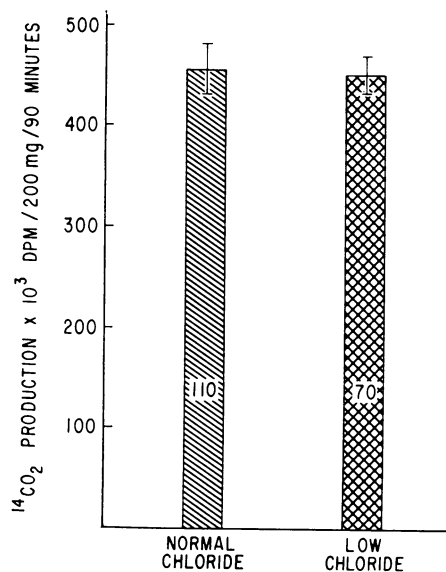


FIGURE 3 The effect of medium chloride concentration on citrate decarboxylation by renal cortical slices. The numbers within the bars represent medium chloride concentrations in each group while the brackets depict ± 1 SE of the mean.

TABLE IV  
Creatinine Clearance, Urinary pH, and Renal Citrate Content in KCL-Depleted Rats Infused with either Dextrose or Mannitol Solutions\*

	C <sub>cr</sub> ml/min	Urinary pH	Renal cortical citrate content μmol/g wet weight
Dextrose infused, n = 12	1.35 ± 0.29	6.83 ± 0.12	0.075 ± 0.008
Mannitol infused, n = 13			
Control	1.40 ± 0.19	6.88 ± 0.14	
Experiment 1	2.02 ± 0.16†‡	7.06 ± 0.05	0.049† ± 0.005
Experiment 2	1.68 ± 0.18	7.10 ± 0.05	
Experiment 3	1.84 ± 0.20†‡	7.08 ± 0.05	

\* Each value represents the mean ± SEM.

† Statistically significant differences from dextrose-infused animals ( $P < 0.05$ ).

‡ Statistically significant differences from control values in mannitol-infused rats ( $P < 0.05$ ).

meq/kg dry weight, respectively, compared to  $312 \pm 18$  meq/kg of dry weight in nondepleted control animals. Muscle and renal potassium content did not significantly differ between dextrose and mannitol-infused rats ( $P > 0.4$ ).

The effect of the dextrose and mannitol infusions on extracellular conditions in potassium chloride-depleted rats is shown in Table III. Arterial pH before and after infusion was unchanged in either group. Mannitol infusion had no effect on the serum potassium level but did lower extracellular bicarbonate concentration significantly ( $P < 0.01$ ). Neither serum creatinine nor blood citrate levels were significantly affected by mannitol infusion. The result of acute mannitol infusions

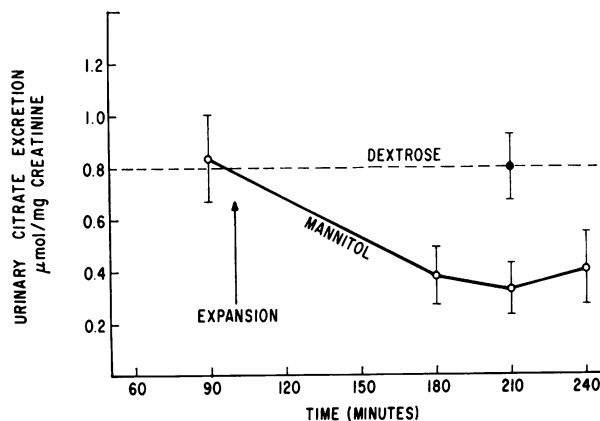


FIGURE 4 The effect of mannitol-induced volume expansion on urinary citrate excretion. The closed circle is the value obtained after 210 min of dextrose infusion while the opened circles are values obtained in the mannitol-infused rats. The dashed horizontal line represents the value for the dextrose-infused group. The brackets depict  $\pm 1$  SE of the mean.

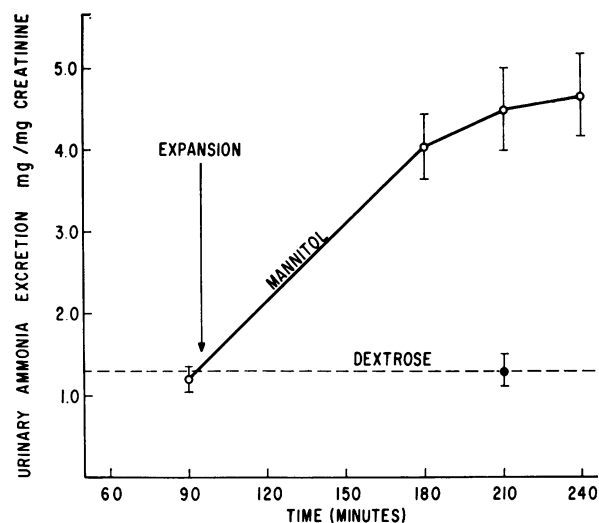


FIGURE 5 The effect of mannitol-induced volume expansion on urinary ammonia excretion. Conventions in this figure are the same as for Fig. 4.

on extracellular conditions, therefore, was similar to that previously shown in the rats chronically given sodium chloride. Serum bicarbonate concentration and arterial  $PCO_2$  fell, metabolic alkalosis was maintained, and blood citrate levels were unaffected. A significant difference is that mannitol infusion decreased the serum chloride concentration while sodium chloride administration raised serum chloride concentration to normal levels.

As was seen with sodium chloride ingestion, mannitol infusion lowered urinary citrate excretion. This is shown in Fig. 4. Citrate excretion in each of the experimental periods is significantly reduced compared to control period values or to excretion in the dextrose-infused animals ( $P < 0.01$ ). Fig. 5 shows the effect on ammonia excretion. In this case, however, urinary ammonia was significantly elevated in each of the mannitol experimental periods compared to control period values or to results obtained in dextrose-infused animals ( $P < 0.001$ ). Citrate and ammonia excretions during the control period were similar in the two groups ( $P > 0.5$ ). The alteration in citrate excretion after mannitol infusion is probably secondary to changes in the filtered load of citrate as experiments employing radioactive labeled citrate indicate that the citrate in the urine comes from extrarenal citrate (13). Thus, urinary citrate essentially represents the net sum of filtered citrate less reabsorbed citrate (14). Table IV, however, shows that changes in filtered load do not account for the observed decrease in urinary citrate excretion. Glomerular filtration rate, calculated from the endogenous creatinine clearance, was increased during mannitol infusion compared both to control period

TABLE V  
Skeletal Muscle, Cardiac, and Liver Cell pH in KCl-Depleted Rats Infused  
with either Dextrose or Mannitol Solutions\*

	Skeletal muscle		Cardiac muscle		Liver	
	K	pH <sub>DMO</sub>	K	pH <sub>DMO</sub>	K	pH <sub>DMO</sub>
	meq/kg dry weight		meq/kg dry weight		meq/kg dry weight	
Dextrose infused, <i>n</i> = 12	304 ±9	6.88 ±0.03	273 ±8	7.10 ±0.03	336 ±8	7.14 ±0.03
Mannitol infused, <i>n</i> = 13	312 ±11	6.93 ±0.03	282 ±9	7.15 ±0.02	282† ±8	7.14 ±0.02

\* Values represent the mean ± SE of the mean.

† Differs significantly from dextrose-infused animals ( $P < 0.01$ ).

values and to the filtration rate of the dextrose-infused animals. This increase was statistically significant only in periods two and four ( $P < 0.05$ ). This change in filtration rate combined with the slight increase in blood citrate (Table III) shows that filtered citrate increased rather than decreased during mannitol infusion. Thus, decreased urinary citrate excretion is probably due to increased tubular reabsorption of filtered citrate. Further proof that the alterations in urinary citrate excretion reflect altered renal citrate metabolism is given in Table IV. The data show that renal cortical citrate content in the mannitol-infused rats was significantly reduced compared to that found in the control rats ( $P < 0.001$ ). Increased urinary ammonia excretion similarly appears to be secondary to changes in renal metabolism rather than to a non-specific effect of the mannitol infusion. Urinary ammonia excretion ordinarily rises when urinary pH falls (15). The data presented in Table IV show that urinary pH rose rather than fell during mannitol infusion. The rise was not significant, however, compared to control period values or to values obtained in the dextrose-infused rats ( $P < 0.1$ ). Thus, chronic NaCl ingestion and acute mannitol infusion in KCl-depleted rats reduce renal cortical citrate content, lower urinary citrate excretion, and increase urinary ammonia excretion.

*Effect of mannitol-induced volume expansion on muscle, liver, and cardiac pH.* Although renal tubular cell pH cannot be directly determined in vivo, skeletal muscle, liver, and cardiac muscle cell pH may be calculated from distribution of the weak acid 5,5-dimethyl-2,4-oxazolidinedione (DMO) (16). Table V shows the effect of mannitol infusion on the intracellular pH of these three tissues. Extracellular potassium concentration was approximately 2.5 meq/liter (Table III) in these animals. The concentration of potassium in each of the three tissues (Table V) demonstrates that a higher intracellular to extracellular gradient existed

at the conclusion of the infusion in each of the tissues studied. Membrane selectivity was thus maintained and the DMO distribution method for measuring cell pH may be employed (16). It is apparent that mannitol infusion did not lower intracellular pH in any of the three tissues. Indeed, there was a tendency in skeletal and cardiac muscle for intracellular pH to increase. This increase was not statistically significant ( $P > 0.05$ ). As shown in Table III, the slight elevation of cell pH cannot be due to a change in extracellular pH for final arterial blood pH in the mannitol-infused rats was slightly lower than the final blood pH of the dextrose-infused animals. The rise in cell pH might be explained by the decreased  $\text{CO}_2$  tension in the mannitol-infused rats since carbon dioxide readily penetrates cell membranes and might influence cell pH more than bicarbonate (17). Citrate content was determined in liver and gluteal muscle tissue. No differences were found between dextrose and mannitol-infused animals. This is consistent with the absence of significant intracellular pH differences since tissue citrate content apparently is a function of intracellular pH (5).

## DISCUSSION

These experiments extend previous work which demonstrated that administration of sodium chloride to potassium chloride-deficient rats alters renal citrate metabolism (1). Whether sodium chloride altered renal citrate metabolism by changing extracellular chloride concentration or extracellular volume was not, however, examined in the earlier study. The present results show that the alteration in renal citrate metabolism is not due to changes in serum chloride concentration since volume expansion with mannitol had the same effect as sodium chloride on extracellular acid base status and renal citrate metabolism despite having an opposite effect on serum chloride concentration. Rather, it appears that increased circulating volume is responsible

for the altered renal metabolism. Although circulating volume was not measured in the tube fed rats, weight loss in the NaCl-repleted animals over the 3 days of feeding was 10 g less than in K-depleted or  $\text{KHCO}_3$  rats. There was sufficient scatter in the data to make this difference nonsignificant ( $P > 0.05$ ,  $< 0.1$ ). Most studies, however, show only small increases or decreases in extracellular volume after chloride repletion or depletion (18), so the inability to obtain significant data under the conditions of the present experiments is not surprising. In addition, expansion of extracellular volume in rats decreases renal bicarbonate reabsorptive capacity (19). Thus, the increase in urinary pH during mannitol infusion and the fall in serum bicarbonate after NaCl mannitol administration is consistent with an increased circulating volume.

In addition to its effect on renal citrate metabolism volume expansion increased ammonia excretion. The latter probably reflects increased renal ammonia production for two reasons. First, the increase was accompanied by a simultaneous rise in urine pH in the mannitol-infused rats.  $\text{P}_{\text{NH}_3}$  is in equilibrium between peritubular and luminal fluid (20), so the concentration of total urinary ammonia ( $\text{NH}_3$  plus  $\text{NH}_4^+$ ) varies directly with the pH gradient between peritubular and luminal fluid; the greater the gradient the higher the urinary ammonia excretion. As blood pH was constant the gradient must have fallen. Less of the relatively nondiffusible  $\text{NH}_4^+$  is then trapped in the tubular lumen, and urinary ammonia excretion should fall if renal ammonia production is constant (15, 21). Elevated urinary ammonia in these circumstances indicates increased renal ammonia production. This mechanism probably explains the decreased ammonia excretion seen in the rats given potassium bicarbonate. A second reason why increased production is probably responsible for the increased ammonia excretion is that renal blood flow and glomerular filtration rate ordinarily rise after volume expansion (22). Endogenous creatinine clearance increased in the rats infused with mannitol so renal blood flow probably also increased. As peritubular blood traps ammonia increased renal blood flow should increase ammonia transport into renal venous blood and decrease the amount of ammonia available for urinary excretion especially when the urine is only weakly acid and urine flow rate is high. Increased renal blood flow plus a rise in urinary pH indicate that elevated urinary ammonia excretion after NaCl or mannitol administration is due, therefore, to increased renal ammonia production.

The volume expansion-induced alterations in renal citrate and ammonia metabolism cannot, obviously, be explained by changes in chloride concentration or by a nonspecific effect of mannitol. Three possibilities, not

mutually exclusive, may explain the results: the renal metabolic changes could be due to the observed isohydric reduction in extracellular bicarbonate; they could be secondary to a decrease in renal tubular cell potassium concentration; or the changes could represent a response to a reduction in renal tubular cell pH. Supporting the first possibility are the many studies showing that varying extracellular bicarbonate concentration at a constant extracellular pH alters intermediary metabolism in a variety of tissues (23, 24). Indeed, Kamm, Fruisz, Goodman, and Cahill (25) have shown that gluconeogenesis in rat renal cortical slices varies inversely with the bicarbonate concentration of the medium at a constant extracellular pH of 7.40. Since ammonia and glucose production by rat renal cortical slices obtained from metabolically acidotic animals is greater than that seen in slices obtained from alkalotic animals (26), it is possible the increased ammonia production in rats given NaCl or mannitol is due to the decreased bicarbonate concentration. Citrate metabolism also appears to be bicarbonate dependent. Adler (27) has demonstrated that the citrate content of rat diaphragm muscle is decreased in an iso-pH system when bicarbonate concentration is lowered. Furthermore, Simpson (4) has shown that under isohydric conditions *in vitro* citrate decarboxylation by renal mitochondria proceeds more rapidly as bicarbonate concentration is progressively lowered. This apparently is due to increased transport of citrate into the mitochondria (28). Increased citrate decarboxylation intramitochondrially would decrease renal citrate content and increase reabsorption of citrate, the results observed in the present study. Other work of Simpson (4), however, is not in accord with his observations in isolated mitochondria. Rabbit renal cortical slices incubated at a constant external pH of 7.39 and at bicarbonate concentrations varying from 10 to 50 meq/liter showed identical citrate decarboxylation rates. These slices, however, were quite responsive to alterations in external pH as the citrate decarboxylation rate varied inversely with the pH of the bathing medium. If the whole kidney responds like the slice then a change in bicarbonate concentration occurring isohydrically would not be expected to alter renal citrate metabolism. The exact role of the decreased bicarbonate concentration, therefore, remains unclear.

A second possibility is that the altered renal metabolism is a consequence of altered intracellular potassium concentration. When normal animals are made alkalotic, urinary citrate excretion (29) and renal citrate content (2) increase while renal ammonia production and excretion decrease (30). In potassium deficiency, however, urinary citrate excretion falls (12), and urinary ammonia excretion rises (30) despite the presence



of an extracellular alkalosis. This has been attributed to coexisting intracellular acidosis and extracellular alkalosis in potassium depletion. Because the changes in pH are accompanied by a fall in tissue potassium concentration, some investigators believe that the renal metabolic alterations are due to decreased intracellular potassium concentration rather than renal intracellular acidosis. In the present experiment, however, renal potassium content did not decrease further after sodium chloride ingestion or mannitol infusion. In addition, Adler, Anderson, and Zett (5) have shown that the citrate content of potassium-depleted rat diaphragm muscle in vitro is a function of cell pH (calculated from DMO distribution) and not the tissue potassium content. Thus, a fall in diaphragm muscle cell pH is accompanied by a decrease in tissue citrate content without any change in cellular potassium. It seems unlikely, therefore, that changes in intrarenal potassium concentration account for the renal metabolic alterations although changes in potassium concentration within specific areas of the cell cannot be ruled out.

Finally, the alterations in renal citrate and ammonia metabolism may reflect reduced renal tubular cell pH. Acidosis apparently increases renal decarboxylation of exogenous citrate leading to a decrease in urinary citrate excretion and renal citrate content while the converse occurs in alkalosis (4). The observed changes in renal citrate content and excretion thus are compatible with renal cell acidosis. Indeed, Crawford, Milne, and Scribner (12), as well as other investigators (28), have postulated that renal citrate metabolism reflects intracellular pH, using as supporting evidence for this hypothesis the decreased citrate excretion and renal cortical citrate content found in potassium deficiency alkalosis (12) and the known decrease in muscle cell pH which occurs simultaneously (31, 32). In muscle there is direct evidence supporting the concept that citrate metabolism is a function of cell pH not of extracellular acidity. Rats acutely administered potassium chloride intraperitoneally develop extracellular metabolic acidosis, intracellular metabolic alkalosis, and elevated skeletal muscle citrate content (33). Ordinarily, tissue citrate decreases in acidosis (2), so the change in citrate content presumably reflects the intracellular not the extracellular pH state. Also, as mentioned previously, Adler and coworkers (5) have been able to relate citrate metabolism in diaphragm muscle to intracellular pH, not to cell potassium content or extracellular pH. Renal ammonia production is also felt to reflect intracellular rather than extracellular pH conditions. As is the case with renal citrate metabolism this hypothesis is based on indirect evidence obtained in abnormal metabolic states since renal cell pH has only been determined in an isolated renal tubular cell

preparation (34). Most investigators agree, nevertheless, that both hydrogen ion secretion and renal ammonia production are regulated by intracellular pH. Both, for example, are increased in potassium deficiency despite a concomitant metabolic alkalosis (35). Also glutamine oxidation and presumably ammonia production in renal cortical slices and mitochondria is directly related to changes in pH (36). Finally, Lowance, Garfinkel, Mattern, and Schwartz (37) demonstrated that hypotonic volume expansion in dogs with chronic metabolic acidosis increases net acid excretion and restores plasma bicarbonate concentration to normal. The increased net acid excretion probably was due in part to an increase in ammonia excretion. Although it is unclear how volume expansion might decrease renal cell pH, the data are consistent with the hypothesis that volume expansion lowers renal tubular cell pH.

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