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Citrate therapy for polycystic kidney disease in rats

GEORGE A. TANNER and JUDITH A. TANNER

Department of Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, Indiana, USA

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Background. Few treatments are available to slow the progression to renal failure in autosomal dominant polycystic kidney disease (PKD). In an animal model of PKD, the male heterozygous Han:SPRD rat, intake of a solution of potassium citrate plus citric acid (KCitr) from age one to three months prevented a decline in glomerular filtration rate (GFR). The present study tested whether this beneficial effect is sustained and explored handling of citrate and ammonia in normal and cystic kidneys.

Methods. Rats were provided with tap water or citrate solutions to drink, and clearance and survival studies were performed.

Results. The GFRs of rats with PKD that consumed KCitr from one month of age were normal at six months of age, while those of their counterparts on water were about one third of normal. Long-term KCitr treatment extended the average life span of rats with PKD from 10 to 17 months. Compared with normal rats, water-drinking rats with PKD had higher plasma [citrate], renal cortical [citrate], and fractional excretion of citrate, and lower rates of renal citrate consumption, ammonia synthesis, and ammonia excretion. Cortical P_{NH3} was not elevated in cystic kidneys. Intake of Na_3 citrate/citric acid solution or K_3 citrate solution, but not ammonium citrate/citric acid solution, prevented a decline in GFR in three-month-old rats with PKD.

Conclusions. Rats with PKD show abnormal renal handling of citrate and ammonia. Citrate salts that have an alkalinizing effect preserve GFR and extend survival.

Autosomal dominant polycystic kidney disease (ADPKD) is an inherited disorder that afflicts more than 500,000 people in the United States alone and millions more worldwide. This disease is accompanied by the formation and enlargement of numerous fluid-filled epithelial sacs (cysts) in both kidneys and eventual development of renal failure. There is currently no specific treatment to halt or slow the progression of renal failure in patients with PKD [1].

In 1991, Kaspareit-Rittinghausen, Deerberg, and Wcislo discovered a mutant strain of Sprague-Dawley rats with autosomal dominant PKD, the Han:SPRD strain, in a laboratory animal-breeding facility in Hanover, Germany [2]. Homozygous animals with altered PKD genes develop massively enlarged cystic kidneys and die at about three to four weeks of age. Heterozygous males and females develop a slowly progressive cystic disease, which is much more severe in males than in females. Death from renal failure in male heterozygotes occurs at a median age of about 6 [2] or 17 months [3] in different colonies. The heterozygous Han:SPRD rat with PKD may be a useful model for human autosomal dominant PKD [2–5], even though the abnormal gene in the rat [6] may not correspond to the *PKD-1* or *PKD-2* genes that are defective in almost all patients with ADPKD.

We recently demonstrated that if heterozygous male Han:SPRD rats with PKD were provided with a solution of potassium citrate/citric acid (abbreviated "KCitr") to drink, starting at one month of age, then the glomerular filtration rate (GFR) was sustained at a normal level and the kidneys showed less cystic disease when the rats were three months old [7]. By contrast, littermate rats with PKD that drank tap water had a GFR one half of normal, large kidney cysts, and extensive renal interstitial disease. The present study had two purposes: (*1*) to ascertain whether the benefits of KCitr therapy would be sustained beyond three months, and (*2*) to examine the mechanism of this beneficial effect.

To assess the effectiveness of KCitr administration beyond three months of age, we treated Han:SPRD rats with KCitr for longer times in two sets of experiments. First, we treated rats, starting at the age of one month, and measured renal function at six months of age. Second, we treated rats from the age of one month until their deaths to see whether this treatment prolonged survival.

To gain some insight into the mechanism of KCitr therapy, we examined several parameters. First, we measured blood and urine pH values to determine whether KCitr intake affected these variables. Second, we hypothesized that renal citrate handling might be abnormal in rats with PKD, based on a previous report that citrate excretion is abnormally elevated in these rats [8]. We therefore measured plasma and renal tissue citrate levels and renal reabsorption and consumption of citrate; the

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effects of KCitr treatment on these parameters were also studied. Third, since Torres et al had suggested that increased ammonia levels contribute to damage in cystic kidneys, we determined renal cortical tissue P_{NH3} and ammonia production and excretion [9, 10]. Fourth, we tested the idea that KCitr's beneficial effect is dependent on the well-known interaction between citrate and calcium ions in the body [11]. Finally, we substituted ammonium or sodium ions for potassium ions in the citrate solution and also gave K_3 citrate (tripotassium citrate without citric acid) to determine whether the beneficial effect of the KCitr solution was related to its potassium content or to its alkalinizing effect.

METHODS

Animals and solutions

All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments were performed on heterozygous male Han:SPRD rats with PKD and their normal littermates. The breeding stock was obtained from the Polycystic Kidney Program at the University of Kansas. All animals were allowed free access to a diet containing 24% protein and 6% fat (Teklad 6% mouse/rat diet 7002; Harlan, Madison, WI, USA). In most experiments, rats were provided with either a solution of 55 mmol/L tripotassium citrate/67 mmol/L citric acid (KCitr) or tap water to drink beginning at one month of age, and they were studied at six months of age or long-term survival was followed. In other experiments, normal rats or rats with PKD were provided with solutions of either (*1*) 82.5 mmol/L diammonium citrate/39.5 mmol/L citric acid, (*2*) 55 mmol/L trisodium sodium citrate/67 mmol/L citric acid, or (*3*) 55 mmol/L K_3 citrate to drink from one month of age, and they were studied at three months of age. The milliequivalents of citrate in all citrate solutions were the same. Drinking solutions were made up in tap water.

Clearance studies

Before experiments, six-month-old rats were deprived overnight of food but had free access to water or citrate solution. They were intraperitoneally anesthetized with the thiobarbiturate Inactin (130 mg/kg body weight; Byk Gulden, Konstanz, Germany). Each animal was placed on a heated table, and rectal temperature (monitored with a probe) was kept at 37°C. The trachea was cannulated, and a slow flow of moistened 35% O_2/65% N_2 was passed over the opening of the cannula. A femoral vein was cannulated for infusions. One milliliter of 6 g/100 mL fraction V bovine serum albumin in 0.9% NaCl was administered intravenously during the surgical preparation. This was followed by a priming dose (0.2 mL/100 g body weight) and constant intravenous infusion at 3 mL/hour of a solution containing 3% polyfructosan (Laevosan Co., Linz, Austria), 5.5 to 11 mg/mL sodium *p*-aminohippurate (PAH), 2 g/100 mL bovine serum albumin, 24 mmol/L $NaHCO_3$, and 125 mmol/L NaCl. A femoral artery was cannulated for blood sampling and for measuring blood pressure with a transducer (Gould-Statham, Hato Rey, Puerto Rico). The abdomen was opened via a midline incision. The left ureter was cannulated, and a cannula was inserted into the bladder to drain urine from the right kidney. A number 25 needle, connected to a short length of Silastic tubing, was inserted into the left renal vein for blood sampling.

Renal clearance measurements were made as follows: About one hour after the left ureter had been cannulated, we collected two 30-minute urine samples under water-equilibrated light mineral oil. Urine volume was determined by weighing, assuming a density of one. Urine pH was measured immediately at room temperature using a 9802BN micro-pH combination electrode (Orion Research, Beverly, MA, USA). Arterial and renal venous blood samples (0.60 to 0.85 mL) were collected at the beginning and end of the urine collections. When determinations of plasma ammonia levels were made, blood samples were collected in iced syringes. Plasma and blood cells were separated immediately. Plasma proteins were precipitated with iced 10% trichloroacetic acid. Supernatants were frozen, and analyses were completed within a few hours. An arterial blood sample (0.25 mL) was also collected anaerobically for measurement of blood gases and pH using an IRMA series 2000 blood system (Diametrics Medical, St. Paul, MN, USA) or model 1400 blood gas electrolyte analyzer (Instrumentation Laboratories, Lexington, MA, USA). In some experiments, a terminal blood sample (0.25 mL) was collected for plasma calcium and phosphate determinations.

The clearance studies in three-month-old rats were done in exactly the same way as in our previous study on rats of this age [7].

Long-term survival

Survival studies were performed on 18 male heterozygous rats with PKD that drank water and on six male heterozygous rats that drank KCitr solution starting at one month of age. Three normal rats on KCitr intake since one month of age were sacrificed when 21 months old.

Tissue collection

At the end of most clearance experiments, one kidney (usually the left) was rapidly removed. Cortex and medulla were separated, and wet and dry weights (samples heated in an oven at 120°C for 16 hours) were determined. In other experiments, samples for tissue calcium were obtained after separating kidney cortex and medulla. Samples for tissue citrate were obtained by rapidly slicing off a piece of cortex, freeze-clamping it in liquid nitrogen, and then homogenizing the sample in iced 10%

perchloric acid. The samples were centrifuged, and the supernatants were then filtered through a 10,000 molecular weight cutoff UltraFuge filter (Micron Separations, Westboro, MA, USA).

Histology

The remaining kidney was fixed by retrograde aortic perfusion with a solution of 3% paraformaldehyde, 137 mmol/L NaCl, 2.7 mmol/L KCl, 1.5 mmol/L KH_2PO_4, 4 mmol/L Na_2HPO_4, and 2 mmol/L picric acid at a perfusion pressure of 150 to 170 mm Hg. The kidney was kept in the same fixative solution for several days in the refrigerator and then weighed, sliced with a razor blade, immersed in 0.1 mol/L cacodylate solution (pH 7.25), and embedded in paraffin. Sections were stained with hematoxylin and eosin. The degree of cystic disease (size of cysts, interstitial widening and fibrosis, presence of inflammatory cells) was evaluated blindly using an arbitrary scale of 0 to 4, where 0 represents the normal condition and 4 represents severe changes.

Chemical analyses

Polyfructosan (a synthetic inulin) in plasma and urine was determined by an anthrone method [12]. PAH was determined by Bratton and Marshall's method [13]. Citrate in plasma, urine, and tissue samples was determined spectrophotometrically using a citrate lyase method (Boehringer Mannheim, Indianapolis, IN, USA). Recovery of citrate added to plasma averaged 99 ± 6% (N = 11). When kidney tissue was analyzed for citrate, we observed a 100 ± 5% (N = 25) recovery of an internal citrate standard at five minutes after adding the citrate lyase, indicating completion of the reaction. The absorbance readings, however, continued to drift appreciably with time, probably because of the presence of nicotinamide adenine dinucleotide (NADH) oxidase activity in kidney cortex extracts. To correct for this "creep," we measured absorbance every 5 minutes for a total of 20 minutes after adding the citrate lyase and extrapolated the absorbance back to time zero [14]. Ammonia was determined using a glutamate dehydrogenase-based kit (Boehringer Mannheim). Recovery of ammonia added to plasma averaged 100 ± 1.4% (N = 4). Phosphate in plasma was determined by the Fiske-SubbaRow method.

Potassium in urine and plasma and calcium in plasma, urine, and kidney tissue were determined using an atomic absorption spectrophotometer (model 951; Instrumentation Laboratory, Wilmington, MA, USA). Tissue samples were homogenized in a solution containing 4% (vol/vol) butanol, 0.2 mol/L HCl, and 36 mmol/L $LaCl_3$ [15] using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY, USA). Recovery of calcium added to tissue samples averaged 103 ± 3% (N = 9). Samples and standards were always prepared in the same matrix solutions.

Calculations

The GFR was calculated from the rate of excretion of polyfructosan divided by the plasma polyfructosan concentration. Renal plasma flow (RPF) was calculated from the PAH clearance divided by the PAH extraction ratio ((arterial minus renal venous plasma [PAH]) ÷ arterial plasma [PAH]). Renal blood flow (RBF) was calculated from this formula: RBF = RPF/(1 − hematocrit). Renal citrate consumption was calculated from RPF × (arterial plasma [citrate] − renal vein plasma [citrate]) minus urinary citrate excretion rate. Tubular reabsorption was calculated from the filtered load minus the excretion rate. Peritubular uptake of citrate was calculated from the renal citrate consumption minus the rate of tubular citrate reabsorption. Fractional excretion was calculated from the excretion rate divided by the filtered load. Renal ammonia production was calculated from the RBF × (renal venous blood [ammonia] − arterial blood [ammonia]) plus the urinary excretion rate. The partial pressure of cortical tissue ammonia (P_{NH3}) was estimated from the renal vein total NH_4^+ concentration and arterial blood pH [16, 17] and this formula:

$$P_{NH3} = (\text{total } NH_4^+ \times 22.09)/(10^{pK - pH} \times \alpha)$$

where the pK for NH_4^+ is 9.02. The pH of renal venous and arterial blood are assumed to be the same, and the solubility coefficient (α) for ammonia is 0.91 [18].

Statistical methods

Data presented are means ± SD. They were analyzed by two-way analysis of variance (ANOVA), after a preliminary test for homogeneity of variances. Individual groups were compared with the Bonferroni method. If variances were heterogeneous, the Kruskal–Wallis test and Dunn's test were used to compare means. A P value of less than 0.05 was considered significant.

RESULTS

Overall function in six-month-old rats

The effects of KCitr consumption in six-month-old normal rats and rats with PKD are summarized in Table 1. The most remarkable finding is that GFR was completely normal in rats with PKD that had been treated with KCitr, whereas GFR was only 37% of normal in rats with PKD that had consumed tap water. RBF also was normal in the rats with PKD that had been treated with KCitr and was about half of normal in untreated rats with PKD. KCitr treatment had no effect on body weight or blood pressure, in agreement with our earlier findings at three months of age [7].

A comparison of water-drinking normal rats and water-drinking rats with PKD (Table 1) shows that the animals with cystic disease had heavier kidneys, higher

Table 1. Effects of KCitr consumption on function in six-month-old normal rats and rats with PKD

	Normal rats		Rats with PKD	
	Tap water	KCitr	Tap water	KCitr
Body weight *g*	464 ± 28 (14)	446 ± 25 (13)	462 ± 29 (13)	464 ± 10 (12)
Kidney weight *g*	1.55 ± 0.14 (14)	1.59 ± 0.15 (13)	2.64 ± 0.40 (13)[c]	2.83 ± 0.28 (12)[e]
Kidney cortex % H_2O	78.9 ± 0.4 (10)	80.2 ± 0.6 (9)[c]	87.5 ± 0.6 (9)[c]	84.4 ± 0.5 (7)[e,g]
Kidney medulla % H_2O	83.0 ± 0.6 (10)	84.0 ± 0.6 (9)[b]	85.0 ± 0.7 (9)[c]	83.9 ± 0.5 (7)[f]
MABP *mm Hg*	107 ± 6 (14)	106 ± 7 (13)	116 ± 10 (13)[a]	119 ± 6 (12)[e]
Hematocrit *% cells*	47 ± 1 (14)	44 ± 1 (13)[a]	39 ± 4 (13)[b]	43 ± 1 (12)
GFR *μL/min per 100 g body weight*	392 ± 31 (14)	448 ± 43 (13)[a]	146 ± 67 (13)[c]	418 ± 37 (12)[g]
$\dot{V}$ *μL/min per 100 g body weight*	6.8 ± 1.7 (14)	6.7 ± 1.7 (13)	8.4 ± 3.6 (13)	9.3 ± 2.4 (12)[d]
PAH extraction	0.88 ± 0.02 (14)	0.84 ± 0.04 (13)	0.37 ± 0.17 (13)[b]	0.72 ± 0.05 (12)
Renal blood flow *mL/min*	18.5 ± 2.5 (14)	18.5 ± 2.3 (13)	9.7 ± 2.3 (13)[c]	17.0 ± 1.4 (12)[g]

Values are means ± SD (number of rats). Kidney data are for the left kidney. Abbreviations are: PKD, polycystic kidney disease; KCitr, potassium citrate/citric acid solution; MABP, mean arterial BP; $\dot{V}$, urine flow rate.

[a] $P < 0.05$, [b] $P < 0.01$, and [c] $P < 0.001$ compared with normal rats on tap water

[d] $P < 0.05$ and [e] $P < 0.001$ compared with normal rats on KCitr

[f] $P < 0.01$ and [g] $P < 0.001$ compared with rats with PKD on tap water

kidney water contents, higher blood pressure, lower hematocrit, and lower PAH extraction. The increased kidney size and water content reflect cyst fluid accumulation.

KCitr treatment did not prevent overall renal enlargement in rats with PKD (Table 1). The histology of cystic kidneys in untreated and treated rats was, however, quite different (Fig. 1). In tap water-drinking rats with PKD, there was severe cystic disease, with numerous large cysts, interstitial widening and fibrosis, and numerous inflammatory cells; the disease score averaged 4 ± 0 ($N = 7$). In KCitr-treated rats with PKD, the disease score averaged 2.6 ± 0.4 ($N = 7$), and in no case was cystic disease judged to be severe (a score of 4).

In the normal rats, KCitr treatment had no significant effect on any of the variables measured (Table 1) or on renal histology, except for about a 10% increase in GFR, a small increase in cortex and medulla water contents, and a fall in hematocrit. In rats with PKD, KCitr treatment overall tended to produce more normal renal function and structure (Table 1 and Fig. 1).

Long-term survival

KCitr treatment significantly prolonged the life of rats with PKD (Fig. 2). The average survival time of rats that drank tap water was 288 ± 39 days ($N = 18$). The average survival time of rats with PKD on KCitr was 506 ± 33 days ($N = 6$). The KCitr-treated animals lived well beyond the time all of the untreated rats with PKD had died (Fig. 2). Histologic examination of kidneys from old KCitr-treated rats with PKD demonstrated marked cystic changes; hence, this treatment does not stop progression of the disease. Normal plasma chemistry values (urea, creatinine, and potassium) were found in three normal rats that had consumed KCitr solution for 20 months (data not shown).

Renal citrate, ammonia, and electrolyte handling in six-month-old rats

Acid-base measurements are presented in Table 2. Rats with PKD that drank tap water showed evidence of a metabolic acidosis (average plasma pH of 7.30 and bicarbonate concentration of 19 mEq/L). KCitr treatment increased blood pH and plasma bicarbonate concentration in the rats with PKD, but did not significantly affect these variables in normal rats. KCitr treatment significantly increased urine pH, by about 0.5 pH unit, in both normal rats and rats with PKD.

Data on renal citrate handling in normal rats and in rats with PKD that had consumed either tap water or KCitr solution are presented in Table 3. It is notable that in the normal rats, the arterial plasma citrate concentration and renal cortical citrate concentration were not affected by increased citrate intake. In fact, KCitr intake in normal rats did not affect any of the parameters measured except for expected increases in urine citrate concentration, excreted citrate, and the fraction of filtered citrate excreted in the urine.

Rats with PKD that were on tap water had an elevated arterial plasma citrate concentration (about twice normal), diminished citrate extraction and consumption, elevated urinary citrate excretion (about 10 times normal), and elevated renal cortical citrate concentration when compared with normal rats on tap water (Table 3). The higher rate of citrate excretion in rats with PKD is not due to a difference in urine pH [19, 20], since both groups that drank tap water had urine pH values about 5.9 (Table 2). A novel finding is that cortical tissue citrate concentration is also elevated in rats with PKD, although to a variable extent. Figure 3 illustrates that this variability is related to the degree of renal impairment in untreated rats with PKD; tissue citrate concentration was inversely related to the GFR.

Increased citrate intake paradoxically led to a lower

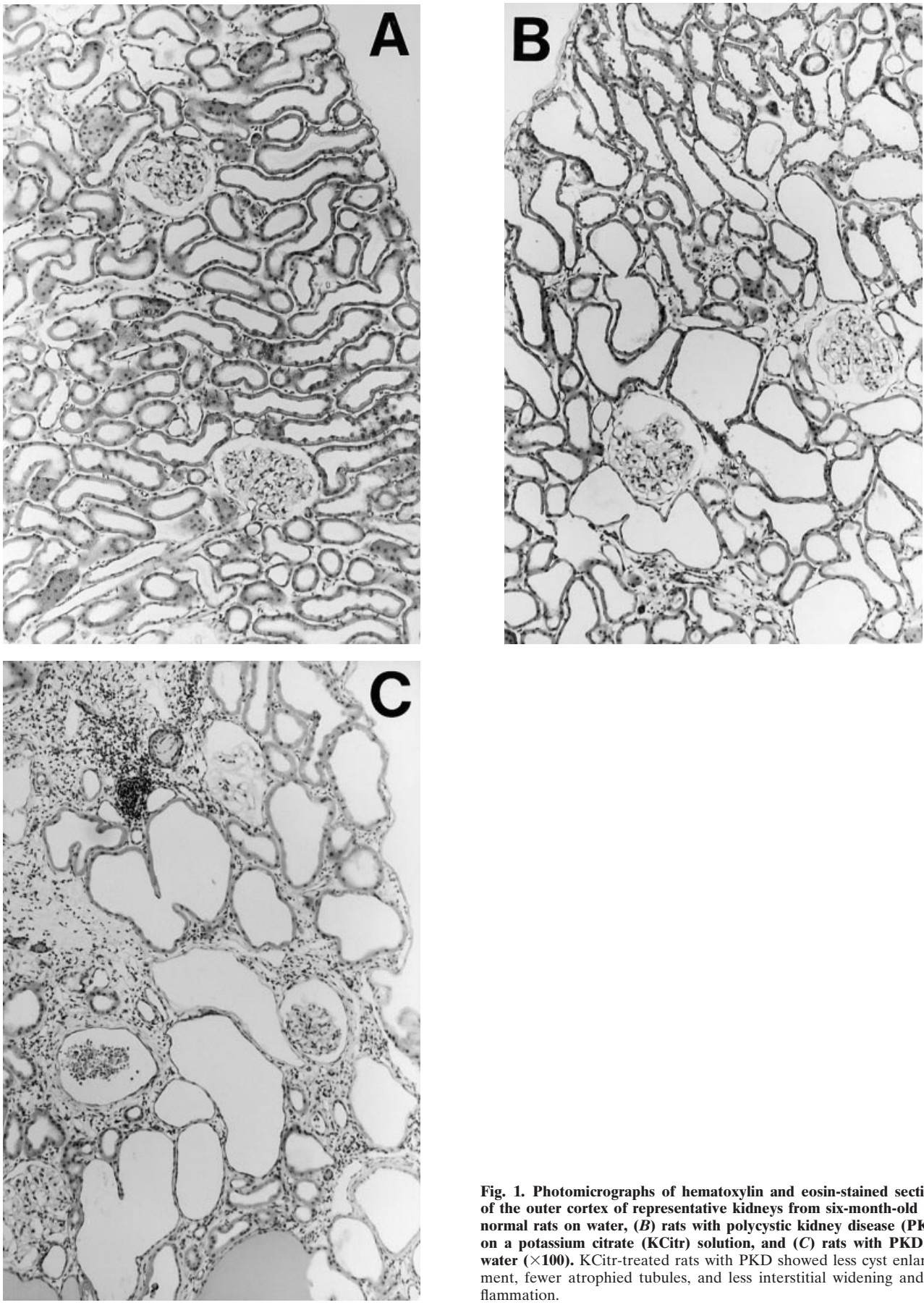

Fig. 1. Photomicrographs of hematoxylin and eosin-stained sections of the outer cortex of representative kidneys from six-month-old (*A*) normal rats on water, (*B*) rats with polycystic kidney disease (PKD) on a potassium citrate (KCitr) solution, and (*C*) rats with PKD on water (×100). KCitr-treated rats with PKD showed less cyst enlargement, fewer atrophied tubules, and less interstitial widening and inflammation.

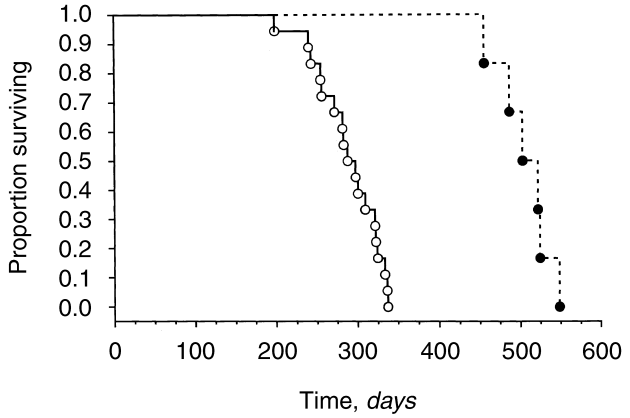

Fig. 2. Survival of male heterozygous rats with PKD drinking tap water (○) or on a KCitr solution (●). The age of death is plotted as a function of time. Treatment with KCitr starting at the age of one month led to an increase in survival time from 288 ± 39 days ($N = 18$) to 506 ± 33 days ($N = 6$).

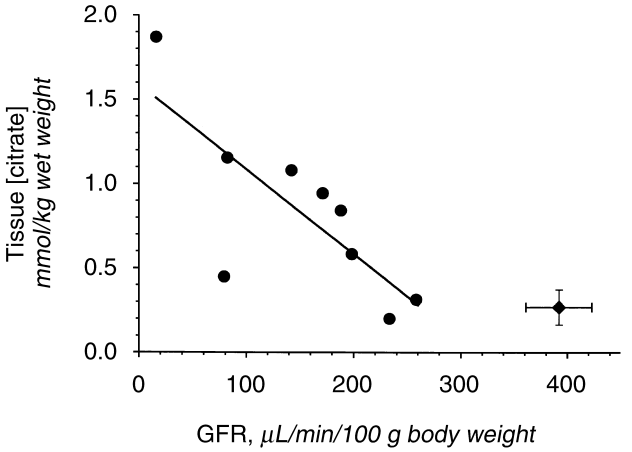

Fig. 3. Inverse relationship between cortical [citrate] and glomerular filtration rate (GFR) in untreated rats with PKD (●). The equation of the least squares line is tissue [citrate] = $-0.00503 \times$ GFR + 1.59 ($r = -0.77$, $P < 0.05$). Average values for eight normal rats consuming water are shown on the right (◆).

arterial plasma citrate concentration and a lower concentration of citrate in cortical tissue of rats with PKD compared with rats with PKD that drank tap water (Table 3). Overall, KCitr treatment of rats with PKD led to a more normal pattern of renal handling of citrate.

Measurements of renal handling of ammonia are summarized in Table 4. KCitr treatment in normal rats led to significant decreases in renal ammonia production and excretion. This may be due to two effects: (*1*) oxidation of citrate to bicarbonate and its consequent alkalinizing effect, and (*2*) inhibition of ammoniagenesis by citrate [21]. Rats with PKD on tap water had lower rates of production and excretion of ammonia than did normal rats on tap water. KCitr-treated rats with PKD had the same rates of production and excretion of ammonia as normal KCitr-treated rats.

Arterial ammonia concentration was lower in the rats with PKD than in normal rats when both drank tap water. Otherwise, there were no statistically significant differences in arterial ammonia concentration, renal venous ammonia concentration, or cortical P_{NH3} among the four groups (Table 4).

Data on electrolyte handling in normal rats and in rats with PKD that drank tap water or KCitr solution are shown in Table 5. Surprisingly, the plasma potassium concentration was significantly decreased in both normal rats and in rats with PKD that consumed KCitr when compared with water-drinking rats. Urinary potassium excretion was elevated with KCitr intake, as expected. The fractional excretion of potassium (FE_K) was increased with potassium intake in normal rats; in the rats with PKD treated with KCitr, FE_K was below that observed in untreated rats, most likely because of the higher GFR and filtered potassium load in the KCitr-treated rats.

Plasma calcium was significantly decreased by KCitr consumption in the normal rats, but was not affected by this treatment in the rats with PKD (Table 5). No significant differences in calcium excretion were seen in the four groups. In untreated rats, cortical tissue calcium levels were higher with PKD than in normals. Potassium citrate treatment actually raised the tissue calcium levels in the cortex and medulla of normal rats and in the medulla of rats with PKD. Plasma phosphate concentration was elevated in the rats with PKD on water, but was brought closer to normal in the rats with PKD that had been treated with KCitr. These changes in plasma phosphate level most likely reflect retention of phosphate in animals with severe renal disease (untreated rats with PKD) and adequate phosphate disposal in animals with a normal GFR (KCitr-treated rats with PKD).

Effects of varying drinking fluid composition

Effects of varying the composition of the drinking solutions were determined in clearance studies on three-month-old rats. Figure 4 shows the measurements of left kidney GFR from these new experiments along with results from our previous study on the effects of intake of tap water or KCitr solution [7]. The data are normalized per 100 g body weight. None of the treatments had a significant effect on body weight, and none prevented renal enlargement in rats with PKD (data not shown).

The intake of an ammonium citrate/citric acid solution had no beneficial effect on GFR in rats with PKD; GFR averaged 261 ± 22 μL/min-100 g body weight, about half of normal, and was the same as in water-drinking rats of the same age (Fig. 4). Urine pH in rats consuming ammonium citrate/citric acid solution was 5.77 ± 0.04 ($N = 4$) in normal rats and 5.60 ± 0.09 ($N = 4$) in rats

Table 2. Acid-base measurements in six-month-old normal rats and rats with PKD

	Normal rats		Rats with PKD	
	Tap water ($N = 14$)	KCitr ($N = 13$)	Tap water ($N = 13$)	KCitr ($N = 12$)
Arterial pH	7.38 ± 0.03	7.39 ± 0.06	7.30 ± 0.05[b]	7.39 ± 0.04[d]
Arterial P_{CO_2} *mm Hg*	37 ± 4	40 ± 6	39 ± 5	37 ± 4
Arterial plasma [HCO_3^-] *mEq/L*	22 ± 2.5	24 ± 2.1	19 ± 1.9[a]	22 ± 2.5[c]
Urine pH	5.96 ± 0.22	6.43 ± 0.25[b]	5.89 ± 0.22	6.39 ± 0.24[d]

Values are means ± SD.
[a] $P < 0.01$ and [b] $P < 0.001$ compared with normal rats on tap water
[c] $P < 0.01$ and [d] $P < 0.001$ compared with rats with PKD on tap water

Table 3. Renal citrate handling in six-month-old normal rats and rats with PKD

	Normal rats		Rats with PKD	
	Tap water ($N = 9$)	KCitr ($N = 8$)	Tap water ($N = 8$)	KCitr ($N = 7$)
Arterial [citrate] *mmol/L*	0.086 ± 0.018	0.081 ± 0.013	0.154 ± 0.038[c]	0.077 ± 0.015[g]
Citrate extraction	0.34 ± 0.08	0.33 ± 0.10	0.21 ± 0.08[b]	0.33 ± 0.09
Urine [citrate] *mmol/L*	0.08 ± 0.06	0.70 ± 0.54[c]	0.65 ± 0.33[c]	1.04 ± 0.57
Filtered citrate *nmol/min*	149 ± 31	158 ± 37	107 ± 18[b]	161 ± 39[f]
Excreted citrate *nmol/min*	2.3 ± 2.1	13.4 ± 8.6[c]	25.4 ± 16.9[c]	43.6 ± 20.3[d]
Reabsorbed citrate *nmol/min*	146 ± 30	145 ± 37	82 ± 17[c]	117 ± 24
$FE_{citrate}$ %	1.4 ± 1.0	8.8 ± 5.7[c]	23.0 ± 12.8[c]	26.3 ± 6.7[e]
Citrate consumption *nmol/min*	257 ± 66	267 ± 137	141 ± 57[b]	202 ± 68
Peritubular citrate uptake *nmol/min*	110 ± 62	122 ± 108	59 ± 41	85 ± 73
Cortical [citrate] *mmol/kg wet weight*[a]	0.269 ± 0.104	0.264 ± 0.069	0.825 ± 0.516[c]	0.271 ± 0.071[g]

Values are means ± SD. Kidney data are for the left kidney.
[a] Measurements in the four groups were done in 8, 7, 9, and 7 rats, respectively
[b] $P < 0.05$ and [c] $P < 0.001$ compared with normal rats on tap water
[d] $P < 0.01$ and [e] $P < 0.001$ compared with normal rats on KCitr
[f] $P < 0.05$ and [g] $P < 0.001$ compared with rats with PKD on tap water

with PKD; intake of this solution was associated with an acidic urine pH.

By contrast, substitution of sodium for potassium in the drinking solution yielded results similar to KCitr. GFR in the rats with PKD, 532 ± 96 μL/min-100 g body weight, was normal. Urine pH averaged 6.57 ± 0.23 ($N = 4$) in normal rats and 6.51 ± 0.33 ($N = 4$) in rats with PKD. The renal histology of rats with PKD that had consumed the sodium citrate salt (data not shown) was identical to that seen in rats of the same age that had consumed KCitr [7]. These results clearly indicate that it is the intake of citrate or citric acid, not the provision of extra potassium in the diet, that is responsible for the beneficial effect of KCitr.

With the administration of K_3 citrate (potassium citrate but no citric acid), GFR was maintained as with KCitr. Urine pH in the rats on K_3 citrate solution was 6.08 ± 0.25 ($N = 2$) in normal rats and 5.83 ± 0.35 ($N = 5$) in rats with PKD. Overall, the results demonstrate that alkalinizing citrate salts prevent a decline in GFR in rats with PKD.

DISCUSSION

This study demonstrates that chronic treatment of rats which have PKD with a KCitr solution, starting at one month of age, results in normal GFR and renal blood flow in six-month-old animals (Table 1). These results extend our previous study in which a beneficial effect of KCitr treatment was seen in rats treated from one to three months of age [7]. Long-term KCitr therapy, started at the age of one month, prolongs the survival time of rats with PKD (Fig. 2). We do not know whether initiation of treatment at an older age, when some degree of renal impairment is already present, would also be effective in slowing the progression of PKD.

Renal handling of citrate

We studied renal handling of citrate in an attempt to understand why it was effective. Some of our results were not expected (*1*) citrate concentrations in plasma, renal cortical tissue, and urine were all higher than normal in untreated rats with PKD; and (*2*) KCitr treatment of rats with PKD led to decreases in plasma and tissue citrate levels, probably as a consequence of more normal renal function.

Plasma citrate. Two factors may explain the elevated arterial plasma citrate concentration in untreated rats with PKD (Table 3). First, renal consumption of citrate was decreased. The kidneys are the major site of citrate utilization in the body [22]. Second, rats or patients with

Table 4. Ammonia handling in six-month-old normal rats and rats with PKD

	Normal rats		Rats with PKD	
	Tap water ($N = 5$)	KCitr ($N = 5$)	Tap water ($N = 5$)	KCitr ($N = 5$)
Arterial ammonia *μmol/L*	40 ± 6	37 ± 3	32 ± 4[a]	35 ± 4
Renal vein ammonia *μmol/L*	84 ± 11	77 ± 6	79 ± 19	84 ± 17
Cortical P_{NH_3} *mm Hg* × 10^{-6}	47 ± 6	53 ± 15	33 ± 9	48 ± 12
Ammonia excretion *μmol/min*	0.74 ± 0.12	0.40 ± 0.09[b]	0.28 ± 0.19[c]	0.32 ± 0.07
Ammonia production *μmol/min*	1.67 ± 0.23	1.10 ± 0.26[a]	0.77 ± 0.41[c]	1.14 ± 0.32

Values are means ± SD.
[a] $P < 0.05$, [b] $P < 0.01$, and [c] $P < 0.001$ compared with normal rats on tap water

Table 5. Electrolyte handling in six-month-old normal rats and rats with PKD

	Normal rats		Rats with PKD	
	Tap water	KCitr	Tap water	KCitr
Plasma [K^+] *mEq/L*	3.70 ± 0.30 (9)	2.91 ± 0.45 (8)[b]	4.04 ± 0.56 (8)	3.34 ± 0.17 (7)[d]
Excreted K^+ *μEq/min*	1.59 ± 0.16 (9)	2.95 ± 0.48 (8)[c]	1.78 ± 0.28 (8)	3.16 ± 0.29 (7)[f]
FE_K %	26 ± 4 (9)	51 ± 6 (8)[c]	64 ± 16 (8)[c]	47 ± 8 (7)[d]
Plasma [calcium] *mg/100 mL*	8.60 ± 0.24 (6)	8.01 ± 0.37 (4)[a]	8.64 ± 0.24 (3)	8.47 ± 0.33 (4)
Excreted calcium *μg/min*	0.62 ± 0.32 (9)	0.36 ± 0.12 (6)	1.10 ± 0.59 (6)	0.74 ± 0.26 (8)
Cortex [calcium] *mg/kg wet weight*	95 ± 4 (6)	176 ± 89 (3)[a]	153 ± 8 (3)[a]	181 ± 28 (3)
Medulla [calcium] *mg/kg wet weight*	156 ± 21 (6)	855 ± 677 (3)[c]	222 ± 37 (3)	1001 ± 462 (3)[e]
Plasma [phosphate] *mg/100 mL*	4.97 ± 0.34 (10)	5.37 ± 0.67 (9)	8.70 ± 1.45 (9)[c]	6.04 ± 0.61 (7)[f]

Values are means ± SD (number of animals). Kidney data are for the left kidney.
[a] $P < 0.05$, [b] $P < 0.01$, and [c] $P < 0.001$ compared with normal rats on tap water
[d] $P < 0.05$, [e] $P < 0.01$ and [f] $P < 0.001$ compared with rats with PKD on tap water

renal failure develop secondary hyperparathyroidism, and parathyroid hormone promotes release of citrate from bone and elevates the plasma citrate concentration [11].

In normal rats, plasma citrate was not affected by the level of chronic intake (Table 3). Likewise, patients on long-term intake of potassium citrate do not show an increase in serum citrate concentration [23].

Tissue citrate. The elevated renal cortical tissue citrate levels in untreated rats with PKD (Table 3 and Fig. 3) could be due to both an increase in intracellular citrate concentration and/or an increase in the tubular fluid or urine citrate level. It appears to be mainly due to an increase in intracellular level, since urine concentrations of citrate were elevated in the KCitr-treated rats and yet tissue citrate levels were not increased in these animals (Table 3).

Many factors can influence renal tissue citrate levels, including intracellular pH, citrate production or consumption, or altered citrate transport by luminal and peritubular cell membrane carriers [20]. Although we cannot say why the tissue citrate level was increased in rats with PKD, the finding of Ogborn et al that tissue succinate levels are low suggests impaired mitochondrial metabolism of citrate [8].

Citrate reabsorption and excretion. High urine citrate concentration and elevated citrate excretion in rats with PKD was first reported by Ogborn et al [8]. Our finding that renal cortical tissue citrate is elevated in rats with PKD may provide an explanation for increased citrate excretion. A high level of citrate in proximal tubule cells would diminish the driving force for reabsorption of filtered citrate across the luminal cell membrane and in this way could lead to increased citrate excretion [20].

Although the tenfold higher rate of citrate excretion in rats with PKD compared with normal rats is most impressive (Table 3), it is important to note that only 1% of the filtered load is excreted by the normal rat kidney. Fractional excretion of citrate in the untreated rat with PKD, 23% (Table 3), is similar to fractional excretion of citrate in the normal human kidney, about 10 to 35% [20]. Excessive urinary excretion of citrate in the rat with PKD may contribute to the development of metabolic acidosis, since loss of citrate represents a loss of potential bicarbonate.

Citrate consumption. Citrate is an important metabolic substrate in the kidneys, accounting for about 10% of their energy production [22, 24, 25]. In untreated rats with PKD, renal citrate consumption was lower than normal (Table 3). Decreased GFR and consequent decreased tubular sodium reabsorption in cystic kidneys probably contributes to the decreased citrate consumption, although we cannot rule out an intrinsic defect in citrate utilization.

Citrate and PKD. Citrate handling by cystic kidneys is clearly abnormal. It is still not obvious, however, why citrate treatment is beneficial. Had we known before

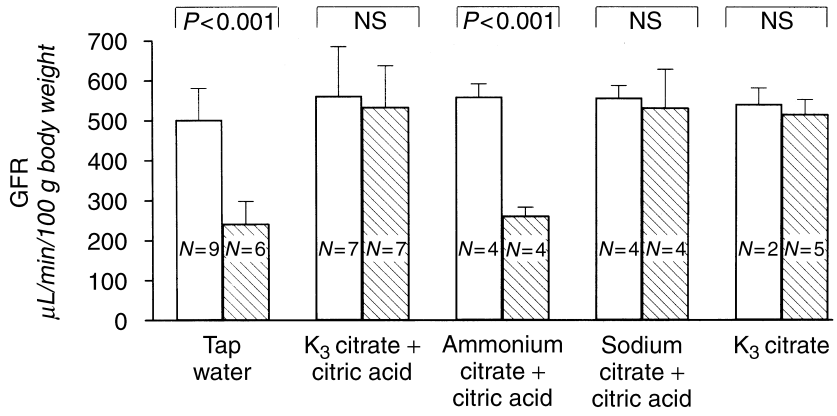

Fig. 4. Summary of effects of various treatments on GFR in three-month-old normal rats (□) and rats with PKD (▨). The data from the rats treated with KCitr or tap water are from our previous study [7]. GFR was preserved in the rats with PKD, which consumed solutions of KCitr (K_3 citrate + citric acid), Na_3 citrate + citric acid, or K_3 citrate alone, but was about half of normal in the rats that drank tap water or the $(NH_4)_2$ citrate + citric acid solution.

undertaking these experiments that plasma and kidney cortex citrate concentrations are elevated in untreated rats with PKD, we might have been dissuaded from administering additional citrate. These surprising results bolster our conclusion (discussed later in this article) that the alkalinizing effect of citrate, rather than some other attribute of citrate, underlies the success of citrate therapy.

Data on citrate handling in patients with PKD are limited. In one study of PKD patients with nephrolithiasis, an abnormally low rate of citrate excretion was seen in 67% of the patients, but this could be due an abnormally low urine pH [26]. Renal citrate handling by PKD patients, as far as we know, has not been systematically studied.

Renal handling of ammonia

The suggestion has been made that increased intrarenal levels of ammonia might contribute to tubulointerstitial injury in chronic renal diseases [27, 28], including PKD [9, 10, 29]. Torres et al reported an elevated ammonia level in human cyst fluid, but whether the fluid was derived from proximal or distal cysts is not clear [10]. Also, cyst fluid total ammonia levels may not reflect interstitial ammonia levels, since fluid within tubules or cysts is generally more acidic and ammonia is trapped as NH_4^+. In the present study, we detected no increase in arterial or renal vein total ammonia concentrations or in cortical tissue P_{NH3} in rats with PKD (Table 4). Therefore, our data do not support the hypothesis that elevated intrarenal ammonia levels are responsible for kidney damage in this disease.

Urinary ammonia production and excretion were below normal in cystic rat kidneys. Preuss et al demonstrated earlier that ammonia excretion was reduced in patients with PKD and postulated that this was secondary to decreased renal ammonia production, as we found in the rat with PKD (Table 4) [30]. Impaired urinary excretion of ammonia could contribute to the metabolic acidosis in untreated rats with PKD (Table 2).

Calcium and citrate

We hypothesized that citrate administration might exert its beneficial effect by preventing precipitation of calcium salts and lowering tissue calcium levels. We found, however, that KCitr treatment for five months led to higher levels of calcium in the kidney, even in normal rats (Table 5). Accumulation of calcium in the rat kidney with citrate intake has been observed before [31] and appears to be a consequence of the elevated urine pH. Deposition of calcium salts in kidney tissue occurs in rats [2] and patients [26] with PKD, and can lead to tissue damage and ischemia. In our study, treatment with KCitr produced only a modest nephrocalcinosis that did not appear to progress; normal rats on KCitr for 20 months had tissue calcium levels the same as in six-month-old rats (data not shown). Nevertheless, the modest elevation in tissue calcium suggests that KCitr does not ameliorate PKD by lowering tissue calcium levels.

Citrate complexes calcium in the urine and inhibits the formation of renal stones [23]. Torres et al found that giving high concentrations of another alkalinizing agent, potassium bicarbonate (200 to 300 mmol/L), caused extensive precipitation of calcium phosphate in medullary collecting ducts [9]. Citrate may be a safer compound to administer than other agents that alkalinize the urine.

Alkalinizing effect of citrate

Data in the present study strongly suggest that the beneficial effect of treatment with KCitr is a consequence of the citrate ion and its oxidation in the body to bicarbonate (alkalinizing effect). Several observations confirm an alkalinizing effect of citrate. In rats with PKD, KCitr administration resulted in an arterial blood pH of 7.39, compared with 7.30 in water-drinking rats (Table 2). Urine pH was significantly more alkaline after the administration of KCitr in both normal rats and rats with PKD (Table 2). Renal ammonia synthesis, which is

inhibited by an alkaline pH, was decreased by KCitr intake in normal rats (Table 4).

The ion-substitution experiments demonstrated that the alkalinizing effect was the factor responsible for the benefits of KCitr treatment. Intake of extra potassium is not the explanation for preservation of GFR, because sodium citrate is just as effective as the potassium salt (Fig. 4). If unmetabolized citrate or citric acid were key, then one would have expected ammonium citrate plus citric acid to have slowed the progression of PKD. Ammonium citrate, when metabolized, does not produce net addition of base to the body; formation of urea from ammonia in the liver, a process that consumes bicarbonate, negates the effect of production of bicarbonate from citrate. Omitting citric acid (K_3 citrate) yielded results similar to KCitr, showing that it is the alkalinizing form of citrate that is effective.

Conclusion

Dietary intake of sodium or potassium citrate in rats with PKD dramatically slows the progression of renal disease. Citrate's beneficial action appears to be due to its alkalinizing effect.

Exogenous citrate is selectively taken up by the kidneys [22], so citrate administration may be a good way to target base to kidney cells. Fortuitously, citrate reduces the risk of urinary calcium stone formation concomitant with administration of other bases. Therefore, it may be an ideal alkalinizing agent in PKD.

There has been extensive clinical experience with citrate, but not, as far as we know, as an agent to slow the progression of inherited PKD. Igarashi et al found that alkali therapy with citrate was of benefit in stabilizing the size and number of renal cysts in one patient with distal renal tubular acidosis [32]. It did not help in three other patients with this disease [33]; this might be explained by lack of patient compliance or by diets too acidifying to have been adequately alkalinized by the amount of alkali prescribed. Citrate therapy is commonly used nowadays in patients for the treatment of a variety of stone-forming disorders [34]. In the early part of the 20th century, it was recommended for treating patients with chronic nephritis [35], but fell into disuse, probably because excessive intake of sodium or potassium salts may be dangerous in patients with impaired renal function. Whether an alkalinizing diet in conjunction with judicious citrate therapy will slow the progression of renal disease in patients with PKD is a question that deserves to be studied.

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Reprint requests to George A. Tanner, Ph.D., Department of Physiology and Biophysics, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, Indiana, USA.
E-mail: gtanner@iupui.edu

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