Renal Cystic Disease and Ammoniagenesis in Han:SPRD Rats[1,2]

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(J. Am. Soc. Nephrol. 1994; 5:1193–1200)

ABSTRACT

Cyst formation in conditions associated with increased renal ammoniagenesis (hypokalemia, distal renal tubular acidosis, renal mass reduction) and experimental links between increased ammoniagenesis and interstitial inflammation have suggested a role for ammonia in the pathogenesis of polycystic kidney disease (PKD). To explore this hypothesis, Han:SPRD rats, a PKD model that affects male more severely than female animals, have been used. Heterozygous cystic (Cy/+) and homozygous normal (+/+) male and female offspring of Cy/+ rats were divided at 3 wk of age into control groups drinking water and experimental groups drinking 300 mM NH_4Cl, 300 mM $KHCO_3$, 200 mM $KHCO_3$, 200 mM KCl, 200 mM $NaHCO_3$, or 200 mM NaCl. At 2 months of age, the rats were kept fasting from 8:00 p.m. to 8:00 a.m. in metabolic cages and urine samples were collected under mineral oil. The rats were then weighed and anesthetized for the collection of blood and kidneys. The administration of 300 mM NH_4Cl, and to a lesser extent that of 200 mM NaCl, was accompanied by an increase in the urinary excretion of ammonia and aggravation of the renal cystic disease. On the other hand, the administration of 300 mM $KHCO_3$, 200 mM $KHCO_3$, or 200 mM $NaHCO_3$ lowered the urinary excretion of ammonia and markedly reduced the severity of the cystic disease and interstitial inflammation. The administration of 300 mM $KHCO_3$, and to a lesser extent that of 200 mM $KHCO_3$, resulted in the precipitation of calcium phosphate in the medullary collecting ducts. These observations are consistent with the hypothesis that renal ammoniagenesis or the metabolic processes linked to it play a role in the pathogenesis of PKD and demonstrate a protective effect of alkali administration on the development of cystic disease in Han:SPRD rats.

Key Words: *Autosomal dominant polycystic kidney disease, renal ammoniagenesis, acidosis, alkalosis, Han:SPRD rats*

Polycystic kidney disease has a complex pathogenesis that includes abnormalities in the proliferation of the tubular epithelial cells, fluid secretion, and remodeling of the extracellular matrix (1–6). The possibility that ammonia is involved in the pathogenesis of polycystic kidney disease was suggested (7) after the observation of an association between chronic hypokalemia and acquired renal cysts (8,9). Acquired renal cysts have also been observed in chronic renal failure, both clinically (10–12) and experimentally (13), and in patients with distal renal tubular acidosis (14,15). Increased renal ammoniagenesis, either in absolute terms or relative to the number of surviving nephrons (16–18), is common to these conditions. Abnormalities in the urinary excretion of ammonia have been described in autosomal dominant polycystic kidney disease (ADPKD) (19,20). To investigate a possible link between alterations in renal ammoniagenesis and the development of renal cystic disease, we have used Han:SPRD rats, a recently characterized model of ADPKD (21,22).

METHODS

Experimental Animals

Han:SPRD rats were obtained from the polycystic kidney program at the University of Kansas Medical Center. The animals used in this study were the offspring from heterozygous rats. The rats with homozygous disease (Cy/Cy) were recognized at 1 wk of age by the marked renal enlargement, died of uremia at 3 to 4 wk of age, and were not used in this study. The remaining homozygous normal (+/+) and heterozygous diseased (Cy/+) animals were divided into different experimental groups designed to alter ammonia production or to control for the administration of sodium or potassium. The severity of the cystic disease differs in male and female Cy/+ rats. Impairment of renal function is noticeable by 8 wk of age in male Cy/+ rats, whereas female Cy/+ rats have a milder disease (22).

Experimental Groups

At 3 wk of age, Cy/+ and +/+ Han:SPRD rats were randomly divided into control groups drinking water and experimental groups drinking 300 mM NH_4Cl, 300 mM $KHCO_3$, 200 mM $KHCO_3$, 200 mM $NaHCO_3$, 200 mM KCl, or 200 mM NaCl. Only female rats, which have milder renal cystic disease, received 300 mM NH_4Cl, and only male rats, which have more severe renal cystic disease, were given 300 mM $KHCO_3$. All rats were fed a standard rodent diet containing 23% protein (Purina Mills Inc., Richmond, IN).

[1] Received January 24, 1994. Accepted April 20, 1994.

[2] Presented at the 26th Annual Meeting of the American Society of Nephrology, November 14–17, 1993.

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1046-6673/0505-1193$03.00/0
Journal of the American Society of Nephrology

Experimental Protocol

At 2 months of age, the rats were placed in metabolic cages and kept fasting from 8 p.m. to 8 a.m. for the collection of urine under mineral oil. After the completion of the 12-h urine collection, the rats were weighed and anesthetized with Inactin (Promonta, Hamburg, Germany), 100 mg/kg body wt ip. Heparinized blood samples were obtained by cardiac puncture, the abdomen was opened, and the kidneys were removed, placed in preweighed containers with 4% paraformaldehyde, weighed, fixed overnight at 4°C, and embedded in paraffin for histologic studies.

Laboratory Methods and Morphologic Analysis

Plasma and urine creatinine concentrations were measured by an adaptation of the Jaffe reaction to an automatic chemical analyzer (23). Urinary ammonia was measured by the Berthelot method (24). Four-micrometer transverse tissue sections including cortex, medulla, and papilla were stained with hematoxylin and eosin and von Kossa stains (25). These sections were graded without knowledge of group assignment as to the extent of the cystic changes (0, absence of cysts; 1, 2, 3, and 4, cysts in <20%, 20 to 40%, 40 to 60% and >60% of renal cortex, respectively) and to the extent and severity of the interstitial infiltration by inflammatory cells (0, absence of infiltrates; 1, focal, mild; 2, focal, moderate; 3, diffuse, mild; 4, diffuse, moderate or severe).

Statistical Analysis

Comparisons of the means between control and experimental groups were made by use of the *t* test. All *P* values reported are two tailed, and the conventional cutoff of 0.05 was taken to reflect statistical significance.

RESULTS

The weights, concentrations of plasma bicarbonate, urinary excretions of ammonia, and creatinine clearances of the male and female Cy/+ control and experimental rats are summarized in Table 1. Male Cy/+ rats drinking 300 or 200 mM $KHCO_3$ and female Cy/+ rats drinking 300 mM NH_4Cl or 200 mM NaCl had retarded growth as compared with the control animals. Plasma bicarbonate concentrations were significantly lower in the groups drinking 300 mM NH_4Cl or 200 mM NaCl and in female rats given 200 mM KCl and were significantly higher in the male rats drinking $KHCO_3$ or $NaHCO_3$ and in the female animals given $NaHCO_3$. Urinary excretions of ammonia were markedly increased in the rats drinking NH_4Cl and to a lesser extent in those drinking NaCl and were significantly reduced in the groups treated with $KHCO_3$ or $NaHCO_3$. No significant changes in the urinary excretion of ammonia were detected in the groups receiving 200 mM KCl. Creatinine clearances were significantly reduced in the 300 mM NH_4Cl group. No significant differences in creatinine clearance were detected between the control and the remaining experimental groups.

Because of the small number of +/+ rats (22 male and 19 female animals divided in 2 control and 10 experimental groups), comparisons between individual Cy/+ and +/+ groups were limited (results not shown). The weights of Cy/+ rats receiving 300 mM $KHCO_3$, 200 mM $KHCO_3$, or 300 mM NH_4Cl were significantly lower than those of +/+ rats. The concentrations of plasma bicarbonate of male control and female NH_4Cl Cy/+ rats were significantly lower than those of +/+ rats. No significant differences in the urinary excretion of ammonia were detected between Cy/+ and +/+ rats. Creatinine clearances of male Cy/+ rats in the control and 200 mM NaCl groups and of female Cy/+ rats in the 300 mM NH_4Cl group were significantly reduced as compared with those of +/+ rats.

TABLE 1. Characteristics of male and female Cy/+ Han:SPRD rats in the control and experimental groups[a]

Rat	*N*	Weight (g)	Plasma Bicarbonate (mEq/L)	Urine NH_4 (μmol/h per 100 g body wt)	Creatinine Clearance (mL/min per 100 g body wt)
Male					
Control	5	237 ± 39	21.3 ± 0.5	9.7 ± 2.3	0.87 ± 0.31
$KHCO_3$ (300 mM)	7	156 ± 22[b]	24.5 ± 1.7[b]	4.5 ± 1.5[b]	1.25 ± 0.31
$KHCO_3$ (200 mM)	5	191 ± 21[b]	23.7 ± 0.6[b]	5.2 ± 0.7[b]	1.09 ± 0.14
$NaHCO_3$ (200 mM)	5	263 ± 13	25.5 ± 3.3[b]	5.0 ± 0.8[b]	1.25 ± 0.20
KCl (200 mM)	8	229 ± 25	21.4 ± 1.2	12.9 ± 2.6	1.01 ± 0.16
NaCl (200 mM)	4	242 ± 11	19.4 ± 0.6[b]	16.7 ± 2.7[b]	0.90 ± 0.26
Female					
Control	5	189 ± 9	23.5 ± 2.0	15.4 ± 2.0	1.16 ± 0.22
NH_4Cl (300 mM)	5	104 ± 38[b]	17.7 ± 4.3[b]	83.7 ± 44.6[b]	0.50 ± 0.22[b]
$KHCO_3$ (200 mM)	5	168 ± 20	23.8 ± 1.6	3.4 ± 1.1[b]	1.00 ± 0.08
$NaHCO_3$ (200 mM)	4	192 ± 20	27.9 ± 2.9[b]	6.0 ± 0.6[b]	1.26 ± 0.08
KCl (200 mM)	5	182 ± 6	20.9 ± 1.0[b]	15.2 ± 3.8	1.03 ± 0.29
NaCl (200 mM)	6	167 ± 8[b]	20.4 ± 1.9[b]	27.2 ± 7.2[b]	0.80 ± 0.33

[a] Values are mean ± SD.
[b] $P < 0.05$ as compared with control.

The relative kidney weights and histologic scores of male and female Cy/+ are shown in Table 2. Males had significantly higher relative kidney weights than did females. The administration of 300 mM NH_4Cl to female Cy/+ rats resulted in marked renal enlargement. The administration of 200 mM NaCl to male and female Cy/+ rats caused renal enlargement of a lesser degree than that observed after the administration of 300 mM NH_4Cl. On the other hand, the administration of 300 mM $KHCO_3$, 200 mM $KHCO_3$, and 200 mM $NaHCO_3$ consistently resulted in a marked reduction in the size of the kidneys. The administration of 200 mM KCl had no significant effect. For the purpose of comparison, the relative kidney weights of male and female +/+ rats in the control group were 0.81 ± 0.02 and 0.78 ± 0.04 g/100 g body wt. The administration of 300 mM NH_4Cl to female +/+ rats and of 300 mM $KHCO_3$ to male +/+ rats was accompanied by a slight increase in relative kidney weight (results not shown).

Macroscopic and microscopic examinations of the kidneys confirmed the marked aggravation of renal cystic disease caused by the administration of 300 mM NH_4Cl (Figures 1 to 3; Table 2) and to a lesser extent by the administration of 200 mM NaCl, as well as the striking protective effect afforded by the administration of 200 or 300 mM $KHCO_3$ or 200 mM $NaHCO_3$ (Figures 4 to 6; Table 2). The administration of $KHCO_3$ or $NaHCO_3$ was accompanied by a marked reduction not only in the number of cysts, but also in the density of cellular infiltrates in the interstitium (Figure 6). The administration of 300 mM $KHCO_3$ was accompanied by the intraluminal deposition of calcium phosphate in the medulla, which was more marked in Cy/+ rats than in +/+ rats (Figure 7). The intraluminal precipitation of calcium phosphate was less prominent in the rats drinking 200 mM $KHCO_3$ and was not observed in the animals receiving 200 mM $NaHCO_3$.

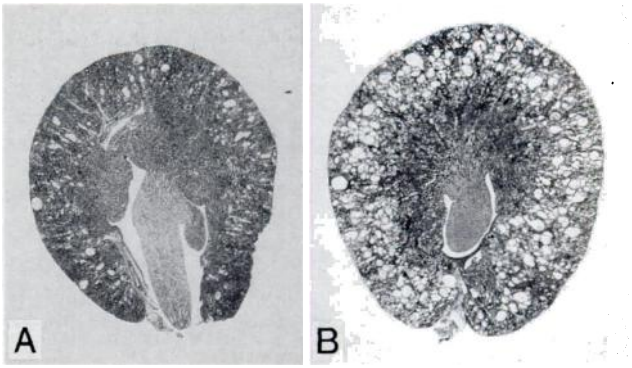

Figure 1. Representative transverse sections of kidneys from female Cy/+ Han:SPRD rats from the control group (A) or from the group drinking 300 mM NH_4Cl (B). Note the marked aggravation of the renal cystic disease observed in the rat drinking 300 mM NH_4Cl.

DISCUSSION

This study clearly shows that the development of inherited renal cystic disease can be markedly altered by environmental or dietary factors. Although the rats were not pair fed, the observations of this study cannot be explained by different caloric or protein intakes. Interventions causing similar degrees of growth retardation had opposite effects on the development of renal cystic disease, whereas consistent results were obtained by interventions that did not cause any growth retardation. Dietary changes accompanied by reduced urinary excretions of ammonia markedly attenuated the development of renal cystic disease, whereas those accompanied by increased urinary excretions of ammonia had the opposite effect. Thus, this study also supports the hypothesis that the ammonia or metabolic processes linked to renal ammoniagenesis may play a role in the pathogenesis of

TABLE 2. Relative kidney weights and histologic scores of male and female Cy/+ Han:SPRD rats in the control and experimental groups[a]

Rat	N	Kidney/Body Wt × 100	Cystic Dilation	Interstitial Inflammation
Male				
Control	5	2.10 ± 0.27	2.80 ± 0.45	2.80 ± 0.45
$KHCO_3$ (300 mM)	7	1.48 ± 0.23[b]	1.43 ± 0.53[b]	0.86 ± 0.38[b]
$KHCO_3$ (200 mM)	5	1.19 ± 0.12[b]	1.20 ± 0.45[b]	1.20 ± 0.45[b]
$NaHCO_3$ (200 mM)	5	1.20 ± 0.10[b]	1.40 ± 0.55[b]	1.20 ± 0.45[b]
KCl (200 mM)	8	1.95 ± 0.16	2.63 ± 0.52	2.38 ± 0.74
NaCl (200 mM)	4	2.95 ± 0.13[b]	3.25 ± 0.29	2.88 ± 0.25
Female				
Control	5	1.39 ± 0.11	1.80 ± 0.45	1.20 ± 0.45
NH_4Cl (300 mM)	5	2.81 ± 0.51[b]	3.40 ± 0.55[b]	2.10 ± 0.22[b]
$KHCO_3$ (200 mM)	5	1.13 ± 0.07[b]	1.10 ± 0.22[b]	0.80 ± 0.27
$NaHCO_3$ (200 mM)	4	1.13 ± 0.07[b]	1.10 ± 0.22[b]	0.80 ± 0.45
KCl (200 mM)	5	1.50 ± 0.07	1.80 ± 0.45	1.20 ± 0.45
NaCl (200 mM)	6	2.20 ± 0.31[b]	2.50 ± 0.45[b]	1.83 ± 0.41[b]

[a] Values are mean ± SD.
[b] $P < 0.05$ as compared with control.

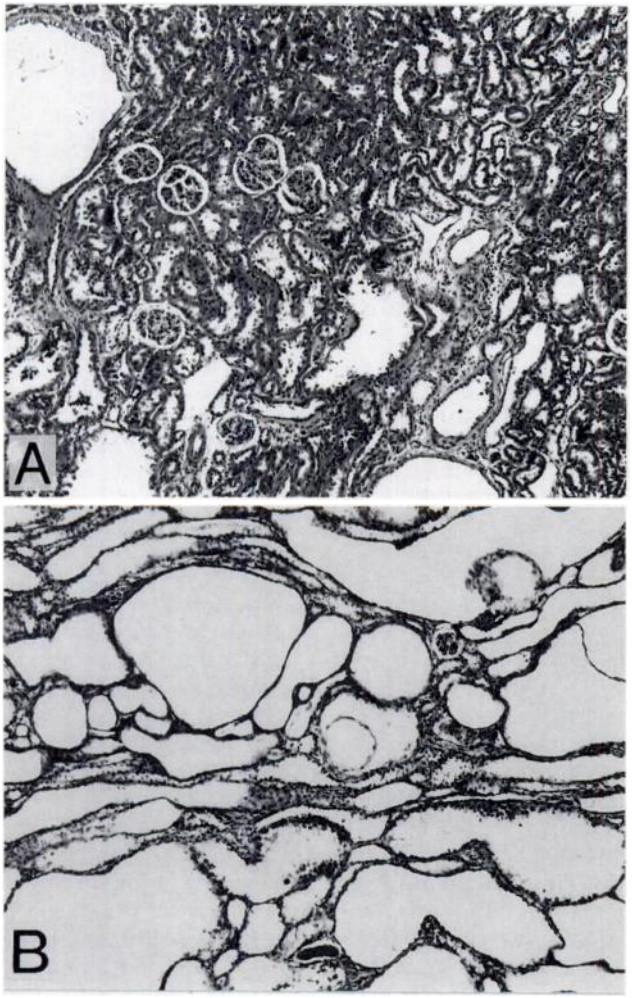

Figure 2. Four-micrometer kidney sections from female Cy/+ Han:SPRD rats from the control group (A) or from the group drinking 300 mM NH_4Cl (B). Note the extensive cystic disease in the rat drinking 300 mM NH_4Cl. Hematoxylin and eosin, ×100.

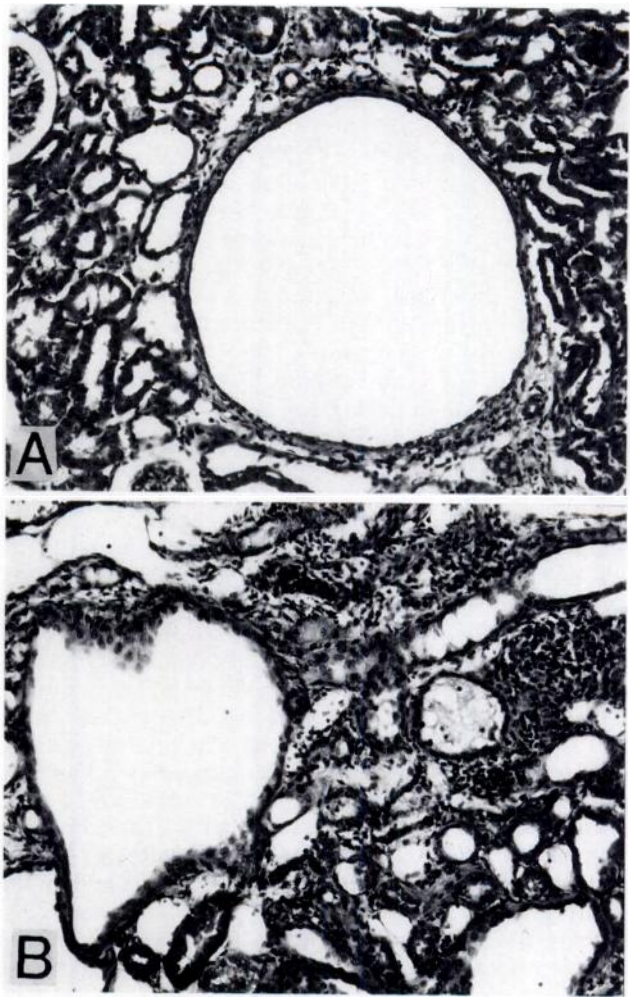

Figure 3. Four-micrometer kidney sections from female Cy/+ Han:SPRD rats from the control group (A) or from the group drinking 300 mM NH_4Cl (B). Note the presence of inflammatory cell infiltrates in the rat drinking 300 mM NH_4Cl. Hematoxylin and eosin, ×200.

polycystic kidney disease. Consistent with these results are the observations by Cowley *et al.* of worse renal cystic disease in Han:SPRD Cy/+ rats fed NH_4Cl or a potassium-deficient diet (26).

The use of alkali in the treatment of renal diseases is not new. It was recommended by Richard Bright, and it was an accepted therapy for many years (27). A number of experimental animal studies at the turn of the century claimed that the administration of alkali could partially prevent the nephrotoxic effects of certain anesthetics (28) or metallic compounds such as uranium nitrate (29,30). In a prospective study of patients with scarlet fever, it was found that the prophylactic administration of large amounts of sodium bicarbonate and potassium citrate reduced the frequency of scarlatinal nephritis (31). The protective effect of alkali administration has been confirmed many years later in a number of renal conditions including subtotal nephrectomy (32) and hypokalemic nephropathy (33). The results of our study indicate that the autosomal dominant model of renal cystic disease in Han:SPRD rats is also markedly susceptible to changes in acid base balance and that pathologic changes can be markedly attenuated by the administration of alkali.

Insufficient understanding of the mechanisms by which changes in acid base metabolism could affect the development of renal disease has likely inhibited the interest of clinicians in this potential form of therapy. Common to many renal diseases, including those where the administration of alkali has been shown to be protective, as well as many renal cystic diseases, is the presence of interstitial cellular infiltrates and fibrosis. Because free base ammonia can activate complement by disrupting a reactive internal thioester bond within the alpha subunit of the third

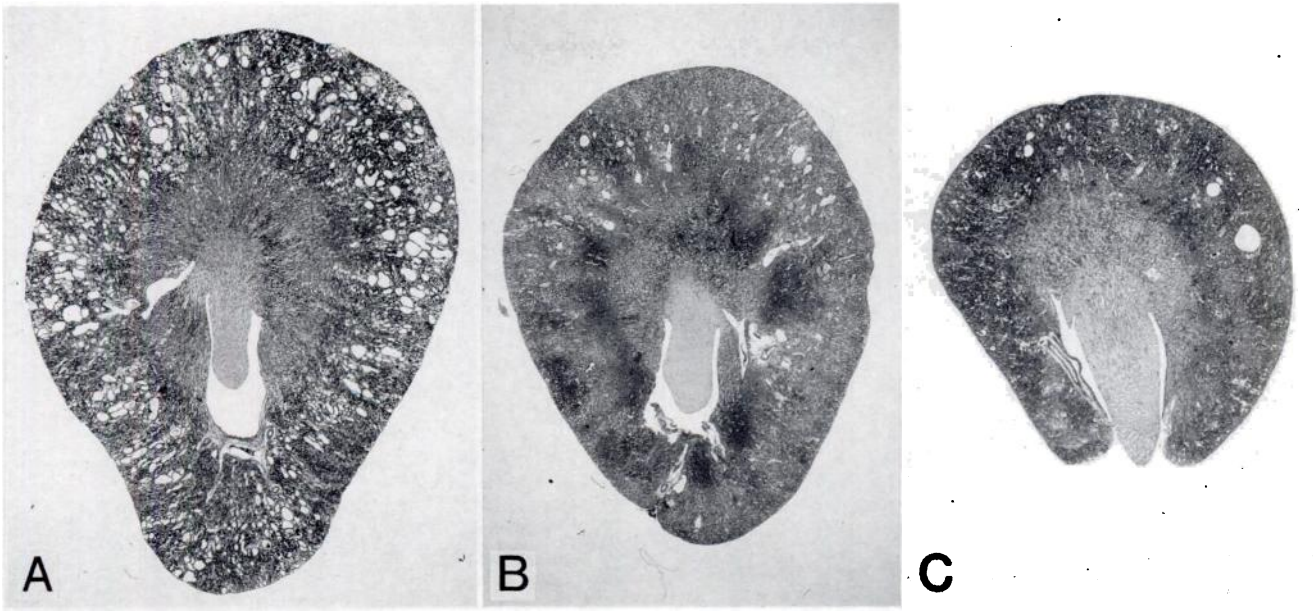

Figure 4. Representative transverse sections of kidneys from male Cy/+ Han:SPRD rats from the control group (A), from the group drinking 200 mM $NaHCO_3$ (B), or from the group drinking 200 mM $KHCO_3$ (C). Note that the severity of the cystic disease is much less in the rats drinking 200 mM $NaHCO_3$ or 200 mM $KHCO_3$.

component of complement (34), it has been proposed that the enhanced cortical production of ammonia associated with renal mass reduction (32), chronic hypokalemia (33), and the dietary deficiency of antioxidants (35) may be responsible for the development of interstitial inflammation and fibrosis in these conditions. A similar mechanism may be operative in polycystic kidney disease because patients with this disease may have a defect in the transfer of ammonia to the final urine (20) analogous to that observed after subtotal nephrectomy (36).

The results of our study indicate that the administration of acids and alkalis has a marked effect not only on the development of interstitial inflammation, but also on cyst formation. Alkali administration was previously found to reduce cystic tubular dilation in chronic hypokalemic nephropathy and after subtotal nephrectomy (32,33). The enhanced renal production of ammonia could be linked to cyst formation by a number of mechanisms. The local generation of autacoids, cytokines, and growth factors as a result of the ammonia-induced complement activation and inflammation in the renal interstitium may contribute to abnormal growth and/or fluid secretion by the tubular epithelium. In nonrenal cells, ammonia can stimulate DNA (37), RNA, and protein synthesis (37,38) and decrease the rate of protein (39–41) and glycosaminoglycan (42,43) degradation. In rabbit proximal tubular cells, ammonia results in an increase in RNA and protein content, stimulation of protein synthesis, and inhibition of protein degradation, without change in DNA synthesis (44,45). Finally, metabolic factors linked to renal ammoniagenesis, rather than to ammonia *per se*, may be important. Glutamine oxidation by the mitochondrial phosphate-dependent glutaminase pathway is an important source of ATP in the proximal tubular epithelial cells (46,47). Extracellular ATP is a mitogen for a number of mammalian cells (48–52), and mitochondrial drugs that deplete the ATP content can result in growth inhibition and cell differentiation (53,54). The addition of exogenous adenine nucleotides to isolated rabbit kidney tubules enriched in proximal segments increases the cell content of ATP (55), and the mitogenic effect of adenine nucleotides on proximal tubular epithelial cells greatly exceeds that of other growth-promoting agents (56,57). We have also found that ATP has a strong mitogenic effect on cyst-derived epithelial cell cultures (V.E. Torres, D.K. Mujwid, unpublished observation).

It is uncertain to what extent the observations in this study are relevant to human renal cystic disease. Nevertheless, certain observations are consistent with a role for renal ammoniagenesis or a metabolic process linked to renal ammoniagenesis in the pathogenesis of acquired renal cystic disease and ADPKD. Studies in animal models with reduced renal mass and in humans with chronic renal disease have shown that, although the absolute excretion of ammonia is reduced, the excretion of ammonia per nephron unit is increased (16–18). In the remnant kidney model, a defective trapping in the renal medulla causes a reduction in the urinary excretion of ammonia, despite an enhanced production and concentration in the renal cortex (36). It seems likely that the high production and concentration of ammonia also occur in the surviving nephrons of dialysis patients because stand-

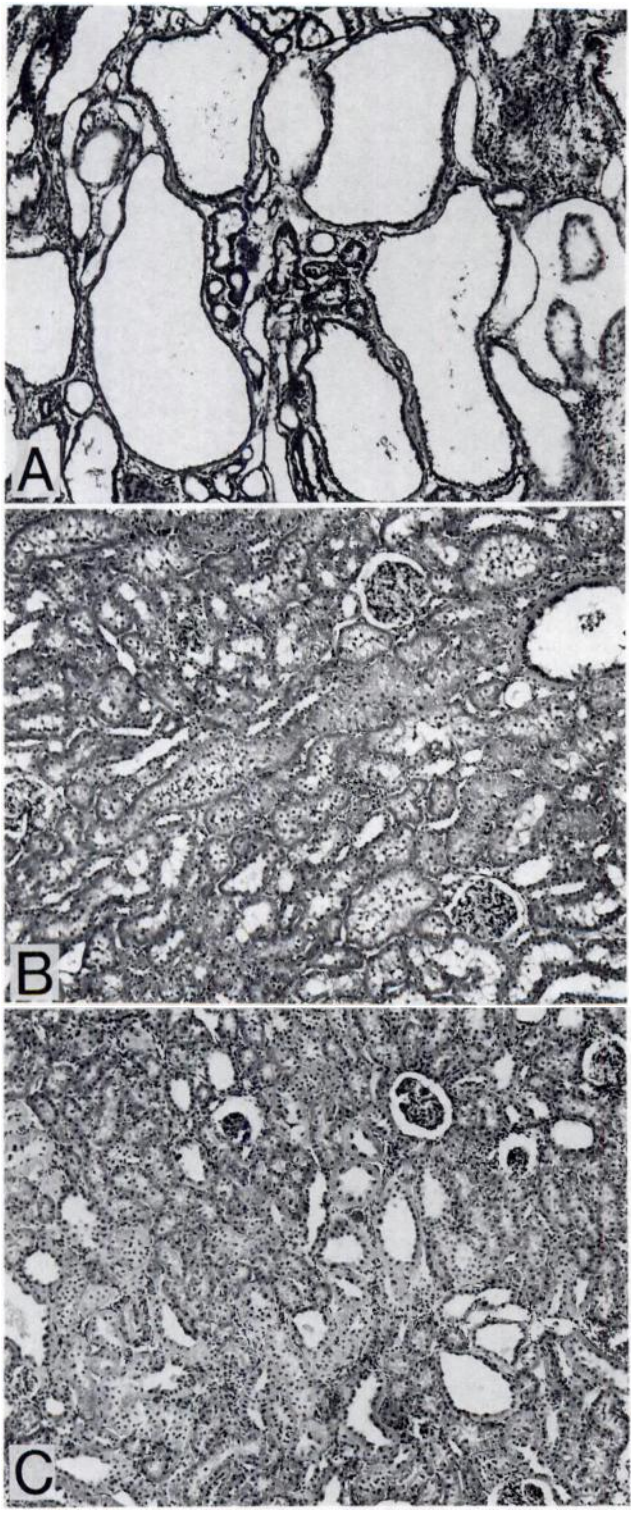

Figure 5. Four-micrometer kidney sections from male Cy/+ Han:SPRD rats from the control group (A), from the group drinking 200 mM $NaHCO_3$ (B), or from the group drinking 200 mM $KHCO_3$ (C). Note that the severity of the cystic disease is much less in the rats drinking 200 mM $NaHCO_3$ or 200 mM $KHCO_3$. Hematoxylin and eosin, ×100.

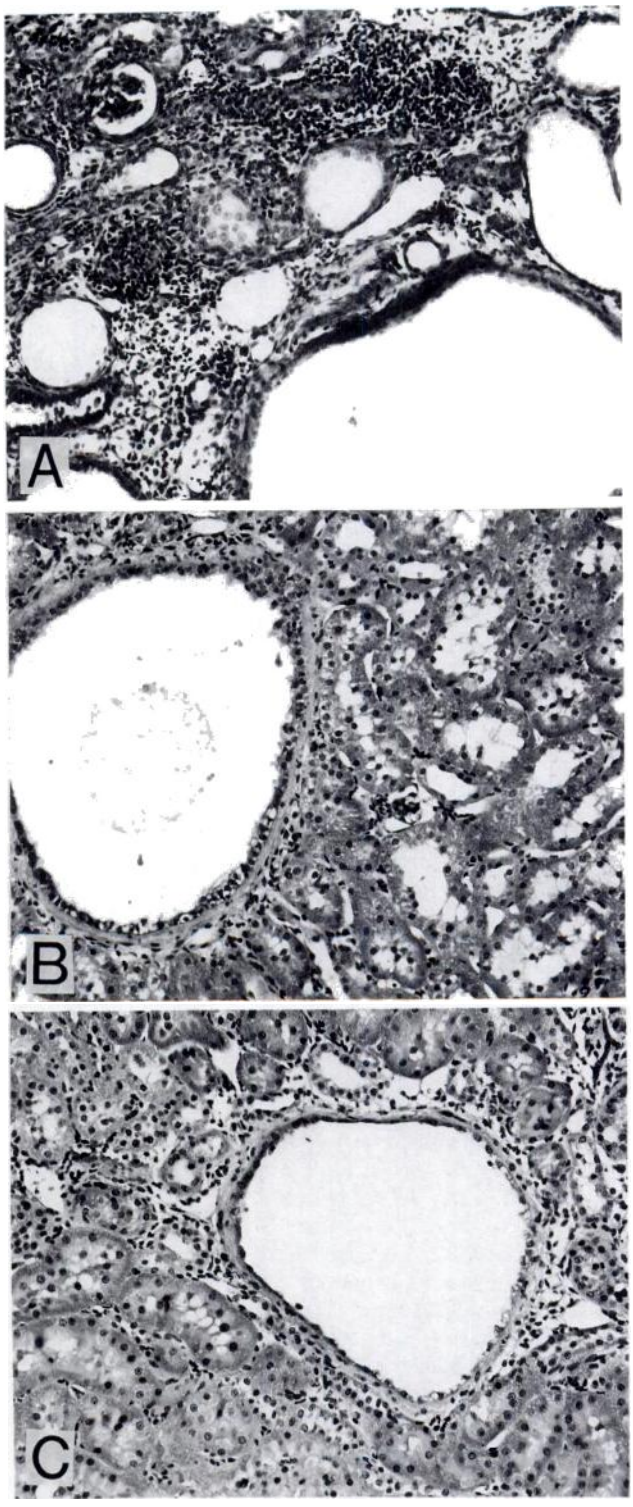

Figure 6. Four-micrometer kidney sections from male Cy/+ Han:SPRD rats from the control group (A), from the group drinking 200 mM $NaHCO_3$ (B), or from the group drinking 200 mM $KHCO_3$ (C). Note that the severity of the inflammatory cell infiltrates is much less in the rats drinking 200 mM $NaHCO_3$ or 200 mM $KHCO_3$.

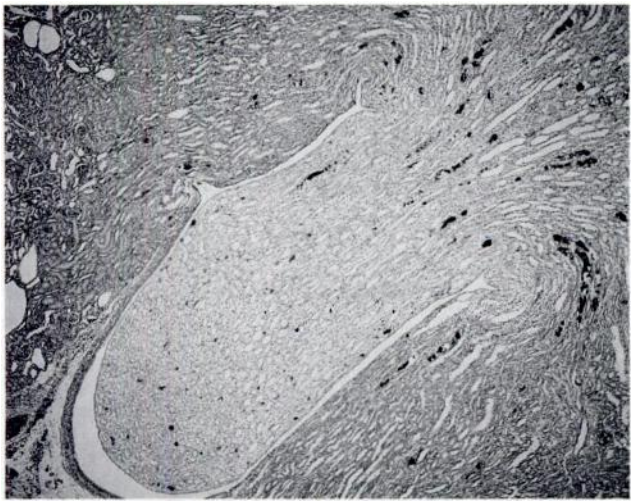

Figure 7. Four-micrometer tissue section from a male Cy/+ Han:SPRD rat drinking 300 mM $KHCO_3$. Note the mild renal cystic disease and the presence of extensive precipitation of calcium phosphate in the medullary collecting ducts. von Kossa, ×25.

ard bicarbonate and acetate dialysates do not completely correct uremic acidosis (58). In ADPKD, the disruption of the normal corticomedullary vascular-tubular architecture by cysts is likely responsible for the renal concentration defect that is the earliest functional abnormality in this disease (59). ADPKD patients with normal GFR cannot transfer ammonia normally to the urine, likely also because of the loss of the corticomedullary concentration gradient (20). To compensate for this transport defect, the cortical production of ammonia may be increased in ADPKD patients at an earlier stage of renal insufficiency and contribute to the progression of the disease.

Note added in proof: Additional experiments have been performed to determine the effects of lower concentrations of $NaHCO_3$. The administration of 75, 150, and 200 mM $NaHCO_3$ significantly reduced the increase in renal size of cystic over that of nonaffected rats by 43, 62, and 70%, respectively.

ACKNOWLEDGMENTS

Research supported by Grant DK44863 from the NIH.

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