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Renal ammonia in autosomal dominant polycystic kidney disease

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Renal ammonia in autosomal dominant polycystic kidney disease. Recent studies have suggested that defective medullary trapping of ammonia underlies the acidosis associated with renal failure and sets in motion maladaptive compensatory mechanisms that contribute to the progression of renal disease. Since a renal concentrating defect is an early functional abnormality in autosomal dominant polycystic kidney disease (ADPKD), defective medullary trapping and urinary excretion of ammonia may also occur early and have important pathophysiological consequences. The urinary pH and excretions of ammonia, titratable acid, and bicarbonate, were measured during a 24-hour baseline period and following the administration of ammonium chloride (100 mg/kg body wt) in ADPKD patients with normal glomerular filtration rate and in age- and gender-matched healthy control subjects. The distal nephron hydrogen ion secretory capacity was assessed during a bicarbonate infusion. Ammonia, sodium, pH, C3dg, and C5b-9 were measured in cyst fluid samples. The excretion rates of ammonia during the 24-hour baseline period and following the administration of ammonium chloride were significantly lower, and the relationship of ammonia excretion to urinary pH was significantly shifted downward in ADPKD. No difference in the increment of urinary pCO_2 (Δ pCO_2) or the peripheral blood-urine pCO_2 gradient (U-B pCO_2) between ADPKD patients and control subjects was detected during a sodium bicarbonate infusion. Calculated concentrations of free-base ammonia in cyst fluid samples exceeded those calculated from reported concentrations of ammonia in renal venous blood of normal subjects. C3dg and C5b-9 were detected in some cyst fluids. The urinary excretion of ammonia is reduced in ADPKD patients with normal glomerular filtration rate. This reduction is not explained by a lower production of ammonia in the renal cortex or by a defect of proton secretion in the collecting ducts. It is likely due to an impaired renal concentrating mechanism and reduced trapping of ammonia in the renal medulla. It may contribute to the pathogenesis of nephrolithiasis and, more importantly, to the progression of the interstitial inflammation and cystic changes seen in ADPKD.

The urinary excretion of ammonia (free-base ammonia or NH_3 and ammonium ion or NH_4^+) plays a central role in the maintenance of acid-base balance, since the bicarbonate necessary to neutralize the daily hydrogen ion load results from the production of ammonia by the proximal tubules and excretion into the urine [1–3]. The excretion of ammonia into the final urine is a complex process that requires active reabsorption in the thick ascending loop of Henle, countercurrent concentration in the renal medulla and diffusion trapping by protonation in the collecting duct lumen. A low urinary excretion of ammonia underlies the inappropriately low urine pH and uric acid nephrolithiasis associated with gout [4–6] and the acidosis of renal failure [7–9]. The marked reduction in the urinary excretion of ammonia seen in uremia is due to defective trapping of ammonia in the renal medulla despite an enhanced ammoniagenesis and an increased concentration of ammonia in the renal cortex [10]. It has been suggested that this compensatory enhancement of ammonia production in the cortical tissue contributes to the progression of renal injury in renal insufficiency [11, 12].

Individuals with autosomal dominant polycystic kidney disease (ADPKD) develop a renal concentrating defect prior to any reduction in glomerular filtration rate [13–16]. Gabow et al found a close correlation between the severity of the concentrating defect and the structural abnormality. They suggested that the disruption of the vascular tubular architecture of the renal medulla is responsible for this early functional abnormality in ADPKD [16]. If the loss of a normal corticomedullary concentration gradient in ADPKD also results in a defective urinary excretion of ammonia, it could have serious pathophysiological consequences.

To determine whether patients with ADPKD can produce and transfer ammonia to the urine normally, we studied the urine pH and the urinary excretion of ammonia, titratable acid, and bicarbonate in baseline conditions and following an acute ammonium chloride challenge in ADPKD patients with normal glomerular filtration rate and in age- and gender-matched healthy control subjects. We also assessed their distal nephron hydrogen ion secretory capacity during a bicarbonate infusion. To estimate the renal concentration of ammonia in ADPKD we analyzed cyst fluids from patients with ADPKD and different levels of glomerular filtration rate. The results of these studies provide support to the hypothesis that patients with ADPKD cannot transfer ammonia normally to the urine and that this defect contributes substantially to the pathophysiology of this disease.

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Methods

Patients

Eight patients with ADPKD and normal serum creatinine, and eight age- and gender-matched healthy control subjects were studied in the Clinical Research Center for three days. The diagnosis of ADPKD was based on a family history of this disease and the demonstration of multiple bilateral renal cysts by computerized tomography. The average renal volume measured by computerized tomography was 1151 cc/1.73 m^2 BSA (range 718 to 1702 cc/1.73 m^2) [17]. All subjects were on no medications for at least two weeks and were eating their regular unrestricted diet. On the days of the study the subjects consumed a diet containing 30 kilocalories and 1 g of protein/kg body weight, 130 mEq of sodium, and 80 mEq of potassium daily. These studies were approved by our Institutional Review Board, and the participating subjects signed an informed consent form.

Baseline studies and ammonium chloride challenge

Every morning at 7 a.m. blood pressure was taken in the sitting position. On the first day of the study blood was obtained for determination of creatinine and electrolytes, and urine collected under a thin layer of mineral oil in a glass container kept in ice from 7 a.m. to 3 p.m., 3 p.m. to 11 p.m., and 11 p.m. to 7 a.m. for determinations of pH, ammonia, titratable acid, bicarbonate, net acid excretion, creatinine, and urea. Meals were given at 8 a.m., noon, and 6 p.m. The baseline protein intake was estimated using the formula: protein intake (g/24 hr) = 6.25 × (urea [g 24 hr]/2.14 + 0.031 × body wt [kg]) [18]. On the second day of the study, the participants drank 200 cc of water at 6 a.m. and remained fasting until noon. At 7 a.m. venous blood was obtained into a heparinized tube for examination of acid base balance. Between 7 a.m. and 8 a.m. 100 mg NH_4Cl/kg body wt in the form of a 10% ammonium chloride syrup were administered p.o. and during the duration of the test 100 cc of water were administered every hour [19]. From 8 a.m to 10 a.m. and from 10 a.m. to noon urine was collected under a thin layer of mineral oil in glass containers kept in ice for determinations of pH, ammonia, titratable acid, bicarbonate, and net acid excretion. At the end of the second urine collection venous blood was obtained for examination of acid base balance. Estimated protein intakes, creatinine clearances, and excretion rates were expressed per 1.73 m^2 of body surface area.

Determinations of urine delta pCO_2 (Δ pCO_2) and urine-blood pCO_2 (U-B pCO_2) gradients in alkaline urines

Seven ADPKD patients and seven control subjects returned for a third day of the study after several weeks. At 7 a.m. urine was collected under a thin layer of mineral oil for examination of baseline urine pCO_2, and venous blood was obtained for examination of acid-base balance. An intravenous infusion of 5% sodium bicarbonate at 1.5 ml/min was started, and urine and blood were collected every 30 minutes for examinations of pH, bicarbonate, and pCO_2. These measurements were performed within five minutes after each collection. After each 30 minute collection, the rate of bicarbonate infusion was increased by 1.5 ml/min to a maximum rate of 6 ml/min if needed. The infusion of bicarbonate was terminated when the urine pH reached 8.0. Urine Δ pCO_2 values were calculated by subtracting the baseline urine pCO_2 from the timed collection pCO_2 [20]. Urine-blood pCO_2 gradients were calculated by subtracting the peripheral blood pCO_2 from the timed collection pCO_2 [21, 22].

Analysis of cyst fluids

Fifty-four cyst fluids were obtained from nine ADPKD patients. Four of these patients had a normal serum creatinine, three had moderate renal insufficiency (serum creatinine concentrations between 2 and 5 mg/dl), one patient had end stage renal failure (serum creatinine 12.4 mg/dl), and one patient had been on hemodialysis for two years. The samples were obtained by percutaneous needle aspiration in one patient, at the time of surgical cyst decompression for pain in five patients, and at the time of bilateral nephrectomy for massive renal enlargement in three patients. Cysts sampled were large (>3 cm in diameter), superficial and totally or partially contained within the renal cortex. Twenty samples from one patient were obtained under a thin layer of mineral oil, in glass containers kept in ice and processed immediately. In the remaining patients cyst fluids were collected in plastic tubes kept in ice, frozen within three hours at −20°C and analyzed within two months from the time of the collection. Cyst fluid measurements included pH, bicarbonate, ammonia, sodium, potassium, and osmolality. The pH values of cyst fluids not obtained under oil and kept frozen before measurement were calculated from the bicarbonate concentrations using the Henderson-Hasselbalch formula and assuming an initial CO_2 concentration of 1.2 mmol [23]. The concentrations of ammonia in three cyst fluids kept frozen at −20°C for three months (6.9, 25.6, and 96.0 μmol/dl) were not different from those measured immediately after collection under mineral oil (5.7, 27.2, and 97.1 μmol/dl) using split sample aliquots. The concentrations of a breakdown product of C_3 (C3dg) and of the terminal complement complex (C5b-9) were measured in some cyst fluids. Cyst fluids were classified as "nongradient" when the sodium concentration was $\geq$ 120 mEq/liter and as "gradient" when it was $\leq$ 100 mEq/liter.

Analytical methods

Urine pH was measured with a Copenhagen Radiometer pHmeter. Blood pH and pCO_2 were measured using an Instrumentation Laboratories gas analyzer (Lexington, Massachusetts, USA). Urinary ammonia was measured by the Berthelot method [24]. Urine titratable acidity was measured by titration with 0.1 M NaOH up to a pH of 7.40. Urinary bicarbonate was measured with a Natelson microgasometer [25]. Net acid excretion was calculated as: ammonia + titratable acid − bicarbonate. Cyst fluid ammonia was measured using Kodak Ektachem Clinical Chemistry Slides (NH_3/AMON) and a Kodak Ektachem 700 Analyzer [26]. Concentrations of free-base ammonia (NH_3) were estimated from total ammonia concentrations and pH values with a transformation of the Henderson-Hasselbalch equation: $[NH_3]$ = total ammonia/1 + $10^{PK\text{-}pH}$, using an acidic dissociation constant of 9.02 [27]. Creatinine, urea, osmolality, sodium, and potassium were measured by techniques routinely used in our Renal Function Laboratory [28]. Cyst fluid C3dg and C5b-9 were measured by ELISA [29].

Table 1. Characteristics of the ADPKD patients and control subjects

	ADPKD ($N = 8$)	Control ($N = 8$)	P value
Gender (M:F)	3:5	3:5	NS
Age *years*	33 ± 8	32 ± 7	NS
Body surface area m^2	1.91 ± 0.07	1.75 ± 0.23	NS
Mean arterial pressure *mm Hg*	96 ± 10	80 ± 7	0.004
Estimated protein intake *g/1.73 m^2*	73 ± 16	71 ± 21	NS
Serum creatinine *mg/dl*[a]	0.99 ± 0.14	0.94 ± 0.13	NS
Creatinine clearance *ml/min/1.73 m^2*	107 ± 9	108 ± 21	NS
Plasma sodium *mEq/liter*	141 ± 2	140 ± 2	NS
Plasma potassium *mEq/liter*	4.3 ± 0.3	4.2 ± 0.2	NS
Plasma chloride *mEq/liter*	104 ± 3	105 ± 2	NS
Plasma bicarbonate *mEq/liter*	26 ± 2	25 ± 2	NS

Data are mean ± SD.
[a] Conversion factor to SI units (μmol/liter) = 88.4

Statistical methods

Two sample rank-sum and *t*-tests were used to compare the ADPKD patients and the control subjects for the variables of interest. Within individuals linear slopes were calculated to reflect the relationship between various dependent variables and urinary pH. The means of these slopes were then compared in the two groups. In addition, multiple regression was employed to simultaneously assess the impact of pH, group assignment, and their interaction on various dependent variables. Degree of association between pairs of numeric continuous variables was assessed using linear and rank correlations. All comparisons were two-sided in nature with $P \leq 0.05$ taken as evidence of differences not attributable to chance.

Results

The age, gender, body surface area, mean arterial pressure, estimated protein intake, creatinine clearance, plasma creatinine and electrolytes of the ADPKD patients and control subjects are summarized in Table 1. The mean arterial pressure of the patients with ADPKD was significantly higher than that of the controls although none of these patients had been previously diagnosed or treated for hypertension. No other significant differences were detected. Estimated protein intakes and creatinine clearances were positively correlated ($P = 0.02$).

The urine flow rates and pH values and the urinary excretions of ammonia, titratable acid, bicarbonate, and net acid for the three eight-hour baseline periods and the two two-hour periods following the administration of NH_4Cl are shown in Figure 1. The urinary excretion of ammonia was significantly lower in the ADPKD patients during the sleep/fasting/antidiuretic period (11 p.m. to 7 a.m.) of the first day of the study and during the first two-hour period following the administration of NH_4Cl. The average urinary excretion rate of ammonia during the 24-hour baseline period was lower at 14.5 ± 3.9 μEq/min/1.73 m^2 as compared to 19.9 ± 5.4 μEq/min/1.73 m^2 in the controls ($P = 0.039$ by *t*-test, and 0.082 by rank-sum test). The average urinary excretion rate of ammonia during the four hours following the administration of NH_4Cl was also lower at 33.1 ± 11.1 μEq/min/1.73 m^2 in the ADPKD patients, as compared to 42.3 ± 6.5 μEq/min/1.73 m^2 in the controls ($P = 0.063$ by *t*-test, and 0.028 by rank-sum test). The renal volumes measured by computerized tomography were negatively correlated with the urinary excretions of ammonia at baseline ($r = -0.33$), following the administration of NH_4Cl ($r = -0.52$) and during the sleep/fasting/antidiuretic period ($r = -0.64$, $P = 0.086$), as well as with the intercepts of the regression lines for urinary excretion of ammonia over pH ($r = -0.60$). Excluding an ADPKD patient who was unable to lower the urine pH to ≤ 5.3, these correlation coefficients were −0.36, −0.57, −0.82 ($P = 0.017$) and −0.57.

No significant differences in the rates of titratable acid, bicarbonate, and net acid excretions were detected between patients and controls in the first day of the study. The urine pH was significantly lower in the ADPKD patients during the 3 to 11 p.m. period. No significant differences in urine pH were detected in the 7 a.m. to 3 p.m. and 11 p.m. to 7 a.m. periods, nor was there a significant difference in urine pH, titratable acid, and bicarbonate after the administration of NH_4Cl. One of the eight ADPKD patients was unable to lower the urine pH to 5.3; this patient had the highest ammonia excretion at urine pH values between 6 and 7 in the ADPKD group. The urine flow rate and net acid excretion in the first two hours following the administration of NH_4Cl were significantly higher in the control subjects than in the ADPKD patients. No significant differences between ADPKD patients and controls were detected in the average urinary excretion rates of titratable acid, bicarbonate and net acid during the 24-hour baseline period and during the four hours following the administration of ammonium chloride.

The relationship of the urinary ammonia excretion to urinary pH in the ADPKD patients and control subjects is shown in Figure 2. No significant difference in the slopes of the regression lines was detected using analysis of individual slopes or multiple regression analysis. The analysis of covariance showed an 8.1 ± 1.9 (SEM) μEq/min/1.73 m^2 downward shift in the regression line for ADPKD patients as compared to controls (P value = 0.001).

During the bicarbonate infusion the urine Δ pCO_2 values were positively correlated with the urine bicarbonate concentrations ($P = 0.014$) and negatively correlated with the urine flow rate ($P < 0.001$). These correlations were independent of each other, and there was no significant interaction between them (Fig. 3). Similar correlations were observed for U-B pCO_2. No significant differences were detected between the ADPKD patients and the control subjects. The maximal urine Δ pCO_2 and U-B pCO_2 values were similar in both groups (Table 2). Although not statistically significant, the baseline urine pCO_2 tended to be lower in the ADPKD patients (35 ± 4 torr) than in the control subjects (41 ± 9 torr).

The concentration of ammonia in the cyst fluids ranged from 2.3 to 3,235 μmol/dl. These concentrations were negatively correlated with the cyst fluid pH (Fig. 4) and sodium ($r = -0.64$, $P < 0.001$). The ammonia concentration in "nongradient cyst" fluids (with a sodium concentration ≥ 120 mEq/liter and a pH of 7.2 to 7.4) was 14.3 ± 12.0 (range 3.1 to 51.1 μmol/dl). The estimated mean concentrations of free-base ammonia in the cyst fluids of nine patients with various glomerular filtration rates ranged between 0.08 and 0.42 μmol/dl. The lowest concentrations of free-base ammonia were estimated in one patient with negligible renal function who had been on dialysis for two years. No significant correlation between the concentrations of free-base ammonia and renal function was detected in the remaining eight patients with serum creatinines ranging between 0.8 and 12.4 mg/dl. No significant difference in the

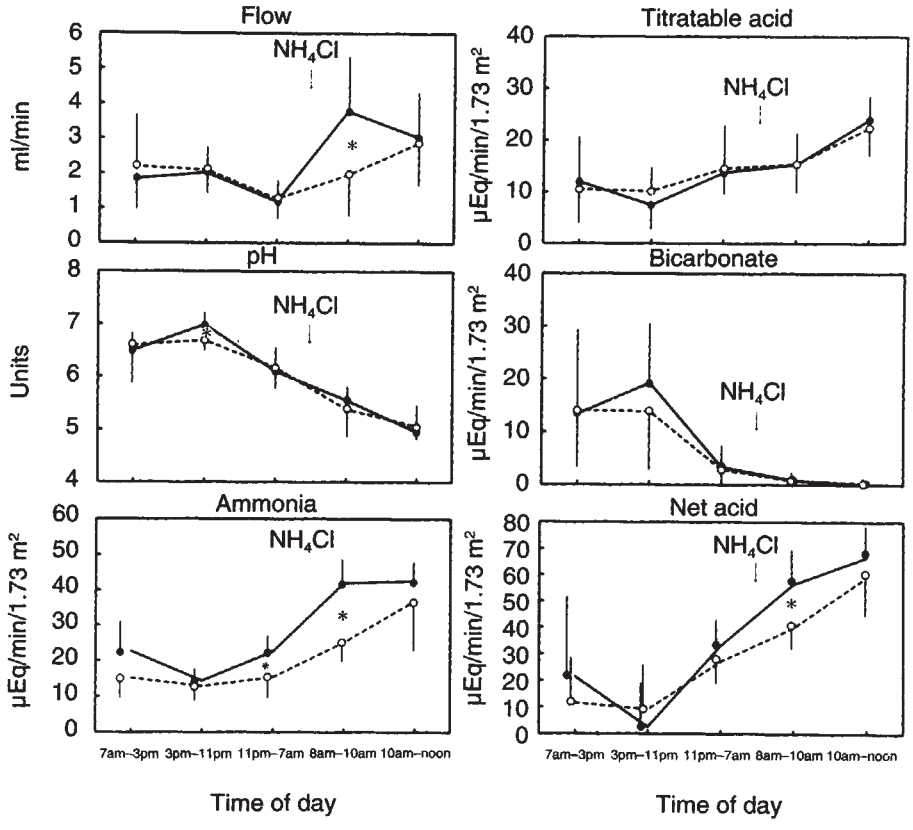

Fig. 1. *Urine flow rates, pH, and excretions of ammonia, titratable acid, bicarbonate and net acid in three consecutive 8-hour collection periods preceding and two consecutive 2-hour collection periods immediately after the administration of ammonium chloride, 100 mg/kg body wt p.o.* The differences in ammonia excretion between ADPKD patients and controls remain significant if the results are not expressed per body surface area.

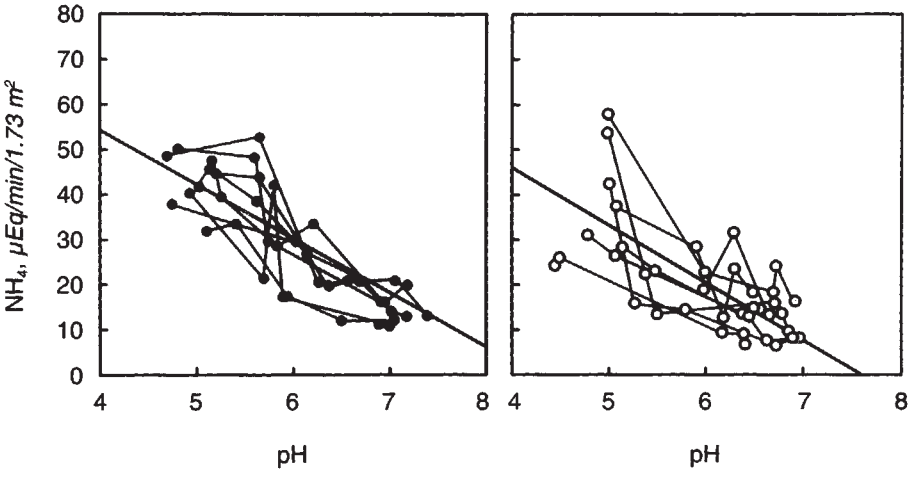

Fig. 2. *Urinary excretion rate of ammonia plotted against urine pH in eight ADPKD patients (○) and eight age- and gender-matched control subjects (●) during three 8-hour periods prior to and two 2-hour periods following the administration of ammonium chloride.* No significant difference in the slopes of the regression lines was detectted using multiple regression analysis. The analysis of covariance assuming a common slope as displayed in the figure showed an 8.1 μEq/min/1.73 m² downward shift in the regression line for the ADPKD patients as compared to control subjects ($P < 0.001$).

concentration of free-base ammonia was detected between gradient (0.19 ± 0.21 μmol/dl) and non-gradient (0.29 ± 0.22 μmol/dl) cyst fluids.

The markers of complement activation C3dg and C5b-9 were measured in renal cyst fluids from three patients. Nineteen of 20 and 13 of 20 cyst fluids from one patient with a serum creatinine of 12.4 mg/dl had detectable levels of C3dg (65 ± 35, range 0 to 124 U/ml) and C5b-9 (4.7 ± 5.2, range 0 to 19.7 U/ml), respectively. One of three cyst fluids in a patient with a serum creatinine of 3.8 mg/dl had detectable levels of C3dg. None of five cyst fluids in the remaining patient with a serum creatinine of 1.0 mg/dl had detectable levels of C3dg or C5b-9. The concentrations of C3dg and C5b-9 were not correlated with pH or concentrations of sodium or ammonia in the cyst fluids.

Discussion

Despite marked enlargement of the kidneys and distortion of the renal anatomy the glomerular filtration rate in ADPKD remains normal until a very late stage of this disease. Early renal functional abnormalities include the loss of urinary concentrating capacity [13–16] and abnormalities in the renal handling of sodium that result in the development of hypertension [30–33]. An impairment of urinary acidification [15, 34–36] and

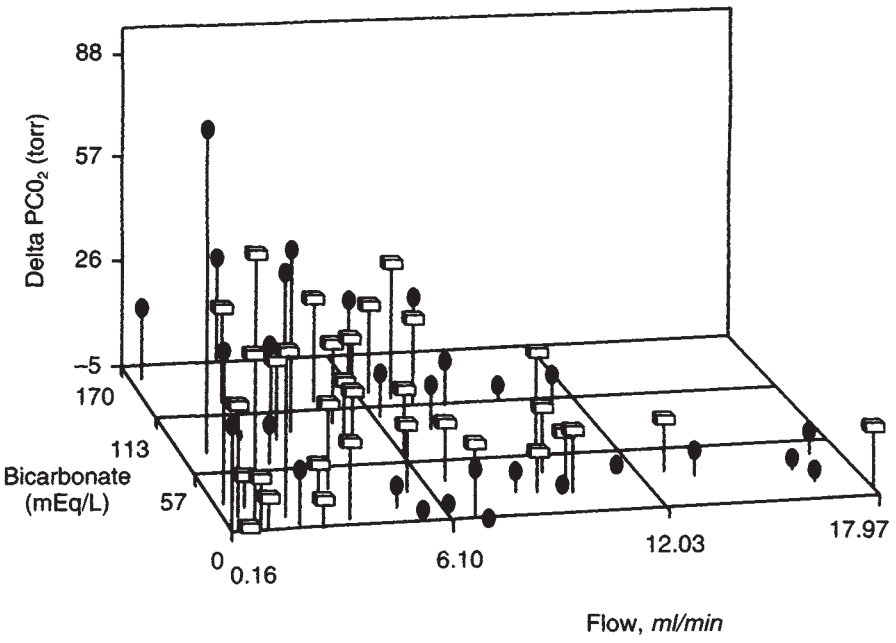

Fig. 3. *Correlation between urine flow rate, pCO_2 increment (Δ pCO_2) and bicarbonate concentration in multiple (2 to 6) urine samples from seven ADPKD patients (open symbols) and seven age- and gender-matched control subjects (closed symbols) during a sodium bicarbonate infusion.* The bicarbonate infusion was terminated when the urine pH reached 8.0. Significant and independent correlations were observed between Δ pCO_2 and bicarbonate concentration ($P = 0.014$) and between Δ pCO_2 and urine flow rate ($P < 0.001$). No difference was detected between ADPKD patients and control subjects.

Table 2. Maximal urine Δ pCO_2 and U-B pCO_2 during sodium bicarbonate infusion in ADPKD patients and control subjects

		Urine					
	Plasma HCO_3 *mEq/liter*	pH	HCO_3 *mEq/liter*	pCO_2 *torr*	Flow *ml/min*	Maximal U-B pCO_2 *torr*	Maximal Δ pCO_2 *torr*
ADPKD							
1	34	8.1	169	64	3.9	19	28
2	31	8.1	127	52	2.2	7	23
3	28	6.5[a]	5[a]	79	0.8	30[a]	43[a]
4	30	6.9[a]	12[a]	73	1.8	22[a]	42[a]
5	35	8.0	110	51	5.5	10	21
6	36	8.0	124	65	4.8	10	25
7	31	6.8[a]	10[a]	68	0.5	14[a]	28[a]
Mean ± SD						16 ± 8	30 ± 9
Controls							
1	35	8.0	115	57	3.3	5	14
2	36	8.0	143	59	8.0	13	19
3	34	7.9[a]	105[a]	59	6.3	7[a]	9[a]
4	27	7.2[a]	24[a]	64	0.3	18[a]	38[a]
5	34	8.2	168	63	2.8	14	27
6	28	7.5[a]	75[a]	126	0.8	85[a]	88[a]
7	37	8.0	148	68	6.2	18	18
Mean ± SD						23 ± 28	30 ± 27

[a] Largest U-B pCO_2 and Δ pCO_2 detected despite continuing bicarbonate infusion to achieve a urine pH $\geq$ 8.0 and urine bicarbonate > 100 mEq/liter

excretion of ammonia [15] have also been reported to occur early in the course of ADPKD.

A defect in urinary acidification as an early manifestation of ADPKD was first noted by Preuss et al [15], but it has not been convincingly demonstrated. This investigator observed that two of four ADPKD patients with creatinine clearances over 73 ml/min were unable to lower their urine pH to 5.3 or below after an acute NH_4Cl challenge as compared to 1 of 12 unaffected family members. Milutinovic et al reported that three of six ADPKD patients with inulin clearances exceeding 86 ml/min were unable to lower the urine pH to 5.3 or below as compared to three of ten family members without ADPKD [34]. García Díaz et al made a diagnosis of distal renal tubular acidosis (RTA) in an ADPKD patient with a creatinine clearance of 34 ml/min, plasma bicarbonate of 14 mEq/liter, and a urine pH of 5.9 [35]. More recently Pabico and McKenna found a defective urinary acidification after an NH_4Cl load in three of four ADPKD patients with an average inulin clearance of 116 ml/min [36]. Contrary to these observations, the urine acidification capacity of seven ADPKD patients in two additional studies was found to be normal [37, 38]. In the present study we did not detect any significant difference in the urine pH between ADPKD patients and age- and gender-matched controls following an acute NH_4Cl challenge, although one of the ADPKD

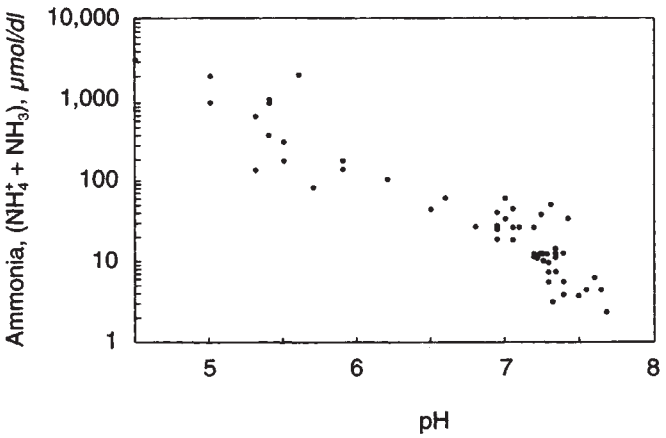

Fig. 4. *Correlation between pH and ammonia concentration in 54 cyst fluids obtained from 9 patients with ADPKD.*

patients was unable to lower the urine pH to 5.3 or below. These results suggest that the majority of ADPKD patients with normal glomerular filtration rate can acidify their urine normally.

To determine whether patients with ADPKD have a defect in proton secretion in the collecting ducts, we measured the Δ pCO_2 and U-B pCO_2 during a sodium bicarbonate infusion [20–22]. Δ pCO_2 and U-B pCO_2 were positively correlated with the urine bicarbonate concentration and negatively correlated with urine flow. In the presence of bicarbonaturia the CO_2 released by nonenzymatic dehydration of carbonic acid in the collecting ducts is trapped in the renal medulla by the countercurrent system [39]. Under conditions of brisk diuresis, however, the medullary trapping of CO_2 and, as a result, the urine Δ pCO_2 and U-B pCO_2 are blunted. Under these conditions no significant difference between the ADPKD patients and the control subjects was observed, which suggests that there is no significant defect of proton secretion in the collecting ducts in the majority of patients with ADPKD and normal glomerular filtration rate.

Preuss et al also reported that the urinary ammonia excretion factored by glomerular filtration rate during a four-day NH_4Cl challenge was lower in seven ADPKD patients with glomerular filtration rates between 44 and 93 ml/min than in six normal control subjects [15]. They argued that, as urine volume plays only a minor role in regulating ammonia excretion and as their patients did decrease the urine pH below 6.5, the major reduction in excretion of ammonia observed in their study had to be secondary to decreased production. This interpretation was based on a simple view of renal transport of ammonia, according to which free-base ammonia produced in the proximal tubules diffuses homogeneously to all renal compartments, and is protonated and trapped as ammonium ion in the collecting ducts.

Neither our study nor previous studies measured the renal production of ammonia. Nevertheless, our determinations of ammonia concentration in cyst fluids provide circumstantial evidence against a decreased renal production to explain the low urinary excretion of ammonia in ADPKD. Renal cysts of the size sampled in our study are disconnected from the nephrons from which they originate [40] and have a rapid fluid turnover [41] The epithelial cysts lining the nongradient cysts cannot maintain a concentration gradient between the surrounding interstitium and the fluid inside the cyst [42, 43]. Therefore, the electrolyte composition of "nongradient cyst" fluids is likely to reflect that of the surrounding interstitial fluid. The concentrations of ammonia in our "nongradient cyst" fluids with pH 7.2 to 7.4 (14.3 ± 12.0 μmol/dl) were higher than those measured in renal venous blood in normal subjects (6.3 ± 0.4 to 7.8 ± 2.2 μmol/dl) [44, 45]. Although "gradient cyst" fluids had higher concentrations of ammonia than "non-gradient cyst" fluids (explained by their lower pH and trapping of ammonia by non-ionic diffusion and protonation), no significant difference in the concentrations of free-base ammonia was detected. This suggests that free-base ammonia freely permeates both types of cysts and that, as sampled cysts were partially or totally contained within the renal cortex, its concentration in the cyst fluids likely reflects that in the cortical tissue. It has been demonstrated that the concentration of free-base ammonia in the renal venous plasma approximates that in the renal cortex [46]. In our cyst fluids the estimated concentrations of free base ammonia (0.08 to 0.42 μmol/dl) also exceeded those estimated in normal renal venous plasma (0.13 ± 0.01 to 0.16 ± 0.04 μmol/dl) [44, 45].

Significant advances in the understanding of the renal transport of ammonia have occurred in the last decade [1–3]. The urinary excretion of ammonia far exceeds that predicted by a simple diffusion trapping model [47] and is contingent on the accumulation of ammonia in the medullary tissue [48]. This accumulation of ammonia in the medullary tissue depends on a number of processes which include apical versus basolateral secretion of ammonia by the proximal tubules, active transport by the $Na^+/K^+/2Cl^-$ cotransporter in the thick ascending loop of Henle, and normally functioning countercurrent multiplication and exchange mechanisms. An altered polarity of the secretion of ammonia in the proximal tubules in ADPKD cannot be ruled out, as the intrarenal renin-angiotensin system is activated in ADPKD [30–33], and angiotensin II has been shown to preferentially stimulate the basolateral secretion of ammonia [49, 50]. The transport of ammonia in the thick ascending loop of Henle might also be abnormal in ADPKD since studies of cyst-derived epithelial cells cultured on permeable membranes have suggested a reverse polarity of $Na^+/K^+/2$ Cl^- cotransporters [51]. More compelling evidence indicates that the countercurrent multiplication and exchange mechanisms are disturbed early in ADPKD. Several studies have demonstrated that a loss of urinary concentrating capacity is one of the earliest manifestations of this disease [13–16]. The architecture of the renal medulla with a close juxtaposition of ascending and descending vasa recta in the inner stripe as well as between the ascending vasa recta and the straight proximal tubules in the outer stripe is essential to maintain a normal concentration gradient [52] and may be altered early in the development of ADPKD. Gabow et al have demonstrated a significant correlation between the reduction in renal concentrating capacity and the severity of the structural disorganization of the polycystic kidneys [16]. Thus, the loss of the corticomedullary concentration gradient due to an alteration of the countercurrent multiplication and exchange mechanisms caused by the structural changes associated with ADPKD is a likely explanation for the abnormal urinary excretion of ammonia in this disease. Consistent with this interpretation is the fact

that the difference in urinary excretion of ammonia between ADPKD patients and control subjects in our study was most clearly demonstrated during the sleep/fasting/antidiuretic period, during which the urinary concentrating mechanisms are more likely to play a role. The significant negative correlation observed during this period between ammonia excretion and renal volume, presumably a reflection of the structural abnormality, also supports this interpretation.

Defects in the urinary excretion of ammonia have also been observed in other renal disorders associated with alterations in medullary structure and/or loss of urinary concentrating capacity such as gout [4–6], sickle cell anemia following the administration of indomethacin [53], and old age [54, 55]. A similar defect may exist in type I or distal RTA, where it has been recently proposed that the transition from incomplete distal RTA, with normal urinary excretion of ammonia, to overt distal RTA, with reduced ammonia excretion, is due to medullary damage resulting from interstitial inflammation and nephrocalcinosis [56].

Abnormalities in the renal transport of ammonia in ADPKD may have substantial consequences. They may explain, for example, the low urinary pH and citrate excretion and the frequency of uric acid calculi reported in some patients with this disease [38]. More importantly, observations in subtotally nephrectomized rats [10, 11], an experimental model with prominent interstitial inflammatory cell infiltrates and tubular dilation [57], suggest that a defect in the renal transport of ammonia may contribute to the progression of the cystic and interstitial renal disease. The low urinary excretion of ammonia in this experimental model is caused by defective trapping in the renal medulla, while the production and concentration of ammonia in the renal cortex are actually increased [10]. A high concentration of ammonia is not innocuous. Free-base ammonia can disrupt a reactive internal thiolester bond within the alpha subunit of the third component of complement (C3) and result in complement activation and interstitial inflammation [11, 58]. The frequent observation of interstitial inflammatory infiltrates between and around the cysts [59, 60], the presence of cytokines in cyst fluids [61], and our preliminary observations that at least some cyst fluids contain markers of complement activation suggest that a similar mechanism could be operating in ADPKD. Renal parenchymal calcifications, which are frequently observed in both ADPKD [62] and end-stage renal failure [63] may be the end result of the interstitial inflammation.

It is also possible that the renal production of ammonia *per se* has an effect on cyst formation. This hypothesis was proposed [64] following the observation of an association between chronic hypokalemia and renal cyst development [65]. It has been known for some time that the chronic administration of ammonium chloride to rats causes renal hypertrophy [66, 67] and that renal hypertrophy develops in a number of conditions (chronic hypokalemia, metabolic acidosis, protein loading) [68] which have in common an enhanced renal ammoniagenesis. 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 [69]. How these changes could lead to cyst formation is not clear, but the hypothesis linking renal ammoniagenesis and cystogenesis has received additional support from observations indicating that the administration of ammonium chloride markedly enhances [70, 71] and the administration of potassium or sodium bicarbonate markedly suppresses [71] the development of autosomal dominant renal cystic disease in Han:SPRD rats.

The normal renal medulla in the antidiuretic state contains concentrations of free-base ammonia which exceed 100-fold those in the renal cortex [72]. The hyperosmolar concentrations of sodium chloride and urea in the medullary interstitium in this state prevent the inflammatory response that would result from ammonia-induced activation of the alternate complement pathway [73]. When the renal concentrating capacity is impaired and the production of ammonia is enhanced, as for example in chronic hypokalemia [45] or distal RTA [56], the medullary environment becomes more propitious for the development of interstitial inflammation. In these conditions interstitial inflammation [74, 75], nephrocalcinosis [75–78], and cyst formation [68, 78–80] may occur in the renal medulla. Since ADPKD patients may have an impaired renal concentrating capacity with inappropriately high tissue concentrations of ammonia, medullary interstitial disease similar to that observed in patients with chronic hypokalemia and distal RTA may ensue and explain the distal acidification defect reported in some patients with this disease [15, 34–36].

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