Effect of acidosis on urine supersaturation and stone formation in genetic hypercalciuric stone-forming rats

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Background. We have successively inbred over 45 generations a strain of rats to maximize urine calcium excretion. The rats now consistently excrete 8 to 10 times as much calcium as controls and uniformly form poorly crystalline calcium phosphate kidney stones. In humans with calcium nephrolithiasis, consumption of a diet high in acid precursors is often cited as a risk factor for the development of calcium-based kidney stones; however, the effect of this diet on urinary supersaturation with respect to the common solid phases found in kidney stones has not been determined.

Methods. To determine the effect of the addition of an acid precursor on urine ion excretion, supersaturation, and stone formation, we fed these genetic hypercalciuric stone-forming (GHS) rats 13 g/day of a 1.2% calcium diet with 0.0, 0.5, 1.0, or 1.5% NH₄Cl in the drinking water for 14 weeks (N = 8 for each). Urine was collected and analyzed every two weeks.

Results. As expected, the addition of dietary NH₄Cl led to a progressive fall in urine pH and urine citrate, while urine ammonium increased. Urine calcium and phosphorus increased, while urine oxalate fell. Increasing dietary NH₄Cl led to a fall in supersaturation with respect to CaHPO₄ (brushite) and CaOx and a rise in supersaturation with respect to uric acid. In spite of differences in supersaturation, most rats in each group formed stones that contained calcium phosphate and not calcium oxalate.

Conclusions. Thus, while the provision of additional dietary acids alters urinary ion excretion and lowers supersaturation with respect to CaHPO₄ and CaOx, it does not change the character or rate of stone formation in the GHS rats.

Hypercalciuria is the most common metabolic abnormality in patients with nephrolithiasis [1–6]. Hypercalciuria raises urine supersaturation with respect to the solid phases of calcium oxalate and calcium phosphate, enhancing the probability of nucleation and growth of crystals into clinically significant stones [2, 3]. Many factors modify the propensity to form kidney stones [2, 3]. Among them is ingestion of a diet with plentiful acid precursors such as one containing large amounts of animal protein rich in methionine and arginine [3]. A diet such as this results in mild systemic acidemia and a clear increase in urinary acidity [7, 8]. The slight decrease in systemic pH should increase urinary calcium excretion mediated by a decrease in renal tubular calcium reabsorption [9]. The source of the additional urinary calcium is thought to be derived from the mineral phases of bone [10]. Increasing urinary calcium excretion should increase supersaturation with respect to calcium-containing stones. The increase in systemic acidity will lead to a decrease in urinary citrate excretion [11, 12], which should also increase the likelihood of forming kidney stones as citrate binds urinary calcium, making it unavailable to bind with oxalate or phosphate [2, 3]. However, the decrease in urinary pH will increase the solubility with respect to the solid phases of calcium phosphate but will decrease the solubility with respect to uric acid [3]. The net result of acid precursor ingestion on urinary supersaturation with respect to the principle types of kidney stones is difficult to predict and has not been previously determined.

Through successive inbreeding of the most hypercalciuric progeny of hypercalciuric Sprague-Dawley rats, we have established a strain of rats, each of which excrete abnormally large amounts of urinary calcium [13–26]. The principal mechanism for the excessive calcium excretion in these rats appears to be an increase in intestinal calcium absorption [26]. The increased intestinal calcium absorption appears to be mediated not by an increase in the serum level of 1,25(OH)₂D₃ but by an increase in the number of intestinal vitamin D receptors [25]. When these hypercalciuric rats are fed a very low-calcium diet, their urine calcium excretion remains elevated compared with that of similarly treated control rats, indi-
cating a defect in renal calcium reabsorption and/or an increase in bone resorption [24]. When exposed to increasing amounts of 1,25(OH)_{2}D_{3}, the bone from these hypercalciiuric rats releases more calcium compared with bone of control rats [21], and inhibition of bone resorption with a bisphosphonate substantially decreases the hypercalciuria when these rats are fed a low-calcium diet [15]. In addition, a primary defect in renal calcium reabsorption is observed during carefully controlled clearance studies [19]. We have shown that in addition to the intestine, both the bone and kidney of the hypercalciiuric rats have an increased number of vitamin D receptors [17, 21, 25]. Thus, these hypercalciiuric rats appear to have a systemic abnormality in calcium homeostasis. They absorb more intestinal calcium. They resorb more bone, and they fail to reabsorb filtered calcium adequately. As each of these hypercalciiuric rats forms renal stones, we have termed the rats genetic hypercalciiuric stone-forming (GHS) rats [16, 20, 22]. The stones formed contain only calcium and phosphate, without oxalate and by x-ray diffraction are exclusively poorly crystalline apatite. Calcium transport abnormalities similar to those documented in the GHS rats have been observed in many patients with idiopathic hypercalciuria and nephrolithiasis [3].

In the current study, we utilized the 44th generation of our hypercalciiuric stone-forming rats to determine the effect of increasing dietary acid precursors on urinary ion excretion and supersaturation with respect to the principle crystal types of kidney stones and on kidney stone formation. The addition of dietary NH_{4}Cl led to a progressive fall in urine pH and urine citrate while urine ammonium increased. Urine calcium and phosphorus increased while urine oxalate fell. Increasing dietary NH_{4}Cl led to a fall in supersaturation with respect to CaHPO_{4} (brushite) and CaOx solid phases and a rise in supersaturation with respect to uric acid solid phase. In spite of differences in supersaturation, most rats in each group formed stones that contained calcium phosphate and not calcium oxalate. Thus, while urinary acidification alters ion excretion and supersaturation, it does not change the character or rate of stone formation in the GHS rats.

METHODS

Establishment of hypercalciiuric rats

Adult Sprague-Dawley rats (Charles River Laboratories, Kingston, NY, USA) were screened for hypercalciiuria by placing the rats in individual metabolic cages on a constant amount of a standard calcium diet and by measuring urine calcium excretion. The most hypercalciiuric male and female rats were used to breed the next generation. A similar protocol was used for screening and inbreeding of subsequent generations as described previously [13–26].

Study protocol

Thirty-two 44th-generation female GHS rats, each initially weighing 125 g, were placed in metabolic cages for a total of 14 weeks. Each rat was offered 13 g/day of diet, an amount that we have previously shown is completely consumed by a female rat of this size [15].

The rats were randomly divided into four groups. All rats were continued on 13 g/day of the standard diet for the entire study. Eight rats drank only deionized distilled water (0.0% NH_{4}Cl). Eight rats drank deionized distilled water with 0.5% NH_{4}Cl. Eight rats drank deionized distilled water with 1.0% NH_{4}Cl, and eight rats drank deionized distilled water with 1.5% NH_{4}Cl. We have previously shown that after eight days, the provision of 1.5% NH_{4}Cl in the drinking water of rats leads to a significant reduction of arterial blood pH and bicarbonate [27, 28]. Every two weeks, two successive 24-hour urine collections were obtained. The first 24-hour urine was collected in thymol and was used for all measurements except oxalate, and the second 24-hour collection was collected in concentrated HCl for measurement of oxalate. Both samples were refrigerated at 0°C, and biochemical measurements were determined within two weeks. At the conclusion of the experiment (14 weeks), each rat was killed, and the kidneys, ureters, and bladder were dissected en bloc and mounted on radiographic film. Any rat that ate less than 12 g of food or drank less than 15 mL of water on any day of the study was eliminated from further analysis.

Chemical determinations

Calcium was measured by reaction with arsenazo III and then determined photometrically at 650 nm [29]. Creatinine was determined by a modification of the Jaffe method by formation of a creatinine-picolate complex [30]. Inorganic phosphorus was measured by a reaction with ammonium molybdate to form a colored phosphomolybdate complex [31]. Uric acid was measured after oxidation by uricase to produce allantoin and hydrogen peroxide [32]. Magnesium was determined by combination with calmagite [33]. Ammonia was determined by coupled enzyme system using glutamate dehydrogenase and nicotinamide adenine dinucleotide phosphate [34]. Sodium was determined by a selective electrode [35] and potassium using a valinomycin membrane attached to a potassium electrode [36]. Chloride was measured by colorimetry using a silver/silver chloride electrode [37]. Oxalic acid and citric acid were measured with an anion chromatography system (Dionex Corporation, Sunnyvale, CA, USA) using sodium hydroxide as the eluent with peaks detected by conductivity. Aqueous calibration standards were used and chromatograms were ana-
lyzed using Peaknet software (Dionex Corporation). pH was measured by an ion selective electrode. Sulfate was measured by turbidity after barium precipitation [38].

**Urinary supersaturation**

The calcium oxalate ion activity product was calculated using the computer program EQUIL developed by Finlayson and associates [39–41]. The computer program calculates free ion concentrations using the concentrations of measured ligands and known stability constants. Ion activity coefficients are calculated from ionic strength using the Davies modification of the Debye-Hückel solution to the Poisson-Boltzman equation. The program simultaneously solves for all known binding interactions among the measured substances. Oxalate, phosphorus, calcium, and urate ion activities were used to calculate the free-ion activity products. The free ions in solution are considered to be in an equilibrium with the dissolved calcium oxalate governed by a stability constant (K) of $2.746 \times 10^3$ mol/L, with the dissolved brushite governed by a K of $0.685 \times 10^3$ mol/L and with the dissolved uric acid governed by a K of $3.981 \times 10^-7$ mol/L. The value of calcium oxalate in a solution at equilibrium with a solid phase of calcium oxalate, the solubility of calcium oxalate, is $6.16 \times 10^-6$ mol/L. The value of brushite in a solution at equilibrium with a solid phase of brushite, the solubility of brushite, is $3.981 \times 10^-7$ mol/L. The value of uric acid in a solution at equilibrium with a solid phase of uric acid, the solubility of uric acid, is $2.61 \times 10^-4$ mol/L. The relative supersaturation for calcium oxalate is calculated as the ratio of the free-ion activity product of calcium and oxalate in the individual urine to the solubility of calcium oxalate. The relative supersaturation for brushite is calculated as the ratio of the free-ion activity product of calcium and phosphate in the individual urine to the solubility of calcium phosphate. The relative supersaturation for uric acid is calculated as the ratio of the free-ion activity product of monohydrogen urate and hydrogen in the individual urine to the solubility of uric acid. Ratios of 1 connote a sample at equilibrium, above 1 supersaturation, and below 1 undersaturation.

The ability of this computer program to predict accurately the saturation of urine or other solution with respect to the solid phase is excellent [14–16, 23, 24, 39, 42, 43]. With a series of 20 artificial solutions, the equilibrium calcium concentration and the extent of calcium precipitation were predicted with average errors of 5 ± 9% and 5 ± 8% (mean ± SD), respectively [39]. We have used this computer program previously and found excellent correspondence between calculated and experimentally measured saturation in urine and blood [14–16, 18, 22–24] and in bone culture medium [42–44].

**X-ray diffraction**

The stones were finely powdered and put in a glass capillary to be examined in an x-ray diffraction Debye-Sherrer powder camera using Cu K radiation (1.5418 Å) with an exposure time of four hours at 40 KV and 25 ma. The recorded diffraction patterns were compared with a standard hydroxyapatite pattern.

**Statistical analysis**

All values are expressed as mean ± SE. Tests of significance were calculated by analysis of variance with the Bonferroni correction for multiple comparisons using conventional computer programs (BMDP; University of California, Los Angeles, CA, USA). $P < 0.05$ was considered significant.

**RESULTS**

**Included rats**

As indicated in the Methods section, any rat that ate less than 12 g of food or drank less than 15 mL of water on any day of the study was eliminated from further analysis. In the 0.0 and 0.5% NH$_4$Cl groups, none of the rats met this exclusionary criteria (Fig. 1). However, in the 1.0% NH$_4$Cl group, two of the eight rats met this criteria and were excluded from further analysis, and in the 1.5% NH$_4$Cl group, six of the eight rats met this criteria and were excluded from further analysis. For completeness, data from the remaining rats in all four groups are shown for the entire study, recognizing that toward the end of the study, there are few remaining rats in the 1.5% NH$_4$Cl group.

**Urinary ion excretion**

Every two weeks, two successive 24-hour urine collections were obtained. The individual urine collections for the 32 rats divided equally into four groups were analyzed separately and were then averaged over weeks 4 to 6, weeks 8 to 10, and weeks 12 to 14.

**pH.** With respect to urine pH, during all collection periods, there was a decrease in urine pH in rats drinking the 0.5, 1.0, and 1.5% NH$_4$Cl compared with those drinking 0.0% NH$_4$Cl (distilled water; Fig. 2A). During weeks 4 to 6, rats drinking the 1.0% NH$_4$Cl had a lower urine pH than rats drinking the 0.5% NH$_4$Cl, and during weeks 8 to 10 and weeks 12 to 14, urine pH was lower in the rats drinking the 1.0 and 1.5% NH$_4$Cl compared with rats drinking the 0.5% NH$_4$Cl. Thus, urine pH fell with the addition of NH$_4$Cl to the drinking water.

**Citrate.** With respect to urine citrate, during each of the three individual time periods, there was a decrease in urine citrate in rats drinking the 0.5% NH$_4$Cl, a further decrease in rats drinking the 1.0% NH$_4$Cl, and an even further decrease in rats drinking the 1.5% NH$_4$Cl com-
pared with rats drinking 0.0% NH₄Cl (Fig. 2B). Thus, urine citrate fell progressively with the addition of NH₄Cl to the drinking water.

Ammonium. With respect to urine ammonium, during each of the three individual time periods, there was an increase in urine ammonium in rats drinking the 0.5% NH₄Cl, a further increase in rats drinking the 1.0% NH₄Cl, and during weeks 8 to 10 and weeks 12 to 14, an even further increase in rats drinking the 1.5% NH₄Cl compared with rats drinking 0.0% NH₄Cl (Fig. 2C). Thus, urine ammonium increased progressively with the addition of NH₄Cl to the drinking water.

Calcium. With respect to urine calcium, during weeks 4 to 6, there was an increase in urine calcium excretion in rats drinking the 0.5% and a further increase in urine calcium excretion in rats drinking the 1.0% NH₄Cl compared with rats drinking 0.0% NH₄Cl (Fig. 3A). Rats drinking 1.5% NH₄Cl had less urine calcium excretion than rats drinking 1.0% NH₄Cl. During weeks 8 to 10, there were no differences in urine calcium excretion between the groups. During weeks 12 to 14, there was an increase in urine calcium excretion in rats drinking the 1.0% NH₄Cl and 1.5% NH₄Cl compared with rats drinking 0.0% NH₄Cl and those drinking 1.0% NH₄Cl.

Oxalate. With respect to urine oxalate, during weeks 4 to 6, there was a decrease in urine oxalate excretion in rats drinking the 1.5% NH₄Cl compared with all other groups (Fig. 3B). During weeks 8 to 10 and weeks 12 to 14, there was a decrease in urine oxalate excretion in rats drinking the 0.5% NH₄Cl, those drinking the 1.0% NH₄Cl, and those drinking the 1.5% NH₄Cl compared with those drinking 0.0% NH₄Cl. There were no differences in oxalate excretion in any of the rats drinking NH₄Cl during these latter two time periods.

Phosphorus. With respect to urine phosphorus, during weeks 4 to 6, there was an increase in urine phosphorus excretion in rats drinking the 1.0% NH₄Cl compared with all other groups (Fig. 3B). During weeks 8 to 10, there were no differences in urine phosphorus excretion between the groups. During weeks 12 to 14, there was an increase in urine phosphorus excretion in rats drinking the 1.0% NH₄Cl compared with rat drinking 0.0% NH₄Cl and those drinking 0.5% NH₄Cl and an increase in urine phosphorus excretion in rats drinking the 1.5% NH₄Cl compared with rats drinking 0.0% NH₄Cl.

Volume. With respect to urine volume, during weeks 4 to 6, there was a decrease in urine volume in rats drinking the 1.5% NH₄Cl compared with rats drinking 1.0% NH₄Cl (Fig. 4A). During weeks 8 to 10 and during weeks 12 to 14, there were no differences in urine volume between rats in any group.

Uric acid. With respect to urine uric acid, during weeks 4 to 6, there was an increase in urine uric acid in rats drinking 1.0% NH₄Cl compared with rats drinking 0.0% NH₄Cl and a decrease in uric acid in rats drinking 1.5% NH₄Cl compared with rats drinking 1.0% NH₄Cl (Fig. 4B). During weeks 8 to 10 and during weeks 12 to 14, there were no differences in urine uric acid between rats in any group.

Chloride. With respect to urine chloride, during each of the time periods, there was an increase in urine chloride excretion in rats drinking 0.5% NH₄Cl, a further increase in urine chloride excretion in rats drinking 1.0% NH₄Cl, and an even further increase in urine chloride excretion in rats drinking 1.5% NH₄Cl compared with rats drinking 0.0% NH₄Cl, except during weeks 4 to 6, when the rats drinking 1.5% NH₄Cl had a greater urine chloride excretion compared only with those rats drinking 0.0% NH₄Cl and those drinking 0.5% NH₄Cl (Fig. 4C).

Urinary supersaturation

CaHPO₄. With respect to the urinary supersaturation of CaHPO₄ (brushite), during weeks 4 to 6, there was a
Fig. 2. Urine pH (A), citrate (B), and ammonium (C) excretion (mean ± SE) in female genetically hypercalciuric stone-forming (GHS) rats. Rats were fed 13 g/day of a normal diet (1.2% calcium) for a total of 14 weeks. Some rats drank only deionized distilled water (0.0% NH₄Cl), while others drank 0.5, 1.0, or 1.5% NH₄Cl ad libitum. Every two weeks, two successive 24-hour urine collections were obtained. The individual urine collections for the rats divided equally into four groups were analyzed separately. The data from week 2 are not shown. The data were then averaged over the next four weeks (weeks 4 to 6), the next four weeks (weeks 8 to 10), and the final four weeks (weeks 12 to 14). *P < 0.05 vs. 0.0% NH₄Cl; +P < 0.05 vs. 0.5% NH₄Cl; and #P < 0.05 vs. 1.0% NH₄Cl.

Fig. 3. Urine calcium (A), oxalate (B), and phosphorus (C) excretion (mean ± SE) in GHS rats eating 13 g/day of a normal diet (1.2% calcium) and drinking deionized distilled water (0.0% NH₄Cl) or deionized distilled water with 0.5, 1.0, or 1.5% NH₄Cl ad libitum. Methods are as in Figure 2 legend. *P < 0.05 vs. 0.0% NH₄Cl; +P < 0.05 vs. 0.5% NH₄Cl; and #P < 0.05 vs. 1.0% NH₄Cl.

CaOx. With respect to the urinary supersaturation of CaOx (calcium oxalate), during weeks 4 to 6, there was a decrease in supersaturation in rats drinking 1.0% NH₄Cl and 1.5% NH₄Cl compared with rats drinking 0.0% NH₄Cl and also rats drinking 0.5% NH₄Cl, while rats drinking 1.5% NH₄Cl had a lower supersaturation than rats drinking 1.0% NH₄Cl (Fig. 5B). During weeks 8 to 10, there was a decrease in supersaturation in rats drinking 1.0% NH₄Cl and 1.5% NH₄Cl compared with rats drinking 0.0% NH₄Cl and rats drinking 1.0% NH₄Cl and 1.5% NH₄Cl had a lower supersaturation than rats drinking 0.5% NH₄Cl. During weeks 12 to 14, there was a decrease in supersaturation in rats drinking 1.5% NH₄Cl compared with rats drinking 0.0% NH₄Cl.

Uric acid. With respect to the urinary supersaturation of uric acid during weeks 4 to 6, weeks 8 to 10, and
weeks 12 to 14, there was an increase in supersaturation in all groups of rats drinking NH₄Cl compared with rats drinking 0.0% NH₄Cl (Fig. 5B). Additionally, during weeks 12 to 14, there was an increase in supersaturation in rats drinking 1.0% NH₄Cl and 1.5% NH₄Cl compared with rats drinking 0.5% NH₄Cl.

**Stone formation**

At the conclusion of the study, seven of eight rats drinking distilled water, seven of eight rats drinking 0.5% NH₄Cl, five of six remaining rats drinking 1.0% NH₄Cl, and two of two remaining rats drinking 1.5% NH₄Cl had radiographic evidence of kidney stone formation. X-ray diffraction patterns of individual calculi from each of the four groups of GHS rats each demonstrates the typical diffraction pattern of poorly crystalline hydroxyapatite.

**DISCUSSION**

In this study, we utilized GHS rats to determine the effect of increasing dietary acid precursors on urinary ion excretion and supersaturation, with respect to the principle crystal types, and on the frequency and type of kidney stone formation. We found that the provision of an acid precursor, NH₄Cl, led to urinary acidification with accompanying hypocitruria and hyperammoniuria. Urine calcium and phosphorus excretion both tended to
increase and urine oxalate decrease. The net result was that urine supersaturation with respect to calcium hydrogen phosphate (brushite) and calcium oxalate actually fell with provision of NH$_4$Cl. Stone formation was almost universal whether or not the rats received the NH$_4$Cl and consisted, in all cases, of a poorly crystalline hydroxyapatite solid phase.

The provision of 1.5% NH$_4$Cl led to the loss of 75% of the rats in that group (Fig. 1). In all cases, the rats reduced their intake to below the required 12 g of food of the 13 g offered. The provision of 1.0% NH$_4$Cl led to the loss of 25% of the rats, while all rats given 0.5 or 0.0% NH$_4$Cl completed the study. It is unclear why the rats given substantial amounts of NH$_4$Cl stopped eating, but this may be related to the associated metabolic acidosis or to the NH$_4$Cl itself. Humans given NH$_4$Cl during the work-up for renal tubular acidosis often report nausea [45].

The fall in urine pH with NH$_4$Cl administration is not surprising as this precursor undergoes metabolism to HCl [45]. The additional hydrogen ions reduce systemic pH and are buffered, in large part, by bicarbonate, resulting in a reduction in serum bicarbonate concentration. The reduction in blood bicarbonate and pH, clinically termed metabolic acidosis, leads to a decrease in urine citrate excretion [11, 12] and an increase in urinary ammonium excretion [46], as confirmed in this study. In this study, we did not measure arterial blood pH and the partial pressure of carbon dioxide to calculate bicarbonate; however, we have done so previously in rats drinking 1.0 and 1.5% NH$_4$Cl and found consistent metabolic acidosis [27, 28]. We have also used NH$_4$Cl to generate metabolic acidosis in mice [47], and others have used it to produce acidosis in humans [8, 48].

Metabolic acidosis previously has been shown to increase urine calcium excretion because of a decrease in renal tubular calcium reabsorption [49]. The source of the urinary calcium must ultimately be bone, as gastrointestinal calcium absorption does not increase with acid administration [8, 50, 51]. Indeed, in vitro studies have shown that bone calcium is released into acidic culture medium [10, 47, 52]. In this study, urine calcium excretion increased in several groups receiving NH$_4$Cl; however, the increase was not as substantial as one might have expected [50]. Perhaps, since the GHS rats already excrete some 8 to 10 times as much urinary calcium as control rats [13–26], further increases in urinary calcium are difficult to achieve. A comparison of the tubular segments involved in the decreased renal calcium reabsorption in the GHS rats, as compared with those involved in the decreased tubular response to metabolic acidosis, will be necessary to resolve this issue. Additionally, since the GHS rats already excrete a substantial amount of their dietary calcium, the potentially reduced amount of calcium present in their bones may not permit augmentation of urinary calcium. Further studies of bone density and/or calcium content in the bones of the GHS rats will be necessary to resolve this issue.

It is unclear why oxalate excretion fell with NH$_4$Cl administration. This fall is not due to greater ex vivo production of oxalate in the less acidic urine from the rats drinking distilled water, as we took care to acidify the urine collections that were used for the urine oxalate measurements (Methods section). Perhaps metabolic acidosis alters oxalate metabolism reducing production; further studies of oxalate metabolism during acidosis would be necessary to address this hypothesis. As expected, urine chloride progressively increased with increasing NH$_4$Cl administration.

The fall in urine supersaturation with respect to the CaHPO$_4$ solid phase during NH$_4$Cl administration appears to be caused by the marked fall in urine pH in spite of the increases in urine calcium and phosphorus. A reduction in urine pH is known to decrease the supersaturation with respect to CaHPO$_4$ [53]. The fall in urine supersaturation with respect to the CaOx solid phase during NH$_4$Cl administration appears principally caused by the fall in urine oxalate excretion. Urine pH has a minimal effect on CaOx supersaturation [54]. Supersaturation with respect to the uric acid solid phase rose markedly with NH$_4$Cl administration clearly because of the decrease in urine pH since uric acid excretion did not change appreciably. A decrease in urine pH is known to increase uric acid supersaturation [55] and is a clear risk factor for the formation of uric acid nephrolithiasis [2, 3].

Although urinary supersaturation with respect to CaHPO$_4$ and CaOx was decreased by the administration of NH$_4$Cl, the number and type of stones formed was not altered; all rats formed stones that consisted of poorly crystalline hydroxyapatite. It is unclear why a decrease in urine supersaturation with respect to a calcium phosphate solid phase, CaHPO$_4$, did not result in a decrease in stone formation. Previously, we have reported that reducing CaHPO$_4$ supersaturation below four to five led to elimination of stone formation in our rats [14]. Perhaps the increasing supersaturation of uric acid provided a nidus for calcium crystal deposition, as suggested by some investigators [56]. We did not, however, find any uric acid in these stones. Further study and analysis of the complex interaction between uric acid and calcium hydrogen phosphate supersaturation will be necessary to understand this complex interaction.

Dietary acid precursors, mainly protein, are often restricted, or oral base administered, in hypercalcemic patients with nephrolithiasis in an effort to decrease urine calcium excretion, increase urine citrate excretion, and thus lower supersaturation with respect to calcium oxalate and calcium phosphate stones [2, 3]. In this study, the metabolic acid load was increased without increasing...
the protein intake, and no effect on calcium stone formation was found. The decrease in oxalate excretion with NH₄Cl may have had a protective effect against calcium oxalate precipitation, which would not occur in humans receiving an acid load from a high protein diet. Perhaps the effect of protein loading to increase urine oxalate excretion [57] combined with the lithogenic effects of acid loading, such as hypercalciuria, are required for the formation of calcium oxalate stones. The current data obtained in hypercalciuric stone-forming rats fed the acid precursor NH₄Cl suggest that a reduction of dietary acid precursors will not lower supersaturation nor alter stone formation. This interesting hypothesis should be tested in hypercalciuric human stone formers.

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