

# Mechanism for Calcium Urolithiasis among Patients with Hyperuricosuria

## SUPERSATURATION OF URINE WITH RESPECT TO MONOSODIUM URATE

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**ABSTRACT** Since monosodium urate (NaU) may play an important etiologic role in the formation of renal stones containing Ca in patients with hyperuricosuria, the current studies were undertaken to define some of the physicochemical factors which determine the formation of NaU. In solutions containing Na, uric acid was rapidly transformed to NaU at pH >6. The results indicated that NaU, and not uric acid, was the stable phase above this pH. A reliable and simple method for the calculation of the state of saturation of urine with respect to NaU was developed from the ratio of concentration products of Na and total dissolved urate ( $U_T$ ) in the ambient fluid before and after incubation of urine with synthetic NaU. The concentration product ratio closely approximated the ratio of activity products of  $Na^+$  and acid urate ion. In contrast, the relative saturation ratio, or the ratio of activity product of original sample and the thermodynamic solubility product of NaU, often differed from the activity product ratio in the individual urine samples. With the concentration product ratio, it was found in 45 urine samples that a critical determinant for the supersaturated state with respect to NaU was the high concentration of  $U_T$ . At  $U_T > 300$  mg/liter, urine samples were invariably supersaturated with respect to NaU. These results suggest that the nidus of NaU could potentially form in the urine of patients with hyperuricosuria and Ca stones.

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## INTRODUCTION

Certain patients with hyperuricosuria may form renal stones containing Ca rather than of uric acid (HU),<sup>1</sup> even though they are normocalciuric and do not have any obvious abnormality of Ca metabolism (1, 2). They have been shown to respond favorably to treatment with allopurinol with reduced incidence of stone formation (1). It has therefore been suggested that hyperuricosuria may be pathogenetically important to the formation of Ca stones.

Recently, two theories have been proposed, which implicate an important etiological role for monosodium urate (NaU), either in a solid phase (3–5) or in a colloidal form (6). In one theory, “seeds” of NaU may contribute to Ca stone formation, by inducing heterogeneous nucleation or epitaxial growth of Ca oxalate and Ca phosphate. Thus, solid seeds of NaU were shown to induce precipitation of Ca salts from solutions which were metastably supersaturated with these salts (3–5). It is theoretically possible that colloidal NaU may participate in heterogeneous nucleation as well. Alternatively, colloidal NaU may promote Ca stone formation, by removing from urine certain mucopolysaccharides which inhibit crystal aggregation of Ca oxalate (6). In support of this theory,

<sup>1</sup> *Abbreviations used in this paper:* APR, Activity product ratio; CPR, concentration product ratio; EHDP, disodium ethane-1-hydroxy-1, 1-diphosphonate; HU, uric acid;  $K_{sp}$ , thermodynamic solubility product; KU, monopotassium urate; NaU, monosodium urate;  $NH_4U$ , monoammonium urate; RSR, relative saturation ratio;  $U_T$ ,  $HU + U^-$  or total dissolved urate.

crystal aggregates of Ca oxalate were found to be more prominent in urine samples of high HU content (7). Thus, NaU may participate in stone formation by promoting nucleation or aggregation of Ca salts.

The validation of above hypotheses requires the demonstration that in the presence of hyperuricosuria, the physicochemical environment of urine is favorable for the formation of crystalline or colloidal NaU. Current studies were therefore undertaken to define physicochemical factors which regulate the formation of NaU. It was found that HU is transformed to NaU in solutions containing Na and with pH >6. Urine specimens with high concentration of total dissolved urate ( $U_T$ ) (>300 mg/liter) were frequently supersaturated with NaU, whereas samples with lower concentrations were usually undersaturated. These studies provide evidence that nucleation of NaU could potentially occur in certain urine specimens with a high  $U_T$  content.

## METHODS

**Phase transition of uric acid to urate salts.** 5 mg of HU (Sigma Chemical Co., St. Louis, Mo.) was incubated in each milliliter of test solutions used at 37°C. Test solutions ranged in pH from 5.0 to 9.0, and contained 150 mmol Na/liter. Those with pH <6.3 were buffered with 50 mmol/liter of acetate and those with pH >6.3 were buffered with 50 mmol/liter of phosphate. The pH of solutions were kept within 0.1 U of original pH by frequent titration with 0.1 N NaOH or 0.1 N HCl. After incubation of the solid phase for 2 h, 1 day, and 7 days, the ambient solutions were separated by filtration of suspensions through 0.05  $\mu$ m Millipore filter, (Millipore Corp., Bedford, Mass.) and assayed for Na and  $U_T$ . A similar technique was used to prepare the filtrates in subsequent experiments. Selected samples of the solid phase were washed in distilled water, air-dried, and analyzed by X-ray diffraction.

Similar incubations of HU were performed in solutions in which K or  $NH_4$  had replaced Na in test solutions and in bases used for titrations. The studies were repeated in solutions which contained EHDP (disodium ethane-1-hydroxy-1, 1-diphosphonate; 2 mg phosphorus/liter).

**Solubility of urates.**  $NaU \cdot H_2O$  was prepared according to the procedure previously described. Monohydrates of monopotassium urate (KU) and monoammonium urate ( $NH_4U$ ) (ICN Pharmaceuticals Inc., Life Sciences Group, Cleveland, Ohio) were washed before use. 10 g of  $KU \cdot H_2O$  (or  $NH_4U \cdot H_2O$ ) were suspended in 500 ml of 150 mM KCl (or  $NH_4Cl$ ) solution, which was titrated to pH 7.0 with KOH (or  $NH_4OH$ ). After 24 h of stirring at 24°C, ambient fluid was replaced with the same vol of fresh 150 mM KCl (or  $NH_4Cl$ ) solution. After another 24 h of stirring, the solid was collected by filtration on a Buchner funnel, washed twice with distilled water, and dried at 80°C for 24 h. The sieved fraction between 44 and 74  $\mu$ m was utilized in the study.

$NaU \cdot H_2O$  was incubated in the same buffer solutions (150 mM Na) and under the same conditions as in incubations of HU. Similarly,  $KU \cdot H_2O$  (or  $NH_4U \cdot H_2O$ ) was incubated in solutions which were 150 mM with respect to K (or  $NH_4$ ).  $NaU \cdot H_2O$  was also suspended in solution at pH 6.7 and 7.4, in which varying amounts of Na were replaced by equimolar amounts of K or  $NH_4$ . Filtrates were prepared

as before and analyzed for  $U_T$ , Na, K, and  $NH_4$ . Activity product of NaU,  $KU$ , or  $NH_4U$  was calculated according to the method of Finlayson et al. (8, 9).

**Calculation of state of saturation with respect to NaU in urine.** Three-to-four 24-h urine samples were collected under refrigeration without acid or preservative from each of eight patients with Ca-containing renal stones and four control subjects. On random diets, patients with stones had normocalciuria (urinary Ca <200 mg/day) and hyperuricosuria (urinary uric acid >650 mg/day). Urinary oxalate was less than 35 mg/day. They were given a diet constant in Na and fluid intake to maintain urinary concentration of Na between 40 and 60 mM. However, dietary purine content was varied from 100 mg to 3 g/day to produce urine samples, which ranged in  $U_T$  concentration from 120 to 520 mg/liter. The pH was removed as a variable by titrating samples to pH 6.4. The actual pH in patients with stones was 6.29  $\pm$  32 SD. The state of saturation with respect to NaU was determined at this pH.

As with brushite and Ca oxalate systems (10), the state of saturation with respect to NaU was assessed by three methods, concentration product ratio (CPR), activity product ratio (APR), and relative saturation ratio (RSR). All urine samples were analyzed for CPR of NaU. Samples were incubated with  $NaU \cdot H_2O$  (5 mg/ml) for 2 days at 37°C, while pH was maintained between 6.3 and 6.5 by frequent titration with 0.1 N NaOH or 0.1 N HCl. After incubation, the ambient fluid was separated by filtration in a Millipore filter (Millipore Corp.) (0.05  $\mu$ m). The original urine samples and the filtrates were analyzed for Na and  $U_T$ . For each sample, CPR was obtained from the ratio of concentration product of Na and  $U_T$  before and after incubation with  $NaU \cdot H_2O$ .

In selected urine samples, the original urine samples and the filtrates were analyzed for Ca, Mg, K,  $NH_4$ , P, oxalate, chloride, sulfate, and citrate as well. From these analyses, the activity product NaU was calculated according to the method of Finlayson et al. (8, 9). The APR was calculated as the ratio of the activity product of original sample and that of the corresponding filtrate. The RSR was also determined from the ratio of original activity product and the thermodynamic solubility product ( $K_{sp}$ ) of NaU. The value obtained in this study in synthetic medium of  $2.79 \times 10^{-5}$  M<sup>2</sup> was assumed to be the  $K_{sp}$ .

For all three methods (CPR, APR, and RSR), the value of 1 indicated saturation, >1 supersaturation, and <1 undersaturation. Under the conditions of the experiment, the values of CPR and APR were independent of variation in the amount of solid phase added or the duration of incubation, since further addition of  $NaU \cdot H_2O$  (up to 20 mg/ml) or longer period of incubation (up to 10 days) did not significantly alter results.

In all incubations involving synthetic media or urine, chloroform was added to control bacterial contamination, six drops initially and two to three drops during subsequent days to each 10 ml of solution used. Under these conditions, uric acid is not oxidized to any significant degree, since it may be recovered almost completely after control incubations (without solid phase). Moreover, allantoin, an oxidation product of uric acid, does not alter the solubility or APR of HU or  $NaU \cdot H_2O$ .

**Heterogeneous nucleation of calcium oxalate.** It was previously shown that solid seeds of  $NaU \cdot H_2O$  induce heterogeneous nucleation of Ca oxalate from solution metastably supersaturated with respect to Ca oxalate (3, 5). The ability of  $KU \cdot H_2O$  (or  $NH_4U \cdot H_2O$ ) to do so was tested in identical conditions. The test solution contained 150 mmol K (or  $NH_4$ ), 5 mmol cacodylate, 0.4 mmol Ca, and 0.4 mmol

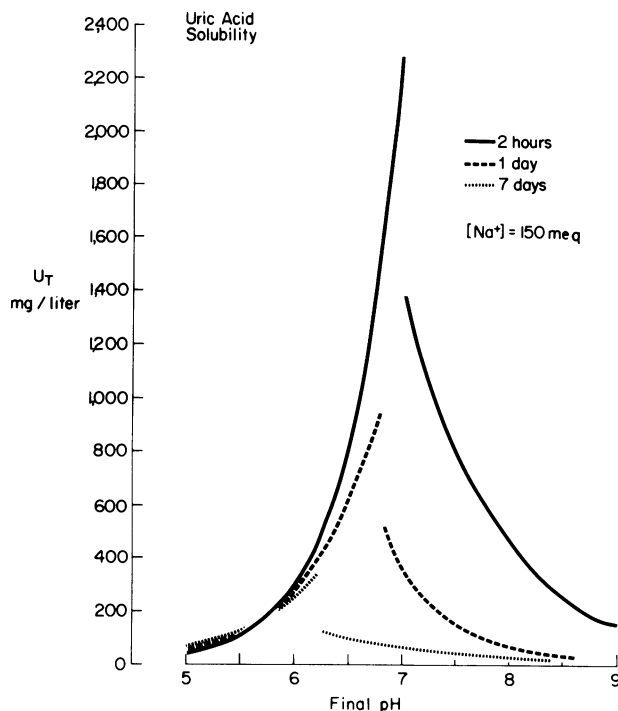


FIGURE 1 The HU solubility in the presence of Na. The concentrations of  $U_T$  in the ambient solution after incubation of HU in 150 mM Na solution for 2 h, 1 day, and 7 days at various pH are shown. The final Na concentration was  $149 \pm 3$  SD mM. For each period of incubation, the best fit, obtained by least squares analysis with the computer, is shown here.

oxalate/liter; it was approximately sixfold saturated with respect to Ca oxalate. After addition of 2 mg of  $KU \cdot H_2O$  (or  $NH_4U \cdot H_2O$ ) to each milliliter of solution used, filtrates were analyzed for Ca and oxalate.

**Analytical methods.** Ca, Mg, Na, K,  $NH_4$ , P, oxalate, chloride, sulfate, and citrate were analyzed according to various techniques as noted before (10).  $U_T$  was determined by the enzymatic method (11).

## RESULTS

**Phase transition of uric acid to urate salts.** The HU solubility was assessed from the concentration of  $U_T$  in the ambient solution after appropriate periods of incubation of HU in synthetic solution containing 150 mM Na (Fig. 1). The HU solubility displayed two patterns: increasing solubility with the rise in pH at low pH ranges, and decreasing solubility with further rise in pH. The critical pH at which this "transition" took place declined with longer duration of exposure to Na; it fell from approximately 7.0 after 2 h to 6.2 after 7 days of incubation. At pH >6, the uric acid solubility in the presence of 150 mM Na fell progressively with time until it approached the "solubility" of  $NaU \cdot H_2O$ . After 7 days

of incubation, the concentration of  $U_T$  in the filtrate approximated that which was obtained at steady state after incubation of  $NaU \cdot H_2O$  in the same solution (Fig. 2). The analysis by X-ray diffraction of the solid phase collected after 7 days of incubation of HU at pH 7.4 indicated the presence of  $NaU \cdot H_2O$ , the same as that found after incubation of  $NaU \cdot H_2O$ . However, at pH 5.2, the predominant crystalline constituent after incubation of HU was still HU, whereas that remaining after incubation of  $NaU \cdot H_2O$  was a mixture of HU and  $NaU \cdot H_2O$ .

The HU solubility in the presence of Na was compared with that obtained in solutions in which equivalent amount of K and NH had replaced Na (Fig. 3). At higher pH ranges, HU solubility decreased rapidly with rising pH. This decrease was most prominent and rapid in the presence of  $NH_4$ . The solid phase obtained after 7 days of incubation of HU in the presence of K (or  $NH_4$ ) at pH 7.4 was identical to the pattern displayed by  $KU \cdot H_2O$  (or  $NH_4U \cdot H_2O$ ) on X-ray diffraction.

These pH-dependent changes in the solubility of HU were not affected by the presence of EHDP.

**Solubility of urates.** When monocationic salts of HU were incubated in solutions containing a fixed amount of respective cation, the solution concentration of  $U_T$  at steady state decreased slightly as the pH was increased from 5.5 to 8 (Fig. 2). However, the concentration of  $U_T$  did not change significantly. The activity products of monocationic salts at steady state were therefore independent of pH at this pH range. Among the three salts tested,  $KU \cdot H_2O$  was found

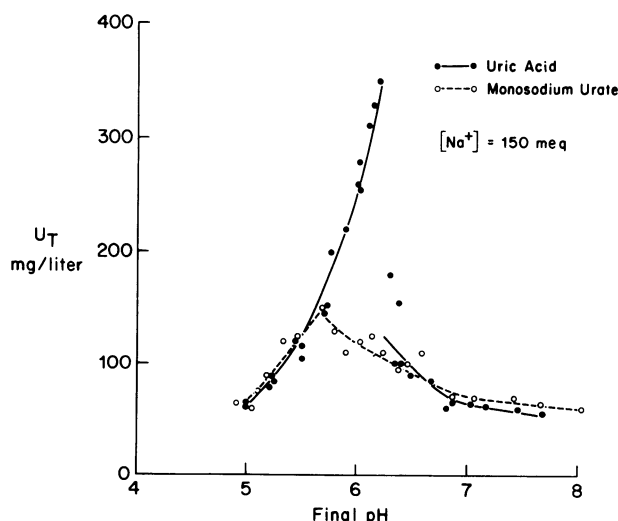


FIGURE 2 The comparison of HU solubility with that of NaU in 150 mM Na solution. After incubation of 5 mg of HU or monosodium urate monohydrate in each milliliter of solution (150 mM Na) used for 7 days, the  $U_T$  in ambient solution was determined.

to be most soluble, and  $\text{NH}_4\text{U}\cdot\text{H}_2\text{O}$  least soluble. The mean value from seven experiments for activity product at steady state ( $K_{sp}$ ), obtained after incubation of the solid phase (5 mg/ml) for 2 days at pH 7.4, was  $9.63 \times 10^{-5} \pm 0.17 \times 10^{-5}$  SD  $\text{mol}^2$  for  $\text{KU}\cdot\text{H}_2\text{O}$ ,  $2.79 \times 10^{-5} \pm 0.15 \times 10^{-5}$   $\text{mol}^2$  for  $\text{NaU}\cdot\text{H}_2\text{O}$ , and  $3.60 \times 10^{-5} \pm 0.14 \times 10^{-5}$   $\text{mol}^2$  for  $\text{NH}_4\text{U}\cdot\text{H}_2\text{O}$ . Essentially the same values were obtained with larger amounts of the solid phase (up to 20 mg/ml) and for longer periods of incubation (up to 10 days).

The solubility of  $\text{NaU}\cdot\text{H}_2\text{O}$ , expressed as the activity product of  $\text{Na}^+$  and  $\text{U}^-$ , was not altered significantly when the incubation medium contained 20–100 mM of K or  $\text{NH}_4$ , and when the Na content varied from 50 to 150 mM.

*Urinary state of saturation with respect to NaU.* Fig. 4 compares the values for RSR, APR, and CPR obtained in each of 16 urine samples. The value of CPR was essentially the same as that for APR in individual samples. However, the intestinal value for RSR sometimes differed considerably from that of APR, even though the mean values of RSR and APR for the whole group were not significantly different from each other.

The activity product of NaU after incubation in urine fluctuated considerably, ranging from  $2.73 \times 10^{-5}$   $\text{mol}^2$  to  $5.02 \times 10^{-5}$   $\text{mol}^2$ . The mean value in 16 samples of  $3.45 \times 10^{-5} \pm 0.70 \times 10^{-5}$  SD  $\text{mol}^2$  was greater than that for  $K_{sp}$  obtained in synthetic medium.

The dependence of CPR of NaU on various urinary

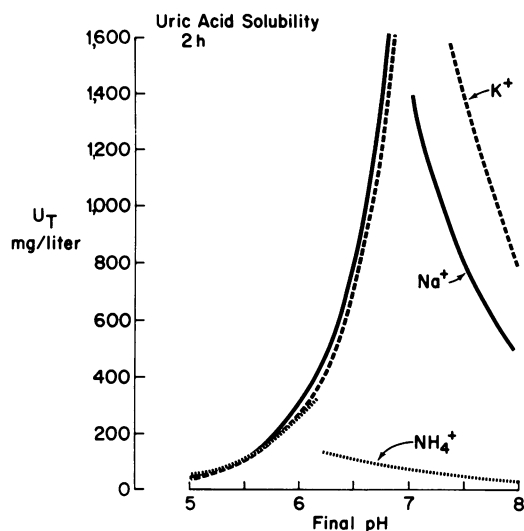


FIGURE 3 The HU solubility in Na solution, compared with that in K or  $\text{NH}_4$ . The filtrate concentrations of  $\text{U}_T$  after incubation with HU for 2 h are shown. The initial concentration of Na, K, or  $\text{NH}_4$  was 150 mM. The final concentrations of Na, K, and  $\text{NH}_4$  were  $149 \pm 2$  SD mM, and  $148 \pm 4$  mM, respectively. As in Fig. 1, the best fits obtained by least squares analysis are presented.

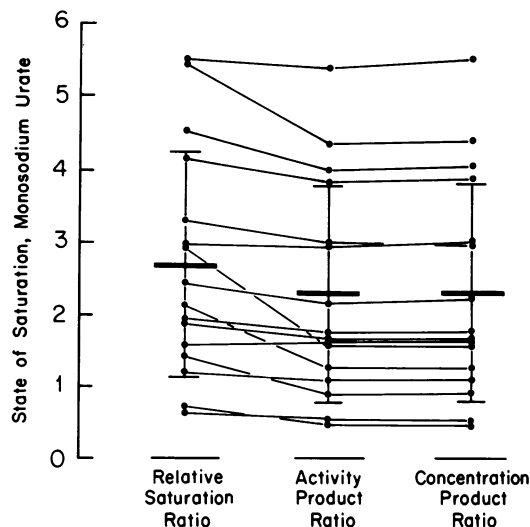


FIGURE 4 The comparison of RSR, APR, and CPR of NaU. Lines connecting points indicate studies in same sample. Horizontal bars indicate mean  $\pm$  SD of 16 urine samples examined.

constituents was examined. The CPR was positively correlated with  $\text{U}_T$  concentration ( $r = 0.90$ ,  $P < 0.001$ ) (Fig. 5). Urine samples with  $\text{U}_T$  concentration  $> 300$  mg/liter were invariably supersaturated ( $\text{CPR} > 1$ ) with respect to NaU. The same dependence was found in patients with nephrolithiasis as in control subjects. The CPR was not correlated with concentrations of Ca, Mg, K,  $\text{NH}_4$ , P, oxalate, chloride, sulfate, or citrate. The dependence of CPR on urinary pH or Na concentration could not be examined since these potential variables were purposely "fixed", by titration of pH to 6.4 initially, and by keeping constant dietary sodium and fluid intake.

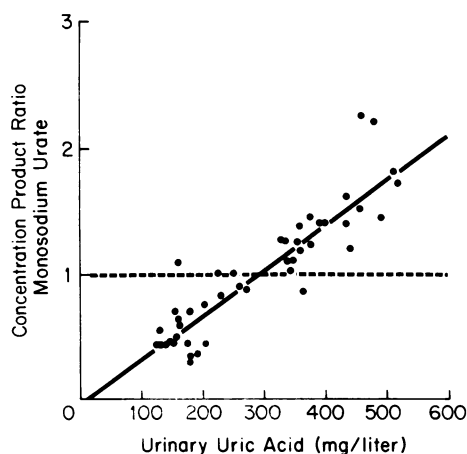


FIGURE 5 The dependence of the CPR of NaU on urinary concentration of HU. Each point represents study in a separate urine sample. The horizontal dashed line indicates saturation with respect to NaU.

In four urine samples, urinary pH was purposely increased from 6.0 to 7.4. The CPR of NaU increased slightly (up to 15%).

*Heterogeneous nucleation of Ca oxalate.* It was previously shown that addition of 2 mg  $\text{NaU} \cdot \text{H}_2\text{O}$  to each milliliter of metastably supersaturated solution (with respect to Ca oxalate) caused a rapid decline in filtrate concentration of Ca and oxalate (3, 5). However, when equal amount of  $\text{KU} \cdot \text{H}_2\text{O}$  or  $\text{NH}_4\text{U}$  was added to the same solution, the filtrate concentration of Ca or oxalate did not change significantly, a finding indicating that the heterogeneous nucleation of Ca oxalate did not occur.

## DISCUSSION

The hypothesis that NaU may be etiologically important in formation of Ca stones is supported by the evidence that either a solid or colloidal form of NaU induces heterogeneous nucleation of Ca oxalate and Ca phosphate (3–5), or plays an important role in the crystal aggregation of Ca oxalate (6, 7). Further validation of this scheme requires the demonstration that the physicochemical environment of urine, passed by patients with hyperuricosuria and Ca stones (1, 2), is “compatible” with the formation of the NaU nidus. Specifically, it is necessary to show that (a) NaU is physicochemically stable in urine, and (b) urine specimens are supersaturated with respect to NaU.

The solubility studies of HU indicated that HU was rapidly transformed to NaU at  $\text{pH} > 6$  in the presence of Na. Since  $\text{pK}$  of the first proton of HU is 5.47 (9), the results suggested that  $\text{pH} > 6$  sufficient amount of acid urate available for the salt formation. Similar conclusion was reached by others (9, 12). In our experience, the urinary pH in patients with hyperuricosuria and Ca stones is invariably  $> 5.5$ , and usually  $> 6$ . Thus, NaU rather than HU may be the preferred or stable phase in these urine samples.

As with brushite and Ca oxalate system (10), several methods were employed for the calculation of the urinary state of saturation with respect to NaU. The RSR compared the activity product of NaU in the original urine sample with the thermodynamic solubility product obtained in synthetic medium. The APR and CPR depended on the solubility of NaU experimentally derived in each urine sample. The activity product of  $\text{Na}^+$  and acid urate at steady state after incubation with  $\text{NaU} \cdot \text{H}_2\text{O}$  varied considerably among urine samples, a finding which indicated that ionic activities may not be accurately determined in complex solutions such as urine. Such errors may be involved in the calculation of activity product of the original urine sample as well as of the filtrate after incubation with the solid phase, and may be “cancelled”

during the determination of APR (10, 13). Thus, APR represented potentially the best method for the estimation of the urinary state of saturation with respect to NaU (10). The results indicated that CPR closely approximated APR, whereas, RSR often did not. Because of the simplicity of the method, CPR was mainly utilized to determine the urinary state of saturation with respect to NaU.

In this study, urinary concentration of Na was kept at approximately 50 mM by regulating dietary Na and fluid intake. Under such conditions, CPR was found to be dependent on urinary concentration of  $\text{U}_\text{T}$ . Urine specimens were invariably supersaturated when the content of  $\text{U}_\text{T}$  exceeded 300 mg/liter. Since this critical  $\text{U}_\text{T}$  concentration was often exceeded, urine samples from patients with hyperuricosuria may often be supersaturated with respect to NaU.

The current study, demonstrating stability of NaU in urine and urinary supersaturation with respect to NaU, provides evidence that the nidus of NaU could potentially form in the urine of patients with hyperuricosuria and Ca stones. Once formed, the nidus of NaU may lead to the formation of Ca stones by inducing heterogeneous nucleation of Ca salts (3, 4) or by absorbing certain urinary inhibitors (6). Indeed, solid seeds of NaU have been shown to cause nucleation of Ca oxalate and Ca phosphate. However, the infrequent appearance of NaU crystals in urine restricts the etiologic role of the solid phase of NaU. The scheme involving colloidal NaU is attractive, since it does not require the crystallization of NaU. Unfortunately, there is as yet no direct experimental evidence that colloidal NaU absorbs inhibitors (6), or participates in heterogeneous nucleation of Ca salts. This study does not discriminate between the role of solid NaU and that of colloidal NaU in Ca stone formation. It indicates that the physicochemical environment of urine from patients with hyperuricosuria and Ca stones is probably conducive to the formation of either solid or colloidal NaU.

Although the phase transition of HU to monocationic salt also occurred in the presence of K or  $\text{NH}_4$ , KU or  $\text{NH}_4\text{U}$  may not play a significant role in the formation of Ca stones. Our study indicates that seeds of  $\text{KU} \cdot \text{H}_2\text{O}$  or  $\text{NH}_4\text{U} \cdot \text{H}_2\text{O}$  are much less effective than the seeds of  $\text{NaU} \cdot \text{H}_2\text{O}$  in inducing heterogeneous nucleation of Ca oxalate. Furthermore, urine specimens are probably seldom supersaturated with respect to KU, because of high solubility of  $\text{KU} \cdot \text{H}_2\text{O}$ .

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