Sodium Urate Accelerates Precipitation of Calcium Oxalate In Vitro (38928)

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A number of clinical observations implicate uric acid and sodium urate in the formation of calcium oxalate kidney stones. Elevated serum levels and urinary excretion rates of these substances are frequent among calcium stone formers (1, 2). Calcium oxalate stones are frequent in patients with gout (3, 4). Allopurinol, a synthetic purine analog that reduces uric acid production by inhibiting xanthine oxidase (5) drastically reduces calcium oxalate stone formation in patients who excrete excessive amounts of uric acid and sodium urate and have no other evident cause for their stones (2, 6).

Lonsdale (7, 8) has theorized that uric acid or sodium urate could promote calcium stone formation because of epitaxial growth of calcium oxalate crystals on a substrate of uric acid or urate crystals. Recently, Pak (9) has shown that sodium urate can initiate crystallization of calcium oxalate from a metastable solution, an observation that strongly supports Lonsdale's view.

The importance of uric acid or sodium urate in calcium oxalate stone formation depends largely upon how quickly either may initiate crystallization. Urine resides in the renal collecting system between 3 and 5 min (10) before it reaches the bladder. If epitaxial growth could produce precipitation within this time interval, it could contribute importantly to kidney stone formation. We report here that sodium urate, but not uric acid, initiates calcium oxalate precipitation within 10 min, and possibly 5 min, from a solution which would otherwise remain stable for 50 min.

Materials and Methods. 25 ml of ¹⁴Clabeled sodium oxalate (400 μ M) in 5.0 mM barbituric acid, 6.0 mM acetate buffer, pH 5.7, adjusted to ionic strength of 300 with KCL was mixed with an equal volume of 10 mM CaCl₂ in the same buffer and at the same ionic strength. The resulting solution, 200 μM oxalate, 5 mM calcium, was shaken at 24° in conical glass tubes. Immediately, and at intervals thereafter, 1 ml aliquots were withdrawn, filtered through 5 μ m pore diameter Millipore discs, and the radioactivity of the filtrate determined in dpm/ml. Because filtered volumes were small, 13 mm diam filter discs were used to minimize fluid losses. Recovery of filtrate was virtually complete; 100 μ l was transferred to a counting vessel. A conventional scintillant mixture (PCS, Amersham Searle) and counting instrument (Packard Tri-carb Liquid Scintillation Spectrometer model 3375) were used. Counting efficiency was assessed by an external standard; in 20 preliminary experiments the accuracy of this method was verified by addition of internal standards to each vial.

The ratio of unprecipitated oxalate at each interval to initial oxalate concentration was assumed to represent the course of calcium oxalate precipitation, there being no other cation present with which oxalate could precipitate. In this system, ¹⁴C-labeled sodium oxalate shaken at 24° remained stable for more than 12 hr. In every experiment, predicted zero time dpm/ml, calculated as one-half of the dpm/ml of the sodium oxalate solution before mixing with $CaCl_2$, was within 0.1% of the observed value, excluding an initial burst of precipitation. For experiments involving addition of solids to the system, crystalline sodium urate, calcium oxalate or uric acid was added to the CaCl₂ solution, prior to mixing with sodium oxalate, at twice the solid to liquid ratios indicated. All of these dissolved to some extent during experiments, so initial ratios were higher than the average over the time interval studied. Filter pore size was not critical, as filters between 0.2

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FIG. 1. Kinetics of Oxalate Precipitation. Unprecipitated oxalate calculated as the ratio of dpm/ml at each interval to that at zero time is shown as a function of incubation time for a solution of calcium chloride (5 mM) and [14C]sodium oxalate (200 μ M) at pH 5.7, ionic strength 300, 24°. This system was studied without added solid phase (\bullet) , with addition of solid sodium urate at 0.1 (\bigcirc) and 2.5 (\blacksquare) mM, and calcium oxalate 0.5 (\triangle) and 2.5 (\triangle) mM initial solid to liquid ratios. Each point is the mean (± 1) SEM) of three experimental values. Predicted zero time dpm/ml was calculated as one-half of dpm/ml in the oxalate solution before mixing (see text). Observed zero time dpm/ml differed negligibly from predicted except for experiments using 2.5 mM calcium oxalate; to avoid crowding, time 0 points are omitted except for this case. At 5 min, the first points shown, unprecipitated oxalate was 98.7 \pm 1.8% for 0.1 mM urate, 97.5 \pm 2.4 for 2.5 mM sodium urate. At 10 min, both differed significantly from the basic system (94.8 \pm 1.5 and 87.9 \pm 3.0, respectively; corresponding P values calculated by unpaired Student's t test were <.05 and <.02, respectively). Subsequent urate points and all calcium oxalate points differed significantly from the basic system and from each other.

and 5 μ m gave nearly identical results. Significance of differences between fractional precipitation of the unseeded system and with addition of solid phase materials was estimated using Student's unpaired t test.

Results. Without added solid phase, the

system began to precipitate, slowly, after 50 min (Fig. 1). Crystalline sodium urate 0.1 mM caused detectable precipitation by 5 min and definite departure from the unseeded system at 10 min (P < .05). At 2.5 mM, sodium urate caused a greater effect; 0.5 mM urate, not illustrated, was intermediate. Calcium oxalate monohydrate at solid to liquid ratios of 0.5 and 2.5 mM was more effective than urate (Fig. 1). The effects of 0.1 mM calcium oxalate were approximately equivalent to 2.5 mM sodium urate and were omitted from Fig. 1 for clarity. At 2.5 mM, calcium oxalate produced 30% precipitation within the few seconds required to obtain a "0-time" sample (Fig. 1).

The effects of crystalline uric acid were first studied at a pH substantially below 5.7, to minimize conversion of uric acid to urate. The pk'a for dissociation of the n-9 proton of uric acid is 5.4 at an ionic strength of 300 (11). At pH 4.4, uric acid could comprise at most 10% of total urate species present, so although some crystalline uric acid might be converted to the sodium salt, the proportion of sodium urate would always be small. In our system at pH 4.4 but otherwise unchanged, oxalate began to precipitate only after 120 min (96% \pm 2 (SEM) unprecipitated). Addition of 2.5 mM solid uric acid produced no detectable effect until 60 min (Table I). Thereafter, precipitation was brisk (percentage of unprecipitated oxalate 90 \pm 3, 82 \pm 3, and 63 ± 75 , 90, and 120 min). Smaller amounts of uric acid had even less effect, as expected (results not shown). Calcium oxalate, though less effective than at pH 5.7, was still a very potent accelerator of crystallization, and sodium urate was also an effective accelerator at pH 4.4, proving that the relative ineffectiveness of uric acid compared to urate was not due to the lower pH at which uric acid was tested (Table I; compare to Fig. 1). Finally, at pH 5.7, solid uric acid had only a slight effect compared to sodium urate (compare Table I and Fig. 1).

Pyrophosphate, a well-established inhibitor of calcium oxalate and calcium phosphate crystallization (12), slowed precipitation of the unseeded system. At pH 5.7,

	Percentage of oxalate remaining in solution at selected time intervals				
	5	10	15	30	60
	(min)	(min)	(min)	(min)	(min)
Uric acid 2.5 mM ^a	100	100	100	100	94 ± 3
Calcium oxalate 0.5 mM ^a	81 ± 1	67 ± 2	51 ± 4	45 ± 2	32 ± 2
Sodium urate 2.5 mM ^a	96 ± 1	86 ± 2	78 ± 1	61 ± 2	43 ± 3
Uric acid 2.5 mM	100	100	100	100	85 ± 2
Sodium urate 0.1 m M + 10 μM PiP	_	99 ± 1	98 ± 1	97 ± 2	91 ± 2
Calcium oxalate 0.5 m M + 10 μM PiP		81 ± 1	53 ± 4	46 ± 1	37 ± 1

TABLE I. EFFECTS OF URIC ACID AND PYROPHOSPHATE UPON CALCIUM OXALATE PRECIPITATION.

^a Solution pH 4.4; in the other entries, pH was 5.7. All results are means of three experiments ± 1 SEM. PiP—pyrophosphate.

pyrophosphate, $10 \ \mu M$, prevented precipitation until 105 min had elapsed. Even then, precipitation was negligible (percentage unprecipitated 99 \pm .1 at 105 min and 97 \pm .4 at 120 min). Sodium urate, 0.1 mM did not initiate precipitation until 15 min had elapsed, and the accelerating effects of urate and calcium oxalate were both much reduced (Table I; compare to Fig. 1)

Because of its potential importance in stone formation, brushite (13) was tested in our system. At solid to liquid ratios of 0.25 and .5 mM, it was without effect at pH 5.7. Also without effect was sodium urate fully dissolved in solution at a concentration of 0.5 mM, pH 5.7.

Discussion. The effectiveness of sodium urate in accelerating calcium oxalate precipitation makes plausible the notion that excessive urinary urate excretion could cause calcium oxalate kidney stones. Even though urine resides in the collecting system of the normal kidney for 5 min or less, urate acts quickly enough that some crystals could be deposited from a urine that would, in the absence of solid phase urate, be stable for a much longer interval However urine contains at least one inhibitor of the urate accelerating effect, pyrophosphate (12), and may contain others; so the effects of urate on calcium oxalate precipitation from urine must be assessed experimentally.

Whether or not urine from hyperuricosuric stone formers contains crystalline sodium urate at solid to liquid ratios of 0.1 mMor greater is also uncertain, although simple quantitative considerations suggest it should.

Thirty-two percent of patients with calcium oxalate renal stones we have studied excreted 800 mg of combined uric acid and urate in a daily urine volume that averaged between 1 and 1.5 liters (2, 6). Among 52 samples from 30 such hyperuricosuric calcium oxalate stone formers, 24-hr urine pH was between 5.7 and 6.3 in 35, between 5.4 and 5.6 in 9, below 5.4 in 5, and above 6.4 in 3, so urate, not uric acid predominated. Spontaneous precipitation of 20 mg/ liter (0.1 mM) of urate would not be surprising under these conditions, as urate solubility is depressed by the high concentrations of sodium and potassium chloride usual in urine so that 800 mg/liter will not remain in stable solution at a pH below 6.4 (11).

Summary. Precipitation of calcium oxalate crystals from a metastable solution can be detected within 10 min if crystalline sodium urate is added at a solid to liquid ratio of 0.1 mM or more. Without urate, precipitation begins after 50 min. Uric acid is not effective. Pyrophosphate inhibits the effects of sodium urate.

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