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# Effects of Sodium Urate and Uric Acid Crystals on the Crystallization of Calcium Oxalate

H.–G. Tiselius

Department of Urology, University Hospital, Linköping, Sweden

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**Summary.** Crystallization of calcium oxalate in the presence of uric acid and sodium urate crystals was analyzed in a metastable crystallization system containing calcium chloride and sodium oxalate (A), in urine highly supersaturated with respect to calcium oxalate (B), and in urine with a high level of metastable supersaturation (C). In system A uric acid crystals in concentrations up to 11.4 mMol/l did not affect calcium oxalate crystallization, neither did sodium urate during the first 6 h in concentrations below 5 mMol/l. In system B neither uric acid nor sodium urate crystals affected calcium oxalate crystallization. However, an increased rate of crystallization was observed with both uric acid and sodium urate in system C, but the effect was less pronounced than with calcium oxalate seed. Urine pre-treated with sodium urate and subsequently analyzed in system A in a concentration of 2%, gave a slightly lower inhibition of calcium oxalate crystal growth. Concerning the crystal size distribution in the same system, larger crystals were observed in several urines pre-treated with uric acid and sodium urate.

**Key words:** Calcium oxalate crystallization, Uric acid, Sodium urate crystals.

## Introduction

Although several authors have emphasized a relationship between hyperuricosuria and calcium oxalate stone disease [6, 17], and treatment with allopurinol apparently has reduced the recurrence rate of calcium oxalate stone formation [1–3, 5, 7, 13, 20–22], a satisfactory explanation for the role of urate in this respect has not been found. The different hypotheses that have been presented include heterogenous nucleation of calcium oxalate on sodium urate [4, 10, 14, 16] or uric acid [4, 6] and binding of crystallization inhibitors either to crystals of sodium urate or uric acid [8, 9, 15] or to colloidal urate [18]. A number of results have been reported on the properties of sodium urate and uric acid with respect to heterogenous crystallization. Most studies have thus shown that sodium urate is more potent than uric acid in inducing calcium oxalate crystallization [4, 11]. On the other hand the risk of forming sodium urate crystals in urine is apparently low. According to some recent results on variations in urine composition during the day, there might be a risk of forming uric acid crystals at least during the early morning hours [24]. Furthermore, in a constant composition system, Koutsoukis et al. [10] recently demonstrated a greater propensity of uric acid than sodium urate to induce calcium oxalate crystallization. For these reasons it was of interest to reinvestigate the properties of sodium urate and uric acid crystals in some calcium oxalate crystallization systems.

## Materials and Methods

### *Metastable Crystallization System*

A metastable crystallization system (system A) was prepared according to principles described previously [18, 23]. The solutions which were saturated with uric acid and sodium urate respectively, contained per litre: 1 mMol of calcium chloride, 0.2 mMol of sodium oxalate, and 10 mMol of a sodium cacodylate buffer adjusted to pH 6.0 in the sodium urate experiment, and to pH 5.0 in the uric acid experiment. The ion-strength was maintained with sodium chloride at a concentration of 0.15 Mol/l. The crystallization was followed by adding ($^{14}$C)-oxalate to the system, giving an isotope concentration of 0.5 $\mu$Ci/l. Crystallization was studied with sodium urate crystals at concentrations of 0.1, 1.0, 2.0, 5.0, and 10.0 mMol/l or uric acid crystals at concentrations of 0.1, 0.6, 1.1, 2.3, 5.7, and 11.4 mMol/l. A control system with 0.15 mMol/l calcium oxalate crystals was always run simultaneously.

### *Higly Supersaturated Crystallization System with Urine*

Forty ml of normal urine was saturated with sodium urate and uric acid respectively, and passed through a Millipore filter (diameter 0.45 $\mu$m) and diluted with physiological saline to give a final volume

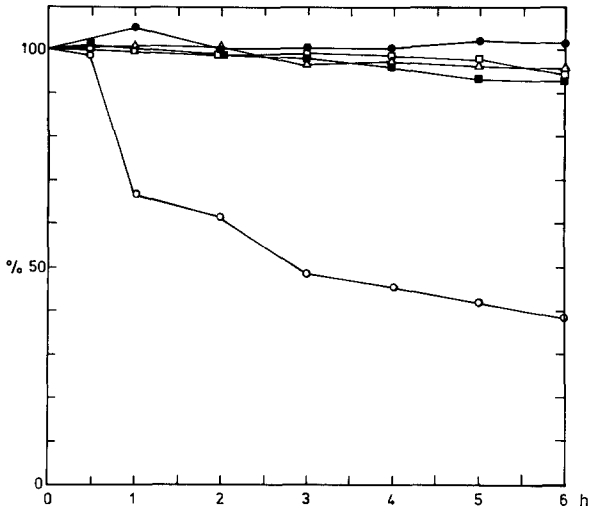


Fig. 1. Per cent of $^{14}$C-oxalate remaining in 50 ml of a solution, metastably supersaturated with respect to calcium oxalate (system A), after the addition of 1 ml of physiological saline (•) or 1 ml of the following crystal suspensions: calcium oxalate 1 mg/ml (○), and sodium urate 1 mg/ml (□), 10 mg/ml (■) and 20 mg/ml (△)

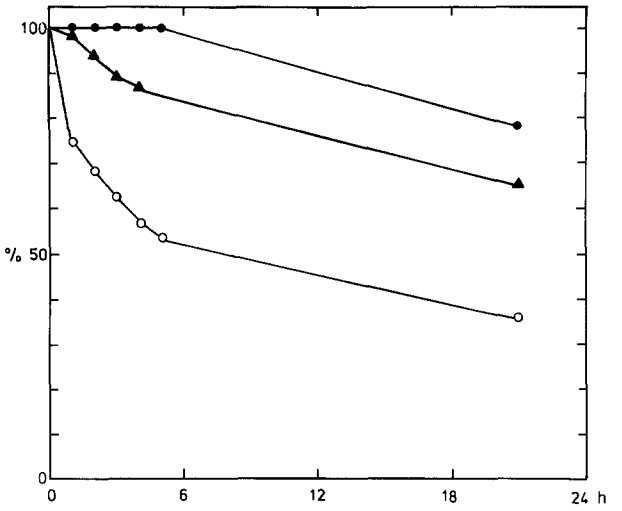


Fig. 2. Per cent of $^{14}$C-oxalate remaining in 50 ml of a solution, metastably supersaturated with respect to calcium oxalate (system A) after the addition of 1 ml of the following crystal suspensions: calcium oxalate 1 mg/ml (○), and sodium urate 50 mg/ml (•) and 100 mg/ml (▲)

of 50 ml. Crystallization was started by addition of sodium oxalate and a trace amount of ($^{14}$C)-oxalate, to increase the oxalate concentration by 0.6 mMol/l (system B). Uric acid crystals with concentrations of 0.1, 0.6, 1.1, 5.7 and 11.4 mMol/l had previously been added to the system. The crystallization was followed by measuring the isotope concentration at pH 4.5, 5.0, and 5.5 at different times after the addition of sodium oxalate. To a similar system sodium urate crystals corresponding to final concentrations of 5.0 and 10.0 mMol/l and uric acid crystals 5.7 and 11.4 mMol/l were added at the same time as calcium oxalate crystals (0.15 mMol/l). The pH was maintained at 4.7–4.8 in the uric acid experiment and 5.5–5.7 in the sodium urate experiment.

*Metastable Crystallization System with Urine*

In another experiment with urine the supersaturation was increased to a level where crystallization started after approximately 1 h (system C). These urine samples were assumed to have a calcium oxalate supersaturation in the upper part of the region of metastable calcium oxalate supersaturation. The amount of 0.01 mMol/l sodium oxalate required for 40 ml of each urine varied between 2.3 and 2.7 ml. The crystallization in this system was studied following the addition of uric acid and sodium urate crystal suspensions, corresponding to concentrations of uric acid between 0.6 and 1.1 mMol/l and of sodium urate between 0.5 and 1.0 mMol/l.

*Adsorption Experiment*

Ten ml of urine was mixed during 45 min at 37 °C with approximately 0.5 g of uric acid and sodium urate crystals in order to study adsorption of inhibitors. The urine was subsequently centrifuged and filtered through a Millipore filter (diameter 0.45 μm). 1 ml of untreated urine or 1 ml of urine pretreated with sodium urate or uric acid crystals was added to system A. The rate of crystallization was determined both from the decrease in isotope concentration and the change in crystal size distribution by Coulter Counter technique (model ZBI with Channelyzer).

## Results

*Crystallization of Calcium Oxalate in the Presence of Sodium Urate Crystals*

Figure 1 demonstrates the decrease in ($^{14}$C)-oxalate during the first 6 h following addition of sodium urate crystals to system A, corresponding to final urate concentrations between 0.1 and 2.0 mMol/l. During the first 4 h the isotope concentration remained at the start level and was similar to that in a crystal-free control. A slight decrease in ($^{14}$C)-oxalate concentration was observed after 5 and 6 h, but apparently without a clear relation to the amount of sodium urate added. When larger amounts of crystals were added (Fig. 2) crystallization of calcium oxalate occurred, although at a much slower rate than in the presence of calcium oxalate seed crystals. A sodium urate crystal concentration of 10 mMol/l resulted in a measurable crystallization within 1 h, and after 21 h 65% of the isotope remained in solution. This should be compared with 78% for 5 mMol/l of sodium urate crystals.

When sodium urate crystals were added together with calcium oxalate crystals in the highly supersaturated system B (Fig. 3) the rate of crystallizations was unaffected by the presence of sodium urate.

*Crystallization of Calcium Oxalate in the Presence of Uric Acid Crystals*

Uric acid crystals in concentrations between 0.1 and 2.3 mMol/l did not result in measurable crystallization in system A (Fig. 4). The same result was obtained even when the

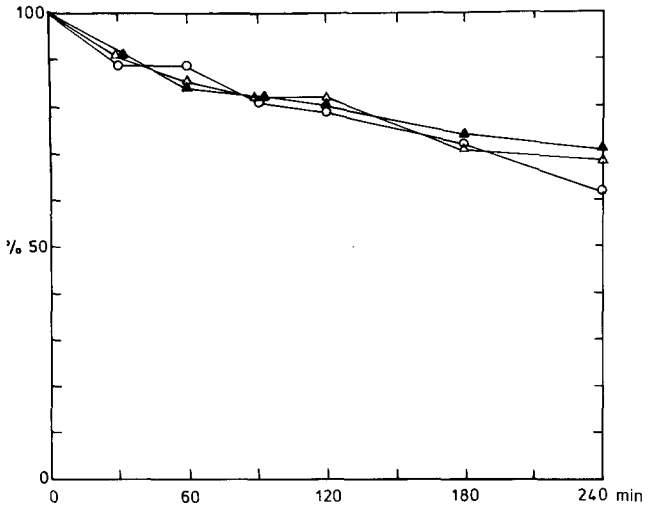


Fig. 3. Per cent of $^{14}$C-oxalate remaining in 40 ml of urine containing crystals of uric acid (▲), sodium urate (△), or no crystals (○), following supersaturation by addition of sodium oxalate (system B)

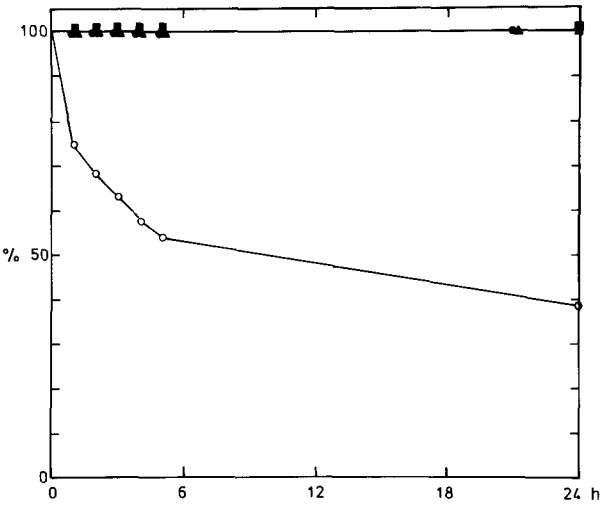


Fig. 5. Per cent of $^{14}$C-oxalate remaining in 50 ml of a solution, metastably supersaturated with respect to calcium oxalate (system A), after the addition of 1 ml of the following crystal suspensions: calcium oxalate 1 mg/ml (○), and uric acid 50 mg/ml (●), 100 mg/ml (△) and 200 mg/ml (■)

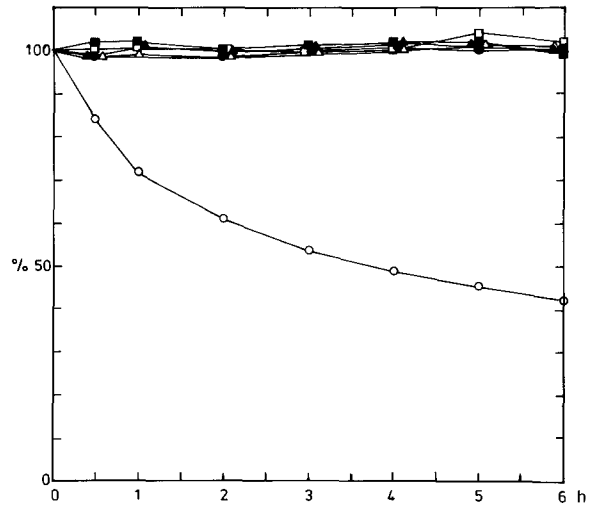


Fig. 4. Per cent of $^{14}$C-oxalate remaining in 50 ml of a solution, metastably supersaturated with respect to calcium oxalate (system A), after the addition of 1 ml physiological saline (●) or 1 ml of the following crystal suspensions: calcium oxalate 1 mg/ml (○), and uric acid 1 mg/ml (□), 5 mg/ml (■), 10 mg/ml (△) and 20 mg/ml (▲)

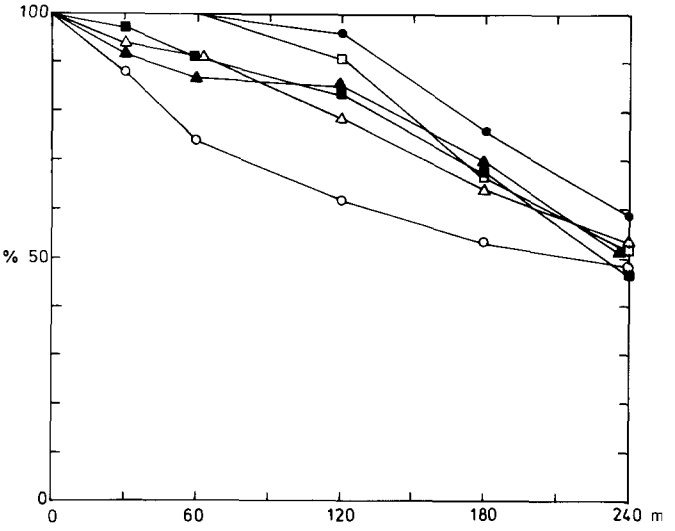


Fig. 6. Per cent of $^{14}$C-oxalate remaining in 40 ml of a urine sample, metastably supersaturated with respect to calcium oxalate (system C) after the addition of 1 ml of physiological saline (●) or 1 ml of the following crystal suspensions: calcium oxalate 5 mg/ml (○), uric acid 5 mg/ml (□), 10 mg/ml (■) and sodium urate 5 mg/ml (△) and 10 mg/ml (▲)

uric acid crystal concentration was increased to 5.7 and 11.4 mMol/l (Fig. 5). When uric acid crystals were added to five different urine samples which subsequently were brought to crystallization by addition of sodium oxalate (system B), no significant effect on the crystallization rate was recorded irrespective of pH.

### *Crystallization of Calcium Oxalate in Highly Metastably Supersaturated Urine*

As is evident from Fig. 6, the rate of crystallization in urine (system C) increased considerably following addition of calcium oxalate crystals, but apparently the presence of uric acid and sodium urate crystals also resulted in an increased rate of crystallization. The same pattern was observed in several urines studied in this way.

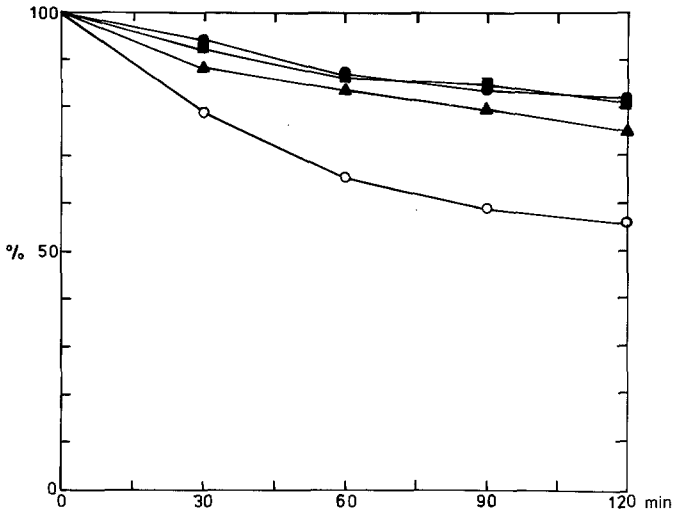


Fig. 7. Mean per cent of $^{14}$C-oxalate remaining in 50 ml of a solution, metastably supersaturated with respect to calcium oxalate (system A) after addition of 2 ml of calcium oxalate seed crystals (1 mg/ml). The system contained 1 ml physiological saline (○), 1 ml of untreated urine (●) or 1 ml or urine preincubated with either uric acid (■) or sodium urate (▲)

*Studies on Adsorption of Crystallization Inhibitors*

Six urine samples pre-treated with sodium urate and uric acid were studied in system A (Fig. 7). Pre-treatment with sodium urate apparently resulted in a slightly lower rate of crystal growth.

With a Coulter Counter, the crystal size distribution was studied in system A with or without pre-treatment with uric acid and sodium urate. More large crystals were recorded in 3 out of 5 urine samples pre-treated with sodium urate, and in 2 out of 5 samples pre-treated with uric acid.

## Discussion

The growth rate in metastably supersaturated solutions of calcium chloride and sodium oxalate was not increased following addition of uric acid seed crystals, even when the amount of seed was so high that it corresponded to a urate concentration of 22 mMol/l. In contrast sodium urate crystals appeared to induce calcium oxalate crystal growth, but only with large amounts of seed (Fig. 2).

However, such concentrations are very unlikely to occur under physiological conditions. The presence of crystals corresponding to urate concentrations below 5 mMol/l did not result in any significant growth of calcium oxalate during the first 6 h. These results are thus in accordance with those presented by Coe et al. [4] but in contrast to the findings by Meyer et al. [11].

In a constant composition system Koutsoukos [10] recorded a significant growth of calcium oxalate with both uric acid and sodium urate. The ability to induce growth was in their experiment most pronounced for uric acid, in accordance with the crystal lattice matching [6, 10]. The differences thus recorded might possibly be explained by different levels of supersaturation in the test systems. When large amounts of sodium urate and uric acid crystals were present in a highly supersaturated *urine* (system B) during the crystallization process (Fig. 3), the rate was unaffected. However, when *urine* was adjusted to a high metastable supersaturation (system C), both uric acid and sodium urate crystals appeared to induce calcium oxalate precipitation.

The adsorption experiments showed a slightly increased growth rate following pre-treatment of urine with crystals of sodium urate, but not with uric acid. Concerning the effects on crystal size distribution the results were not fully conclusive, but larger crystals were observed following pre-treatment with both sodium urate and uric acid. However, in order to draw conclusions in this respect it is probably necessary to know the inhibitor composition in the individual urine sample but unfortunately such information is not available.

The results in this study indicate that the presence of sodium urate or uric acid crystals might increase the rate of calcium oxalate crystallization in urine, but evidently only at levels of high metastable calcium oxalate supersaturation (system C). At a labile supersaturation (system B) no effect was recorded. At lower saturation levels (system A) the crystallization was increased by sodium urate crystals in very high concentrations but not by uric acid. A heterogenous nucleation can explain these effects, but the experimental results are also consistent with a reduction of the inhibitory activity in the presence of sodium urate or uric acid crystals [9, 15, 18, 19].

Inasmuch as the greatest risk of uric acid crystallization occurs in the early morning hours [24], coincident with a high risk of forming a urine supersaturated with respect to calcium oxalate, it might be of value to reduce urate excretion during that period, especially in patients with a high 24 h urate excretion.

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Prof. Dr. H.-G. Tiselius
Department of Urology
University Hospital
S-58185 Linköping
Sweden