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F. Grases · A.I. Villacampa · A. Costa-Bauzá

Ammonium and sodium urates precipitating from synthetic urine and fine structure of urate renal calculi

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Abstract A study of ammonium and sodium urate precipitation in vitro and the fine structure of several urate renal calculi was carried out to contribute to an understanding of the participation of ammonium and sodium urates in urolithiasis. Ammonium urate precipitated in vitro in two different morphologies: a typical spherulite morphology formed at high supersaturation and disorganized needle-like crystals formed at low supersaturation. In all cases sodium urate precipitated in vitro as bundles of curved fibrils, its crystallization being inhibited by calcium in concentrations between 20 and 60 mg/l depending on the sodium urate supersaturation. From a collection of 1300 renal calculi, only three had ammonium urate as their main component (0.2%), three were mixed calculi (0.2%) consisting of ammonium urate and calcium oxalate (two) or uric acid (one), and in one calculus ammonium urate was present as a minor component. Only in a mixed calculus of uric acid and calcium oxalate was sodium urate detected in a very low quantity. The study of the fine structure of the renal calculi constituted mainly by ammonium urate demonstrated similar patterns in which spherulites, needle-like individual crystals and an amorphous mass of ammonium urate with abundant organic matter in non-organized structures coexist. As minor components, struvite or calcium oxalate crystals were found. A general mechanism of the formation of such calculi is proposed.

Key words Ammonium urate · Sodium urate · Crystallization · Renal calculi

F. Grases (✉) · A.I. Villacampa · A. Costa-Bauzá
Laboratory of Urolithiasis Research,
Faculty of Sciences,
University of Balearic Islands,
E-07071 Palma de Mallorca, Spain

Introduction

The incidence of ammonium urate stones has dramatically decreased in industrialized countries due to the efficacy of treatments for infection. Obviously, the presence of ureolithic bacteria that produce ammonium and increase urinary pH, together with high urinary uric acid levels, are the main risk factors for formation of such stones. Nevertheless, formation of ammonium urate calculi in sterile urine has been described [1, 3, 17]. Moreover, different patterns of these calculi were also characterized [1, 9].

Sodium urate is seldom observed in renal calculi in spite of human urine often being supersaturated with monosodium urate [8, 12, 15, 16]. Only exceptionally pure monosodium urate calculi have been described and these were of vesical origin [1]. Monosodium urate could also act as an effective promoter of calcium oxalate crystallization through heterogeneous nucleation processes, according to in vitro experiments [2, 7, 10, 11]. Nevertheless, the presence of sodium urate in the core of calcium oxalate calculi is extremely exceptional.

From this description of accumulated knowledge about the role of ammonium and sodium urates in urolithiasis, it is clear that insufficient information is available to clarify all aspects related to crystallizing behaviour under physiological conditions. For this reason, a study of ammonium and sodium urate precipitation in vitro and the fine structure of several urate renal calculi was carried out to contribute to an understanding of the participation of ammonium and sodium urates in urolithiasis.

Materials and methods

Studies on ammonium urate precipitation from synthetic urine

Solid phases precipitating from synthetic urine at different ammonium and uric acid concentrations and at several pH values were determined. The synthetic urine stock solution was prepared daily

with a composition corresponding to 4-fold the concentrations indicated in Table 1. When experiments at different pH values were performed, the pH of the different solutions was preadjusted to the required value by adding either HCl or NaOH solution, as appropriate. The final pH value was checked and immediately adjusted if necessary, and was continuously monitored during all the experiments, to check that no significant changes were occurred.

One hundred and twenty-five millilitres of synthetic urine stock solution was first brought to 37°C in a water bath. It was then placed in a 600-ml beaker equipped with four wall baffles and stirred by a double-bladed stirrer. The beaker was in a constant temperature water bath at 37°C. Thereafter a solution of 2000 mg/l uric acid (previously thermostated to 37°C) was quickly poured into the beaker and the final volume adjusted to 500 ml (Table 2). The resulting reaction mixture was covered and stirred continuously for 60 min.

Samples were withdrawn from the stirred reaction mixture at predetermined intervals. A drop of suspension was placed on a microscope coverslip and left standing for a few minutes to allow sedimentation of crystals present. Then, liquid was cautiously sucked off using a filter paper, the glass was dried, sputter-coated with gold and observed with a Hitachi S-530 scanning electron microscope (SEM) equipped with energy-dispersive X-ray microanalysis (EDS). The whole volume of reaction mixture was filtered through a membrane filter of pore size 0.45 μm, the filter was dried and the collected precipitate subjected to X-ray diffraction (Siemens D 5000).

Table 1 Final composition of the media used as synthetic urine

Species	Concentration (mg/l)	Molar concentration (mol/l)
Na^+	4873	0.212
K^+	3180	0.081
NH_4^+	782[a]	0.043
Mg^{2+}	72	3.0×10^{-3}
Ca^{2+}	120	3.0×10^{-3}
Cl^-	8590[b]	0.242
SO_4^{2-}	1927	0.020
$H_2PO_4^-$	824	8.5×10^{-3}
HPO_4^{2-}	2522	0.026
$C_2O_4^{2-}$	25	2.8×10^{-4}
$C_6H_5O_7^{3-}$	321	1.7×10^{-3}

[a] When studying the influence of ammonium ions, this value ranged between 900 and 7564 mg/l
[b] When studying the influence of ammonium ions, this value ranged between 8822 and 21 925 mg/l

Studies on sodium urate precipitation from synthetic urine and aqueous calcium-containing solutions

Solid phases precipitating from synthetic urine and aqueous NaCl solutions containing different sodium, uric acid and calcium concentrations at a pH value of 8.0 were determined. Stock solutions of 2000 mg/l uric acid, 165 g/l NaCl and 98.5 g/l $CaCl_2.2H_2O$ were used in the experiments.

All stock solutions were first thermostated to 37°C in a water bath and then placed in a 600-ml beaker equipped with four wall baffles and stirred by a double-bladed stirrer. The final volume was adjusted to 500 ml and the pH value to 8.0 using 5 N NaOH (Table 3). The resulting mixture was covered and stirred continuously for 60 min. Samples were withdrawn from the stirred reaction mixture at the end of the experiment and studied as described above.

Synthetic urine was first brought to 37°C in a water bath. It was then placed in a 600-ml beaker equipped with four wall baffles and stirred by a double-bladed stirrer. The beaker was in a constant-temperature water bath at 37°C. Thereafter a solution of 2000 mg/l uric acid (previously thermostated to 37°C) was quickly poured into the beaker and the final volume adjusted to 500 ml (Table 2). The resulting reaction mixture was covered and stirred continuously for 60 min.

Study of renal calculi

Renal stones classified as ammonium urate renal calculi were selected from our stone collection, containing over 1300 specimens. Renal stones, often in several pieces, were fractured by a scalpel along different planes. One or several fragments of each stone were mounted on a stub, sputter-coated with gold and studied by a SEM equipped with EDS.

Table 2 Experimental conditions and results for ammonium urate precipitation in synthetic urine (see Table 1) at 37°C [*AmU* ammonium urate, *HAP* hydroxyapatite, *BRU* brushite, *STR* struvite, (*t*) thread-like, (*n*) needle-like, (*s*) spherulites, (*b*) big crystals]

[Ammonium][a] (mg/l)	pH	Vol (ml) 2 g/l UA[b]	[Uric acid][a] (mg/l)	Precipitated solid
900	6.7	375	1500	BRU + HAP
1000	6.7	350	1400	HAP
1000	6.7	375	1500	AmU (t) + HAP
1400	6.7	125	500	HAP
1400	6.7	250	1000	HAP
1400	6.7	350	1400	AmU (s) + HAP
1400	6.7	375	1500	AmU (n, s) + HAP
1891	6.7	125	500	HAP
1891	6.7	250	1000	AmU (n) + HAP
1891	6.7	375	1500	AmU (n, s) + HAP
3782	6.7	125	500	AmU (t) + HAP
3782	6.7	175	700	AmU (t) + HAP
3782	6.7	250	1000	AmU (b) + HAP
3782	6.7	275	1100	AmU (b) + HAP
3782	6.7	300	1200	AmU (n, s) + HAP
3782	6.7	325	1300	AmU (n, s) + HAP
3782	6.7	350	1400	AmU (n, s) + HAP
3782	6.7	375	1500	AmU (n, s) + HAP
3782	7.8	250	1000	AmU (n, s) + STR
3782	7.8	375	1500	AmU (n) + STR
7564	6.7	250	1000	AmU (n, s) + HAP

[a] Final concentration
[b] Volume (ml) of 2000 mg/l uric acid (UA) added to the reaction flask (reaction solution total volume = 500 ml)

Table 3 Experimental conditions and results for sodium urate precipitation in aqueous NaCl solution at pH 8.0 and 37°C and different supersaturations (*NaU* sodium urate, (*No precip*. no precipitate formed)

Vol (ml) 2 g/l UA[b]	[Uric acid][a] (mg/l)	[Calcium][a] (mg/l)				
[Na^+] = 9290 mg/l[a] (obtained by adding 71.6 ml of 165 g/l NaCl to the reaction solution)						
		0	100	120		
425	1700	NaU	NaU	NaU		
375	1500	NaU	NaU	No precip.		
250	1000	NaU	NaU	No precip.		
200	800	NaU	NaU	No precip.		
150	600	NaU	NaU	No precip.		
100	400	No precip.				
[Na^+] = 4640 mg/l[a] (obtained by adding 35.8 ml of 165 g/l NaCl to the reaction solution)						
		20	30	40	60	80
375	1500	NaU	NaU	NaU	NaU	No precip.
250	1000	NaU	NaU	No precip.		
200	800	NaU	No precip.			

[a] Final concentration
[b] Volume (ml) of 2000 mg/l uric acid added to the reaction flask (reaction solution total volume = 500 ml)

Results

Ammonium urate precipitating from synthetic urine

Experiments were performed at pH 6.7 and 7.8, at several uric acid and ammonium concentrations, as indicated in Table 2. The experiments lasted a maximum of 1 h. As can be deduced, when experiments were performed at high ammonium urate supersaturation, a typical spherulitic morphology was observed (Fig. 1a), whereas at low supersaturations a structure characterized by the presence of disorganized needle-like crystals was seen (Fig. 1b). Thus, at an ammonium concentration of 3782 mg/l, uric acid concentration of 1000 mg/l and pH 6.7 a spherulitic pattern was obtained (Fig. 1c), whereas in the same conditions but at pH 7.8 a fibrous mass of ammonium urate crystals mixed with struvite (magnesium ammonium phosphate) crystals was formed (Fig. 1d). It is also interesting to observe that at normal urinary uric acid levels (600 mg/l) a high level of ammonium ions (3782 mg/l) is necessary to crystallize ammonium urate, while at normal urinary ammonium ion levels (750 mg/l) the presence of extremely high levels of uric acid (1500 mg/l) is necessary to crystallize ammonium urate during the selected time period (1 h).

Sodium urate precipitating from aqueous calcium-containing solutions

Experiments were carried out at pH 8.0 and at several calcium, sodium and uric acid concentrations, as indicated in Table 3. Thus, using synthetic urine at pH 8.0 and in the presence of 1500 mg/l of uric acid, no solid was formed during the duration of the experiment (1 h). However, in the absence of calcium a precipitate of fine curved needles was obtained, clearly identified as sodium urate by X-ray diffraction (Fig. 2a), demonstrating the importance of calcium in preventing the crystallization of sodium urate. The results of these experiments are summarized in Table 3. As can be observed, at normal sodium levels (4640 mg/l) and in the presence of 800 mg/l uric acid, calcium concentrations above 20 mg/l prevent sodium urate precipitation and at higher uric acid concentration (1500 mg/l) calcium concentrations above 60 mg/l also prevent sodium urate formation. As can be seen in Fig. 2b, in all cases the crystalline morphology of the sodium urate crystals obtained is similar and corresponds to bundles of curved fibrils.

Urate renal calculi

From a collection of 1300 renal calculi, only three had ammonium urate as their main component (0.2%), three were mixed calculi (0.2%) consisting of ammonium urate and calcium oxalate (two) or uric acid (one), and in one calculus ammonium urate was present as a minor component. Only in a mixed calculus of uric acid and calcium oxalate was sodium urate detected in a very low quantity (Fig. 3) as curved needles.

The structure of the calculi mainly constituted by ammonium urate was similar to that found in pure hydroxyapatite calculi [6]. Thus, important amounts of organic matter in non-organized structures were detected. In these structures spherulites of ammonium urate coexist (Fig. 4a, b) with well-developed spicule-shaped ammonium urate crystals (Fig. 4b, c, d) and layers of amorphous material, structureless macroscopically (Fig. 5a) but on detailed inspection revealed to be composed of fused spherulites of ammonium urate (Fig. 5b). In the mixed calculi, the part containing ammonium urate exhibited a structure similar to the above description.

Discussion

Studies on in vitro urate precipitation

From the results on the in vitro ammonium urate crystallization it can be deduced first that in normal urodynamic situations (experiments were followed for 1 h) precipitation of supersaturated ammonium urate in the upper urinary tract is very difficult and, given normal values of urinary ammonium ion, extremely improbable. Only when a notable increase in urinary ammonium takes place, as a consequence of some ureolithic infection or other pathologies that notably enhance ammonium production, in the presence of important amounts of uric acid at pH values near to 7, will the precipitation of ammonium urate occur in reasonable periods of time. This fact explains the low incidence of this type of calculus. It must be also considered that, bearing in mind the notable metastability of the ammonium urate solution, during long periods of time no precipitation would be produced in normal supersaturated urine, but in situations of prolonged urinary stasis, solid ammonium urate could be formed.

Two morphologies of ammonium urate crystals were observed: spherulites (Fig. 1a, c) obtained at high supersaturation, and well-developed needle-like individual crystals forming a fibrous mass (Fig. 1b, d) obtained at low supersaturation. This can easily be explained given that the higher the supersaturation values attained, the more favourable the homogeneous nucleation processes and consequently the smaller the crystals formed, implying a poor crystallinity.

Experiments on sodium urate precipitation demonstrate the extraordinary metastability of the solutions in artificial urine, mainly due to the presence of calcium ions. This explains why, although human urine is often supersaturated with respect to monosodium urate, these crystals are seldom formed in normal urine in the upper urinary tract, and when formed they appear in the bladder due to the longer time of urine residence. This again shows the importance of kinetic factors in stone-forming processes. Thus, although some crystals of sodium urate could be formed in a supersaturated urine in the bladder, they would be eliminated as asymptomatic crystalluria without any trouble. The presence of very low quantities of calcium (30 mg/l) prevents sodium urate precipitation, at times longer than 1 h, in the presence of 800 mg/l uric acid, 4640 mg/l sodium ions and pH 8.0. Consequently it is clear that formation of sodium urate crystals in the urinary tract must be associated with low calcium excretion. In all cases, the morphology of the sodium urate crystals obtained was similar and corresponded to well-developed large curved fibrils (Figs. 2a, b). This morphology indicates that, during their formation, the supersaturation attained was not sufficient to produce homogeneous nucleation and likewise demonstrates the importance of the ''incubation time'' with regard to sodium urate crystallization in biological fluids, as emphasized by some authors [8].

It is necessary to emphasize that the in vitro results presented here demonstrated for both cases (ammonium and sodium urate) the noteworthy metastability of their solutions – such that even when large amounts of uric acid and ammonium or sodium are excreted, the formation of stones in the upper urinary tract requires prolonged urinary stasis. Moreover, from the physiological viewpoint, some important metabolic differences distinguish upper urinary tract urate stone-formers from bladder urate stone-formers. Normally, upper urinary tract urate stone-formers have high ammonium or sodium excretion in the urine. In fact, as the in vitro study demonstrates, this is probably a necessary condition but not sufficient in an important number of cases. Obviously, for a complete explanation of the formation of urate stones in the upper urinary tract both pathophysiological and physicochemical aspects must be considered together.

Studies on the fine structure of urate-containing renal calculi

The study of the fine structure of the renal calculi mainly constituted by ammonium urate demonstrated similar patterns in which spherulites, needle-like individual crystals and an amorphous mass of ammonium urate with abundant organic matter coexist. As minor components, struvite or calcium oxalate crystals were found. The presence of abundant bacterial detritus and erythrocytes in all cases demonstrated the infectious origin of these calculi.

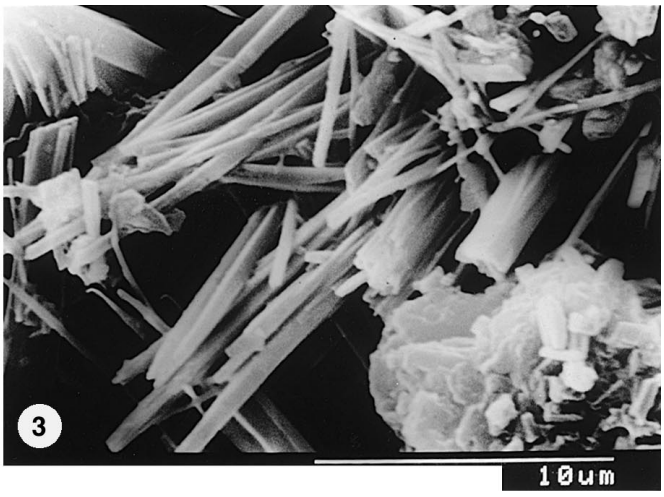
On the basis of the reported results, the following mechanism for the formation of this type of calculius can be deduced: bacterial attack on the urothelium producing abundant organic matter and bacterial detritus is responsible for the retention of deposits of such material in cavities with poor urodynamic efficacy, serving as a template for the development of ammonium urate crystals in appropriate conditions. At elevated ammonium urate supersaturation the formation of spherulitic and ''amorphous'' ammonium urate structures will take place, whereas at low supersaturation crystalline spicule-shaped forms will occur. Thus, the coexistence of spherulites and spicule-shaped crystals in the same renal calculus can easily be explained by considering fluctuations in the uricuria (for example due to changes in the diet) or/and in urinary pH values (due to changes in the infection process, for example as a result of a medical treatment). These processes must lead to the formation of non-organized structures such as those observed in these renal calculi. The presence of struvite is obviously due to the appropriate situation for the precipitation of ammonium magnesium phosphate as a consequence of the conditions generated by the infection [6]. Due to the permanent urinary supersaturation relative to calcium

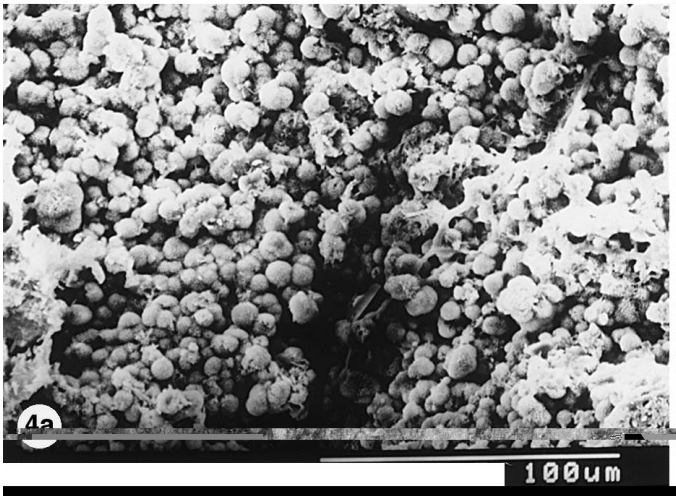
Fig. 1a–d Morphology of ammonium urate precipitated in synthetic urine containing 3782 mg/l ammonium ions. **a** Spherulites obtained in the presence of 1300 mg/l uric acid and pH 6.7. **b** Needle-like crystals obtained in the presence of 500 mg/l uric acid and pH 6.3. **c** Spherulites obtained in the presence of 1000 mg/l uric acid and pH 6.7. **d** Fibrous mass of ammonium urate crystals obtained in the presence of 1000 mg/l uric acid and pH 7.8. A crystal of magnesium ammonium phosphate is also seen. [The presence or absence of the corresponding substances and ions was previously proved by X-ray diraction and confirmed by energy-dispersive X-ray microanalysis (EDS)]

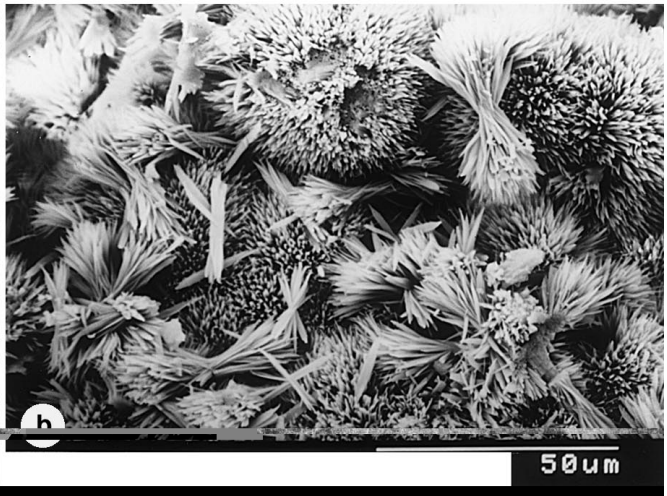
Fig. 2a, b Morphology of sodium urate crystals precipitated in the presence of 9290 mg/l sodium ions, 1500 mg/l uric acid and pH 8.0, **a** in the absence of calcium and **b** in the presence of 60 mg/l calcium. (The presence or absence of the corresponding substances and ions was previously proved by X-ray diraction and confirmed by EDS)

Fig. 3 Sodium urate crystals detected in a mixed calculus of calcium oxalate monohydrate–uric acid. (The presence or absence of the corresponding substances and ions was previously proved by X-ray diffraction and confirmed by EDS)

Fig. 4a–d Scanning electron microscopy images of ammonium urate renal calculi with **a** ammonium urate spherulites, **b** spherulites in various stages of development mixed with spicule-shaped ammonium urate crystals, **c** spicule-shaped ammonium urate crystals, **d** spicule-shaped ammonium urate crystals mixed with bacteria. (The presence or absence of the corresponding substances and ions was previously proved by X-ray diffraction and confirmed by EDS)







oxalate [13], the formation of these crystals on a solid in permanent contact with urine is not surprising.

Formation of mixed uric acid–ammonium urate or calcium oxalate–ammonium urate calculi can be explained as follows: Formation of an initial calcium oxalate or uric acid calculus according to previously described mechanisms [4, 5] causes an infection and, as a consequence, due to the urine composition, an ammonium urate concretion is generated by the mechanism described above.

Sodium urate was detected only in minimum amounts in a mixed uric acid–calcium oxalate monohydrate calculus (Fig. 3). This result can be explained by considering that sodium urate crystals are seldom formed in urine due to the high metastability of its solutions, in part as a result of the presence of some urine compo-

Fig. 5 a Layers of amorphous compact ammonium urate alternating with layers of spherulites. **b** Detail of a compact layer. (The presence or absence of the corresponding substances and ions was previously proved by X-ray diffraction and confirmed by EDS)

nents such as calcium ions. On the other hand, urinary stasis may cause sodium urate crystallization and, thus, some pure sodium urate vesical calculi have been described [1]. Urinary alkalization therapy using, for example, sodium bicarbonate, can usually achieve dissolution of uric acid stones. However, very favourable conditions for sodium urate crystallization through the rise in urinary pH and sodium concentration are generated. Probably this was what provoked the formation of the sodium urate crystals observed in the above-described calculus. Obviously, in a case in which the stone surface is covered with sodium urate crystals, it is no longer sensitive to the chemolytic action of basifiers [14].

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