URIC ACID CALCULI

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Abstract

The study of the composition and structure of 41 stones predominantly composed of uric acid was complemented by in vitro investigation of the crystallization behaviour of uric acid. The solubility of anhydrous (UAA) and dihydrated (UAD) forms of uric acid in urine-like liquor at 37°C was calculated as a function of pH using reported dissociation constants and solubility products. UAD precipitates from urine under physiological conditions when the medium is supersaturated with respect to this compound, though UAA represents the thermodynamically stable, and hence, less soluble, form. Solid UAD in contact with liquid, transforms into UAA within 2 days. This transition is accompanied by development of hexagonal bulky crystals of UAA and the appearance of cracks in the original prismatic UAD crystals. Uric acid calculi can be classified into three distinctly different groups, differing in outer appearance and inner structure. Formation mechanism of individual types of uric acid calculi was inferred from results of performed investigation. Thus, type I includes stones with a little central core and a compact columnar UAA shell, and stones with interior structured in alternating densely non-columnar layers developed around a central core; both of these stones are formed mainly by crystalline growth at low uric acid supersaturation. Type II includes porous stones without inner structure and stones formed by a well developed outermost layer with an inner central cavity; type II stones are formed mainly by sedimentation of uric acid crystals generated at higher uric acid supersaturation. Type III includes stones whose structure and mechanism of formation is a mixture of types I and II.

Key Words: Anhydrous uric acid, calculi, crystallization, dihydrated uric acid, energy dispersive X-ray analyzer, scanning electron microscopy.

Introduction

Calculi predominantly composed of uric acid represent a sizable proportion of human kidney stones, around 13% [16]. However, considerably smaller attention was devoted to their investigation compared to other kinds of concretions, such as calcium oxalate or phosphatic uroliths. A relatively simple and frequently effective medical treatment of uric acid calculi based on systematic alkalization of urine, increased fluid intake and a low purine diet [1, 20] is probably partially accountable for such remarkable lack of interest. Furthermore, uric acid crystallizes from an aqueous solution according to the prevailing reaction conditions as an anhydrous compound (UAA), a dihydrate (UAD) or a mixture of both modifications [2], and dihydrate readily transforms into the anhydrous salt [11, 12]. These properties of uric acid substantially complicate analysis of processes taking place at calculogenesis, and this may represent an additional reason for the absence of in-depth studies of uric acid calculi. As a result, information available on morphology, structure and formation mechanism of uric acid calculi is considerably limited [5, 15].

Contemporary knowledge concerning uric acid calculi can be summarized as follows. Uric acid occurs most often in calculi in a pure state, rarely accompanied by urates, phosphates or calcium oxalate. Uric acid calculi largely form pebble-like objects with a smooth, but not polished surface, though uneven and globular surface is also occasionally encountered. A cross-section of uric acid stone usually shows concentric layers of microcrystalline material forming typical laminations with radial striation converging to the core area. Less frequently, some calculi exhibit a course granular interior without a sign of lamination, texture or preferred orientation of constituting crystals. The calculus core is commonly composed of randomly oriented plate-like crystals of uric acid. Calculi of uric acid contain also around 2 wt.% of filamentous organic matrix forming a fine meshwork uninterrupted by the crystal border. It is believed that organic matrix is incorporated in uric acid crystals and does not exist between columnar crystals constituting radial striation [13, 14, 16, 17, 18].

Different conditions of crystallization are held responsible for generation of diverse groups of uric acid
stones. These calculi largely consist of UAA, but around 20% contain significant amount of UAD, and 3% comprise over 95% of UAD [11]. In the case of stones constituted by UAA, it is not known whether dihydrate precipitated first, and later underwent transformation into anhydrous substance, or UAA was formed directly, or if both processes participated in uric acid stone formation. Formation mechanism of these calculi is suspected to be similar to that which gives rise to calcium oxalate monohydrate concretions [17]. It should be emphasized, however, that quoted conclusions are based on observing a limited number of specimens, and therefore must be regarded as tentative.

In vitro studies of the crystallization behaviour of uric acid demonstrated that UAA is the stable phase from the thermodynamic viewpoint. Temperature, pH, present admixtures, and the concentration of a liquid medium determine the nature of solid phase actually precipitating under given conditions. As a rule, UAD, sometimes accompanied by the anhydrous form, precipitates readily under physiological conditions [2, 19]. However, UAD in contact with a liquid phase undergoes a gradual transition into UAA. The transition rate, depending on both reaction conditions and the size of transforming crystals, is reported for physiological conditions to be relatively slow [2, 3] and rather fast [11]. Both conclusions, though contradictory, were called upon to substantiate and explain observations. As evident, insufficient information is available on the structure, formation mechanism and composition of uric acid calculi. Moreover, uric acid crystallizing behaviour under physiological conditions is far from clear, and even hitherto performed in vitro studies have shed little light on existing serious discrepancies and uncertainties. In order to contribute to the better understanding of uric acid calculogenesis, a thorough investigation of composition, morphology and inner structure of 41 uric acid calculi was carried out, and a first in vitro attempt of a crystallization study of uric acid under physiological conditions was carried out.

Experimental

Uric acid crystallization

Precipitation of uric acid from the artificial urine [8] at pH 5.5 and 4 was studied at 37°C. The precipitation was carried out in the beaker equipped with 4 baffles and mixed throughout each experiment with a double-bladed stirrer. Three solutions, A (62.5 ml), B (375 ml) and C (62.5 ml), preheated to 37°C were, in the succession introduced into the beaker, situated in a constant temperature water bath. Solution A contained 44.08 g/l NaSO₄·10H₂O, 5.84 g/l MgSO₄·7H₂O, 18.56 g/l NH₄Cl, 48.62 g/l KCl and 4.16 g/l CaCl₂·3.5H₂O; solution C consisted of 10.6 g/l NaH₂PO₄·2H₂O, 75.28 g/lNaH₂PO₄·12H₂O, 52.2 g/lNaCl, 4.0 g/l trisodium citrate dihydrate and 0.30 g/l C₆H₈O₆, while solution B contained uric acid in an amount appropriate to achieve a predetermined uric acid concentration in a resulting mixture and a quantity of NaOH ensuring complete dissolution of the substance. All solutions were prepared by dissolving pure (analytical grade) chemicals in distilled water.

Immediately after introducing solution C, the reaction mixture pH was adjusted to 4 or 5.5 by addition of concentrated HCl. Then an induction time, i.e., time elapsed between an instant of establishing supersaturation through pH adjustment and the appearance of crystals, was determined. Crystal appearance was detected by the naked eye observing the beaker content against a black background, perpendicular to the coherent light beam illuminating the reaction mixture. The detection of bright sparkles in the liquid, signifying the presence of crystals, was regarded as a termination of the induction period. Each experiment was repeated three times, and the observations were performed by two different people to ensure the reproducibility of the presented results.

The transformation of UAD to UAA was studied in contact with both air and a mother liquor. UAD crystals were precipitated from the artificial urine, isolated by filtration and subjected to thermogravimetry (TG) and X-ray diffraction analysis (XRD). Thereafter, crystals were put in a desiccator filled with either silica-gel or water when transformation in a dry or 100% humid air respectively was studied. The desiccator was placed in a constant temperature box kept at 4, 20 or 40°C. The solid was regularly sampled and analyzed by TG and XRD. When studying the transformation taking place in contact with a mother liquor, UAD crystals were precipitated from the artificial urine containing 500 and 1500 mg/l of uric acid at pH 5.5. A part of suspension was filtered, and the solid phase thus obtained was analyzed. The remaining volume of suspension was kept at 37°C, agitated for 12 hours and then left still. In selected time intervals, the suspension was sampled, a drop of suspension was situated on a microscopic cover glass and left still for a while to allow present crystals to deposit on the glass. Then liquid was removed by filtration paper, sediment sputtered with gold and observed by a Hitachi (Tokyo, Japan) scanning electron microscope (SEM). At the experiment end, the solid phase was isolated from suspension on a Millipore (Molsheim, France) filter, carefully dried in a desiccator over silica gel, and without delay, subjected to TG and XRD.

Uric acid calculi

Forty-one spontaneously passed or surgically removed calculi from 34 patients (from several patients, more
than one stone was investigated) composed of uric acid as a major component ("pure" uric acid calculi) were studied. If other minor components were present, they were not detected in the first classification performed by stereoscopic microscopy plus infrared spectroscopy. No calculi with ammonium or sodium urate or calcium oxalate as major components were included.

The stone external morphology was first observed using an optical stereomicroscope and photographically documented. Then every stone was cut by a razor into approximately two halves, usually lengthwise first. One half was retained for further inspection, while the other was cut again perpendicularly to the longer axis. Thus the inner stone structure could be observed in two mutually perpendicular planes, and this permitted its reconstruction with a high degree of fidelity. The overall texture of the cut planes was observed under a low magnifying stereomicroscope; the macroscopic structure of the stone interior was reconstructed, recorded and photographically documented. Thereafter, these fragments were metallized, their macroscopic structure was observed, and the fine structure of selected areas was studied by SEM equipped with an energy dispersive X-ray analyzer (EDS).

A foreign solid phase, when present in a studied calculus, was identified by the EDS through a semi-quantitative analysis on Ca, Mg and P. The solid substance containing only Ca was regarded as calcium oxalate. Magnesium and phosphorus were in no case detected. When neither of these elements was present, the analyzed solid was regarded as of organic nature.

Calculi composition was determined by infrared spectrometry and XRD. Fragments of calculi not used for the structure observation were powdered and immediately subjected to XRD.

**Physicochemical calculations of uric acid solubility**

Uric acid (H$_2$U) in aqueous solution dissociates according to

$$H_2U \leftrightarrow H^+ + HU^- $$

Further dissociation, and hence a concentration of U$^2-$ ion is negligible in our experimental pH range [21]. The first dissociation constant, $K_{d1}$, and the solubility product, $K_{sp}$, of uric acid are defined as

$$K_{d1} = a(H^+)a(HU^-)/a(H_2U) \quad (1)$$

$$K_{sp} = a(H^+)a(HU^-) \quad (2)$$

where $a(X)$ represents the activity of a component X. Solubility, S, is defined as the total (analytical) concentration of uric acid, i.e.,

$$S = [H_2U] + [HU^-] \quad (3)$$

where square brackets denote molar concentration. Combining expressions (1) to (3), and realizing that $pH = -\log a(H^+)$ yields

$$S = K_{sp}/K_{d1} [1 + (K_{d1}/f10^{-pH})] \quad (4)$$

where $f$ is the activity coefficient of HU$^-$ ions. Expression (4) also applies in the case of dihydrate, since the water activity, rigorously appearing in the expression for $K_{sp}$, can be regarded as equal to one because of a low solute content in urine.

The relative supersaturation was calculated as

$$\sigma = [c(H_2U)/S] - 1 \quad (5)$$

where $c(H_2U)$ represents the initial total molar concentration of uric acid, and S is the solubility of UAA or UAD in moles per liter pertinent to the actual pH reaction mixture.

The solubility of both compounds was calculated at 37°C as a function of pH and plotted in Figure 1 using $K_{sp}$ values 2.25 x 10$^{-9}$ and 9.87 x 10$^{-10}$ mol$^2$l$^{-2}$ for UAD and UAA respectively [2], and $K_{d1} = 4.2 x 10^{-6}$ mol/l [21].

**Results**

**Uric acid crystallization**

Precipitation of uric acid was induced by a sudden decrease of reaction mixture pH to 4 or 5.5, respectively, caused by an addition of HCl. The total initial concentration of uric acid in a reaction mixture varied between 170 and 1500 mg/l. The experimentally determined induction time as a function of initial supersaturation is plotted in Figure 2. In all cases, only UAD crystals either relatively thin and elongated (Fig. 3a), or agglomerates of thin plates (Fig. 3b), were detected in the reaction mixture 4 hours after the experiment onset when initial solution contained 170 (pH = 4.0) or 1500 mg/l (pH = 5.5) of uric acid, respectively. However, the solid phase separated from suspension after 8 hours of experiment duration, contained already some thick hexagonal crystals of UAA (Fig. 4a). UAD crystals were still present in suspension when 36 hours elapsed from the experiment onset, but almost disappeared during after another 12 hours, i.e., 48 hours after the suspension origin. XRD confirmed that solid phase after 48 hours of contact...
with liquid consisted only of UAA. At low supersaturation (220 mg/l of uric acid and pH = 5.5), UAA crystals are directly formed (Fig. 4b). XRD confirmed that the solid phase formed in such conditions consisted only of UAA.

The conversion of UAD to UAA in contact with dry air was completed within 4 days at 4°C and 3 hours at 40°C. The phase transformation proceeding in contact with either wet air at 20°C or a mother liquor at 37°C was finished in 2 days. Elongated prismatic UAD crystals, during transformation on both dry and wet air, partially disintegrated into numerous, irregularly bound small crystals of UAA (Fig. 3d). However, when the transition took place in contact with a mother liquor, a quantity of new thick hexagonal crystals of UAA developed (see above), though deep longitudinal cracks evolved in some prismatic crystals whose original shape was nevertheless retained (Fig. 3c).

Uric acid calculi

All studied calculi were composed of UAA, sometimes in a mixture with a small amount of UAD, as determined by XRD. However, abundant presence of crystals exhibiting large cracks, a typical sign of dihydrate crystals transformed into anhydrous compound, in some calculi points to preferential formation of UAD during a certain stage(s) of calculogenesis. Calcium oxalate monohydrate (COM) crystals forming the calculus core or disseminated in the stone mass were sometimes detected, and in one case, even a continuous COM layer in the calculus interior was observed. Exceptionally small spiky globules of ammonium urate were also observed by SEM. Calcium and magnesium phosphates were not detected in the studied stones.

Uric acid stones can be classified, according to their structure, into 3 distinctly different types:

**Type I** This type of calculus is formed by crystalline growth of uric acid particles and can comprise little or no large particles deposited from urine. According to the character of both the core and interior of the urolith, this type of calculi can be further divided into 2 subgroups:

**(Ia)** Type Ia stones were developed from a core situated either approximately in the calculus center or essentially on its surface, by a regular crystalline growth. The central core consisted of either several well developed and only loosely connected UAA crystals (Fig. 5a), or a small COM concretion (Fig. 5b). The external core was mostly represented by a small COM concretion. A compact stone interior was formed by seemingly columnar crystals radiating towards the calculus periphery (Fig. 5c). Columnar crystals were flat plate-like crystals stuck together. Laminations, concentric in the case of the central core, and oval when the core is externally situated, were often visible. The laminations, boundaries between adjacent columnar...
layers, were composed of disoriented small crystals of UAA. UAA, largely a predominant component of this sort of stones, evidently crystallized directly from urine and was not formed secondarily as a result of UAD phase transformation. UAD, transformed in various degrees to UAA, rarely formed the calculus interior. These calculi, exhibiting an outer appearance of pebbles in the case of centrally situated core, and of papillary COM stones for externally placed core (Figs. 6a and 6b), were only several millimeters in diameter. The surface was smooth but not polished and was covered by an organic matter.

(IIa) Type Ib calculi were similar to the Ia type with a centrally situated core, but exhibiting a porous interior (Fig. 5d). A stone core was represented by relatively large UAA crystals, largely unconnected, assembled without any apparent order. A porous calculus interior was composed of small crystals of UAA originating on the core. The interior was apparently structured into alternating densely and sparsely populated non-columnar layers, creating an impression of concentric laminations. Individual crystals of both UAD and UAA were observed only exceptionally. An organic matter was present in a substantially increased amount compared to the Ia type. The Ib type stones were infrequent, with incidence around 5%.

Approximately 53% of examined stones belonged to type I.

Type II Consisting of stones formed predominantly by sedimentation, Type II can be subdivided into two groups based on differences in the appearance of the calculus outer layer. Crystalline growth, in addition to sedimentation, plays an often significant role in forming this type of calculi.

(IIa) Type IIa were stones with a porous interior formed by a material of an organic origin, crystals of UAA and COM, of shape and size characteristic for particles

Figure 3. Thin elongated (a) and agglomerated (b) plate-like crystals of uric acid dihydrate crystallizing at 37°C from the artificial urine when initial solution contained 170 or 1500 mg/l of uric acid, respectively. (c) Longitudinal cracks developed in UAD crystals during phase transition to UAA. (d) UAD crystals partially disintegrated as a result of phase transition taking place on air. Bars = 50 (a), 100 (b), 20 (c) and 50 (d) µm.
appearing in urine during crystalluria, and smaller crystalline objects with a regular internal structure distributed randomly in a calculus volume (Figs. 6c, 7a and 7b). No prevailing inner structure, even over a limited area, could be distinguished. The calculus outer part constituted of a thin, rather compact, layer of a crystalline material. This layer mostly formed a smooth, lower and side surface of a calculus. The stone’s upper irregular surface, characterized by frequent occurrence of globules, was usually formed by a thicker layer of crystalline material arranged without much order. The outer layer enveloping stone and the calculus interior contained particles of comparatively large size that could only be formed by a subsequent growth of initially small crystals deposited from urine. The main constituent of the IIa type stones, UAA, was accompanied by typical COM particles disseminated in the calculus mass and by large blocks of originally UAD that were later transformed into UAA, as signified by the occurrence of the parallel longitudinal cracks (Fig. 7b). A modification of the IIa type of calculi, typified by an absence of a thin outer layer and by a substantially increased content of organic matter in comparison to regular calculi of this type, was also occasionally encountered.

(IIb) Type IIb stones were formed by a well-developed dense outermost layer predominantly composed of comparatively large blocks of what was originally UAD and later transformed into UAA, as indicated by the appearance of cracks in the crystalline mass. This layer created a type of a “shell” (Fig. 6d), with an inner space either empty or partially filled by particles, i.e., crystals and organic debris, deposited from urine during calculogenesis. Crystalline particles often obviously grew after deposition, since their actual size could not develop during a relatively short mean residence time of urine in the kidney.

The type II calculus represented roughly 39% of the studied uric acid concretions.

Type III An intermediary type between the growth and sedimentary type of calculi was also observed. One part of such a stone was obviously of sedimentary origin displaying features characteristic of type II, whereas the rest closely resembled I type. Occurrence of such a modified type was about 8% of the observed stones.

The outer surface of the majority of the observed uric acid stones was covered by largely inhomogeneous layer of an organic matter divided into isolated pieces. However, an organic matter was rarely detected in the interior of the Ia type of calculi, contrary to the Ib type and sedimentary calculi.

The color of uric acid stones varied from grayish to deep brownish orange, without any obvious connection to their inner structure, i.e., the calculus type. Moreover, coloration of the stone interior was not homogeneous, and often successive concentric layers on the cross-section exhibited different shades of orange.

Discussion

Calculated solubility of dihydrate and anhydrous uric acid, plotted in Figure 1 as a function of pH, basically corresponds to a similar plot of uric acid (not further defined) solubility in the whole urine [6]. All crystallization experiments reported herein were carried out from solutions supersaturated with respect to both UAD and UAA, as transpires from Figure 1. UAD was always the only solid phase precipitating from these systems, though supersaturation with respect to UAA was invariably higher than that to UAD. Therefore, the energy barrier for nucleation of dihydrate in a urine-like liquor had to be substantially lower compared to that for the anhydrous substance. Thus, UAD is the only solid phase formed from urine supersaturated with respect to this compound. However, when urine is supersaturated just in respect of UAA (Fig. 1, domain 1), dihydrate cannot appear and only anhydrous compound may be formed.
Uric acid calculi

The transformation of UAD to UAA under physiological conditions is fast enough, (two days for small crystals at the outside), to account for a conspicuous absence of a larger amount of dihydrate in the studied calculi. UAD, that might have been initially formed in abundance, had to transform into UAA either in vivo during calculus evolution, or subsequently, in vitro during urolith storage before analysis.

The mechanism of formation of uric acid stones can be deduced from their inner structure. The stones of type I, i.e., of the growth type, originate obviously on a “core” formed by either several large crystals of UAA, a relatively large sedimentary body consisting of numerous crystals or a small COM concretion. This core is retained in an open cavity of the kidney, which is freely accessible to fresh urine. On the core, which is in continuous contact with fresh urine supersaturated with respect to UAA, but undersaturated with respect to UAD, a compact crystalline layer, predominantly composed of UAA, starts to develop. Columnar crystals constituting this layer form the majority of the stone interior. This signifies a relatively low supersaturation prevailing during stone generation, and consequently, a comparatively slow crystal growth. However, a periodic appearance of the concentric laminations composed of small disoriented crystals implies an occurrence of transitional short episodes of suddenly increased urine supersaturation. Long periods of high supersaturation would result in a formation of abundant new UAA or UAD crystals, and that would cause a complete breakdown of the columnar structure. The stone interior, in this case consisting of small, largely disorganized and loosely arranged crystals, was observed in a few calculi. A developing urolith, which is not attached to the cavity wall, incessantly “rocks” due to constant body movement. This ensures an unrestricted access of supersaturated urine, i.e., a uniform supply of the stone building material, to the whole

Figure 5. The core of Ia type calculi consisting of (a) few well-developed and loosely connected UAA crystals, (b) small COM concretions (marked by arrows, confirmed by EDS), (c) Columnar UAA crystals forming the interior of the Ia type stones (confirmed by EDS). (d) A cross-section of calculus of the type Ib. Bars = 50 µm (a), 200 µm (b and c) and 1 mm (d).
Stones of the Ib type, with the core situated near the surface, apparently developed on a small papillary COM concretion. This concretion serving as a template for uric acid crystallization thereafter becomes surrounded by a shell composed of either UAA or UAD. UAD preferentially forms the initial urolith component if urine is supersaturated with respect to both forms of uric acid. The crystalline shell develops through regular crystalline growth and not by sedimentation, as indicated by its orderly and compact structure. UAD, even if representing the urolith initial component, undergoes a phase transition into UAA during later stages of stone development, since UAD was not detected by XRD in the studied uroliths. This transition cannot proceed by a solution-mediated mechanism, owing to a continuously renewing stone surface by its crystalline growth. Hence, the temporarily uppermost crystalline layer is not in contact with urine for a sufficiently long period, at least 24 hours, for such transition to take place. The phase transition occurs later in the bulk of solid calculus causing cracks development in the stone mass.

The sedimentary stones, i.e., the type II, exhibited a more complex structure than calculi of type I. This complexity results from a superposition of three independent processes: crystalline growth, sedimentation and solution-mediated phase transformation, proceeding successively and/or simultaneously during a part or the whole period of calculogenesis. Both subgroups of the type II calculi started first by developing crystalline deposits on the wall of an open cavity in the kidney, consisting of either UAA formed only on the cavity bottom or UAD covering the whole cavity. Particles present in urine, i.e., crystals of uric acid, calcium oxalate or organic debris, deposited under favorable conditions in the cavity. These particles obviously remained in intimate contact with urine for a considerable period, and therefore, could grow further, and in the case of UAD, could also later transform by solution-mediated process into bulky hexagonal crystals of UAA. Thus the cavity interior became filled with a mixture of large

**Figure 6.** (a) A cross-section of calculus of the Ia type. (b) Papillary uric acid calculus of the Ib type. (c) A cross-section of calculus of the Ib type. (d) A cross-section of calculus of the IIb type.
and often intergrown UAA crystals, particles of COM, organic material and UAA crystals appearing in urine during crystalluria, according to the actual conditions prevailing in the kidney. The stone interior was porous without any ordered structure due to this chaotic and random pattern of its formation. When this cavity was full of material, the compact layer over the top part of the stone developed, closed the calculus interior and practically terminated its further development. However, individual smaller globules could be subsequently formed on this layer, giving rise to a frequently encountered nodular upper surface of a stone.

Based on performed in vitro experiments combined with solubility calculation, a region of pH at which uric acid stones can be formed in the kidney from urine with varying content of uric acid can be roughly estimated. Uric acid concentration in urine varies between 170 and 500 mg per liter for the normal population [10], exceeds 550 mg per liter for hyperuricosuric patients [7] and reaches maximally 1200 mg per liter [1]. Figure 2 demonstrates that a minimum relative supersaturation for nucleating uric acid crystals in the urine-like liquor is around 1. These crystals are too small, around 10 μm, to sediment within the given time span into a cavity. Therefore, prevailing urinary supersaturation for forming sedimetary uroliths must be higher than this value. Since the actual necessary value of supersaturation is not known, let us assume that $\sigma = 1.5$ would be sufficient for nucleation of uric acid crystals and their subsequent growth to about 100 μm in size. Taking into account that UAD crystals are always formed by nucleation in the urine, a urinary pH at which required supersaturation (with respect to UAD) is achieved for a certain concentration of uric acid can be calculated from eq. (4). These values of urinary pH, at which the relative supersaturation equals 0, i.e., urine is just saturated with respect to UAD (curve b), 1 (curve c) or 1.5 (curve d), respectively are plotted as a function of uric acid concentration (Fig. 1). Three resulting curves, (b) to (d), delineate four domains, 1 to 4, with distinctly different characteristics related to uric acid stone formation. New crystals of uric acid (in fact, of UAD) cannot be formed in urine, and hence, a calculus cannot originate under any circumstances, within the region 1. In the domain 2, new crystals of uric acid originate in the kidney, but their size is insufficient for being deposited in a cavity, and therefore, they are voided as crystalluria. The domain 3 can be regarded as a transient one, since some of newly formed crystals can reach a size adequate for their retaining in the kidney, and thus, the formation of a new stone is feasible. Conditions prevailing in the last domain 4 ensure uric acid stone formation, since new crystals of uric acid formed in urine reach large enough sizes to be deposited in a cavity, forming there a sediment.

The above considerations relate mainly to the formation of sedimentary, i.e., the type II, calculi. Conditions for generating the growth, i.e., the type I, stones are different. Urine has to be just supersaturated with respect to UAA or UAD, according to the component forming the stone and no minimal level of supersaturation is requisite for their origin. The curve (a) in Figure 1 shows the UAA solubility in urine as a function of pH. In the region between the (a) and (c) curves, type I stones, composed of UAA, can develop if a suitable growth center, i.e., some large crystals of uric acid or COM concretion, were already present. The “growth type” stone, consisting of UAD, could only have formed in region 2 if an adequate template was available. The main characteristics and etiological factors of the different types of uric acid calculi according to the proposed classification are summarized in Table 1.

The above conclusions concerning the pH range in which uric acid stone can be generated represent just the first approximation of the reality due to uncertainties in the solubility calculation. However, these results are supported by an observed absence of calcium phosphate particles in uric acid calculi, since calcium phosphates can start to
precipitate from urine rich in phosphates at a pH over 6, but usually around 7 and more [9], where urine already becomes undersaturated with respect to both forms of uric acid. Besides, according to clinical experience, the uric acid uroliths form from urine of pH 5.5 and less [1], and this roughly corresponds, actually within a few tenths of a unit, to the calculated boundary of the domain 4 in Figure 1, where a risk of this calculi formation is acute.

The question why uric acid stones are not formed by all those with low urinary pH remains to be solved. As probably hyperuricosuria and certainly low urinary pH are not a prerequisite for uric acid lithiasis [1], absence of inhibitors of uric acid crystallization in urine of the respective stone-former seems to be a viable explanation. However, to our knowledge, uric acid crystallization has not been studied yet from this view-point, and therefore, the mere existence of such inhibitors is, at present, purely speculative.

The observed structure of uric acid stones suggests why treatment of already formed uric acid stones based on their dissolution by alkalization of urine is not always successful. It seems obvious that large stones with a compact inner structure, i.e., type Ia, are difficult, if not impossible, to dissolve. Stones with a porous interior, especially those with a thin or non-existent outermost layer, are prone to dissolution, and therefore, should respond easily to alkalization. According to the statistical occurrence of individual stone types and considering around 50% of the type I stone as soluble, about 30% of uric acid stones should not positively respond to the alkalization treatment. Unfortunately, we are not aware of any reported data concerning this problem, and hence, no comparison with reality can be made.

References

Uric acid calculi


Discussion with Reviewers

A. Rodgers: In type Ib stones, could the porous nature arise from organic mucoid material that has dried up?

Authors: Effectively, the majority of the pores observed in the uric acid stones, classified as Ib, can be attributed to organic mucoid that has dried with time.

A. Rodgers: In stones of type IIa, where UAA and COM occur randomly, are you able to speculate as to the initiating mechanism, i.e., does the primary event involve UAA or COM crystallization?

Authors: The morphology and arrangement of the COM particles in stones classified as type IIa seems to indicate that they have two different origins: (a) some individual COM crystals probably have been developed in urine as crystalluria and due to their size have sedimented on UAA crystals. In such cases, it is possible that colloidal uric acid acted as heterogeneous nucleant of COM crystals. (b) Some COM aggregates have developed by epitaxy on sedimented UAA crystals.

A. Hesse: Have your investigations shown that UAD requires an especially acid urine (pH < 5.5), also in comparison to UAA?

Authors: Our studies indicate that UAD formation requires high uric acid supersaturation that depends on both, urinary pH and uric acid concentration (Fig. 1). Effectively, when urinary pH decreases the UAD supersaturation increases. UAA only can develop directly in an urine supersaturated with respect to this phase but undersaturated with respect to UAD.

A. Hesse: Can you confirm that whetstone-shaped crystals in the uric acid sediment are composed of UAD?

Authors: In the uric acid sediment, we have observed the two phases: UAA and UAD. Normally, UAA crystals appear as hexagonal or whetstone-shaped (lenticular) crystals. UAD crystals (normally observed in low pH urines) appear as irregular or rectangular shaped crystals.

A. Hesse: Are you in possession of any studies which confirm that alkalization renders compact uric acid stones (type: growth/Transformation) resistant to chemolitholysis?

Authors: At this moment, we are studying this problem, and unfortunately, we still can not offer definitive...
conclusions. Nevertheless, it seems a commonly accepted fact that an important percentage of uric acid stones are really resistant to chemolitholysis by systematic alkalinization. Now it is necessary to establish the causes of this.

**E. Prien:** I do not know why the authors hypothesize that some calculi of type I or Ia might be difficult to dissolve with alkali alone, as they are hypothetically precipitated from patient urine with the least degree of supersaturation (region 1 in Fig. 1). Also they admit there is no data on this problem.

**Authors:** Precisely, the development of the calculi of type Ia is consequence of the formation of columnar UAA crystals, only possible in patient urine with the least degree of supersaturation, generating very compact structures without any pore. Due to their compactness, the alkaline urine can not penetrate into the calculus, making its dissolution very difficult.