Urol Res (1999) 27:297–305

INVITED EDITORIAL

Richard A. Ashby · John P. Byrne · Ákos Z. György

Urine is a saturated equilibrium and not a metastable supersaturated solution: evidence from crystalluria and the general composition of calcium salt and uric acid calculi

Received: 18 November 1998 / Accepted: 25 March 1999

Abstract A computer algorithm is described which allows urine to be modelled as a saturated equilibrium solution with respect to any combination of the solids calcium oxalate, calcium hydrogen phosphate (brushite), amorphous calcium phosphate, uric acid, sodium hydrogen urate and ammonium hydrogen urate. It is demonstrated that this model of urine, unlike the widely accepted metastable supersaturated solution model, explains the long-known calcium salt crystalluria versus pH curves of both non-stone-forming and stone-forming urine. Further, the saturation model accounts for why most "infection" stones do not contain calcium oxalate and why most "urate" stones are composed solely of uric acid and not admixed with alkali metal hydrogen urate salts. The supersaturation model of urine cannot explain satisfactorily these well-known phenomena. For example, the supersaturation model predicts that virtually *all* "infection" stones should contain calcium oxalate along with calcium phosphate and, perhaps, struvite.

Key words Urolithiasis · Crystalluria · Calcium salts · Uric acid · Urates · pH · Urine equilibrium

Introduction

A novel urinary risk index for calcium salt urolithiasis has been described recently [2, 20, 21] prompted by the widespread observation of crystals in the urines of many stone-formers and especially of non-stone-forming normals [1, 4, 6, 7, 14, 16–18, 23–26, 29, 36–40, 42]. The risk index was the moles per litre of calcium solids – calcium oxalate (CaOxS) plus calcium hydrogen phosphate (brushite) – divided by the activity of the free citrate^{3-} ion (AC3). Urine must be modelled as a saturated solution in order to estimate the calcium solids content for use in this index. The index has been satisfyingly successful in separating stone-formers from non-stone-formers (≥95% [21]) and in devising effective calcium stone prevention strategies [2, 20, 21]. Over a 5-year treatment period, the average stone formation rate fell from 4.2 stones per 3 years per patient to just two reported episodes in now 155 patients [21]. This success rate, based on the saturation model, made it logical to expand the model to include uric acid, its alkali metal salts and amorphous calcium phosphate in order to explain better the observed qualitative and quantitative aspects of crystalluria [23–25, 36] in both normals and stone-formers, and formed stones in the latter [13].

An algorithm will be described which allows the computation of the equilibrium condition in urine where the soluble ion equilibria are satisfied simultaneously with the solubility equilibria (a SATURATION model) pertaining to any combination of the solids CaOxS, brushite, amorphous calcium phosphate (ACP), uric acid (HUS), sodium (NaUS) and ammonium hydrogen urate (NH_4US). The resultant computer program is a modification of SEQUIL [2].

The saturation model explains why:

(i) CaOxS crystals disappear generally from non-stone-forming and stone-forming urine above a pH of about 6.5: the supersaturation model predicts their existence up to a pH of 7.5 and above;
(ii) most "infection" stones, struvite/apatite mixes which result from urea-splitting organisms resulting in high ammonia and pH urines, do not contain CaOxS: the supersaturation model predicts all such stones should contain CaOxS;
(iii) NaUS crystals occur in slightly acidic to alkaline urine but do not occur in the same urine when the pH is near the low end of normal where only HUS crystals occur: the supersaturation model predicts

R.A. Ashby (✉) · A.Z. György
Department of Medicine, The Royal North Shore Hospital, St. Leonards, 2065, Australia

R.A. Ashby · J.P. Byrne
Department of Chemistry, Materials and Forensic Science, University of Technology, Sydney, Australia

NaUS crystals to be present at all pHs in hyperuricosuria;

(iv) NH_4US crystals occur in slightly acidic to alkaline urine without accompanying NaUS crystals when urinary [NH_4^+] is elevated but [Na^+] is normal: the supersaturation model predicts NH_4US crystals to be present at all pH levels accompanied by NaUS crystals in hyperuricosuria;

(v) most ''urate'' calculi are comprised of uric acid, without an admixture of alkali metal urate salts, but sometimes with CaOxS: the supersaturation model predicts a more prevalent admixture of NaUS and/or NH_4US crystals in ''uric acid'' stones;

(vi) calcium urate, a fairly insoluble compound, is not a prevalent component of crystalluria and calculi: the supersaturation model predicts its presence at all pH values above 5.5 in hyperuricosuria.

The calcium phosphates in urine

Before describing the algorithm, the problem of the precipitation of apatite needs consideration. Hydroxyapatite does not precipitate initially from urine, it forms gradually via other calcium phosphate salts [32]. The solubility product for hydroxyapatite of 2.35×10^{-59} used normally in calculating urinary relative saturation ratios (RSR) [9, 43] was obtained from a highly crystalline solid form of hydroxyapatite which had to be synthesized under very stringent chemical conditions [30] which certainly do not exist in urine. Indeed, if this value were the operational solubility product for apatite in urine, then urine should display apatitic crystalluria (which it does not) between pH 5 to pH 6 because calculated RSRs rise from near 5 at pH 5 to 10 000 at pH 5.5 to 10^7 at pH 6 (see Table 4). Apatitic calcium phosphate (ACP) precipitates directly and spontaneously from synthetic solutions in two highly hydrated amorphous forms: ACP1 with a solubilty product of 2.3×10^{-11} and ACP2 with a solubility product of 3.3×10^{-12} [12]. These appear to be the forms of calcium phosphate which are commonly observed to precipitate spontaneously when urine is made alkaline, and easily redissolved when it is acidified, around pHs of 6.5 to 7.

ACP2 was chosen for incorporation in our model because its time frame of existence [12] (30 s to 1 h) seems to be reasonable for the time it takes urine to reach the bladder from the glomerulus prior to excretion. This form of calcium phosphate is in addition to brushite used in our previous model [2], and which of the two precipitates is essentially determined by the pH, as will be discussed subsequently.

The algorithm

First, the soluble ion equilibria are satisfied in the well-established iterative manner of EQUIL2 [43], EQUIL93 [9] and SEQUIL [2]. The total concentrations (M) of Ca, Mg, NH_3, Na, K, P, S, citrate, urate, oxalate and pH are required as input data.

The results of this computation can be considered as providing the first estimates of the concentrations of the soluble species in equilibrium with the solids. Necessarily, these estimates will be overestimates and, therefore, the first estimates of the solids content must be underestimates compared with their true equilibrium values.

The calcium solids content

The urine is now tested as to its saturation with respect to brushite and ACP2. If it is saturated in both, then a decision has to be made as to which solid should precipitate. Co-precipitation of these solids requires the following equilibria be satisfied simultaneously.

For brushite:

$$CaHPO_{4(s)} \Longleftrightarrow Ca^{2+}_{(aq)} + HPO^{2-}_{4(aq)}$$

$$K_{SPB} = [Ca^{2+}][HPO_4^{2-}]\gamma_{2\pm}^2$$

For ACP2:

$$Ca(PO_4)_{0.74}H_{0.22} \Longleftrightarrow Ca^{2+} + 0.74PO_4^{3-} + 0.22H^+$$

$$K_{SPA} = [Ca^{2+}][PO_4^{3-}]^{0.74}H^{0.22}\ \gamma_{2\pm}\gamma_{3\pm}^{0.74}$$

where K_{SPB} and K_{SPA} are, respectively, the solubility products of brushite and ACP2; the terms in the square brackets are, as usual, the molar concentrations of the appropriate ions in the urine; and the $\gamma_{n\pm}$ are the charge ($n\pm$) dependent activity coefficients.

Thus,

$$\frac{[Ca^{2+}][HPO_4^{2-}]\gamma_{2\pm}^2}{[Ca^{2+}][PO_4^{3-}]^{0.74}H^{0.22}\gamma_{2\pm}\gamma_{3\pm}^{0.74}} = \frac{K_{SPB}}{K_{SPA}}$$

for the co-precipitation of the solids. If [PO_4^{3-}] is replaced by [HPO_4^{2-}] using the latter's acid dissociation constant (K_a),
where

$$K_a = \frac{[H^+][PO_4^{3-}]\gamma_{3\pm}}{[HPO_4^{2-}]\gamma_{2\pm}}$$

then the preceding equation reduces to $H^{0.52}HPO_4^{0.26}\gamma_2^{0.26}/K_a^{0.74} = K_{SPB}/K_{SPA}$ Therefore, if the concentrations of ions in solution (urine) are such that the left-hand side (LHS) of this equation is greater than the right-hand side (RHS), which is a constant, then brushite will precipitate in favour ACP2 and vice versa if LHS < RHS. If the latter condition prevails then small quantities of Ca^{2+} and P in the mole ratio of 1/0.74 are removed successively, and called ACP2, until the solubility product relationship for ACP2 is achieved. If the former condition prevails, then the urine is tested as to its saturation with respect to CaOxS and brushite, that is, whether conditions 1 and 2 are satisfied simultaneously:

$$\text{Condition 1} \quad [Ca^{2+}][Ox^{2-}]\gamma_{2\pm}^2 > K_{SPO} \tag{1}$$

$$\text{Condition 2} \quad [Ca^{2+}][HPO_4^{2-}]\gamma_{2\pm}^2 > K_{SPB} \tag{2}$$

K_{SPO} is the solubility product of CaOxS.

If both conditions 1 and 2 are satisfied then the total calcium solids content T (CaOxS and brushite) is calculated from Eq. (3):

$$\begin{aligned} T^2 &- ([Ca^{2+}] + [Ox^{2-}] + [HPO_4^{2-}])T \\ &+ ([Ca^{2+}][Ox^{2-}] + [Ca^{2+}][HPO_4^{2-}]) \\ &- (K_{SPO} + K_{SPB})/\gamma_{2\pm}^2 = 0 \end{aligned} \tag{3}$$

The separate quantities of CaOxS and brushite are then calculated from Eqs. (4) and (5):

$$([Ca^{2+}] - T)([Ox^{2-}] - CaOxS)\gamma_{2\pm}^2 = K_{SPO} \tag{4}$$

$$([Ca^{2+}] - T)([HPO_4^{2-}] - \text{brushite})\gamma_{2\pm}^2 = K_{SPB} \tag{5}$$

Note that Eq. (3), the addition of Eqs. (4) and (5), is quadratic in T and the smaller of the two roots is the required value of T.

If only condition 1 is satisfied, then CaOxS is calculated from Eq. (6), and brushite is estimated from Eq. (7) if only condition 2 is satisfied:

$$([Ca^{2+}] - CaOxS)([Ox^{2-}] - CaOxS)\gamma_{2\pm}^2 = K_{SPO} \tag{6}$$

$$([Ca^{2+}] - \text{brushite})([HPO_4^{2-}] - \text{brushite})\gamma_{2\pm}^2 = K_{SPB} \tag{7}$$

Again, note that Eqs. (6) and (7) are quadratic in the appropriate solid and the smaller of the roots, in each case, is the required quantity of solid. Equation (6) is used to estimate CaOxS if ACP2 is the precipitable phosphate.

Uric acid and urate salt solid content

There are three important points to be considered before this part of the algorithm is described.

The first consideration relates to the soluble ion pairs involving the hydrogen urate ion (U^-) and the solubility products of NaUS, KUS and NH_4US. The stability constants for the ion pairs involving the U^- ion with the cations Ca^{2+}, Mg^{2+}, Na^+, K^+ and NH_4^+, respectively, were all set to zero. Babic-Ivancic et al. [5] suggested a near-zero value for the stability constant of $CaU^-_{(aq)}$ and, therefore, zero could be assumed for the stability constant of $MgU^-_{(aq)}$ because Ca^{2+} and Mg^{2+} ions generally form complexes, pertinent to urinary chemistry, of similar stabilities [3]. Finlayson and Smith [19] saw no evidence for the existence of $NaU_{(aq)}$ in solution so, in all likelihood, the same can be assumed for $KU_{(aq)}$ and $NH_4U_{(aq)}$. However, if any of these ion pairs were found to be of significance, which is unlikely, then the program can handle them in the initial soluble ion equilibrium routine.

The 37°C values used for the solubility of uric acid ($K_{sol} = 2.72 \times 10^{-4}$), the dissociation constant of uric acid ($K_a = 3.63 \times 10^{-6}$) and the solubility product of NaUS ($K_{SPNaU} = 5.23 \times 10^{-5}$) were those reported by Brown et al. [10]. Their K_{SPNaU} value is similar to the value (4.65×10^{-5}) calculated from the 37°C solubility of NaUS reported by Delatte [14], after making corrections for activity effects, but differs from the value (2.79×10^{-5}) reported by Pak et al. [31]. The values used for the solubility products of KUS ($K_{SPKUS} = 1.16 \times 10^{-4}$) and NH_4US ($K_{SPNH_4US} = 7.57 \times 10^{-6}$) were also calculated from Dellatte's [14] reported solubilities for the appropriate solids, and not those reported by Pak et al. [31], because of the internal inconsistency displayed between the solubility products and claimed solubilities of the compounds NaUS, KUS and NH_4US by these latter workers.

The second consideration relates to the co-precipitation of HUS with NaUS (or NH_4US). There is a single, unique, $[Na^+]$ (or $[NH_4^+]$) at which HUS and NaUS (NH_4US) co-precipitate once the pH has been fixed. If the $[Na^+]$ (or $[NH_4^+]$) is less than this value then only HUS can precipitate; if it exceeds this value then only NaUS (or NH_4US) will precipitate. This follows from the equilibrium considerations, appropriate to the case of NaUS/HUS,

$$HUS_{(s)} \Longleftrightarrow HU_{(aq)} \quad K_{sol} = [HU] \tag{8}$$

$$HU_{(aq)} \Longleftrightarrow H^+_{(aq)} + U^-_{(aq)} \quad K_a = \frac{[H^+][U^-]}{[HU]}\gamma_{1\pm} \tag{9}$$

$$NaUS_{(s)} \Longleftrightarrow Na^+_{(aq)} + U^-_{(aq)} \quad K_{SPNaU} = [Na^+][U^-]\gamma_{1\pm}^2 \tag{10}$$

For the three equilibria to be satisfied simultaneously (i.e. co-precipitation of HUS and NaUS), then

$$[Na^+] = (K_{SPNaU}[H^+])/(K_i\gamma_{1\pm}) \tag{11}$$

where [HU] is the concentration of undissociated uric acid and K_i is the product $K_{SOL}K_a$. So, if the pH is fixed and $[Na^+]$ is greater than $([H^+]K_{SPNaU})/(K_i\gamma_{1\pm})$ (Eq. 11), then NaUS will precipitate until its solubility product is no longer exceeded. This process will remove U^- from solution, which, in turn, will reduce [HU] and take HUS into solution to satisfy the equilibria (8–10). The net result is to leave the solution undersaturated in HUS, that is, there will be a precipitate of NaUS only. Of course, the reverse of the foregoing processes will occur if $[Na^+]$ is less than $([H^+]K_{SPNaU})/(K_i\gamma_{1\pm})$ with the result that only HUS will precipitate. Similar considerations apply to the case of the co-precipitation of NH_4US with HUS, and indeed, KUS with HUS and calcium hydrogen urate (CaU_2S) with HUS. However, because of the relatively high solubilities of KUS [14] and CaU_2S [5], and competing equilibria, their solubility products will not be exceeded in any but an extremely exceptional urine. The program, therefore, computes only values for the RSRs for these compounds and not the quantities of solid, which of course are most commonly zero.

The third consideration relates to the possible co-precipitation of NaUS and NH_4US. If the $[Na^+]$/

$[NH_4^+]$ ratio is greater than the ratio KSPNaU/$KSPNH_4U$, then only NaUS can precipitate even though the urine sample may appear to be saturated with respect to both solids. The reverse process will occur (i.e. precipitation of NH_4US only) if the $[NH_4^+]/[Na^+]$ ratio is greater than the ratio $KSPNH_4U$/KSPNaU. Co-precipitation of NaUS and NH_4US can only occur if the urine is saturated with respect to both solids *and* the ratio $[Na^+]/[NH_4^+]$ is less than the ratio KSPNa/$KSPNH_4U$.

That part of the algorithm for the computation of solid uric acid and hydrogen urate salts will be described now.

The urine is tested according to Eq. (11) to ascertain whether HUS or NaUS (NH_4US) should precipitate.

If only HUS should precipitate then the quantity of HUS is calculated from

$$HUS = [HU] - K_{SOL} \tag{12}$$

where [HU] is the concentration of HU in solution after the soluble ion equilibria have been satisfied.

If only NaUS and/or NH_4US should precipitate then the solution is tested via the $[Na^+]/[NH_4^+]$ ratio and appropriate K_{SP} ratios to determine whether only one or other, or both, of the salts should precipitate.

If both should precipitate then TUS (TUS = NaUS + NH_4US) is calculated from Eq. (13):

$$\begin{aligned} TUS^2 &- ([Na^+] + [NH_4^+] + [U^-])TUS \\ &+ ([U^-][Na^+] + [U^-][NH_4^+]) \\ &- (KSPNaU + KSPNH_4U)/\gamma_{1\pm}^2 = 0 \end{aligned} \tag{13}$$

Equation (13) is the sum of Eqs. (14) and (15):

$$([Na^+] - NaUS)([U^-] - TUS)\gamma_{1\pm}^2 = KSPNaU \tag{14}$$

$$([NH_4^+] - NH_4US)([U^-] - TUS)\gamma_{1\pm}^2 = KSPNH_4U \tag{15}$$

and the smaller of the two roots is the required value of TUS. NaUS and NH_4US are calculated from Eqs. (14) and (15) once TUS is known. If the urine is saturated with respect to only one or other of NaUS and NH_4US then the appropriate quantity of salt is computed from either Eq. (16) or (17):

$$([Na^+] - NaUS)([U^-] - NaUS)\gamma_{1\pm}^2 = KSPNaU \tag{16}$$

$$([NH_4^+] - NH_4US)([U^-] - NH_4US)\gamma_{1\pm}^2 = KSPNH_4U \tag{17}$$

At this stage, the first iteration through the calculation of the solids content is complete.

Computation of equilibrium

The second iteration starts by subtracting the quantities (mole/litre) of CaOxS, brushite, ACP, HUS, NaUS and NH_4US, determined in the first iteration, from the appropriate starting total concentrations of Ca, Ox, P, Na, urate and NH_4^+. The soluble ion equilibria are again satisfied but with the now reduced input total concentrations of Ca, Ox, P, Na, urate and NH_4. The solids content is again evaluated, as in the first iteration, and the quantities of solid, so determined, added to those previously found.

The process is repeated and, as the number of iterations increases, the concentrations of the soluble species fall and the total quantities of solids increase, but more slowly the greater the iteration number. This leads to a natural convergence of the program to the condition of saturated equilibrium.

Equilibrium is deemed to have occurred when the calculated quantity of *each* solid in the current iteration is less than 0.01% of the total accumulated for *each* solid in all previous iterations, and of course, appropriate solubility products are not exceeded.

Test of attainment of equilibrium

True equilibrium occurs when the activity of a solid in solution equals its solubility product and, for a 1:1 solid, the [ion pair] corresponding to the solid is a constant given by

$$[\text{ion pair}] = K_{STAB} \cdot K_{SP} \tag{18}$$

where K_{STAB} and K_{SP} are, respectively, the stability constant of the ion pair and the solubility product. Both these conditions can be expressed in terms of the RSR of the solution with respect to the solid where

$$RSR = [\text{ion pair}]/(K_{STAB} \cdot K_{SP}) \tag{19}$$

or

$$RSR = [X^{n+}][Y^{n-}]\gamma_{n\pm}^2/K_{SP} \tag{20}$$

Naturally, RSR = 1 exactly by either formula for a solution saturated with respect to a particular solid.

On exit from the program, RSRs in solution are calculated from Eqs. (19) and (20) to check how close the true equilibrium condition had been computed.

Discussion

Performance of the program

Tables 1–3 demonstrate the performance of the program in determining the solid content of urine. For each table, a particular input urinary chemistry was taken and the pH varied between 5 and 7.5 in steps of 0.5 pH units to show how the program responded to the precipitation of a variety of solids.

Notice that, in each table, the computed RSRs on exit from the program generally never differ from 1 by more than $\pm 1\%$ when the urine is saturated with respect to a particular solid (a non-zero amount of solid is calculated). This occurs even when the urine is saturated simultaneously with respect to more than one solid species. Naturally, the computed RSR on exit is always

less than one when the urine is not saturated with respect to a particular solid species.

It can be concluded, therefore, that the program does, indeed, compute a good approximation to the saturated equilibrium condition.

Relationship to stone formation and crystalluria

Normal urine

Table 1 is for the urine of non-stone-forming males [35] with a normal total urate. At pH values below about 5.8, both CaOxS and HUS precipitate; between 5.8 and 6.0 CaOxS can precipitate with a small quantity of NaUS; at about pH 6.0 and above brushite precipitates with CaOxS but above pH 6.5 ACP 2 precipitates in preference to brushite and the urine becomes undersaturated in CaOxS. Not until pH > 7.2 is the RSR of struvite exceeded and at no pH does NH_4US precipitate. As might be expected, the pH at which the urine becomes undersaturated with respect to CaOxS (because of the precipitation of ACP2) rises as the total oxalate concentration, T_{ox}, increases. For this urinary chemistry, and that of Table 2, when T_{ox} = 0.149 mM, the pH = 6.7. If T_{ox} is increased to 0.3 mM then the pH rises to 7.1 and to 7.3 when T_{ox} = 0.4 mM.

Even though CaOxS does precipitate (crystalluria), CaOxS stone formation (aggregation of crystals [2]) is unlikely to occur from the urines of Tables 1, 2 and 3 provided the pH is not kept at 5.2 or lower, because the calcium stone salt risk index falls within the non-stone-forming region as discussed in Ashby and Györy [2].

Table 1 Solid content of a non-stone-forming urine: saturation model (Input total (mM): NH_4 = 17, Ca = 4.34, Mg = 3.18, Na = 118, K = 42, P = 20.5, S = 15.8, citrate = 2.3, urate = 1.5, oxalate = 0.149, RSRs ≥ 0.99 (1–1%) are shown in **bold**)

	pH					
	5.0	5.5	6.0	6.5	7.0	7.5
CaOxS (mmol/l)	0.114	0.113	0.089	0.040	0.000	0.000
RSR CaOxS	**1.001**	**1.001**	**1.000**	**1.000**	0.567	0.275
Brushite (mmol/l)	0.000	0.000	1.793	2.908	0.000	0.000
RSR brushite	0.283	0.786	**1.000**	**1.000**	0.703	0.403
ACP2 (mmol/l)	0.000	0.000	0.000	0.000	3.711	4.018
RSR ACP2	0.086	0.328	0.602	0.913	**0.995**	**0.990**
HUS (mmol/l)	1.094	0.804	0.000	0.000	0.000	0.000
RSR HUS	**0.999**	**1.000**	0.632	0.202	0.006	0.002
NaUS (mmol/l)	0.000	0.000	0.478	0.601	0.611	0.613
RSR NaUS	0.160	0.505	**1.000**	**1.000**	**0.997**	**0.997**
NH_4US (mmol/l)	0.000	0.000	0.000	0.000	0.000	0.000
RSR NH_4US	0.152	0.478	0.946	0.942	0.932	0.931
RSR CaU_2S	0.012	0.115	0.238	0.121	0.047	0.023
RSR struvite	0.000	0.003	0.019	0.099	0.461	**1.624**

Table 2 Solid content of a urine with elevated urate concentration: saturation model (Input total (mM): the same as those used in Table 1 except urate = 4.0)

	pH					
	5.0	5.5	6.0	6.5	7.0	7.5
CaOxS (mmol/l)	0.114	0.114	0.090	0.040	0.000	0.000
RSR CaOxS	**1.001**	**1.000**	**1.000**	**1.000**	0.525	0.276
Brushite (mmol/l)	0.000	0.000	1.799	2.913	0.000	0.000
RSR brushite	0.284	0.786	**1.000**	**1.000**	0.731	0.407
ACP2 (mmol/l)	0.000	0.000	0.000	0.000	3.741	4.018
RSR ACP2	0.086	0.328	0.601	0.911	**1.000**	**0.995**
HUS (mmol/l)	3.596	3.304	0.000	0.000	0.000	0.000
RSR HUS	**1.000**	**1.001**	0.646	0.207	0.006	0.002
NaUS (mmol/l)	0.000	0.000	2.959	3.069	3.093	3.099
RSR NaUS	0.160	0.506	**1.000**	**1.000**	**1.000**	**1.000**
NH_4US (mmol/l)	0.000	0.000	0.000	0.000	0.000	0.000
RSR NH_4US	0.152	0.478	0.965	0.961	0.944	0.952
RSR CaU_2S	0.001	0.115	0.246	0.125	0.045	0.023
RSR struvite	0.000	0.003	0.019	0.100	0.496	**1.633**

Table 3 Solid content of urine of infected-stone-formers: saturation model (Input total (mM): NH_4 = 30, Ca = 3.68, Mg = 1.79, Na = 95, K = 28, P = 14.7, S = 13.5, citrate = 2.02, urate = 3.0, oxalate = 0.146)

	pH					
	5.0	5.5	6.0	6.5	7.0	7.5
CaOxS (mmol/l)	0.112	0.112	0.104	0.070	0.000	0.000
RSR CaOxS	**1.001**	**1.000**	**0.999**	**1.000**	0.672	0.354
Brushite (mmol/l)	0.000	0.000	0.669	1.929	0.000	0.000
RSR brushite	0.190	0.524	**0.999**	**1.000**	0.864	0.381
ACP2 (mmol/l)	0.000	0.000	0.000	0.000	3.006	3.315
RSR ACP2	0.063	0.236	0.640	0.973	**0.992**	**0.990**
HUS (mmol/l)	2.596	2.309	0.000	0.000	0.000	0.000
RSR HUS	**1.000**	**1.001**	0.402	0.129	0.041	0.013
NaUS (mmol/l)	0.000	0.000	0.000	0.000	0.000	0.000
RSR NaUS	0.139	0.413	0.523	0.526	0.523	0.527
NH_4US (mmol/l)	0.000	0.000	2.358	2.425	2.439	2.442
RSR NH_4US	0.273	0.861	**1.000**	**1.000**	**0.999**	**0.992**
RSR CaU_2S	0.011	0.103	0.122	0.063	0.026	0.011
RSR struvite	0.000	0.002	0.015	0.078	0.391	**1.277**

Uric acid

Elevation of the total urate to 4 mM (hyperuricosuria), in otherwise the same urine, shows the same precipitates at similar pHs (compare Tables 1 and 2). Again, notice that the urine does not co-precipitate HUS and NaUS at any pH shown. In fact, the only pH at which the two solids can precipitate simultaneously is calculated to be 5.8 [cf. Eq. (11)]. If the pH is varied by only ±0.01 pH units away from 5.8 then only HUS (below) or NaUS (above) will precipitate. NH_4US never precipitates. It may be of interest to note here that, as might be expected, the pH at the cross-over between HUS and NaUS precipitation drops as the $[Na^+]$ increases. It is

pH = 5.55 for $[Na^+]$ = 200 mM rising to pH = 6.15 for $[Na^+]$ = 50 mM, assuming $\gamma_{1\pm} = 0.75$, which is typical for urine. It is independent of total urate.

Most "urate" stones contain only uric acid (no NaUS nor NH_4US) [5, 13] and it has been observed that uric acid stone formation is associated more often with urine whose pH is maintained towards the low end of the normal range (<5.5) rather than an elevated total urate content [27]. The urines of Tables 1, 2 and 3 will precipitate uric acid with no NaUS nor NH_4US crystals if the pH stays below about 5.8, and this would seem to be the explanation for the clinical observations related to uric acid stone formation [15, 27, 33]. In other words, a persistently acidic urine should lead to persistent uric acid crystalluria (no NaUS nor NH_4US crystals), probably more pronounced the lower the pH, which in turn may lead to uric acid stone formation. Mixed CaOxS/HUS stones would seem to result from a urine which stays at the acidic end of the normal range *and* which possesses a depressed AC3. Indeed, calculations using SEQUIL [2] with the average urinary chemistry of gouty diathesis patients [27] who had formed CaOx stones, with or without uric acid admixture, showed that the urines were deficient in AC3 and fell in the CaOx stone formation region as discussed previously [2, 20, 21]. Non-stone-forming urine may generate uric acid crystalluria if it becomes too acidic, but normal diurnal variations in pH should result in the dissolution of any uric acid crystals that may have lodged in the urinary tract, which is in accord with the well-known fact that, indeed, formed uric acid stones can be dissolved by alkalinizing urine [22].

The urine of Table 2 has RSRs for NaUS and NH_4US ranging from 1.6 to 4.6 and 1.5 to 4.3, respectively, as the pH changes from 5 to 8, when modelled as a supersaturated solution (see Table 4). The RSR of HUS ranges from 9.8 at pH 5 to 2.5 at pH 6. The supersaturated model of urine, therefore, would seem to predict mixed stones (HUS + NaUS + NH_4US) from urine whose pH is maintained at the low end of the normal range and which has an elevated total urate, although such mixed stones are not known and stones containing NaUS are a rarity [13, 14].

Infection stones

The urinary chemistry of Table 3 comes from "infection stone" -formers [35] with total urate set to 3 mM. The change in the nature of the precipitates with pH is similar to the two previous urines, except that now NH_4US, rather than NaUS, precipitates when the pH reaches about 5.8. Whether NaUS or NH_4US precipitates is governed by the conditions described previously, but it is probably of interest here to note that NH_4US will precipitate only if $[NH_4^+]/[Na^+] > 0.145$, independent of activity effects, meaning that for $[NH_4^+]$ = 10 mM, $[Na^+]$ must be less than 69 mM (unlikely), for $[NH_4^+]$ = 30 mM, $[Na^+] < 207$ mM (probable) and for $[NH_4^+]$ = 40 mM, $[Na^+] < 276$ (highly probable) – otherwise, NaUS precipitation is favoured. Again, the cross-over pH for precipitation of NH_4US rather than HUS decreases as $[NH_4^+]$ increases, being pH 6.0 if $[NH_4^+]$ = 10 mM dropping to pH 5.4 if $[NH_4^+]$ = 40 mM, assuming $\gamma_{1\pm} = 0.75$, and it is independent of total urate.

At urinary pHs > 7 (see Tables 1–3), where "infection stones" form, the saturation model of urine accounts for the occurrence of calcium phosphate stones devoid of CaOxS, but with struvite, provided the pH is high enough (near 7.3 or above for struvite) and total oxalate is not excessively high (<0.4 mM for pH 7.3). Many, if not most, "infection" stones do not contain CaOxS [11, 13 and our own experience].

The urines of Tables 2 and 3, when modelled as supersaturated solutions (Tables 4, 5), on the other hand, are supersaturated in CaOxS at all pHs with RSRs near 4.5 at pH 5, dropping to near 3.6 at pH 8. Thus, the supersaturation model of urine would seem to predict that *all* infection stones should contain CaOxS, which is contrary to general observations [13]. Indeed, it is also difficult to account for the existence of the pure calcium phosphate stones which are characteristic of high pH urine in renal tubular acidosis [25] by the supersaturation model, but such stones follow naturally from the saturation model.

Table 4 RSRs of various urinary solids for urine with elevated total urate: supersaturation model (Input total (mM): the same as used in Table 2)

RSR	pH					
	5.0	5.5	6.0	6.5	7.0	7.5
CaOxS	**4.390**	**4.290**	**4.160**	**3.970**	**3.970**	**3.620**
Brushite	0.290	0.803	**1.979**	**3.794**	**5.355**	**6.010**
ACP2	0.090	0.340	**1.200**	**3.350**	**7.690**	**15.000**
Apatite	**4.540**	**8.0e+3**	**9.0e+6**	**6.0e+9**	**2.0e+12**	**2.0e + 14**
HUS	**9.800**	**5.730**	**2.470**	0.880	0.290	0.090
NaUS	**1.580**	**2.900**	**3.930**	**4.380**	**4.520**	**4.550**
NH_4US	**1.490**	**2.740**	**3.700**	**4.110**	**4.220**	**4.230**
CaU_2S	**1.230**	**3.870**	**6.610**	**7.700**	**7.750**	**7.430**
Struvite	0.000	0.000	0.021	0.120	0.520	**1.840**

Table 5 RSRs of various urinary solids for infected-stone-forming urine: supersaturation model: (Input total (mM): the same as used in Table 3)

RSR	pH					
	5.0	5.5	6.0	6.5	7.0	7.5
CaOxS	**4.490**	**4.370**	**4.210**	**4.000**	**3.800**	**3.630**
Brushite	0.200	0.540	**1.340**	**2.660**	**3.873**	**4.430**
ACP2	0.060	0.024	0.900	**2.540**	**5.900**	**11.700**
Apatite	**1.200**	**2.0e+3**	**3.0e+6**	**2.0e+9**	**5.0e+11**	**6.0e+13**
HUS	**7.410**	**4.330**	**1.870**	0.670	0.220	0.070
NaUS	0.970	**1.790**	**2.440**	**2.730**	**2.820**	**2.840**
NH_4US	**2.030**	**3.740**	**5.070**	**5.650**	**5.810**	**5.840**
CaU_2S	0.640	**2.000**	**3.470**	**4.670**	**4.070**	**3.910**
Struvite	0.000	0.000	0.020	0.101	0.460	**1.650**

Saturation versus supersaturation

Blomen et al. [8] have demonstrated that CaOxS "seed" crystals begin to grow, albeit slowly, as soon as the RSRs of test solutions of CaOx exceed *one* and calcium phosphate crystals are known to be "seeds" for CaOxS precipitation [25, 28, 41]. Why, then, does not calcium phosphate, which precipitates obviously from neutral and mildly alkalized urine [14], always "seed" the formation of CaOxS from a supersaturated urine at pH > 6.5 (Table 4)? Why do not "infection" stones always contain CaOxS? How do pure calcium phosphate stones form? Why, then, does not uric acid "seed" the formation of NaUS and NH_4US in acidic urine in which total urate is elevated? Why do not more "urate" stones contain urate salts, including CaU_2S (see Tables 4, 5), with the ubiquitous uric acid? The observations that most "infection stones" do not contain CaOxS, that pure calcium phosphate stones do exist and that most "urate" stones contain only uric acid (no urate salts) is strong evidence in favour of the saturation model of urine, particularly in the way precipitation of the various solids occurs.

Further support for a saturation, rather than supersaturation, model of urine comes from its ability to explain the well-known, and oft-referred to, calcium salt crystalluria/pH curves of Robertson et al. [14, 36, 40]. The supersaturation model predicts CaOx crystalluria at all urinary pHs from 5 to 8 because the RSRs of normal urine with respect to CaOx fall by only about 20% over this pH range. On the other hand, the saturation model shows that a normal urine becomes undersaturated with respect to CaOx around pH 7 and, therefore, cannot display oxalate crystalluria above this pH. Given that Robertson et al.'s [36] and Hallson and Rose's [25] crystalluria curves show that oxalate crystalluria disappears at pHs over about 6.5, then the saturation model just described appears not to match experimental observations closely enough, although it is decidedly better than the supersaturation model. The reason for the lack of a closer fit between model and observation may simply result from the fact that above a pH about 6.5 the calculated mole ratio of phosphate to oxalate solid is approximately 70:1 or greater, which means that oxalate particles could easily have been overlooked in the microscopic examination of urines [25, 36]. Indeed, Hallson and Rose [25] have shown, by chemical analysis as distinct from microscopic examination, very small quantities of oxalate in predominantly phosphatic crystalluria in some urines up to around pH values of 7. Nevertheless, the saturation model is the answer to the quandary expressed in Delatte's [14] statement: "There is still no satisfactory explanation for the scarcity of calcium oxalate crystals in neutral or alkaline urine [according to the supersaturation model]." The saturation model also readily explains the conditions for the occurrence of gross NaUS crystalluria (elevated pH and total urate [44]) and the fact that NH_4US crystals are observed in both weakly acidic and alkaline urine *without* accompanying NaUS crystals [14] when urinary $[NH_4^+]$ is elevated (see Table 3), observations that the supersaturation model has great difficulty in explaining.

The quantification of observed calcium oxalate and phosphate crystalluria adds further weight to the argument in favour of a saturation model of urine. The volumes of CaOxS and calcium phosphate crystals at, respectively, pH 5 and 7 measured by Robertson et al. [36] with a Coulter counter, convert to near 0.1×10^{-3} and 2×10^{-3} moles/litre of precipitated oxalate and phosphate, respectively. Similar quantities of oxalate and phosphate in crystalluria were found by Hallson and Rose [24, 25] using centrifugation and direct chemical analysis. The measured quantities should be compared with those calculated by SEQUIL: see Tables 1–3. When making the comparison, however, one should bear in mind the facts that crystals smaller than about 2.5 μm diameter could not have been included in the measurements of Robertson et al. [36] as they are smaller than the detection limit of the Coulter counter, and, in all likelihood, the experimental procedures adopted by Hallson and Rose [24, 25] would have missed particles as small as, or smaller than, about 0.5 μm diameter. That is, both groups of workers must have underestimated the total quantity of material in crystalline particles. Of course, the estimation, by calculation, of total quantities of material in crystalluria cannot be done under the supersaturation hypothesis. The agreement between measured and calculated quantities, by SEQUIL, must surely be proof positive that "supersaturated" urines are not metastable solutions but rather solutions which have moved well towards the true saturated equilibrium condition.

Conclusion

It would appear that modelling urine as a saturated solution offers a more satisfactory explanation for stone formation and crystalluria than the widely accepted supersaturation model.

The supersaturation model predicts mixed component "urate" stones from low pH urine (HUS + NaUS + NH_4US) and mixed-component "calcium salt" stones from high pH urine (CaOxS + calcium phosphate) unless either special inhibitors to precipitation of one or other species are proposed, or one solid, the dominant one, does not seed the other. On the other hand, the saturated solution model can explain, in a straightforward way, the existence of uric acid stones devoid of urate salts from low pH urine and calcium phosphate stones, with or without struvite, but devoid of CaOxS, from high pH urine; these are the usual occurrences. In fact, the formation of both uric acid and "infection stones" would seem to result from major disturbances in the kidney's ability to vary the pH of urine. Non-stone-forming urine may result in uric acid or calcium phosphate crystalluria if it becomes too acidic or too alkaline, respectively, but the normal

diurnal variation in urinary pH should redissolve any of these crystals which may lodge in the urinary tract, hence preventing stone formation.

Further, if the saturated equilibrium model is the more appropriate description of the behaviour of urine than the supersaturation model, as has been demonstrated, then it follows that the pre-eminent reason(s) for stone formation must lie in a factor(s) other than simply the degree of supersaturation of urine [34, 35, 41]. Indeed, while some [34, 35, 41] have shown that the urine of the average calcium salt stone-former had an RSR greater than that of the urine of the average non-stone-former, the overlap of the ranges of RSRs was considerable. Others have reported [27, 41], however, no significant difference between the RSRs of the urines of average stone-formers and non-stone-formers. Also, there are many non-stone-forming individuals, for example pregnant women [29], whose urinary RSRs are pathological. That is, the RSRs are as high as or higher than those of female stone-formers, but pregnancy is not associated specifically with stone formation. Significantly, the urine of pregnant women showed crystalluria similar to non-stone-formers' urine [29, 36], that is, finely dispersed small crystals.

These well-documented and accepted facts are not consistent with the supersaturation model of urine and stone formation. The inconsistency between facts and model is removed when urine is modelled as a saturated equilibrium solution, particularly in respect of calcium salts, and stone formation attributed to an unstable colloidal (crystal-containing) solution (i.e. urine in which significant agglomeration of calcium salt crystals occurs) where the stability is most likely controlled by citrate^{3-} ion activity [2, 20, 21].

References

1. Andrews JC, Yrabro CL, Golby RL (1955) Studies on the mechanism of formation of renal calculi. J Urol 73:930
2. Ashby RA, Györy AZ (1997) A thermodynamic equilibrium model for calcium salt urolithiasis: clinical application. Exp Nephrol 5:246
3. Ashby RA, Sleet RJ (1992) The role of citrate complexes in preventing urolithiasis. Clin Chim Acta 210:157
4. Azoury R, Robertson WG, Garside J (1987) Observations on in vitro and in vivo calcium oxalate crystalluria in primary calcium stone formers and normal subjects. Br J Urol 59:211
5. Babic-Ivancic V, Furedi-Milhofer H, Brnicevic N, Markovic M (1992) Precipitation and solubility of calcium hydrogen urate hexahydrate. J Res Nat Inst Stds Tech 3:365
6. Baumann JM, Ackermann D, Affolter B (1989) The influence of hydroxyapatite and pyrophosphate on the formation product of calcium oxalate at different pHs. Urol Res 17:153
7. Berg C, Tiselius HG (1989) The effects of citrate on hydroxyapatite induced calcium oxalate crystallisation and on the formation of calcium phosphate crystals. Urol Res 17:167
8. Blomen LJMJ, Will EJ, Bijvoet OLM, Van der Linden H (1983) Growth kinetics of calcium oxalate monohydrate. II. The variation of seed concentration. J Cryst Growth 64:306
9. Brown CM, Ackermann DK, Purich DL (1994) EQUIL93: a tool for experimental and clinical urolithiasis. Urol Res 22:119
10. Brown WE, Gregory TM, Furedi-Milhofer H (1987) Computed phase diagrams for the system sodium hydroxide–uric acid–hydrochloric acid–water. J Cryst Growth 83:588
11. Cambell R, Griffith DP (1987) Infected (struvite) calculi. In: Rous SN (ed) Stone disease. Grune and Stratton, Orlando, p 161
12. Christoffersen MR, Christoffersen J, Kibalczyc W (1990) Apparent solubilities of two amorphous calcium phosphates and of octacalcium phosphate in the temperature range 30–42°C. J Cryst Growth 106:349
13. Daudon M, Donsimoni R, Hennequin C, Fellahi S, Moel G, Paris M, Troupel S, Lacour B (1995) Sex and age-related composition of 10 617 calculi analysed by infrared spectroscopy. Urol Res 23:319
14. Delatte LC (1983) Crystalluria. In: Roth RA, Finalyson B (eds) Stones: clinical management of urolithiasis. Williams and Wilkins, Baltimore, p 21
15. deVries A (1983) Clinical management of uric acid lithiasis. In: Roth RA, Finlayson B (eds) Stones: clinical management of urolithiasis. Williams and Wilkins, Baltimore, p 228
16. Dyer R, Nordin BEC (1967) Urinary crystals and their relation to stone formation. Nature 215:751
17. Elliott JS, Rabinowitz IN, Silvert M (1976) Calcium oxalate crystalluria. J Urol 116:773
18. Elliott JS, Rabinowitz IN (1980) Calcium oxalate crystalluria: crystal size in urine. J Urol 123:324
19. Finlayson B, Smith A (1974) Stability of the first dissociable proton of uric acid. J Chem Eng Data 19:94
20. Györy AZ, Ashby RA (1997) Equilibrium vs supersaturated urine hypothesis in calcium salt urolithiasis: a new theoretical and practical approach to a clinical problem. Scanning Microsc 11:1
21. Györy AZ, Ashby RA (1999) Calcium salt urolithiasis: a new approach to diagnosis and management. Clin Nephrol. 51:197
22. Halabe A, Sperling O (1994) Uric acid nephrolithiasis Miner. Electrolyte Metab 20:424
23. Hallson PC, Rose GA (1976) Crystalluria in normal subjects and in stone formers with and without thiazide and cellulose phosphate treatment. Br J Urol 48:515
24. Hallson PC, Rose GA (1988) Procedure for the measurement of calcium oxalate and phosphate crystals in urine. Br J Urol 62:199
25. Hallson PC, Rose GA (1989) Measurement of calcium phosphate crystalluria: influence of pH and osmolality and invariable presence of oxalate. Br J Urol 64:458
26. Hermann U, Schwille PO (1992) Crystalluria in idiopathic recurrent calcium urolithiasis. Urol Res 20:157
27. Khatchadourian J, Preminger GM, Whitson PA, Adams-Huet B, Pak CYC (1995) Clinical and biochemical presentation of gouty diathesis: comparison of uric acid versus pure calcium stone formation. J Urol 154:1665
28. Kok DJ (1997) Intratubular crystallization events. World J Urol 15:219
29. Mainkrantz P, Holley JL, Parks JH, Lindheimer MD, Nakagaway Y, Coe FL (1989) Gestational hypercalciuria causes pathological urine calcium oxalate supersaturations. Kidney Int 36:108
30. McDowell H, Gregory TM, Brown WE (1977) Solubility of $Ca_5(PO4)_3OH$ in the system $Ca(OH)_2$–H_3PO_4–H_2O at 5, 15, 25 and 37°C. J Res NBS 81 A:273
31. Pak CYC, Waters O, Arnold L, Holt K, Cox C, Barilla D (1977) Mechanism for calcium urolithiasis among patients with hyperuricosuria. J Clin Invest 59:426
32. Pak CYC (1981) Potential etiologic role of brushite in the formation of calcium (renal) stones. J Cryst Growth 53:202
33. Pak CYC (1997) Medical management of nephrolithiasis: a new, simplified approach for general practice. Am J Med Sci 313:215
34. Parks JH, Coward M, Coe FL (1997) Correspondence between stone composition and urine supersaturation in nephrolithiasis. Kidney Int 51:894

35. Robertson WG, Peacock M, Nordin BEC (1968) Activity products in stone-forming and non-stone-forming urine. Clin Sci 34:579
36. Robertson WG, Peacock M, Nordin BEC (1969) Calcium crystalluria in recurrent renal-stone formers. Lancet II:21
37. Robertson WG, Peacock M (1972) Calcium oxalate crystalluria and inhibitors of crystallisation in recurrent renal stone formers. Clin Sci 43:499
38. Robertson WG, Peacock M, Nordin BEC (1971) Calcium oxalate crystalluria and urine saturation in recurrent renal stone-formers. Clin Sci 40:365
39. Robertson WG, Scurr DS, Bridge CM (1981) Factors influencing the crystallisation of calcium oxalate in urine: critique. J Cryst Growth 53:182
40. Rodgers AL, Spector M (1987) Crystalluria. In: Rous SN (ed) stone disease. Grune and Stratton, Orlando, p 47
41. Tiselius H-G (1997) Estimated levels of supersaturation with calcium phosphate and calcium oxalate in the distal tubule. Urol Res 25:153
42. Werness PG, Bergert JH, Smith LH (1981) Crystalluria. J Cryst Growth 53:166
43. Werness PG, Brown CM, Smith LH, Finlayson B (1985) EQUIL2: a basic computer program for the calculation of urinary supersaturation. J Urol 134:1242
44. Wilson DM (1987) Uric acid lithiasis. In: Rous SN (ed) Stone disease. Grune and Stratton, Orlando, p 109