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EQUILIBRIUM VERSUS SUPERSATURATED URINE HYPOTHESIS IN CALCIUM SALT UROLITHIASIS: A NEW THEORETICAL AND PRACTICAL APPROACH TO A CLINICAL PROBLEM

A.Z. György[1*] and R. Ashby[2]

[1]Department of Medicine and Renal Medicine, The Royal North Shore Hospital and University of Sydney, and [2]Department of Chemistry, University of Technology, Sydney, Australia.

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Abstract

It is proposed that urine is better modeled as a true equilibrium rather than in a supersaturated/metastable state, and that the free citrate^{3-} ion plays a major role in maintaining dispersion of the solid particles (reduced agglomeration). Published urinary chemistries, in con-junction with the computer program SEQUIL, have been used to formulate a novel risk index for calcium stone formation independent of the traditional clinical classification of the stone former. Applying the risk index to three consecutive 24 hour urine samples of 58 untreated Ca stone formers showed that 55 (96%) **patients** produced at least one abnormal urine (4% "idiopathic" stone formers), while 49% were "idiopathic" according to conventional urinary parameters. Traditional single urinary parameter assessment in patients, Ca/cr (Ca to creatinine ratio), oxalate/cr or citrate/cr ratios showed that 91%, 91% and 89% of the normal **urines** respectively were abnormal according to SEQUIL, while taking all three together 81% of normal urines were abnormal by SEQUIL. Treatment regimes have been devised using the computer program to return an abnormal urine to the normal according to the proposed risk index. In most urines, two or more factors had to be changed simultaneously. Clinically there has been only one recurrence in 36 months, whereas, before they had 4.4 stone episodes every 3 years.

Key Words: Kidney stone disease, metastable urine, urine equilibrium, calcium oxalate, calcium phosphate, crystallization, agglomeration, calculated risk index (SEQUIL), therapy.

*Address for correspondence:
A.Z. György
Department of Medicine
The Royal North Shore Hospital
St. Leonards 2065, Australia
Telephone number: 61 2 9926 7238
FAX number: 61 2 9436 3719
E-mail: azgy@medicine.su.oz.au

Introduction

In this paper we present an approach to kidney stone disease which is based on thermodynamic complex ion equilibria in urine. In this tutorial, we are not concerned with the causes of individual urine abnormalities but rather with the ability to predict which urine is likely to form stones and to devise a therapeutic regimen for each patient.

Conventional calculations using chemical analysis of urine samples and known complex ion equilibria based on the theory that urine of calcium stone forming patients is supersaturated and in a metastable state does not separate these patients from normal controls adequately and does not correlate well with severity of stone disease (Ashby and György, 1997). We describe an adaptation of such calculations based on the hypothesis that urine is in a true equilibrium state, the free citrate^{3-} ion playing a major role in maintaining dispersion of the solid particles (reduced agglomeration). A computer modeling programme (SEQUIL) allows calculation of a risk index using the quantity of total solid Ca oxalate (CaOx) and phosphate per litre of urine divided by the free citrate^{3-} activity (AC3) for individual urines and patients. Twenty four hour urinary volume calcium, oxalate, phosphate, citrate, magnesium, sodium, potassium and pH measurements are needed for the calculations as input data. In SEQUIL, in its novel saturated equilibrium mode, the soluble ion equilibria (Finlayson, 1977; Robertson *et al*., 1968) and solubility equilibria are satisfied simultaneously allowing the maximum number of moles of solid CaOx and brushite in a particular urine sample to be estimated. This is achieved by removing excess calcium, oxalate and or HPO_4^{2-} ions, as the appropriate solids, until the solubility equilibria are satisfied, that is saturation has been reached. The moles of calcium salts, CaOx plus brushite, were calculated after each iteration of the program, and summed, until convergence was reached in the normal manner (Werness *et al*., 1985). That true computed equilibrium was reached was checked by calculating the concentrations of the aqueous ion pairs, CaOx(aq) and $CaHPO_4$ (aq) at convergence. Theoretically, at equilibrium:

$$[\text{ion pair}] = K_{SP} \cdot K_{STAB} \quad (1)$$

where [ion pair] is the molar concentration of the ion pair. K_{SP} and K_{STAB} are, the appropriate solubility product and soluble ion pair stability constant, respectively (Finlayson, 1977). Computed ion pair concentrations differed by no more than 0.1% from the true value (KSP.KSTAB) at convergence. The limit for the proposed index, as calculated by SEQUIL that is, when too much solid is produced to be suspended effectively (stone formation) is based on an empirical "inhibition test" for urines (Pak and Fuller, 1986), whereby, soluble oxalate was added to a urine sample until "visible spontaneous precipitation occurred at 3 hours" (Ashby and György, 1997). This was set at 37 ± 4 with a new and improved iteration subroutine, with an AC3 of 2.0×10^{-5}. At an AC3 of 2×10^{-5} and above, the solid/AC3 ratio remains constant. Thus, "Normal" level is below 29, "Danger" level between 29-45 (mean ± 2 standard deviations, SD) and "Abnormal" level above 45. However, there is another possible danger limit (stone formation) and that is, where AC3 is so low that even normal, or below normal, quantities of urinary solids cannot be dispersed effectively (Ashby and György, 1997). This AC3 value was computed using SEQUIL to be 0.65×10^{-5} (and the solid/AC3 ratios for points between 0.65×10^{-5} and 2×10^{-5} were interpolated from the equation given by these two points) from the urinary chemistry of the pretreatment idiopathic hypocitraturic stone formers (Pak and Fuller, 1986) who had prodigious rates of stone formation. Thus, this risk index allows a prediction to be made for each patient, and each urine sample as to the likelihood of stone forming propensity.

Of a total of 105 patients so far examined, 58 were untreated calcium stone forming patients. Of these untreated patients, SEQUIL predicted 96% (55) to be abnormal in at least one urine sample (3 consecutive samples collected). Three **patients** had normal urines by SEQUIL and are thus truly "idiopathic". Whether more than 3 urine collections would have resulted in abnormal values in these patients is unknown, but is a distinct possibility due to the variability in the three collections. Twelve normal subjects have so far been tested and only one showed abnormal urines. This may be due to the fact that, at this stage, we are only estimating urinary ammonium and not measuring it. Ammonium could significantly interact with citrate and alter the calculated "free" $citrate^{3-}$. The normal urines of stone forming patients and the abnormal urines of normal volunteers could, in fact, be defining a small but specific subgroup where the absence or presence, respectively, of other (organic) inhibitors may play a significant role not defined by citrate. Using the Ca/Cr (Ca to creatinine), oxalate/cr or citrate/cr ratios or 24 hour excretions normally obtained from "routine" investigations indicated that 90, 91 and 86% of the normal **urines**, respectively, were abnormal according to SEQUIL. Clinically (average 4.4 stone episodes per 3 years prior to present investigation), there has been one recurrence in 36 months. Individual urine volume or concentration of calcium, oxalate, citrate, etc. were altered in every patient and SEQUIL used to recalculate the results until their urines became normal. The changed item therefore became "therapy needed". In 47% of the patients, a single therapy (Ca, volume, citrate or Ox) was sufficient to return their urines to normal according to SEQUIL, 36% needed two changes, while 13% three and 4% remained untreated. In only 5% of patients was increasing urine volume alone a successful treatment regimen but was needed in a further 35% as adjunct therapy in combination with other measures (Table 1).

Table 1. Type of therapy devised by SEQUIL*.

Single therapy	**47%**
Increase urinary citrate	35
Increase urinary volume	5
Decrease Ca with Thiazide & diet	5
Decrease uric acid	2
Double therapy	**36%**
Citrate & volume	13
Citrate & Thiazide	13
Volume & Thiazide	9
Citrate & oxalate	1
Triple therapy	**13%**
Citrate, Thiazide & volume	11
Oxalate, Thiazide & volume	2
No therapy	**4%**

*SEQUIL was used to manipulate the original abnormal urine values of the 55 untreated calcium stone formers in order to return their urine chemistry to the normal range.

On applying SEQUIL to patient groups in five recent important papers, with various kidney stone disease problems, showed excellent prediction of abnormalities and treatment successes (Table 2). Absent urine values from these publications were replaced with values from our own normals. In 1270 patients (Levy *et al*., 1995), 20 of 21 group means were abnormal by SEQUIL, cystinurics were normal. In primary hyperoxalurics (Milliner *et al*., 1994), the untreated group was abnormal but following therapy with orthophosphate and pyridoxine they became normal according to SEQUIL.

In a comparative study of crystal growth inhibition in control subjects and patients with accelerated nephrolithiasis who had all their treatment suspended except citrate (Porile *et al*., 1996), no difference in urinary inhibitor was found between the normals and the patients. SEQUIL

Table 2. Values of calculated free citrate^{3-} (AC3) at equilibrium and the solid/AC3 ratio in patients in the present paper and others.*

	Clinical diagnosis	Dietary calcium	AC3 x 100,000	Solid/ AC3	Comment by SEQUIL
Present paper	previously untreated, abnormal by SEQUIL (55)	unrestricted Ca	0.992	11.84	Abnormal
	previously untreated, normal by SEQUIL (3)	unrestricted Ca	3.92	8.39	Normal
	previously treated, abnormal by SEQUIL (47)	unrestricted Ca	1.05	102.48	Abnormal
	Normal controls (12)	unrestricted Ca	2.312	12.29	Normal
Levy *et al*. [4]	Absorptive I hypercalciuria	restricted Ca	0.675	6.56	Abnormal
	Absorptive II hypercalciuria	restricted Ca	1.142	9.87	Danger
	Renal hypercalciuria	restricted Ca	0.562	3.92	Abnormal
	Hyperparathyroid hypercalciuria	restricted Ca	1.12	9.6	Danger
	Unclassified hypercalciuria	restricted Ca	0.906	10.02	Abnormal
	Fasting hypercalciuria	restricted Ca	0.815	13.12	Abnormal
	Hyperuricosuric	restricted Ca	0.945	8.97	Danger
	Gouty diathesis	restricted Ca	0.764	21.15	Abnormal
	Enteric	restricted Ca	0.338	19.13	Abnormal
	Primary oxaluria	restricted Ca	0.465	38.16	Abnormal
	Dietary oxaluria	restricted Ca	0.842	13.16	Abnormal
	RTA complete	restricted Ca	0.045	0	Abnormal
	RTA incomplete	restricted Ca	0.667	9.99	Abnormal
	Chronic diarrhhoea	restricted Ca	0.49	24.31	Abnormal
	Idiopathic hypocitraturia	restricted Ca	0.609	18.65	Abnormal
	Hypo magnesuria	restricted Ca	1.09	13.01	Danger
	Infection stones	restricted Ca	0.726	8.1	Abnormal
	Cystinuria	restricted Ca	1.14	0	Normal
	Low urine volume (0.821 l)	restricted Ca	2.12	144.25	Abnormal
	Low urine volume (1.821 l)**	restricted Ca	1.3	13.87	Danger
	No metabolic abnormality	restricted Ca	1.44	3.96	Normal
	Difficult to classify	restricted Ca	1.17	7.99	Danger
Millner *et al*. [5]	Normals		2.056	7.28	Normal
	Before treatment		1.541	103.71	Abnormal
	After treatment		4.261	15.56	Normal
Kok *et al*. [3]	Normals		2.056	7.28	Normal
	Patient 1		0.371	0	Abnormal
	Patient 2		0.624	13.62	Abnormal
	Patient 3		0.664	16.04	Abnormal
	Patient 4		0.789	3.67	Danger
	Patient 5		0.482	1.9	Abnormal
	Patient 6		0.794	0.36	Danger
Rudman *et al*. [9]	Normals		1.48	1.11	Normal
	Gasto II		0.497	23.37	Abnormal
	Gastro III		0.413	43.44	Abnormal

*The urinary values not reported in the above papers were substituted by values obtained from our normals.
**Urine volume increased by SEQUIL; increasing further (4.821 l) did not result in a “normal” urine.

correctly predicted the controls to be normal and patients to be abnormal indicating that citrate was the important agglomeration inhibitor and that agglomeration is the more important phenomenon. In patients with gastrointestinal malabsorption and kidney stone disease (Rudman *et al*., 1980), the two groups with malabsorption were abnormal by SEQUIL. For all these comparisons, we used our own normal values for urinary constituents where insufficient data was given. Most interestingly, where agglomeration rate was measured physically in 7 patients (Kok *et al*., 1986), all were predicted to be either in the Danger or Abnormal area by SEQUIL. Experimental manipulation of urinary citrate in one patient was faithfully reproduced by the computer programme SEQUIL.

Conclusions

In conclusion, this approach has a high positive predictive value for calcium stone patients and allows individualization of therapy which may, but not necessarily does, include changing Ca, citrate or urine volume individually or in some combination. It strongly supports the equilibrium theory as more appropriate for Ca urolithiasis, as would be expected if agglomeration, and not just crystal formation, is of major significance in the development of Ca stones.

References

Ashby R, György ÁZ (1997) A thermodynamic equilibrium model for calcium salt urolithiasis: Clinical application. Exp Nephrol **5**: 246-252.

Finlayson B (1977) Calcium metabolism in renal failure and nephrolithiasis. In: Calcium Stones: Some Physical and Clinical Aspects. David DS (ed.). John Wiley and Sons, New York. pp 337-382.

Kok DJ, Papapoulos SE, Bijvoet OLM (1986) Excessive crystal agglomeration with low citrate excretion in recurrent stone-formers. Lancet **i**: 1056-1058.

Levy L, Adams-Huetr B, Pak CYC (1995) Ambulatory evaluation of nephrolithiasis: An update of a 1980 Protocol. Am J Med **98**: 50-59.

Milliner DS, Jeffrey T, Eickholt BS, Bergstrahl EJ, Wilson DM, Smith LH (1994) Results of long term treatment with orthophosphate and pyridoxine in patients with primary hyperoxaluria. N Engl J Med **331**: 1553-1558.

Pak CYC, Fuller C (1986) Idiopathic calcium oxalate nephrolithiasis successfully treated with potassium citrate. Ann Intern Med **104**: 33-37.

Porile JL, Asplin JR, Parks JH, Nakagawa Y, Coe FL (1996) Normal calcium oxalate crystal growth inhibition in severe calcium oxalate nephrolithiasis. J Amer Soc Nephrol **7**: 602-607.

Robertson WG, Peacock M, Nordine BEC (1968) Activity products in stone-forming and non-stone-forming urine. Clin Sci **34**: 579-594.

Rudman D, Dedonis JL, Fountain MT, Chandler JB, Gerron GG, Fleming GA, Kutner MH (1980) Hypocitraturia in patients with gastrointestinal malabsorption. N Engl J Med **303**: 657-661.

Werness PG, Brown CM, Smith LH, Finlayson B (1985) EQUIL2: A basic computer program for the calculation of urinary supersaturation. J Urol **134**: 1242-1244.

Discussion with Reviewers

D.J. Kok: If I understand correctly, you propose that the urine be considered as a suspension of calcium-salt particles in equilibrium with the solution and you simulate this particle formation in your calculations. Did you apply specific filtration steps and measure the concentrations of free ions in your calculations?

J.P. Kavanagh: Have authors made any attempt to measure solid Ca, oxalate or phosphate and compare it with the predicted value?

Authors: Of course, the conventional hypothesis has been based on some (not many) filtration and Coulter counter comparisons. The concentrations of calcium, oxalate, phosphate, etc., used for the thermodynamic calculations had been obtained after either centrifugation or filtration and crystal sizes determined by Coulter counter techniques. All of these methods, employed in the older publications, have cut off points of 3-4 μ^c below which smaller crystals, if present, may not have been removed from the supernatants or filtrates which were then subsequently acidified (redissolved) to obtain the ionic concentrations for the calculations. These techniques could, therefore, clearly lead to overestimations of element concentrations as being in an ionic and soluble form. It is quite possible that, in fact, urine of all people contain crystals of sizes even down to 0.4 µm and not just down to 1 µm as required by thermodynamic definitions of the equilibrium state. These crystal are in an insoluble state, but which, on preparation for measurement of soluble element concentration, are dissolved again to contribute as "free" ions. Since this paper does not address this problem but presents a new hypothesis as applied to patients, inclusion of such discussion is beyond its scope.

D.J. Kok: What happens to your calculations when other types of calcium phosphates are formed, e.g., hydroxyapatite?

Authors: Hydroxyapatite crystals begin to form and can become of significance at pH's of about 7 and above and very few urines are at such pH's. However, even then, the apatite would only replace the brushite with a similar mass and this would not significantly alter the results as

evidenced by the good separation of normals and abnormals.

D.J. Kok: How does SEQUIL compare to risk indices which combine several urine parameters, like the indices proposed by Tiselius?.
Authors: Professor Tiselius is in fact looking at this himself with our program.

A.L. Rogers: What urinary data are required as input for SEQUIL?
Authors: Twenty four hour urinary volume calcium, oxalate, phosphate, citrate, magnesium, sodium, potassium (all as mmol/l), and pH measurements are needed for the calculations as input data.

Reviewer IV: How may one obtain a copy the SEQUIL program?
Authors: SEQUIL can be obtained by writing to A.Z. Györy (address on page 261).