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Urine Saturation with Calcium Salts in Normal Subjects and Idiopathic Calcium Stone-Formers Estimated by an Improved Computer Model System

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Summary. The state of saturation of urine with calcium salts has been estimated by means of a computer model system whose accuracy has been improved by the use of stability constants of 31 complexes which were re-determined at 37 °C and at the actual ionic strength of urine. The experimental determination of the concentration solubility products of calcium oxalate monohydrate (CaOx) and of calcium hydrogen phosphate dihydrate (bsh) allows an expression of the saturation degree as free concentration product ratio β_{CaOx} and β_{bsh}. Morning urine samples from 50 healthy controls and 50 idiopathic calcium stone-formers and 24 h urines from 40 normal subjects and 192 stone-formers, taking normal diet were investigated by this technique. From our results urine supersaturation with calcium oxalate salts seems to play an important role in calcium stone disease. Hypercalciuria and hyperoxaluria seem to be the main pathological features in this regard. The data concerning β_{bsh} values have not confirmed previous reports in which this parameter was found to be increased in stone-formers.

Key words: Calcium stone disease, Calcium oxalate, Brushite, Urine saturation, Stability constants, Soluble complexes, Computer system.

Introduction

The genesis of stones in the urinary tract is currently believed to derive from the homogeneous or heterogeneous nucleation of a crystal nidus, followed by crystal growth and crystal aggregation processes [9, 10, 14, 15, 18]. An essential condition for a stone to form and grow is that urine be supersaturated with respect to the precipitating phases [5]. Calcium oxalate and calcium phosphate are the calcium salts important in calcium stone disease [8, 11].

Two general approaches have been so far proposed for the estimation of the state of saturation, the so-called ab initio calculation and the semi-empirical determination of the solubility of the salts concerned directly performed in urines. When these methods are compared the correlation of the results has been reported to be very low [4, 12].

In previous papers [1, 2] we described an equilibrium-based computer model system for the calculation of free ion concentration improved by the use of stability constant values redetermined at 37 °C and at different ionic strength. The experimental determination of the solubility of calcium oxalate monohydrate and of brushite, at the same ionic strength and temperature, allowed an estimate of the state of saturation with respect to these salts.

The full method was applied to the estimation of the state of saturation of the above calcium salts in urines from control healthy subjects and patients with a history of calcium nephrolithiasis, all studied as out-patients while taking a normal diet.

Methods and Material

The method for the calculation of free concentration of constituent ions was published elsewhere [2]. Thirty-one complex species formed by Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, NH_4^{+}, and H^{+} with $Citrate^{3-}$, $Oxalate^{2-}$, HPO_4^{2-}, $Sulfate^{2-}$ were considered. The state of saturation, free concentration product ratio, was expressed as:

$$\beta_{CaOx} = c_{Ca} \cdot c_{Ox} / K^{I}_{sp,CaOx} \quad \text{and}$$

$$\beta_{bsh} = c_{Ca} \cdot c_{HPO_4} / K^{I}_{sp,bsh}$$

where c is the calculated free concentration and K^{I}_{sp} the concentration solubility product of calcium oxalate monohydrate and of brushite as experimentally determined at the actual ionic strength (I) of the urine specimen.

Fresh urine samples were obtained from 50 controls (27 males and 23 females) and 50 idiopathic calcium stone-formers (25 males and 25 females) who had been instructed to cease fluid intake for at least 12 h before the urine collection.

24 h urine samples were obtained from 192 calcium stone-formers (132 males and 60 females) and from 40 healthy controls (25

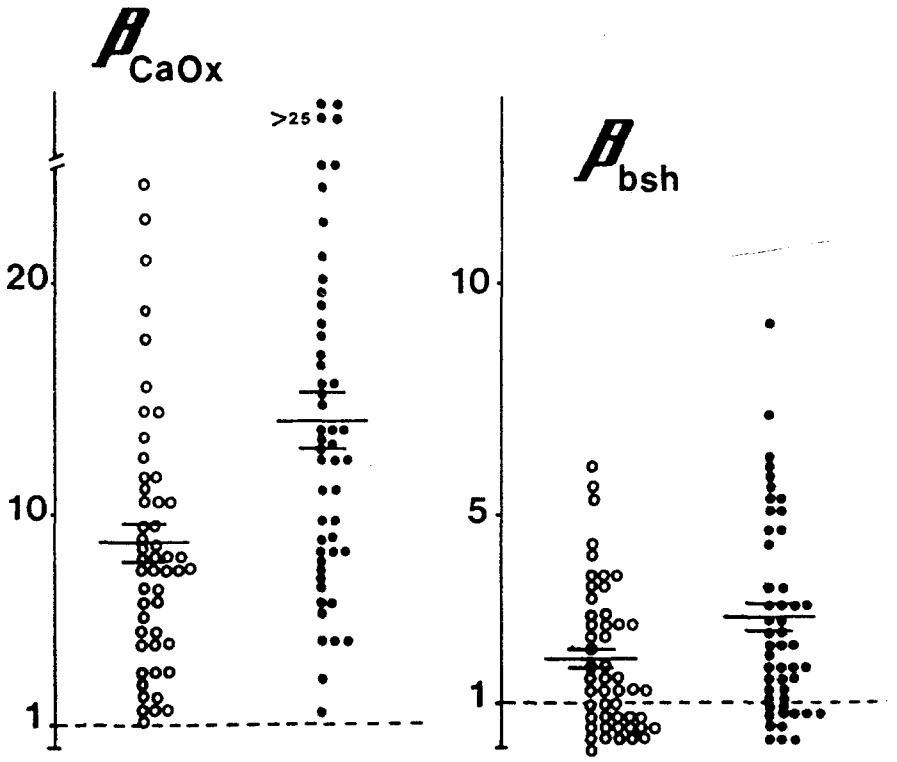

Fig. 1. Saturation degrees with respect to calcium oxalate monohydrate (β_{CaOx}) and brushite (β_{bsh}) in fasting morning urines from 50 controls (○) and 50 idiopathic calcium stone-formers (●). Horizontal bars indicate mean ± 1 SEM. The dropped lines denote saturation

Table 1. Excretion of the main urine constituents determined on 24 h-urines from healthy subjects and idiopathic calcium stone-formers at home

	healthy subjects (n = 40)	stone-formers (n = 192)	p
pH	6.15 ± 0.07	5.94 ± 0.03	< 0.01
Volume (ml/24 h)	1.213 ± 78	1.555 ± 41	< 0.005
Calcium (mmol/24 h)	4.46 ± 0.25	6.52 ± 0.39	< 0.05
Magnesium (mmol/24 h)	3.40 ± 0.25	3.76 ± 0.14	n.s.
Sodium (mmol/24 h)	178.0 ± 11.4	182.0 ± 4.6	n.s.
Potassium (mmol/24 h)	56.0 ± 2.8	51.0 ± 1.2	n.s.
Ammonium (mmol/24 h)	27.4 ± 2.2	37.6 ± 0.92	< 0.001
Oxalate (mmol/24 h)	0.30 ± 0.03	0.46 ± 0.02	< 0.001
Citrate (mmol/24 h)	3.80 ± 0.24	3.71 ± 0.13	n.s.
Phosphate (mmol/24 h)	23.2 ± 1.2	25.6 ± 0.8	n.s.
Sulfate (mmol/24 h)	18.4 ± 1.0	20.4 ± 0.5	n.s.
Chloride (mmol/24 h)	170.0 ± 10.1	180.0 ± 5.0	n.s.
I (mmol/l)	0.229 ± 0.01	0.197 ± 0.004	< 0.01

males and 15 females), all taking normal diet and fluid intake. None of the subjects studied had urinary tract infection at the time of the study. Renal function was within normal range in all cases.

Results

The urine saturation with $CaOx \cdot H_2O$ and with brushite from fasting samples are shown in Fig. 1: the samples from stone-formers are more supersaturated with respect to both salts, the difference being more signficant for $CaOx \cdot H_2O$ ($p < 0.001$ for β_{CaOx} and $p < 0.05$ for β_{bsh}).

In Table 1 the net daily excretions of the urine constituents and of 24 h urine volume are shown. Significant differences were found in oxalate and calcium excretion according to our own previous findings [7].

In Fig. 2 the estimation of the urine saturation from 24 h samples with the considered calcium salts is shown: a significant difference was found in the β_{CaOx} which was increased in the stone-formers ($p < 0.001$). There was no

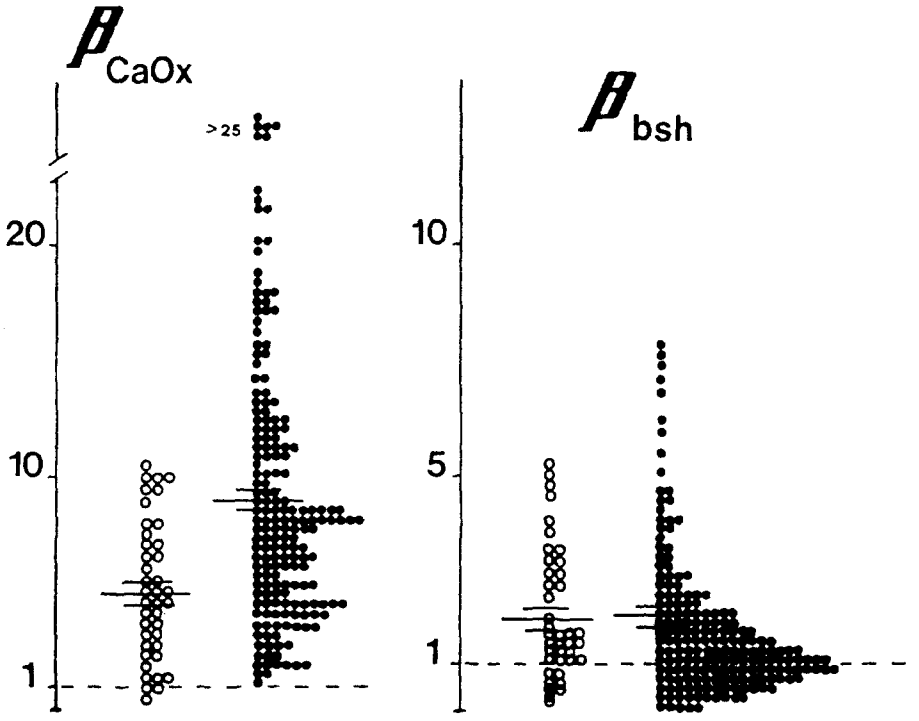

Fig. 2. Saturation degrees with respect to calcium oxalate monohydrate (β_{CaOx}) and brushite (β_{bsh}) in 24 h urines from 40 controls (○) and 192 idiopathic calcium stone-formers (●). Horizontal bars indicate means ± 1 SEM. The dropped lines denote saturation

Table 2. Urine saturation with calcium oxalate (β_{CaOx}) and brushite (β_{bsh}) in 24 h samples from single and recurrent idiopathic calcium stone-formers

	single stone-formers (n = 55)	recurrent stone-formers (n = 137)	p
β_{CaOx}	8.28 ± 0.74	9.37 ± 0.49	n.s.
β_{bsh}	1.55 ± 0.20	1.74 ± 0.13	n.s.

difference in the saturation with brushite. When the patients are subdivided into single and recurrent stone-formers, no difference between the two subgroups was found (Table 2).

Discussion

In this study the state of saturation with $CaOx \cdot H_2O$ and with $CaHPO_4 \cdot 2H_2O$ were estimated by means of a method which should represent an improvement compared to previous ones. This method accounts for urinary temperature, ionic composition and ionic strength.

A check of the method was made [2] by comparing the calculated free Ca^{2+} concentrations to those directly measured by an ion selective electrode (Philips IS 561-Ca^{2+} electrode): the mean difference of the two values, performed on all the fasting urine samples, was about ± 5% with errors not higher than ± 11%. The results of the estimation of the urine saturation with respect to calcium oxalate has been compared in selected samples with those obtained by a previous computer calculation.

Robertson's method, with the value of 1,900 $dm^3 \cdot mol^{-1}$ for the calcium oxalate complex stability constant [16], was chosen because it was available and because it provided quite similar results when compared by Pak et al. [12] to other computer calculations (namely Roberton's 7070 and Finlayson's methods).

Although a significant direct correlation was found between the values of our own and of the Robertson's method ($y = 0.506\,x + 0.151$; $r = 0.978$; $n - 2 = 34$; $p < 0.001$), Robertson's values were two fold higher than ours.

The differences can be explained by the use of the different stability constant values. Our results show that urine ionic strength under basal conditions and under fluid restriction is such that the Davies' equation for activity coefficients [3] is inaccurate.

In fact if ${}^T\beta_{pqr}$ is the thermodynamic value ($I \to 0$) of the formation constant for a general reaction and a_i and f_i are the activities and the activity coefficients respectively, of the ion, the expression can be written:

$$ {}^T\beta_{pqr} = \frac{a_{[M_pL_qH_r]}}{a_M^p \cdot a_L^q \cdot a_H^r} $$

$$ = \frac{f_{[M_pL_qH_r]}}{f_M^p \cdot f_L^q \cdot f_H^r} \cdot \frac{c_{[M_pL_qH_r]}}{c_M^p \cdot c_L^q \cdot c_H^r} = F_{pqr} \cdot \beta^I_{pqr} $$

Table 3. Values of F_{pqr} (see test) at I = 0.3 mol · dm^{-3} and t = 37 °C, as determined by our approach ($F_{pqr,exp}$) and as calculated by the Davies' equation ($F_{pqr,Davies}$)

Complex	$F_{pqr,exp}$	$F_{pqr,Davies}$
$[Ca(Cit)]^-$	37.2	46.6
$[Mg(Cit)]^-$	30.2	46.6
[Ca(Ox)]	10.7	12.7
[Mg(Ox)]	10.7	12.7
$[Ca(HPO_4)]$	8.9	12.7
$[Mg(HPO_4)]$	9.3	12.7
$[Ca(SO_4)]$	9.3	12.7
$[Mg(SO_4)]$	9.3	12.7
[Na(Ox)]	2.6	3.6
[K(Ox)]	2.3	3.6

where $F_{pqr} = \frac{f_{[M_pL_qH_r]}}{f_M^p \cdot f_L^q \cdot f_H^r}$ and β^I_{pqr}

is the formation constant expressed as free concentration ratio (c's with subscript). In previous methods the factors F_{pqr} were calculated by Davies' equation [3], while in our method the factors were experimentally determined [2].

Table 3 exemplifies the differences which can be found when a comparison between the two methods is made. Our values of F_{pqr}, are always significantly lower; the formation constants are therefore always higher, as $\beta^I_{pqr} = {}^T\beta_{pqr}/F_{pqr}$. This accounts for lower values of free calcium and oxalate concentrations and then of β_{CaOx}.

According to previous reports [6, 17] and also in this study, stone-formers investigated under normal diet showed significantly increased degrees of saturation with respect to $CaOx \cdot H_2O$ both on their fasting morning urines and on 24 h collections.

Hypercalciuria is the main cause of the urine oversaturation in the stone-former group, when studied in the fasting state. The state of saturation with $CaOx \cdot H_2O$ was significantly increased in the stone formers as compared to controls. From the 24 h excretion values listed in Table 1 the differences between controls and patients are more relevant for oxalate than for calcium.

Of some interest are the findings of increased phosphate, sulfate, ammonium and hydrogen ions excretion in stone-formers compared to normals; as suggested for calcium and oxalate excretion, the changes could be related to high protein intake. When patients are grouped, on the basis of calcium and oxalate daily excretion, the highest degrees of saturation with $CaOx \cdot H_2O$ are observed in hypercalciuric and hyperoxaluric patients.

The difference in β_{bsh} values between controls and stone-formers are slight in the fasting samples whereas no difference exists in the 24 h urines. From this study the previous reports [11, 13] of decreased levels of urine supersaturation in normal subjects are not confirmed.

The results of this study support the hypothesis that a significant relationship exists between urine environment, from a physico-chemical point of view, and the formation of calcium containing renal stones. Calcium oxalate salts are likely to be more frequently involved in the pathogenesis of idiophatic calcium stone-disease than is brushite.

As β_{CaOx} is significantly correlated to net calcium and net oxalate excretion, these are the features that should be relevant in lithogenesis, while lesser importance should be attributed to other urine components.

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