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# Cystine Crystalluria and Urinary Saturation in Cystine and Non-Cystine Stone Formers

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Summary. It has been suggested recently that the first step in the formation of calcium oxalate stones appears to be crystallisation. This step is said to depend on the state of saturation of the urine. This hypothesis was checked in cystine stone formers.

Cystine crystalluria was found in 83% of 24 urine samples from cystine stone formers (CSF) but in none of the 400 control samples and appears to be a good guide in the diagnosis of cystine lithiasis. Urinary cystine saturation was constantly higher in CSF than in non-cystine stone formers (NCSF) who exhibited undersaturated urine with respect to cystine. There was almost no overlap between these 2 groups. Crystals were never found in undersaturated urine and were always present when the saturation was above 1. There appears to be a good correlation between the level of urinary saturation and the presence of crystalluria and there is no need for any additional factor such as a defective inhibitor. The study underlines the limits of a therapeutic regimen of a high fluid intake and alkalinisation of the urine.

Key words: Cystine stone, Crystalluria, Urinary saturation.

## INTRODUCTION

Cystine stones are uncommon and account for about 1% of all renal stones (10, 17). As cystinuria is almost constantly present in cystine stone formers (CSF), cystine stone formation is an interesting and simple model in which to study the pathophysiology of metabolically induced renal calculi. New data on the physical aspects of calcium stone formation have been recently reviewed (7, 15). According to the current theory, the nucleation of the crystal nidus is the first step in stone formation, depending on the state of saturation of the urine. In calcium oxalate stone formers a good correlation was found between crystalluria and the urinary calcium oxalate saturation (19). Among control subjects, urine samples were undersaturated or only slightly supersaturated (14, 18, 19). It is, however, accepted that even in normal people, the urine may be supersaturated with calcium oxalate (14, 19), octocalcium phosphate, hydroxyapatite (17) or brushite. The presence in normal urine of effective inhibitors is thought to prevent more frequent stone formation (8).

This theory has been applied mainly to calcium stone formation. In cystine stone formers cystine crystalluria has been recognized for many years (6, 16) as well as an increased excretion of cystine. This paper reports the results of a combined study of cystine crystalluria and urinary saturation in cystine stone formers.

## MATERIALS AND METHODS

Ten ml of freshly voided urine was centrifuged at room temperature (165 g for 10 min). Nine ml of the supernatant were removed and crystals resuspended in the 1 ml remaining (Vortex-mixer). One aliquot of this suspension was immediately screened for cystine crystals under a light microscope (Leitz Dialutz). Crystalluria was graded, using a 1 $mm^3$ Malassez counting cell, from 0 to 3 : Grade 0 = no crystals, Grade 1 = up to 10 crystals/$mm^3$, Grade 2 = from 10 to 30 crystals/$mm^3$, Grade 3 = over 30 crystals/$mm^3$.

A 2 h urine collection was made in the fasting state ($U_1$: 7 to 9 a.m.), after a 200 ml water load ($U_2$: 9 to 11 a.m.), and after a standard meal ($U_3$: 11 a.m. to 1 p.m., $U_4$: 1 to 3 p.m.).

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The cystine content of the meal was 1340 mg. Urine volume and pH (Radiometer pH meter 27) were immediately recorded. Cystine concentrations were determined by ion exchange chromatography. The relative urinary cystine saturation (RS) was calculated from the nomogram established by Marshall and Robertson (12). According to this nomogram, the relative urinary cystine saturation can be calculated from the pH and the urinary cystine concentration.

The studies of crystalluria were performed in cystine stone formers (CSF) n = 6, non-cystine stone formers (NCSF) n = 80 and normal volunteers n = 20.

A further study of crystalluria and urinary saturation was performed in CSF (n = 6) and NCSF controls (n = 15). All CSF (mean age: 33.9) had a history of active stone disease with more than 1 new stone formed every year, the last one within the preceding 6 months. All had elevated urinary cystine excretion (from 1.12 to 3.57 mM per 24 h) and normal renal function (inulin clearacne above 1.3 ml/s/1.73 $m^2$). There was only 1 female in the CSF group. None was receiving Penicillamine at the time of the study.

## RESULTS

### Crystalluria

Cystine crystals were never seen in NCSF (320 urine samples) or in normals (80 samples). Typical cystine crystals (Fig. 1) were found in 20 of 24 urine samples (83%) in CSF with the following repartition: Grade 0: n = 4, Grade 1: n = 5, Grade 2: n = 6, Grade 3: n = 9.

### Urinary Cystine Saturation (Fig. 2, Table 1)

In controls subjects (NCSF) the urinary cystine saturation (RS) was always below -0.8, with most of the values below -2. In CSF, RS was always above -0.9 with 18 of 21 samples above 0.

The 200 ml water load ($U_2$) decreased RS in NCSF although the difference was not significant (mean decrease = 0.1). In CSF, a decrease was evident in 1 patient, no decrease occurred in 3 and in the others no conclusion could be drawn (Table 1).

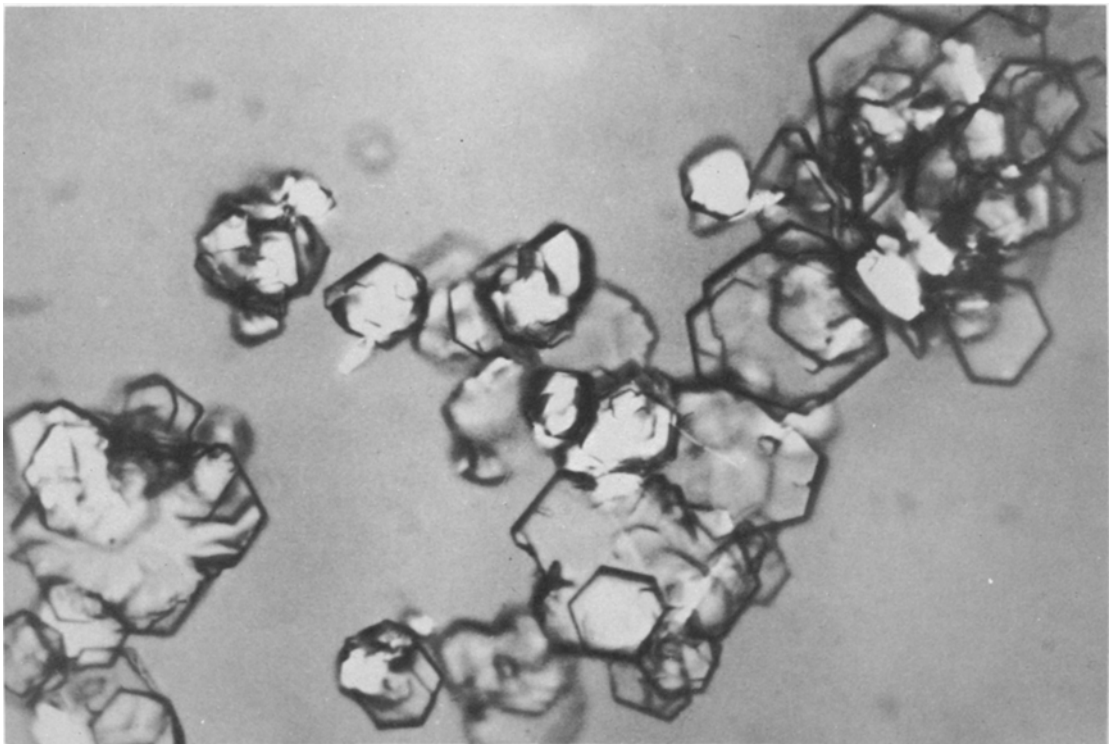

Fig. 1. Cystine crystals in urine

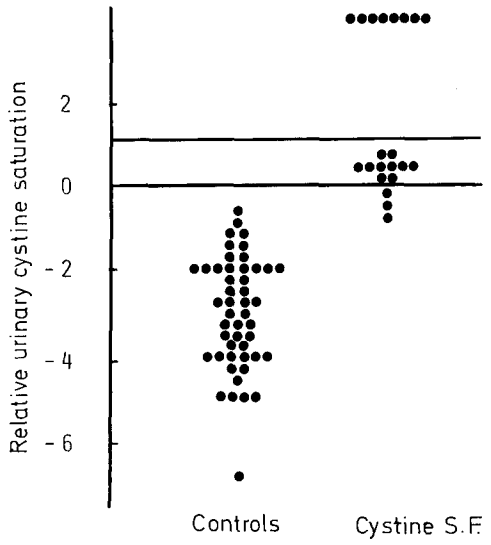


Fig. 2. Relative urinary saturation in non-cystine stone formers (controls) and cystine stone formers

Table 1. Relative cystine saturation in CSF (n = 6) and controls (n = 15). For $U_1$, $U_2$, $U_3$, $U_4$, see methods

| | | Cystine relative saturation | | | |
|---|---|---|---|---|---|
| | | $U_1$ | $U_2$ | $U_3$ | $U_4$ |
| Controls | | -3.19 | -3.05 | -3.00 | -2.76 |
| CSF | 1 | ---- | -0.4 | 0.1[b] | 0.5[a] |
| | 2 | 0.4[a] | 0.4[a] | 0.4[a] | 0.4[a] |
| | 3 | 2.0[a] | 2.0[a] | 2.0[a] | 2.0[a] |
| | 4 | 2.0[a] | 2.0[a] | 2.0[a] | 2.0[a] |
| | 5 | 0.3[a] | -0.9[a b] | 0.3[a] | 0.5[a] |
| | 6 | -0.7 | ----[a] | ----[a] | 0.2[a] |

[a] Samples with crystals

[b] Samples in which there is disagreement between the study of crystalluria and the value of RS (see text for discussion)

Table 2. Cystine excretion rate (pMol/s) in controls (NCSF, mean values) and CSF

| | | Cystine excretion rate (pMol/s) | | | |
|---|---|---|---|---|---|
| | | $U_1$ | $U_2$ | $U_3$ | $U_4$ |
| Controls | | 1.1 | 1.3 | 1.2 | 1.5 |
| CSF | 1 | ---- | 23 | 22 | 23 |
| | 2 | 13 | 21 | 13 | 15 |
| | 3 | 452 | 485 | 438 | 182 |
| | 4 | 430 | 429 | 433 | 431 |
| | 5 | 11 | 26 | 15 | 35 |
| | 6 | 12 | ---- | ---- | 35 |

The post-prandial values ($U_4$) were higher than fasting values in NCSF (mean increase = 0.4, $p < 0.05$, paired t test) due to an increase in the cystine excretion rate without significant change in the urinary volume. No such changes, both in RS and cystine excretion, could be documented in CSF (Table 2).

## Correlation Between Crystalluria and Relative Saturation (RS)

This relationship is shown in Fig. 3 (see also Table 1). Sixty-nine urine samples were available for study, 21 in CSF and 48 in NCSF. Urine from NCSF contained no crystals and exhibited negative urinary saturation. In CSF, 17 samples showed both crystalluria and supersaturation (RS above 0). One sample contained few crystals (Grade 1) with a RS value of -0.09. Two had no crystals with RS values of -0.4 and 0.1. One sample had no crystals and a negative RS.

## DISCUSSION

### Crystalluria

Routine examination revealed crystals in 26% of Dahlberg's cases (2) and Ettinger (6) reports

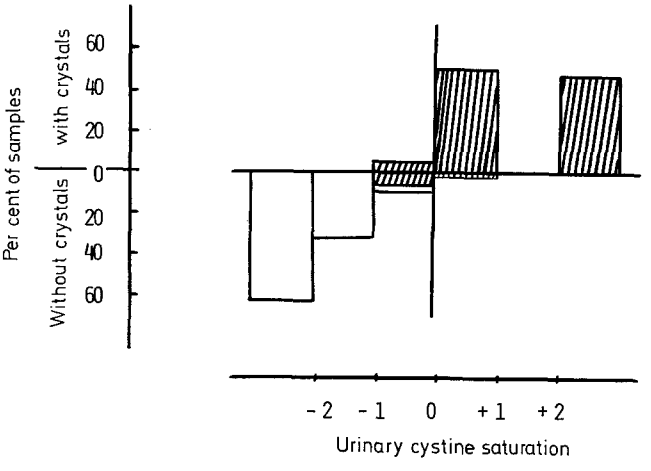


Fig. 3. Correlation crystalluria - saturation: The total number of samples (69) divided into samples with crystals (upper part, n = 18) and samples without crystals (lower part, n = 51). The samples were then classified depending on their RS value (lower scale). Open bars = NCSF, hatched bars = CSF

crystalluria in 6 of 12 patients during their "control period". Thus, the finding of cystine crystals in 83% of the urinary samples in CSF is a rather high figure. Since crystalluria depends on the amount of cystine present in the urine (6), the level of cystine excretion is an important variable to consider: subjects with a high level of cystine excretion should exhibit crystalluria more often than others. The mean 24 h urinary excretion of cystine was 1.34 $\pm$ 77 mM (mean $\pm$ SEM) in our patients (normals: below 0.1 mM) with 2 patients eliminating more than 1 mMol/ 24 h.

On the other hand, repeated examination of urine might be an important factor, especially in subjects with moderate cystinuria. Three of the 4 samples without crystals were obtained from the same subject (Table 1, subject 1). Our protocol included examination of 4 urine samples from each patient, in the fasting ($U_1$), hydrated ($U_2$) and post-prandial state ($U_3$ and $U_4$); we believe this might be a crucial factor.

## Relative Saturation

Because of the way in which it is calculated (11), the relative cystine saturation (RS) depends mainly on pH and cystine concentration. Both Marshall's nomogram (11) and the curve of cystine solubility (4) agree on 7 as the critical value for urinary pH: alkaline pH increases cystine solubility and decreases RS.

In controls, the RS was constantly below 0, due to a low rate of cystine excretion (Table 2). The maximal rate was 3.5 pmol/s in 1 patient. Even in acid urine, a RS of 0 would be reached only for an excessively low urinary flow rate (2,5 µl/s). Thus urine from non-cystinuric subjects is always undersaturated with respect to cystine. This situation is quite peculiar since normal urine may be oversaturated with respect to other substances involved in renal stones.

In CSF, the RS was constantly higher than in NCSF. This was not due to a pH dependent effect. In our CSF, 13 of 24 (54,2%) of urinary pH values were above 7, versus 6 of 48 (12,5%) in controls. Thus, the pH of urine from CSF would tend rather to decrease RS. Since urinary flow rates were not significantly different in CSF and controls, the higher values of RS found in CSF were due exclusively to increased cystine excretion. The mean value of the cystine excretion rate was 175 pmol/s in CSF versus 1.3 pmol/s in controls.

Although this study involved only a few patients, it is obvious from Fig. 2 that CSF could be divided into 2 groups: One with RS constantly above 2, 1 with RS between 0 and 1. Harris and coworkers (9) classified cystinuric patients in homozygous and heterozygous subjects. The distinction between these 2 groups can conveniently be made by measuring the amount of cystine excreted: More than 1 mMol per g of creatinine for homozygotes, less than 1 mMol/g for heterozygotes (1). Only homozygotes are thought to form stones, although this point has been disputed since 5 of Dahlberg's cases formed stones while they were considered as heterozygotes or compound heterozygotes (2, 13). The mean 24 h urinary cystine excretion was 3.57 mMol in our patients with RS above 2 (homozygous subjects) and 0.59 mM in patients with RS between 0 and 1 (supposed heterozygotes). This would confirm that even heterozygotes might form stones.

The post-prandial values of urinary cystine saturation were higher than fasting values in controls (mean difference = 0.4, $p < 0.05$, paired t test). This was due to an increase in the cystine excretion rate (mean difference 28 pmol/s). Such an increase did not occur in CSF. Dent (3) reported that after a 5 g oral cystine load, cystine excretion increased in controls, but not in all cystinurics. Although Morin (12) and Rosenberg (20) reported lower changes in plasma cystine levels in cystinurics after a 0.5 mmol/kg oral load, suggesting a defective intestinal transport, there is, however, no conclusive evidence that plasma levels of cystine might influence cystine excretion significantly.

## Correlation Between Crystalluria and Saturation

Assuming that the theory for calcium stone development is applicable to cystine stones, spontaneous crystallisation occurs for values of RS above 1. For values below 0, there is no spontaneous crystallisation and solubilisation of preformed crystals is possible. Between 0 and 1, the urine may support the growth of preformed crystals but spontaneous crystallisation can not occur (12, 15, 19). Accordingly, crystals should always be found in urine samples with RS >1, never in RS < 0, and could be found in samples with RS between 0 and 1, depending on the RS of the preceding period.

Fig. 3 shows that almost all (50 out of 51) samples with cystine RS below 0 do not contain crystals; in contrast, from the 18 samples in which the RS was positive, only 1 had no crystals. These findings support the theory of a close relationship between saturation and crystalluria although the semiquantitative evaluation of crystalluria does not allow a more precise study of this correlation.

This was not the case in Robertson's experience (19) nor in ours (11) in calcium oxalate stone formers since both calcium oxalate crystals and supersaturated urine were documented in normals, suggesting the presence of defective

inhibitory activity in calcium oxalate stone formers. According to our results, there is no need for such a defective inhibitory factor in cystine stone formation.

Two exceptions occurred: One sample from a CSF contained few crystals (Grade 1) with a RS of -0.9. It was collected after hydration which increased the urinary flow rate from 6,2 µl/s to 41,8 µl/s. Since the volume of crystalluria decreased from Grade 2 to Grade 1, together with RS (from + 0.3 to -0.9), we suppose that the preformed crystals were undergoing actual solubilisation (Table 1, subject 5, period $U_1$ and $U_2$). The other sample contained no crystals with an RS value close to 0 (RS = 0.1). As the preceding sample exhibited a negative RS (Table 1, subject 1) no spontaneous crystallisation occurred.

It should be considered, however, that crystals were found in urine with an RS value between 0 and 1. When this occurred in the first sample ($U_1$), it is conceivable that higher RS values might have occurred at night, explaining the persistence of crystalluria. In one case however, such an explanation is not acceptable (subject 1, $U_4$) suggesting that crystallisation might actually occur for values of RS between 0 and 1.

## Practical Consequences

The treatment of cystine lithiasis usually includes adequate oral fluid intake and urinary alkalinisation. This regimen is efficient in one third (13) to two thirds (5) of patients. It is conceivable, although disputed (13), that the treatment will have more chance of being effective in patients with moderate cystinuria and values of RS below 1. In our study, the mean cystine concentration was 1.9 µmol/ml in samples with RS between 0 and 1. Since at pH 7.5, RS values below 0 (thus dissolution of crystals) are obtained for a cystine concentration below 1.75, an increased urinary volume and alkalinisation is thought to prevent stone formation in this group, providing that this treatment is followed even at night (4). The 200 ml water load seemed to decrease the cystine saturation. However, this load appears to be a small one, since it would lead to an approximate 24 h urinary output of 2.4 l.

In contrast, the mean urinary cystine concentration in samples with RS above 2, averaged 29.8 µmol/ml. Under these circumstances the urinary flow rate would have to be increased sixteenfold to reach undersaturation. These patients should receive additional treatment designed to decrease the urinary cystine excretion.

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