CALCIUM OXALATE CRYSTALLURIA AND INHIBITORS OF CRYSTALLIZATION IN RECURRENT RENAL STONE-FORMERS

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SUMMARY

1. The particle size distributions of calcium oxalate crystals were measured at 37°C in fresh urine from recurrent, idiopathic stone-formers and their controls under the same conditions of dietary and fluid intake. The crystals excreted by the controls were small and belonged to a unimodal distribution, whereas those excreted by the stone-formers belonged to a distribution which contained a second peak of much larger particles. The proportion of large crystals in the urines of the stone-formers was significantly higher than in the urines of the controls.

2. The difference in the proportion of large particles passed by the two groups was accentuated by adding a small quantity of sodium oxalate to their diets. Whereas the controls continued to excrete only small crystals of calcium oxalate, the stone-formers passed most of their crystals as large particles.

3. Further investigations showed that the urines of the controls contained a potent inhibitor of the growth and aggregation of calcium oxalate crystals in vitro and that the inhibitor was deficient in the urines of the recurrent stone-formers.

4. It is suggested that the inhibitor in normal urine may allow calcium oxalate to be passed harmlessly in the form of small particles, whereas the lower inhibitory activity in the urines of the recurrent stone-formers is insufficient to prevent the growth of the primary crystals into the large aggregates seen in these urines. By blocking the formation of abnormally large crystals and aggregates the inhibitor may therefore play an important role in preventing crystalluria leading to stone formation.

Key words: calcium oxalate crystalluria, renal stone formation.

The passage of crystals in the urine has been recognized as a common feature of renal stone disease for many centuries. However, an examination of random fresh urine samples collected and maintained at 37°C from non-recurrent idiopathic stone-formers and normal healthy controls showed that crystals were not common in either group. The only crystals which were
observed were octahedral crystals of calcium oxalate dihydrate and an 'irregular' form of basic calcium phosphate (Dyer & Nordin, 1967). A recent intensive study confined to recurrent idiopathic stone-formers, who were forming predominantly calcium oxalate stones several times each year, showed that they frequently passed crystals of calcium oxalate in their urine and did so more often than their controls under the same conditions of dietary and fluid intake (Robertson, Peacock & Nordin, 1969). At least in part this could be accounted for by the higher excretions of calcium and oxalate and the higher supersaturation concentrations of calcium oxalate in the urines of the recurrent stone-formers (Robertson, Peacock & Nordin, 1971). The differences in calcium oxalate crystalluria and urine saturation between the stone-formers and their controls were accentuated by adding small amounts of sodium oxalate to the diets of both groups (Robertson et al., 1969, 1971).

In addition light microscopy studies on the fresh warm urine samples showed that the calcium oxalate crystals excreted by the recurrent stone-formers were mainly large octahedral crystals of calcium oxalate dihydrate, often in aggregates up to 200 μm in diameter, whereas those excreted by the controls were small particles with little or no aggregation (Robertson et al., 1969). They suggested that the difference in size of the crystals passed by the stone-formers and their controls might be caused by the presence in normal urine of an inhibitor of crystallization which is deficient in the urine of recurrent stone-formers. This concept has recently received some qualitative support from the work of Dent & Sutor (1971), who reported that crystals of calcium oxalate grown in vitro in urine from stone-formers tended to be large and mainly of the dihydrate, as were those shown to be present in fresh urine from recurrent stone-formers (Robertson et al., 1969). However, crystals grown in the urine from normal subjects were small and of the monohydrate, similar to those in normal urine (Robertson et al., 1969). These authors also suggested that this difference in crystal size and type observed in their experiments in vitro was evidence that normal urine contained some inhibitor of crystal growth.

The present paper attempts, first to define qualitatively the difference in crystal size of calcium oxalate crystals passed in vivo by recurrent stone-formers and their controls, and secondly to measure the ability of urine to inhibit the growth and aggregation of calcium oxalate crystals in an in vitro system.

**MATERIALS AND METHODS**

* Determination of the size of calcium oxalate crystals in the urine of recurrent stone-formers and controls

The patients were six male idiopathic stone-formers, aged between 24 and 54 years, who were passing predominantly calcium oxalate stones at least twice per year. All had kidney stones at the time of the study, but were otherwise healthy and had no known metabolic disorder other than idiopathic hypercalciuria. [The mean (± 1 SEM) 24 h urinary excretion of calcium of these patients was 380 ± 39 mg/24 h compared with 238 ± 14 mg/24 h in our normal controls.] There was no evidence of urinary tract infection and all had normal renal function. These patients we have defined as recurrent idiopathic stone-formers (Robertson et al., 1969, 1971). They were investigated in a metabolic ward on a constant basal intake of calcium (1000 mg/day), oxalate (120 mg/day) and phosphate (1200 mg/day). Fluid intake was controlled at 1300 ml/day and given at fixed times.
The controls were six healthy male members of staff who were between 24 and 51 years old. They were investigated in the metabolic ward under the same conditions of dietary and fluid intake as the patients, except that they were allowed home after the evening meal.

All subjects emptied their bladders before going to bed and on the following day urine samples were collected into warm Dewar flasks at 09.00, 11.30, 14.30 and 17.00 hours. Similar collections were made on subsequent days to study the effect on calcium oxalate crystalluria of a single oral dose of sodium oxalate (5 mg/kg body wt) added to the basal diet.

Calcium oxalate crystalluria between the crystal sizes 3.8 and 48.6 μm was measured in each sample immediately after voiding by using a model B Coulter counter with a model M volume-converter attachment (Coulter Electronics Ltd, Dunstable, Beds.) as described by Robertson (1969). The sample was maintained at 37°C during the crystal-counting procedure. The type of crystal counted was identified by light microscopy.

**Measurement of the inhibition of calcium oxalate-crystal growth and aggregation by urine**

The patients were ten male recurrent idiopathic stone-formers, who were currently passing calcium oxalate-containing stones several times per year. The controls were ten healthy male laboratory and medical staff. All subjects were studied on a free diet. They emptied their bladders at approx. 08.00 hours and a urine sample was collected 2 h later. The urine was immediately filtered through a Millipore filter (0.45 μm pore size).

The test consisted of incubating pure calcium oxalate crystals in a solution metastable with respect to calcium oxalate, i.e. in a solution sufficiently supersaturated with calcium oxalate to allow added crystals of calcium oxalate to grow, but insufficiently supersaturated to allow spontaneous precipitation of calcium oxalate in the absence of seeding crystals. The solution initially contained calcium (1 mmol/l), oxalate (0.2 mmol/l), sodium chloride (0.15 mol/l) and was buffered at pH 6.0 with sodium cacodylate (10 mmol/l). A 10 ml portion of a calcium oxalate crystal suspension (1 g/l) was added to 500 ml of the metastable solution. The growth and aggregation of the added crystals was measured by using the Coulter counter described above to monitor the change in the particle-size distribution of calcium oxalate crystals with time (Fig. 1).

The effect on the growth curve of calcium oxalate crystals of adding urine from stone-formers and controls to the metastable solution to give a final concentration of urine of 5% (v/v) was studied.

**RESULTS**

**Size of calcium oxalate crystals in the urine of recurrent stone-formers and controls**

The particle size distributions of calcium oxalate crystals excreted by a recurrent stone-former and a normal control on the basal diet in a typical experiment are shown in Fig. 2. Whereas the normal control excreted his calcium oxalate as a unimodal distribution of small particles the stone-former had in addition a second peak of much larger particles.

To compare the proportion of large crystals passed by the two groups a dividing line was drawn at 12.2 μm and the percentage of the total volume of calcium oxalate particles larger than 12.2 μm calculated for each subject. The comparison between the percentage of calcium oxalate crystals larger than 12.2 μm in the urines of the stone-formers and their controls is given in Fig. 3. This shows that a significantly greater proportion of the calcium oxalate crystals excreted by
Fig. 1. Particle size distributions of calcium oxalate crystals in a metastable solution of calcium oxalate showing the progress of growth and aggregation with time. •, Zero time; ▲, 70 min; ×, 120 min; ■, 240 min.

Fig. 2. Particle size distributions of calcium oxalate crystals in the fresh urine at 37°C of a recurrent stone-former (●) and a control (○), (a) on the basal diet and (b) after an oral supplement of sodium oxalate.
Urinary inhibitors of crystallization

the stone-formers were larger than 12.2 μm in diameter \((P<0.01)\). Figs. 2 and 3 also show that this difference in the size of crystals excreted by the stone-formers and their controls is accentuated by adding a small quantity of sodium oxalate to the diets of both groups. Whereas the normal controls showed no increase in the percentage of calcium oxalate particles larger than 12.2 μm, the stone-formers showed a marked increase in the proportion of large particles

(Fig. 3. Percentage of calcium oxalate crystals larger than 12.2 μm in diameter in the fresh urine at 37°C of the recurrent stone-formers (●) and their controls (○), on the basal diet and after an oral supplement of sodium oxalate. \((P<0.001)\). The difference between the percentage of large particles in the urines of the stone-formers and their controls after the addition of sodium oxalate to their diets was highly significant \((P<0.001)\).

Inhibition of calcium oxalate crystal growth and aggregation by urine

The growth and aggregation of calcium oxalate crystals in the standard metastable solution of calcium oxalate was measured by determining the particle size distribution of crystals at different times as shown in Fig. 1. The data can be presented on a single curve by plotting the increase in the number of particles of one particular diameter (for example 20 μm) against the time of incubation. The growth curve calculated in this way in a simple metastable solution is shown in Fig. 4, together with the growth curves obtained when urine from a normal subject and from a recurrent stone-former are added to the system to give a final concentration of urine of 5% \((v/v)\). The growth and aggregation rate of calcium oxalate crystals in the metastable solution containing 5% normal urine was markedly decreased indicating that normal urine contains some factor which inhibits the crystallization of calcium oxalate. The decrease
Fig. 4. Increase in the number of aggregates of calcium oxalate crystals of 20 \( \mu m \) in diameter in relation to the time of incubation in a metastable calcium oxalate solution containing 5\% (v/v) 0.15 \( M \) sodium chloride (■); in a metastable solution containing 5\% urine from a recurrent stone-former (▲); and in a metastable solution containing 5\% urine from a normal control (●).

Fig. 5. Increase after 4 h of incubation in the number of aggregates of calcium oxalate crystals of 20 \( \mu m \) diameter in the metastable solutions containing 5\% urine from the recurrent stone-formers and their controls as a fraction of the increase in the number of aggregates in the metastable solution containing 5\% (v/v) 0.15 \( M \) sodium chloride in place of urine.
Urinary inhibitors of crystallization

in the rate of growth and aggregation in the metastable solution containing 5% urine from the recurrent stone-former was much less, suggesting that there is less inhibitory material in the stone-forming urine than in the normal urine.

The rate of growth and aggregation of calcium oxalate crystals in the urines of ten recurrent calcium oxalate stone-formers and their controls are shown in Fig. 5. The growth rate is expressed as the increase in the number of particles of 20 μm diameter after 4 h in the system containing 5% urine as a fraction of the increase in the number of particles of 20 μm diameter after 4 h in the system containing 5% of a sodium chloride solution (150 mmol/l) in place of urine. Fig. 5 shows that the fractional growth rate in the metastable solutions containing 5% urine from the stone-formers was significantly higher \( (P < 0.005) \) than the growth rate in the metastable solutions containing 5% normal urine.

DISCUSSION

The passage of crystals in the urine has long been considered a feature of renal stone disease, but normal people can also on occasions harmlessly pass particles of calcium salts in their urine (Robertson et al., 1969). This implies that crystalluria alone may not be sufficient to cause a stone to form, although it may be a necessary contributory factor. The critical difference between stone-formers and normals lies in the size, type and frequency with which crystals are passed by the two groups. It has been clearly shown that there is a quantitative difference in the frequency with which crystals are passed and a marked qualitative difference in the size and type between recurrent calcium oxalate stone-formers and their controls (Robertson et al., 1969). The present paper contains a quantitative verification of the latter findings showing clearly (Figs. 2 and 3) that recurrent stone-formers pass significantly larger particles of calcium oxalate than their controls under the same conditions of dietary and fluid intake.

The reason for this difference in the size of the calcium oxalate crystals excreted by the two groups merits consideration. The first possibility is that the larger crystals and aggregates in the urines of the stone-formers may result from the increased crystal growth anticipated from the higher supersaturation concentrations of calcium oxalate in their urines (Robertson et al., 1971). Against this is the evidence that when a small quantity of sodium oxalate is added to the diet of the controls to increase the supersaturation of urine with calcium oxalate to the same level as that in the urines of the stone-formers on their basal diet (Robertson et al., 1971), there is no increase in the proportion of large particles in the urines of the controls (Fig. 3). Thus, although the controls under the stress of an oxalate load pass urines as supersaturated as those of the stone-formers on their basal diet, they do not form the large crystals and aggregates found in the urines of the stone-formers at the same level of supersaturation. This suggests that increased supersaturation alone is not responsible for the difference in the size of crystals passed by the two groups.

The second possibility is that normal urines contain some inhibitor (or inhibitors) of crystal growth and aggregation which is (are) deficient in the urine of the recurrent stone-formers. It is clear from the results of our \textit{in vitro} test system (Figs. 4 and 5) that normal urine does indeed contain some extremely potent inhibitor of crystal growth and aggregation that is active at low concentrations, and that urine from recurrent stone-formers appears to contain less of this material.

It will be interesting to identify the inhibitory material and to determine the factors which affect its excretion in the urine.
REFERENCES


