CALCIUM OXALATE CRYSTALLURIA AND URINE SATURATION IN RECURRENT RENAL STONE-FORMERS

W. G. ROBERTSON, M. PEACOCK AND B. E. C. NORDIN

Medical Research Council Mineral Metabolism Unit, The General Infirmary, Leeds

(Received 17 August 1970)

SUMMARY

1. The degree of saturation with calcium oxalate has been determined in fresh urine samples from six patients with recurrent calcium oxalate-containing renal stones and six normal control subjects who were studied under the same conditions of diet and fluid intake.

2. The degree of saturation of urine with calcium oxalate was significantly higher in the group of stone-formers than in the control series and more often exceeded the amount needed for spontaneous crystallization of calcium oxalate (formation product). This was accounted for by the higher concentration of calcium and oxalate in the urine of the stone-formers.

3. Crystals of calcium oxalate were observed in all freshly examined urines in which the formation product of calcium oxalate was exceeded. Since the formation product of calcium oxalate was exceeded more often in the urines of stone-formers than in the urines of the control subjects, this accounted for the greater calcium oxalate crystalluria of the stone-formers.

4. Addition of a small quantity of sodium oxalate to the basal diets of the two groups resulted in a greater increase in the urine saturation and calcium oxalate crystalluria of the stone-formers, thus accentuating the difference observed between the two groups when they were given the basal diet.

5. Calcium oxalate crystalluria was related quantitatively to the degree of oversaturation of urine with calcium oxalate, although uric acid solubility may play a small role at low pH values.

6. The results are consistent with a 'hyperexcretion–crystallization' mechanism of stone formation.

The simplest hypothesis of renal stone-formation is that it results from the trapping of crystals deposited in the kidney from urine oversaturated with one of the stone-forming salts. Such
oversaturation would follow in turn from the increased excretion of one or more of the constituents of the particular salt formed. Although there is much evidence to support this 'hyper-excretion-crystallization' theory in the formation of cystine stones (Harris & Warren, 1953; Dent & Senior, 1955) and uric acid stones (Henneman, Wallach & Dempsey, 1958; Metcalfe-Gibson, McCallum, Morrison & Wrong, 1965; Rapoport, Crassweller, Husdan, From, Zweig & Johnson, 1967) in man, the evidence for the operation of similar mechanism in the formation of calcium-containing stones is less convincing. In a study of 24 h urines from male idiopathic stone-formers and their controls, Robertson, Peacock & Nordin (1968) found that although these urines were generally supersaturated with calcium oxalate and less frequently with calcium phosphate they were usually not sufficiently supersaturated to cause spontaneous crystallization of these salts, even in the urine from hypercalciuric stone-formers. Indeed a search for crystals in the fresh urine of these stone-formers was invariably negative. However, in a later study which was confined to recurrent calcium oxalate stone-formers, Robertson, Peacock & Nordin (1969) found that under the same conditions of diet and fluid intake these patients excreted more crystalline calcium oxalate than their controls, the difference being mainly due to an increase in crystal size rather than crystal number. This was verified qualitatively by light microscopy which showed that the calcium oxalate crystalluria of the recurrent stone-formers consisted mainly of octahedral crystals of calcium oxalate dihydrate, often in aggregates up to 200 μm in diameter, whereas the calcium oxalate in control urines took the form of very small particles with little or no aggregation. It was noted that the increased crystalluria of the stone-formers was associated with increased urinary concentrations of calcium and oxalate, and the present paper relates the crystalluria to the degree of urinary saturation as defined by the calcium oxalate activity product (Robertson et al., 1968).

METHODS

Patients studied

The patients were six male stone-formers, aged between 24 and 54 years, who were passing calcium oxalate-containing stones at least twice per year. All of the patients had renal stones at the time of study but none had nephrocalcinosis or medullary sponge kidney. Apart from being stone-formers they were otherwise healthy with no known metabolic disorder (other than hypercalciuria). There was no evidence of urinary tract infection and all had normal renal function. These we have defined as idiopathic stone-formers. 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 limited to 1300 ml/day and given at fixed times, 100 ml of water at 09.30 hours, 300 ml of milk and 300 ml of tea at 10.00 hours, 150 ml of tea at noon, 150 ml of tea at 15.00 hours, 150 ml of tea at 18.00 hours and 150 ml of tea at 22.30 hours.

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 diet 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
Crystalluria and urinary saturation of a single oral dose of sodium oxalate (5 mg/kg body weight) added to the basal diet.

Analytical procedures

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., U.K.) as described by Robertson (1969a), crystalluria being measured as the volume of crystals per unit volume of urine (volume-concentration). The sample was maintained at 37° during the crystal-counting procedure. The type of crystal counted was identified by light microscopy.

The saturation of urine with the stone-forming salts was determined as described by Robertson et al. (1968) by using the following analytical techniques. The pH of freshly voided urine was measured with a glass electrode; calcium, magnesium, sodium and potassium were measured simultaneously by atomic-absorption spectroscopy and flame-emission photometry (Dawson, Ellis & Milner, 1968); ammonia (Chaney & Marbach, 1962); phosphate (Chen, Toribara & Warner, 1962); oxalate (Zarembski & Hodgkinson, 1965); citrate (McArdle, 1955); sulphate (Berglund & Sorbo, 1960); uric acid (Feichtmeir & Wrenn, 1955). Creatinine was measured by the standard AutoAnalyzer technique.

The activity products of octacalcium phosphate, calcium oxalate and magnesium ammonium phosphate were calculated by using the procedure described by Robertson (1969b, c). The solubility and formation products of these salts were those quoted by Robertson et al. (1968).

RESULTS

Qualitative relationship between crystalluria and urine saturation

The means and standard errors of the measured ion concentrations and the corresponding octacalcium phosphate, calcium oxalate and magnesium ammonium phosphate activity products in the urines of the recurrent stone-formers and their controls on the basal diet are compared in Table 1. This shows that the stone-formers have significantly higher concentrations of ionized calcium ($P<0.001$), total calcium ($P<0.01$) and oxalate ($P<0.02$) and significantly higher calcium oxalate activity products ($P<0.001$) than their controls under the same conditions of diet and fluid intake. There is no difference, however, between the octacalcium phosphate and magnesium ammonium phosphate activity products of the two groups.

The distributions of the calcium oxalate activity products of the two groups are shown in Fig. 1 in relation to the solubility and formation products of that salt. Those urines are indicated in which crystals of calcium oxalate were observed by light microscopy immediately after voiding. It can be seen first that crystals were present in all urines that exceeded the calcium oxalate formation product and, secondly, that the stone-formers produced crystals more frequently than the controls since their urines more often exceeded the formation product.

The means and standard errors of the measured ion concentrations and the corresponding activity products of the stone-formers and their controls after the oral dose of sodium oxalate are compared in Table 2. This shows that the stone-formers have significantly higher concentrations of creatinine ($P<0.01$), total calcium ($P<0.001$), magnesium ($P<0.001$), sodium ($P<0.01$), phosphate ($P<0.05$), oxalate ($P<0.01$), sulphate ($P<0.01$) and ionized calcium...
**Table 1.** Means (±1 SEM) of the concentrations and activity products in the urines of the stone-formers and their controls on the basal diet

<table>
<thead>
<tr>
<th>Ion</th>
<th>Controls (n = 21)</th>
<th>Stone-formers (n = 24)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/100 ml)</td>
<td>140 ± 13</td>
<td>146 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>pH</td>
<td>5.96 ± 0.16</td>
<td>6.07 ± 0.13</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Calcium (mm)</td>
<td>6.09 ± 0.79</td>
<td>7.8 ± 0.35</td>
<td>NS</td>
</tr>
<tr>
<td>Magnesium (mm)</td>
<td>3.31 ± 0.43</td>
<td>3.78 ± 0.25</td>
<td>NS</td>
</tr>
<tr>
<td>Sodium (mm)</td>
<td>136 ± 12</td>
<td>132 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Potassium (mm)</td>
<td>63 ± 5</td>
<td>59 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Ammonium (mm)</td>
<td>30.4 ± 4.2</td>
<td>26.4 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphate (mm)</td>
<td>24.3 ± 3.3</td>
<td>23.2 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Oxalate (10⁻⁴ M)</td>
<td>2.34 ± 0.19</td>
<td>3.07 ± 0.24</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Citrate (mm)</td>
<td>3.54 ± 0.44</td>
<td>2.62 ± 0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Sulphate (mm)</td>
<td>14.5 ± 1.9</td>
<td>17.3 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Ionized calcium (mm)</td>
<td>2.95 ± 0.45</td>
<td>5.10 ± 0.34</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

- log (octacalcium phosphate activity product) | 47.73 ± 0.56 | 46.50 ± 0.40 | NS |
- log (calcium oxalate activity product)     | 7.82 ± 0.04   | 7.58 ± 0.03   | < 0.001 |
- log (magnesium ammonium phosphate activity product) | 14.75 ± 0.18 | 14.69 ± 0.16 | NS |

NS = not significant.

**Fig. 1.** The distributions of calcium oxalate activity products (expressed in negative logarithms) in the urines of the recurrent stone-formers and their controls on the basal diet. Urines are indicated in which crystals were observed on voiding. ●, +Crystals; ○, −crystals.
Crystalluria and urine saturation

(P<0.001) and also significantly higher calcium oxalate-activity products (P<0.001) than the controls. As observed by Robertson et al. (1969), many of these differences between the ion concentrations in the urines of the stone-formers and their controls are attributable to a 40% increase in the urine flow rate of the controls, an increase not observed among the stone-formers on the same regimen. Thus, when compared with the basal diet, the oral dose of oxalate produces a decrease in the concentrations of most ions (except oxalate) in the urines of the controls.

As observed by Robertson et al. (1969), many of these differences between the ion concentrations in the urines of the stone-formers and their controls are attributable to a 40% increase in the urine flow rate of the controls, an increase not observed among the stone-formers on the same regimen. Thus, when compared with the basal diet, the oral dose of oxalate produces a decrease in the concentrations of most ions (except oxalate) in the urines of the controls.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Normal controls (n = 15)</th>
<th>Stone-formers (n = 20)</th>
<th>P₁ (n=15)</th>
<th>P₂ (n=15)</th>
<th>P₃ (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/100 ml)</td>
<td>102±10</td>
<td>152±12</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>pH</td>
<td>6.06±0.16</td>
<td>6.06±0.14</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium (mm)</td>
<td>4.21±0.72</td>
<td>8.05±0.66</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Magnesium (mm)</td>
<td>2.17±0.24</td>
<td>3.64±0.29</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sodium (mm)</td>
<td>109±7</td>
<td>144±9</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Potassium (mm)</td>
<td>55±5</td>
<td>69±5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ammonium (mm)</td>
<td>20.6±2.2</td>
<td>25.1±2.9</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphate (mm)</td>
<td>19.3±2.8</td>
<td>26.2±1.8</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Oxalate (10⁻⁴ mol)</td>
<td>3.70±0.37</td>
<td>6.17±0.72</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Citrate (mm)</td>
<td>3.11±0.39</td>
<td>2.67±0.30</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Sulphate (mm)</td>
<td>11.3±1.5</td>
<td>18.0±1.3</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Ionized calcium (mm)</td>
<td>1.85±0.39</td>
<td>4.06±0.35</td>
<td>NS</td>
<td>&lt;0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>-log (octacalcium phosphate</td>
<td>48.14±0.79</td>
<td>46.15±0.52</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>activity product)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-log (calcium oxalate activity</td>
<td>7.67±0.06</td>
<td>7.32±0.04</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>product)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-log (magnesium ammonium</td>
<td>14.89±0.26</td>
<td>14.37±0.23</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>activity product)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P₁, Comparison of controls on basal diet and after oral dose of sodium oxalate.
P₂, Comparison of stone-formers on basal diet and after oral dose of sodium oxalate.
P₃, Comparison of stone-formers and controls after oral dose of sodium oxalate.
NS = not significant.

(Table 2, P₁) but no change (except for an increase in the concentration of oxalate) in those of the stone-formers (Table 2, P₂). This observation, although repeated, has not yet been explained. The mean calcium oxalate activity product of the stone-formers after the oral dose of oxalate is significantly higher (P<0.001) than on the basal diet whereas that of the controls is only slightly higher (P<0.05).

The distributions of the calcium oxalate activity products in the two groups are shown in Fig. 2 in relation to the solubility and formation products of that salt. Urines are indicated in which crystals of calcium oxalate were observed on voiding. Comparison of Fig. 2 with Fig. 1 shows the marked effect of the oral dose of sodium oxalate on the saturation of urine from the stone-formers. The parallel increase in calcium oxalate crystalluria has already been demonstrated (Robertson et al., 1969).
Quantitative relationship between crystalluria and urine saturation

Fig. 3 shows the quantitative relationship between the volume of calcium oxalate crystals per unit volume of urine and the calcium oxalate activity products in urines from the stone-formers and their controls on the basal diet and after the oral dose of sodium oxalate. It can be seen that as the oversaturation increases above the formation product of calcium oxalate an

Fig. 2. The distributions of calcium oxalate activity products (expressed in negative logarithms) in the urines of the recurrent stone-formers and their controls after the oral dose of sodium oxalate. Urines are indicated in which crystals were observed on voiding. ●, +Crystals; ○, −crystals.

Fig. 3. The volume-concentration of calcium oxalate crystals in the fresh urines at 37° (pH ≤ 6.0) of the recurrent stone-formers and their controls on the basal diet and after the oral dose of sodium oxalate in relation to the corresponding calcium oxalate activity products (expressed in negative logarithms). The line connects mean values (± 2 SEM). ●, Stone-formers; ○, controls.
Increasing amount of the salt is precipitated. Since the controls did not produce large amounts of crystals even after the oral dose of oxalate, comparison of the two groups is limited to the region immediately above the formation product. In this region there is no difference between the crystalluria of the stone-formers and that of their controls.

**Effect of uric acid saturation on calcium oxalate crystalluria**

Urines with a high uric acid content tended to yield larger volumes of calcium oxalate crystals, an effect which increased with increasing urine acidity. Fig. 4 shows the relationship in the urines from two stone-formers between calcium oxalate crystalluria and uric acid saturation as defined by the ion product $K_{UA}$, where $K_{UA} = \{H^+\} \times [UA]$. In this expression, $\{H^+\}$ is the hydrogen ion activity, and [UA] is the concentration of uric acid in urine. There is a high correlation between calcium oxalate crystalluria and uric acid saturation in this region ($r = 0.8677$). Moreover, this relationship is independent of urine volume since there is no correlation between calcium oxalate crystalluria and creatinine concentration in the same urine samples ($r = 0.003$).

**DISCUSSION**

Although it has previously been shown that urine is generally supersaturated with calcium oxalate and less frequently with calcium phosphate, idiopathic stone-formers (i.e. patients with hypercalciuria as their only demonstrable metabolic abnormality, see the Methods section), have only a tendency to pass more highly supersaturated urines than their controls (Robertson et al., 1968). Their urines do not consistently exceed the upper limit of metastable supersaturation (i.e. the formation product) and consequently do not form crystals. Indeed, a search for calcium oxalate crystals in random fresh urines of these stone-formers was invariably negative.

One of the problems in studying calcium stone-formation in man is that the average re-
currence period is of the order of 9–10 years (Williams, 1963). If stone-formation is a phasic disturbance with such a long intervening period of inactivity, the chance of observing the critical initiation stage by studying random 24 h urine specimens is remote. The present investigations were confined to a small group of male idiopathic stone-formers who were forming calcium oxalate-containing stones several times each year, in order to increase the possibility of observing this critical period. The results showed that under the same conditions of diet and fluid intake these recurrent stone-formers excreted more crystalline calcium oxalate than their normal controls. This increased crystalluria was associated with increased urinary concentrations of calcium and oxalate (Robertson et al., 1969).

Fig. 1 and Table 1 show that as a direct result of their higher urinary concentrations of calcium and oxalate, the recurrent stone-formers but not the controls were consistently passing urine which was sufficiently oversaturated with calcium oxalate to cause spontaneous precipitation of that salt. The urines could remain in the metastable region of supersaturation for some time without precipitation taking place, but there was no difference between the two groups, with respect to the degree of saturation at which crystals first formed. This indicates that urine from the stone-formers did not contain a specific nucleator of calcium oxalate and that urine from the control subjects did not contain an inhibitor for the homogeneous nucleation of calcium oxalate. This has been confirmed by studies in vitro (Robertson, 1969c).

There is a quantitative relationship between the volume-concentration of calcium oxalate crystals and the degree of oversaturation of urine (Fig. 3), i.e. as the oversaturation increases above the formation product of calcium oxalate an increasing amount of salt is precipitated. Thus the significant difference in the activity products of the recurrent stone-formers and their controls probably accounts for most of the difference in calcium oxalate crystalluria between the two groups.

In the region immediately above the formation product, however, where there appears to be little relation between calcium oxalate crystalluria and calcium oxalate saturation, uric acid crystals probably play a small but significant role as nucleators of calcium oxalate precipitation. This effect becomes apparent in urines of low pH because the proportion of urate present as relatively insoluble uric acid is directly related to the acidity of the urine. Uric acid has been shown to affect the crystallization of calcium oxalate in vitro (Mayer, Chase, Farvas, Waidh, Longo, Karp & Zinsser, 1968) a process that is theoretically possible on the basis of epitaxial growth (Lonsdale, 1968).

The effect of the oral dose of sodium oxalate was to produce a small but significant increase \( (P<0.05) \) in the calcium oxalate activity products of the controls and a much larger increase \( (P<0.001) \) in those of the stone-formers. Thus the oral dose of oxalate accentuated the pre-existing difference between the calcium oxalate activity products of the two groups and, as shown earlier (Robertson et al., 1969), also increased the difference between the calcium oxalate crystalluria of the two groups.

Increasing the excretion of calcium, on the other hand, had much less effect than increasing the excretion of oxalate on calcium oxalate crystalluria in the stone-formers (Robertson et al., 1969). Indeed, experiments in vitro have shown that increasing the calcium concentration of an average urine tenfold will not cause calcium oxalate to precipitate whereas increasing the concentration of oxalate to the upper end of the normal range will usually produce crystallization (W. G. Robertson, unpublished results). This confirms the prediction made from theoretical curves by Robertson & Nordin (1969) that hypercalciuria per se may not be as
important in calcium oxalate precipitation as is often thought. However, if the concentration of urinary calcium is very high, only a small increase in urinary oxalate excretion is necessary to produce supersaturation of the urine and precipitation of calcium oxalate.

In the present study the relatively high urine oxalate of the stone-formers combined with their high urinary calcium to produce calcium oxalate activity products above the formation product of that salt. The resulting crystals could then have grown and aggregated under the existing supersaturated conditions and perhaps could have become trapped in the urinary tract. Indeed, the stone-formers frequently complained of 'passing gravel' and three of them had brief attacks of renal colic during the study. The results are therefore consistent with a 'hyperexcretion-crystallization' mechanism of stone-formation similar to that described by Vermeulen and his coworkers (1966, 1967) for oxamide stone-formation in rats.

ACKNOWLEDGMENTS

We thank Dr J. B. Dawson of the Department of Medical Physics, University of Leeds, for the use of his scanning multichannel spectrophotometer, Mrs Margaret Abbott and Mr R. Milner for their technical assistance, and Miss Ursula Sturzenegger for typing the manuscript. We also express our appreciation to Mr P. B. Clark, Mr R. E. Williams and Mr P. H. Smith of the Department of Urology for referring the patients with renal stone disease.

REFERENCES


W. G. Robertson, M. Peacock and B. E. C. Nordin


