Urological Research 4, 161-167 (1976)

Urological Research

A Contribution to the Formation Mechanism of Calcium Oxalate Urinary Calculi

III. On the Role of Magnesium in the Formation of Oxalate Calculi

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Received: August 28, 1975

Summary. The influence of magnesium in vitro on the precipitation of calcium oxalate was investigated. Even at maximum physiological magnesium concentrations a litholytic effect could not be observed, but the retardation of the calcium oxalate crystallization caused by magnesium might be decisive for a reduction in calculi formation. The enlargement of the calcium oxalate crystals and aggregates caused by the retardation of crystallization, however, should be regarded as a contraindicating factor for Mg therapy in oxalate calculous disease. It is safe to say that high magnesium concentrations prevent the conversion into Whewellite of the calcium oxalate calculi substance primarily formed as Weddellite.

Key words: Urinary calculi - Calcium oxalate - Magnesium - Solubility - Crystallization.

The effects of magnesium ions on calcium oxalate solubility and its inhibition of endogenous oxalic acid formation (19, 25, 28) are known from the first studies made by Klemperer and Trischler (18) and from the fundamental in vivo and in vitro experiments carried out by Hammarsten (12).

A high Ca/Mg ratio in association with increased oxalic acid excretion is considered to be a cause for the formation of urinary calculi (22, 27, 30). Treatment with magnesium is aimed at increasing urinary magnesium and so reducing the Ca/Mg quotient.

Numerous authers have reported a significant reduction in recurrence rate after treatment with magnesium (7, 20, 23, 24, 26). Although this effect has been confirmed in animal experiments (1, 3, 16), the rationale of magnesium therapy has been increasingly questioned in recent years (5, 6, 21, 29, 31).

The investigation of the effect of magnesium and other urinary minerals on quantitative and qualitative calcium oxalate precipitation were carried out in an attempt to help clarify the mode of action of magnesium in urinary stone disease.

MATERIAL AND METHOD

Precipitation

The calcium oxalate precipitations were made by the addition of ammonium oxalate to calcium solutions, to which different concentration of cations, organic compounds (cf. 14, 15) and phospate buffer (pH 7) had been added. Precipitation was carried out at 37° C using 2 mmol of NH_4 oxalate unless otherwise stated. Each precipitation was repeated ten times and compared with precipitations from calcium solutions without admixtures.

Series A	I	1000 ml containing 2 mmol Ca^{++} + 20 mmol Mg^{++}
	II	1000 ml containing 2 mmol Ca^{++} + 2 mmol Mg^{++}
	III	1000 ml containing 2 mmol Ca^{++} + 0.2 mmol Mg^{++}
Series B	I	1000 ml containing 2 mmol Ca^{++} + 2 mmol Ni^{++} (Zn^{++})

II 1000 ml containing 2 mmol Ca^{++}
\+ 0.2 mmol Ni^{++} (Zn^{++})

Series C 1000 ml containing 4 mmol Mg^{++}
\+ 0.5 mmol NH_4 oxalate
precipitated with 20 ml of
0.1 m $CaCl_2$ solution

Series D I 100 ml urine containing 2 mmol Ca^{++}
\+ 2 mmol Mg^{++}

II 100 ml urine containing 2 mmol Ca^{++}
\+ 0.4 mmol Mg^{++}

III* 100 ml dialysate of urine from D I

IV* 100 ml dialysed urine from D I

V 100 ml water containing 1 mmol Ca^{++}
\+ 833 mmol urea

*for preparation see previous papers (15)

Series E I 100 ml of aqueous solution containing
Ca^{++} 8 mmol/l ; Mg^{++}
4 mmol/l ; Na^+ 160 mmol/l
K^+ 48 mmol/l ;
Zn^{++} 0.024 mmol/l
Me^{++} 0.0016 mmol/l (Me = Co, Ni, Mn, Cu)
Creatinine 136 mmol/l
Urea 2.8 mol/l

II as above but with 8 mmol/l Mg^{++}

III as above but no Mg^{++}

IV as above but no organic components.

The centrifuged precipitates were weighed after they had been washed several times with NH_4-oxalate solution, methanol, ether, and dried under vacuum. The precipitates were analysed by infrared spectroscopy (UR 20 twin-beam spectrophotometer, Carl Zeiss, Jena).

Dissolving Tests on Synthetic Calcium Oxalate in Mg Solutions

Test Series F: 50 mg of calcium oxalate were suspended in 50 ml of aqueous solutions containing 0.34 mmol and 0.11 mmol of $MgCl_2$ as well as in 50 ml of an aqueous solution containing 0.34 mmol of $MgCl_2$ + 0.34 mmol of $CaCl_2$ each and stirred at 37° C for 5 hours. The suspensions were then cooled to 25° C, the undissolved calcium oxalate was centrifuged and weighed after being washed with methanol and ether and dried under vacuum.

Investigations as to the Crystal Aggregation

The volumes of the precipitates from solutions E I through E IV were determined after weighing in small measuring glasses graduated in 1/10 cm^3 in order to examine the influence of magnesium on the crystallization rate of calcium oxalate. The precipitate density was obtained from the g/cm^3 quotient.

Optical observation of the crystals was made with a polarization microscope "Laboval-pol" (Jenoptik Jena GmbH) at a magnification of 640 x. For taking pictures the precipitations were effected at 60° C so that the crystallites of the precipitates become more clearly visiable.

Analysis of the Foreign Ion Oxalates

In order to check the qualitative composition of the metallic oxalates (Mg, Zn, Ni, Co, Mn, Cu), accurate quantities of the samples were rendered soluble in $HClO_4/HNO_3$ mixtures (1 : 1) by means of a return condenser. The foreign ions were determined complexometrically with disodic ethylene-diamine-tetra-acetate from aliquot parts of the largely neutralized attracking solutions of unknown ions, which were made up to 100 ml. The quantitative determination of C and H was made by combustion analysis. The exact atomic equivalents and thus the molecular formulae of the foreign ion oxalates were obtained from the percentages calculated by taking oxalate oxygen into consideration.

RESULTS

Table 1 shows the results of the calcium oxalate precipitations from aqueous solution mixtures containing 2 mmol of Ca with stepwise addition of magnesium (test series A).

The calcium oxalate precipitations carried out in test series B in the presence of other cations (Zn, Ni) show a precipitation output above the theoretical quantitiy of 2 mmol of Weddellite with a calcium-metal ion quotient of 1 : 1, as indicated in Table 2.

These cations yield oxalate precipitates of low solubility in the highly acid precipitation environment (pH 1) which could be identified as complexes of the general formula

$$Me^{II}\left[Me^{II}\ (C_2O_4)_n\right]^{(2n-2)^-} \cdot x\,H_2O,$$

$$\left[n\ (\text{for Zn, Mn, Co, Cu}) = 4;\ \text{Ni} = 6\right].$$

Such a complex is also known for magnesium (13), but this is more soluble than calcium oxalate (Wh = 8 mg/l, Mg oxalate 300 mg/l).

Conductivity determinations and measurements of the magnetic moments show that calcium in an aqueous solution does not form such complexes (11).

If the formation of a slightly dissociated Mg complex, the addition of calcium ions to the $MgCl_2/NH_4$ oxalate mixture of test series C

Table 1. Test series A - precipitations of calcium oxalate from Ca-Mg mixtures at 37° C with 2 milli mol of NH_4 oxalate

Ca/Mg[a] of the precipitation solution	Calcium oxalate precipitation (mg)	Infrared analysis of the precipitates (Weddellite/Whewellite in %)
0.1	322.2	100/0
1	290.4	10/90
10	285.5	10/100
Blank, without Mg	286.0	0/100

[a] related to 2 mEq of Ca ≙ 328 mg of Weddellite (Wd) or 292 mg of Whewellite (Wh).

Table 2. Test series B - precipitations of calcium oxalate from Ca-Ni and Ca-Zn solutions at 37° C with 2 milli mol of NH_4 oxalate

Me	Ca/Me of the precipitation solution	Calcium oxalate precipitation (mg)	Infrared analysis of the precipitates (Weddellite/Whewellite in %)
Ni	1	357.6	Wd + Ni oxalate
	10	284.9	0/100
Zn	1	342.5	Wd + Zn oxalate
	10	286.9	10/90

should not cause an oxalate precipitation, or only a slight one. However, 80.1 mg of Weddellite were formed. The blank test without Mg resulted in 74.9 mg (≙ 0.5 mg) of Whewellite.

Calcium oxalate precipitations from concentrated urea solutions likewise did not show any solubility effect. Precipitation tests from urine did show a reduction of the calcium oxalate precipitation by some 15 %, but this is irrespective of the magnitude of the Ca/Mg quotient, as indicated in Table 3 (test series D), and has to be attributed to other litholytic factors in the urine. In contrast to that, the theoretical values of 2 mmol of calcium oxalate were obtained from the organic dialysis fractions of high molecular weight (molecular weight > 5.000) and from the organic-mineral dialysis fractions of low molecular weight (Table 3).

The theoretical amounts of calcium oxalate were obtained by precipitation from the simulated urine solutions (solutions E I-IV) which are comparable with normal urine in their absolute and relative mineral composition, independent of the magnesium level.

The higher dilution of the precipitation solutions hinders the stabilising effect of magnesium so that Weddellite was obtained only in model solution E II having a Ca/Mg quotient of 1, whereas all other precipitation solutions E I, III, and IV having quotients > 2 yielded precipitates containing a high share of Whewellite.

Even the addition of urea and creatinine to solution E I did not increase the proportion of Weddellite as is shown by comparing the infrared analysis of the precipitates from solutions E I and E IV. These results are listed in Table 4.

The dissolving tests carried out on 50 mg of synthetic calcium oxalate in test series F showed a solubility effect up to 10 %, but there was none when the tests were carried out in Ca/Mg solutions having a Ca/Mg ratio of 1 (Table 5).

Table 3. Test series D - precipitations of calcium oxalate from Ca solutions of different composition with 2 milli mol of NH_4 oxalate

Precipitation solution	Ca/Mg of the precipitation solution	Ca oxalate precipitation (mg)	Infrared analysis of the precipitates (Weddellite/Whewellite in %)
I (urine)	1	281.7	100/0
II (urine)	5	271.0	50/50
III (dialysis solution)	1	321.2	100/0
IV (dialysed urine)	-	290.4	20/80
V (urea solution)	-	326.2	100/0
Control (Without Mg)	-	285.0	0/100

Table 4. Test series E - precipitations of calcium oxalate from 100 ml of model solution (100 ml ≙ 0.8 mEq of Ca = 131.5 mg of Weddellite or 116.8 mg of Whewellite)

Model solution	Variation of the precipitation solution	Ca oxalate precipitation (mg)	Infrared analysis of the precipitates (Weddellite/Whewellite in %)
I	Ca/Mg = 2	119.2	20/80
II	Ca/Mg = 1	129.5	90/10
III	without Mg	118.0	10/90
IV	Ca/Mg = 2 without organic components	119.0	20/80

Table 5. Test series F - dissolving tests of 50 mg of synthetic Ca oxalate (= 0.34 mEq of Ca) in Mg and Ca-Mg solutions

	Weight (mg) of calcium oxalate after treatment with		
0.34 mEq of Mg	0.11 mEq of Mg	0.34 mEq of Mg 0.34 mEq of Ca	Control
44.2	46.5	49.5	49.6

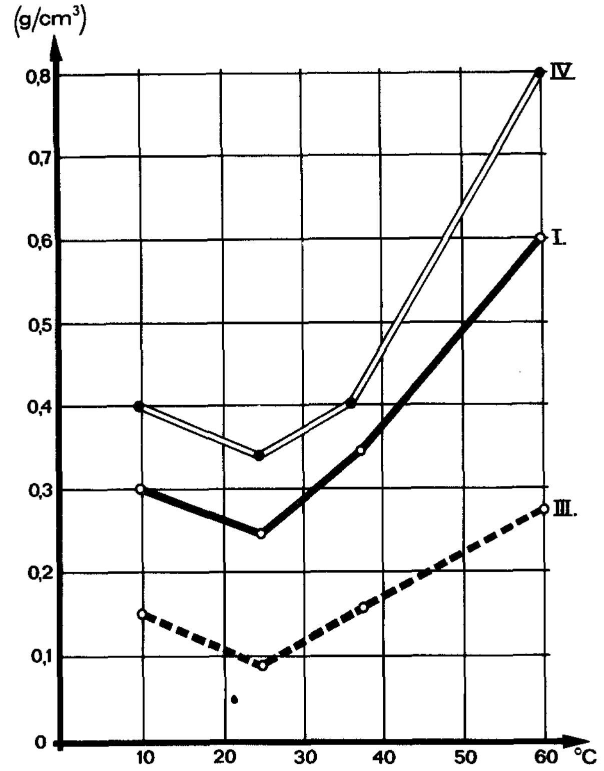

Fig. 1. Precipitate density as a function of the composition and precipitation temperature of the precipitation solution (I, III, and IV correspond to the model solutions of Table 4, Test series E)

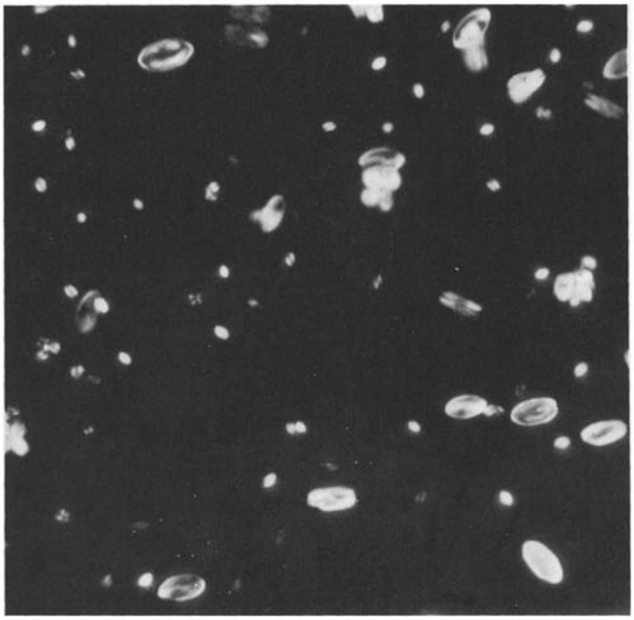

Fig. 2. A mixture of calcium oxalate crystallites from model solutions E III and E IV in polarizing microscope Magnification: 640 x

All precipitation tests showed that the calcium/metal ion quotient influences the hydrate type of the calcium oxalate precipitated (Tables 1, 3, and 4). High proportions of metal ions (Ca/Me = 1) in the precipitation solutions always result in stable Weddellite precipitates. We investigated the stabilising effect in detail using magnesium (15) since all other metal ions tested are present in urine only in very small quantities (Ca : Me $1:1 \times 10^{-3}$ to $1:1 \times 10^{-5}$) and have no influence on the formation of Weddellite.

Since in all precipitation tests magnesium led to a marked retardation of precipitation (varied with concentration max. 4 seconds), we investigated this effect in correlation with the crystal size in test series E. The quotients, i.e. weight in grams per precipitate volume, calculated as the measure of precipitate density (and thus of crystal size) are given in Figure 1 as a function of the composition of solution and precipitation temperature. From these quotients it can be seen that a lower Ca/Mg quotient (solutions E I and E IV) produces crystals of larger size whereas precipitations from solutions poor in magnesium (solution E III) produce very small, poorly aggregated calcium oxalate crystals. Figure 2 shows a mixture of small crystals from solution E III and a mixture of large crystals and spherolites from solution E IV.

DISCUSSION

The precipitation tests described above show that magnesium, even at favourable concentration levels in relation to calcium (Ca/Mg = 1), in vitro has no significant solubility or inhibitor effect. The increasing solubility of calcium oxalate by a factor of 20 under the influence of Mg in vitro obtained by Hammarsten and other authors necessitates a supply of magnesium ions which cannot be reached under physiological conditions. Calculations of the equilibrium of magnesium oxalate ions in the average standard urine showed that a maximum of 40 %, and in urines having higher Mg values a maximum of 50 %, of the oxalate ions are bound by Mg ions (25). The reduction of the oxalate ions to one half merely involves an increase of the calcium ion concentration in the ion product of calcium oxalate (1.3×10^{-7} at pH6) and thus an additional solubility of 3.6×10^{-4} mol/l. (The influence of the complexing agents PO_4^{3-}, SO_4^{2-}, and citrate is eliminated here so that a high proportion of the total Ca is available in ionized form). It is open to question whether oral Mg treatment is useful in cases of oxaluria with regard to prophylaxis against recurrence.

From our experiments and from the experimental and clinical observations of other authors it can be concluded that lack of magnesium alone without other litholytic factors cannot be the cause of the oxalate calculi disease. However, since magnesium therapy has been shown to be effective in stone prophylaxis and reduces calcium excretion in oxalate stone formers with hypercalciuria alternative explanations are required. The inhibiting effect of magnesium in conjunction with vitamin B_6 on the endogenous formation of oxalic acid may be a factor.

It is known that calculi formers excrete larger aggregates of calcium oxalate than normals. Our optical investigations of precipitates show that calcium precipitation solutions having Ca/Mg quotients < 2 yielded a high proportion of crystal aggregates and larger crystals in contrast to calcium solutions containing less magnesium. These findings, however, contrast with the observations made by Fleisch et al. (8) who could not find any aggregating effect of magnesium. It is possible that the retardation of calcium oxalate crystallization caused by the formation of the relatively large Mg-oxalate complex molecules (13), which was observed by us and also by Desmars and Tawashi (5), is of importance for the formation of oxalate calculi. Thus, magnesium could influence the frequency of crystal formation. The higher this frequency, the smaller are the crystals formed. With a lower frequency of formation of nuclei and retardation of crystallization, fewer crystal nuclei will be formed, but these may grow to form large monocrystals or spherolites of a finite size, depending on the absolute value of oversaturation. These structures may be excreted, add to an existing calculus, or aggregate in stagnant urine (9).

We already reported in detail on the influence of magnesium on the precipitation and stabilisation of Weddellite in synthetic solutions and urine (15). Lagergren also stated that the conversion of Weddellite into Whewellite in the urine depends on the magnesium level (4).

The incorporation of magnesium ions into the crystal structure of the Weddellite precipitates is a prerequisite for stabilisation and can be traced by means of the "Langmuir Adsorption Isotherm" depending on their concentration in the precipitation solution (5). While a small Ca/Mg quotient stabilises the primarily formed Weddellite conglomerates, these can grow to form large crystal aggregates, which due to mechanical irritation tend to be excreted compared to Whewellite calculi which have smooth, non-crystalline surfaces.

Acknowledgement. We are particularly grateful to Mrs. G. Beck for technical assistance.

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