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A Contribution to the Formation Mechanism of Calcium Oxalate Urinary Calculi

I. Stabilising Urinary Constituents in the Formation of Weddellite

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Summary. 25 to 30% of calcium oxalate urinary calculi consist of the metastable Weddellite crystal phase. By fractionation of urine it was found that mineral substances are stabilising factors. The stability was checked in dry condition at room temperature, at 38° C and at 110° C. These results could be confirmed by precipitation from synthetic solutions. Mg, Zn, Ni, Co, Mn and Cu individually, and above all in combination, promote the formation of Weddellite. The formation of mixed crystal phases must be considered one of the main factors for the stabilisation of Weddelite in the urinary calculus.

Key words: Urinary calculi - Whewellite - Weddellite - Stabilising factors.

Calcium oxalate is the basic constituent of 65% of all urinary calculi (2) and disease statistics show an increasing incidence of these stones (3). The successful treatment regimes for uric acid and struvite calculi contrast with the results in oxalate calculous disease. The formation factors for oxalate calculi may be different so that a universally applicable model of stone formation cannot be formulated. The metabolic processes which result in an increased excretion of oxalic acid and hypercalciuria have to be examined as well as a variety of other urinary factors contributing to the formation and aggregation of crystals, such as:

The role of magnesium in solubility,
Calcium-citrate complex formation,
Pyrophosphates (and possibly diphosphonates) preventing aggregation.

The physical and chemical methods employed in the analysis of urinary calculi permit the differentiation of two crystal phases of calcium oxalate, which differ in their crystal water content:

1. Whewellite - Calcium Oxalate Monohydrate
2. Weddellite - Calcium Oxalate Dihydrate.

Whewellite is the more stable modification in terms of thermodynamics and forms the main component in 70 to 80% of calcium oxalate stones. Mixed calculi of both hydrate forms are frequently found. Synthetic, analytically pure Weddellite is unstable at room temperature and forms Whewellite by yielding 1 molecule of water. Weddellite urinary calculi and synthetic Weddellite precipitates, however, are stable for an unlimited time.

Up to now, not much attention has been devoted in the literature to the formation of two different crystal phases of calcium oxalate with different stabilities. As a first step in an investigation of the factors influencing the formation of Whewellite or Weddellite, precipitations of calcium oxalate from urine fractions after dialysis and incineration were carried out. Urine was also simulated by means of synthetic solutions.

MATERIAL AND METHOD

Precipitation of Calcium Oxalate

2 mmol of ammonium or sodium oxalate in 2 ml of distilled water were added at one time to

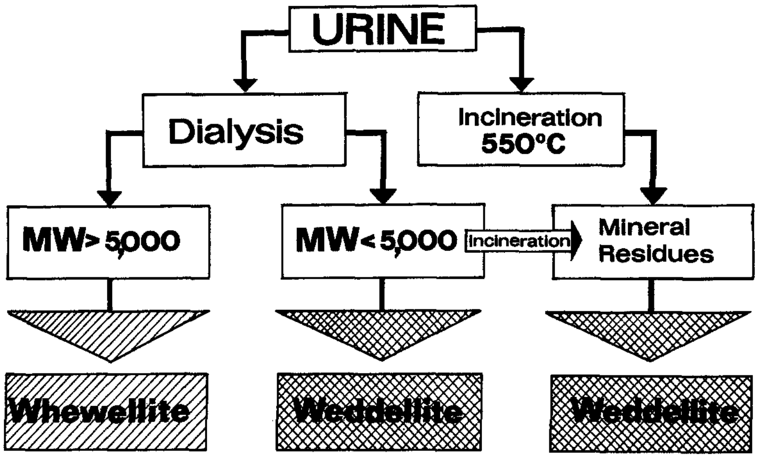

Fig. 1. Precipitation of calcium oxalate from different urine fractions

20 ml of a M/10 $CaCl_2$ solution (or 20 ml of urine or urine fraction). The calcium chloride solution contained various additional substances. The precipitates were removed by centrifugation, washed twice with oxalate-containing water and once each with methanol and ether. Drying was effected in air or under vacuum at room temperature. In the case of experiments deviating from room temperature, all solutions were brought to the same temperature.

Aliquots of 24 hr urine collections from 10 patients with calcium oxalate stones and 10 healthy controls were fractionated. The separation of the compounds of high molecular weight (>5000) was by dialysis against bidistilled water in collodion sleeves (Sartorius Type SM 13200). The dialysate (fraction 2) and the dialysed urine (fraction 1) were diluted or reduced to the initial urine volume.

The mineral constituents were isolated by evaporation of urine to dryness at 100° C and subsequent incineration at 550° C (16 hours) in a muffle furnace. The ash was dissolved in 20 ml of 10 % hydrochloric acid and then made up to the initial volume with distilled water.

Analysis of the Precipitates

The qualitative and quantitative evaluation of the calcium oxalate precipitations was carried out by infrared spectroscopy (4). All chemicals used were of analytical purity.

RESULTS

Precipitation of Calcium Oxalate from Urine

Weddellite precipitates were always obtained in the precipitation at 38° C of calcium oxalate from the untreated urine from healthy controls and patients suffering from urinary calculi. After the separation of the urine samples into fractions,

1. molecular weight >5000 (dialysed urine)
2. molecular weight <5000 (dialysate)
3. mineral constituents (evaporation)

different hydrate phases of the calcium oxalate occur in the precipitations (Fig. 1). The compounds of high molecular weight (fraction 1) contained in the urine did not influence the formation of calcium oxalate. At a temperature of 37° C Whewellite was obtained just as from an aqueous solution of calcium chloride (vide infra). The urine components of low molecular weight (including all metal cations) (fraction 2) promote the formation of Weddellite. Weddellite was also obtained in precipitations from the dissolved ash residue (fraction 3).

Precipiation of Calium Oxalate from an Aqueous Solution

In precipitations of calcium oxalate from an aqueous solution of calcium chloride at 38° C the stable Whewellite was always obtained. If the calcium oxalate precipitation from an aqueous solution was carried out in the presence of certain cations, Whewellite/Weddellite mixtures were formed, and above certain cation concentrations, pure Weddellite precipitates were always formed such as with a Ca: Mg: K concentration ratio of 1 : 20 : 6.

The bivalent Mg, Zn, Ni, Co, and Mn ions had a similar influence. At calcium/metal cation molar concentration ratios of 1 : 0.1 they individually resulted in stable Weddellite precipitates. But these metal ion concentrations were (except for magnesium) above physiological levels. At physiological ratios of $1 : 1 \times 10^{-3}$ for zinc and $1 : 1 \times 10^{-5}$ for nickel, cobalt and manganese, unstable Weddellite precipitations were obtained. This stabilising effect of bivalent cations on Weddellite was increased if several of the specified bivalent ions were simultaneously present during precipitation. The ion combinations are indicated in Table 1.

In all different physiological combinations of molar concentration rations pure Weddellite precipitates were obtained.

Stability Test

The stability tests of the air-dried Weddellite preparations showed different results depending on the precipitation conditions.

At 5° to 10° C, Weddellite precipitates could also be obtained from aqueous solution. These precipitates changed completely into Whewellite within 24 hours at room temperature. At temperatures of 38° C or 110° C this change was completed within 3 to 5 hours. Weddellite urinary calculi, however, were stable for weeks at 110° C. In the dry condition and in the presence of different concentrations of single ions and ion combinations the Weddellite precipitates were also very stable at 37° C. Variations depending on the concentration of metal cations could be observed in the hot-air cabinet at 110° C. In Figure 2 this is shown with magnesium as an example.

At higher concentrations (Ca/Mg 3 : 1 to 1 : 1) magnesium promoted the precipitation of temperature-stable Weddellite. The physiological ion combinations as shown in Table 1 promoted the stability of the dry Weddellite precipitates to such an extent that no change could be observed at 110° C for several weeks.

DISCUSSION

The fact that 16.9 % of all urinary calculi (2) consist of a crystal phase which is metastable as a pure substance is ascribed by us to the participation of additional urine constituents in Weddellite crystal formation (1).

Weddellite precipitates were always obtained from urine at 38° C in vitro. By fractionation of urine it was found that mineral substances preferentially promote Weddellite formation. The effect of magnesium was particularly interesting because this element is used in the prophylaxis of calcium oxalate calculous disease. At the same time it promoted the formation of Weddellite at physiological concentrations. This contradiction gave rise to the investigation of the prophylactic effect of magnesium in detail (5).

Table 1. Molar ion ratios for the precipitation of Weddellite

	Ca	Mg	Zn	Mn	Co	Ni	Cu
I	1	10^{-1}	10^{-3}	10^{-4}	10^{-4}	10^{-4}	10^{-4}
II	1	3×10^{-1}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}
III	1	3×10^{-1}	10^{-3}	10^{-5}	10^{-5}	10^{-5}	10^{-5}

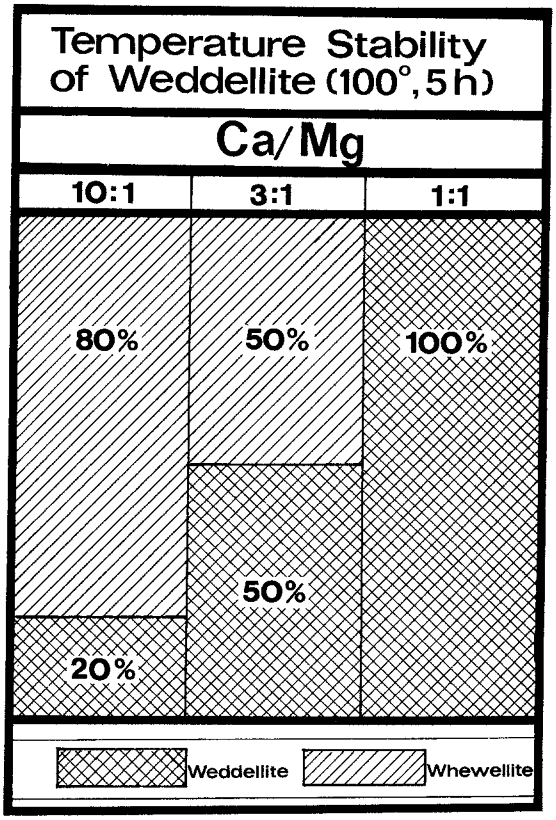

Fig. 2. The influence of magnesium on the temperature stability of Weddellite

According to the available results the formation of mixed crystal phases seems to be the main cause for the stabilisation of Weddellite. The stability of dry Weddellite urinary calculi for years can be explained by these experiments.

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