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# Can the Formation of Calcium Oxalate Stones be Explained by Crystallization Processes in Urine?

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Most investigators agree that stone formation is related to crystallization processes in urine. But despite enormous research in this filed our knowledge has remained fragmentary and stone metaphylaxis is therefore arbitrary. This review discusses the problems which complicate the study of crystallization phenomena in urine.

## Theoretical Aspects of Crystallization Phenomena

Theoretically crystallization processes may be subdivided into nucleation (increase of crystal number), growth (increase of crystal size) and aggregation (increase of particle size and decrease of particle number). Practically, however, these 3 phenomena often occur together and can be generally described as crystallization. Urinary supersaturation with respect to stone forming minerals is the driving force for nucleation and crystal growth. The state of urinary saturation with respect to calcium oxalate is governed by the concentration of the stone forming ions calcium and oxalate, by ionic strength, pH, temperature and by complex formation. Complex ions (e.g. magnesium) reduce free ionic concentrations by the formation of soluble complexes (e.g. magnesium oxalate). A supersaturated solution tends to reach saturation by precipitating the solutes responsible for supersaturation. To overcome the surface tension of crystals to be formed a minimal state of supersaturation (formation product, Fig. 1A) is needed which decreases with induction time (time necessary until measurable crystallization occurs). Promoters are substances with preformed surfaces which induce crystallization (heterogenous nucleation). They reduce both the formation product and the induction time. If crystal seeds of the solid to be formed are used crystallization starts immediately and continues until a state of saturation is reached (secondary nucleation and growth, Fig. 1C). Inhibitors, unlike complex ions influence crystallization processes without changing the apparent solubility. They are assumed to poison growing sites and to change the surface potentials of crystals and of any crystal niduses. Inhibitors increase the formation products for spontaneous nucleation (Fig. 1B) and for secondary nucleation and growth (Fig. 1D) and decrease the rate of crystal growth [23] as well as of crystal aggregation [21]. Some substances (e.g. citrate and magnesium) have an inhibitor as well as a complexor-effect (Fig. 1E).

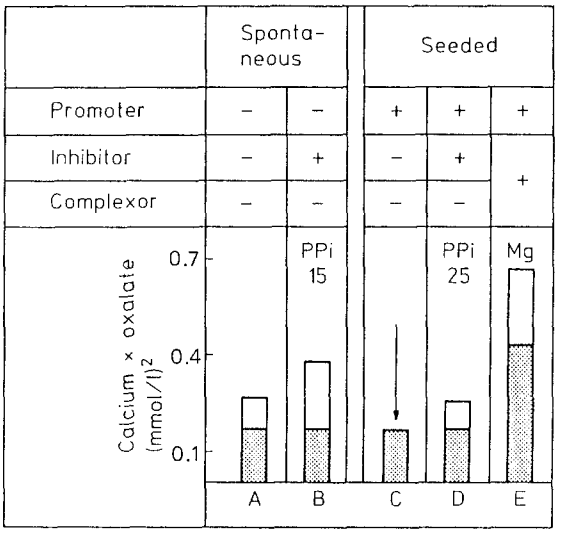


**Fig. 1.** Formation products (▭) for spontaneous nucleation of calcium oxalate and for secondary nucleation and growth of 0.02 mg/ml seeds of calcium oxalate monohydrate compared to apparent solubility products (▨) in a standard solution. Pyrophosphate (PPi) increases in low physiological concentrations (15 μmol/l) the formation product for sponataneous nucleation (*B*) and in high physiological concentration (25 μmol/l) the formation product for secondary nucleation and growth of calcium oxalate (*D*). Magnesium (2 mmol/l) is an inhibitor and a complexor since it increases also the apparent solubility product (*E*)

## Problems Complicating the Study of Crystallization Processes in Urine

Since only the ionic activity (a = free ionic concentration · activity coefficient) of calcium can be measured directly

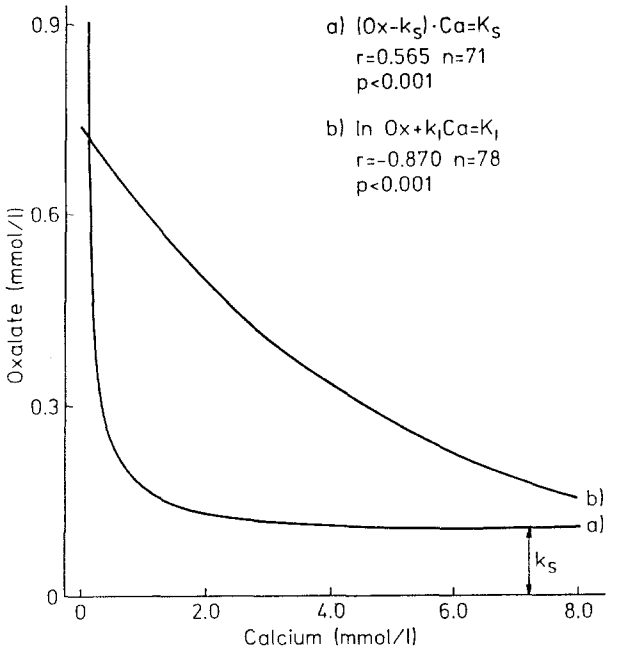


**Fig. 2.** Average urinary oxalate versus calcium concentrations: *a)* after equilibration with 10 mg/ml calcium oxalate monohydrate during 24 h and *b)* necessary to induce secondary nucleation and growth of 0.02 mg/ml calcium oxalate monohydrate within 90 min

the activities of the other stone forming ions must be calculated by computer programs. However, there is neither agreement on the number of relevant complexes which is reported to be from 22 [20] to 31 [15] nor on the stability constants of these complexes. The state of urinary saturation can be expressed as relative saturation, which is the ratio of the activity product of stone forming ions ($AP_{CaOx} = a_{Ca} \cdot a_{Ox}$) and of the thermodynamic solubility product (SP) obtained in artificial solutions: Relative saturation = $AP_{CaOx} \cdot SP^{-1}_{CaOx}$. A simpler approach is to equilibrate urine with a large mass of calcium oxalate. If calcium and oxalate in solution increase the urine is undersaturated and if they decrease the urine is supersaturated. The state of saturation can therefore be expressed as ratio of the concentration products calcium · oxalate before (native) and after equilibration (equ.): $CPR = (Ca \cdot Ox)_{native} \cdot (Ca \cdot Ox)^{-1}_{equ.}$. If AP's are calculated from the data of equilibration experiments and an activity product ratio (APR) is formed, APR equals CPR [17] ($APR = (a_{Ca} \cdot a_{Ox})_{native} \cdot (a_{Ca} \cdot a_{Ox})^{-1}_{equ.}$). But relative saturation for calcium oxalate is more than twofold higher than CPR and APR because also $(a_{Ca} \cdot a_{Ox})_{equ.}$ is more than twice the thermodynamic solubility product. The apparent higher solubility of calcium oxalate in urine than in artificial solutions may be explained by the fact that equilibrium is not attained in equilibration experiments or that computer programs neglect important oxalate complexes. Data from equilibration experiments in Fig. 2a show that even at extremely high urinary calcium concentrations the oxalate concentration does not fall below a minimal value $k_S$. When this constant $k_S$ is subtracted from the total oxalic acid concentration before calculating the activity product at saturation this product reaches the thermodynamic solubility product [3].

In addition to the process of complex formation, inhibitors and promoters complicate the study of crystallization processes in urine. Besides known inhibitors (e.g. magnesium, citrate, pyrophosphate) there are at least 4 poorly specified groups of substances with inhibitory activities namely glycosaminoglycans, acid polypeptides, polyribonucleotide fragments and polyaminoacids [6]. Some substances like glycosaminoglycans are potent inhibitors to aggregation while others like magnesium and citrate in physiological concentrations influence nucleation and growth. Every inhibitor has its own dose-response curve [21] and the effect of the combination of several inhibitors has not been studied in depth. True urinary inhibitor capacity may therefore be estimated only by performing crystallization experiments in whole urine.

Heterogenous nucleation of calcium oxalate in the urinary tract can be induced by such different promoters as hydroxyapatite, uric acid, brushite [16], uromucoid [13], kidney calcification [19] and perhaps by injured urothelium [10]. The effect of the latter two promoters can only be simulated in in vivo experiments. If heterogenous nucleation of calcium oxalate induced by crystalline promoters (e.g. urate) is to be studied, the urine must first be saturated with respect to urate [26] and is thus altered in its original composition. Another approach is to induce secondary nucleation and growth by the addition of a small amount of the crystals being studied [1]. This approach simulates the further growth of trapped calcium oxalate crystals or of a heterogenous promoter already coated with calcium oxalate.

## Current Knowledge of Crystallization Phenomena in the Urinary Tract

Calculations of activity products of calcium oxalate [22] and equilibration experiments (Fig. 2a) reveal that at normal or high calcium concentration urinary saturation with respect to calcium oxalate is mainly governed by urinary oxalate. A similar effect is shown by Briellmann et al. in this issue [7] who studied spontaneous nucleation at high urinary supersaturation. Mild hyperoxaluria had therefore been postulated to be much more important for calcium stone formation than hypercalciuria [22]. However, mild hyperoxaluria is not always found in idiopathic stone formers. Furthermore at physiological oxalate concentrations the limit of urinary metastability to spontaneous nucleation [23] and to secondary nucleation and grwoth of calcium oxalate (Fig. 2b) clearly shows a negative correlation between calcium and oxalate. Thus hypercalciuria, inspite of its week influence on urinary saturation with respect to calcium oxalate markedly increases the risk of crystallization of calcium oxalate in urine.

When crystallization phenomena are relevant for nephrolithiasis they have to occur in the upper urinary tract in

which transit time of urine in the absence of obstruction is in the order of minutes. In this issue Briellmann et al. [7] report that an oxalic acid addition of several mmoles is necessary to induce rapid nucleation of calcium oxalate in urine. Such oxalic acid concentration can hardly be observed in idiopathic stone formers even under extreme conditions [4]. Furthermore, crystal concentrations produced by rapid spontaneous nucleation exceed by far values measured during spontaneous crystalluria [8].

Crystallization processes in the kidney and in the upper urinary tract seem to be induced by heterogenous nucleation. Monosodium urate is a potent promoter of crystallization of calcium oxalate and furthermore favors crystallization by binding glycosaminoglycans in urine [18]. Hyperuricosuria, as discussed by Gill and Rose in this issue [11] is believed to induce the formation of calcium oxalate by these 2 effects. In animal experiments calcium oxalate stone formation can be produced by fixed particle growth on papillary ducts obstructed by calcium oxalate [5]. However, such papillary deposits can only be achieved either by extreme manipulations as ethylenglycol poisoning or by pyridoxine deficiency. Calculations of the maximum growth of calcium oxalate crystals during renal transit time of urine revealed that these crystals would hardly be able to obstruct collecting ducts with diameters of about 200 $\mu$m [9]. Furthermore, the maximum diameters of crystals and crystal aggregates observed in concentrated overnight urine after oral oxalate load were generally below 200 $\mu$m [2]. Crystal adhesion to the urothelium occurs after chemical injury by HCl, non-ionic detergents, or proteolytic enzymes [10]. But up to now there is no information on urothelial injuries in the initial state of idiopathic nephrolithiasis.

Another possibility for fixed particle growth already postulated in 1939 by Randall are papillary calcifications. Systematic examination of 100 randomizedly collected kidneys revealed microscopic or submicroscopic calcifications in 100% [12] possibly consisting of hydroxyapatite. Combined phase- and texture-analyses of about 700 oxalate rich stones disclosed calcium phosphate within or near the central core in more than 70% [14]. In patients tending to form calcium oxalate stones urine is not only supersaturated with respect to calcium oxalate but also to calcium phosphate and a lack of inhibitor capacity to crystal growth was only found in the case of calcium phosphate but not of calcium oxalate [4, 24]. Furthermore, the concurrence of hydroxyapatite crystalluria in such patients [24] emphasizes a possible role of calcium phosphate in the formation of calcium oxalate stones.

The state of research can be summarized as follows: There is some evidence that calcium oxalate concretions start by heterogenous nucleation and fixed particle growth and that calcium phosphate is involved in this process. However, much further research has to be done in order to explain how a microscopic kidney calcification, an encrustation of papillary ducts or a urothelial lesion can lead to a sizeable obstructing concrement. Urinary stones not only consist of crystals, but also of a highly organized matrix [25] often neglected in stone research.

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