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Editorial

How Reliable are the Measurements of Crystallization Conditions in Urine?

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Urolithiasis seems to be related to crystallization processes in supersaturated urine. They comprise nucleation, crystal growth and aggregation and are influenced by promoters, chelators and inhibitors. Promoters probably provide preformed surfaces for heterogenous nucleation and epitactical crystal growth. Chelators form soluble complexes with stone forming ions and thus reduce free ionic activity and urinary supersaturation. Inhibitors are likely to change crystal surfaces and to block growing sites of crystals and crystal niduses, but contrary to chelators do not influence urinary supersaturation. In the last years many methods for the measurement of crystallization conditions in urine have been published and we have studied six of them in our laboratory [3, 4]. The most important impression we had was that results were often more influenced by the test conditions applied than by the composition of the urine to be examined.

Problems begin already with the collection and dilution of urine. Figure 1 shows the results of 2 studies in which urinary inhibitor activity to crystal growth and aggregation of calcium oxalate monohydrate are measured by the method of Robertson et al. [8], but with different techniques of urine collection and dilution. In the study of Robertson et al. [9] (Fig. 1A), the individual urine portions of 8 calcium stone patients and 8 healthy controls were examined with 1% dilution in the test system. A large distribution of values was found. However, there was a significant difference between patients and controls. In our study (Fig. 1B) 24 h urine was collected from 12 stone patients and 9 controls. In order to equalize variations of inhibitor concentrations due to different degrees of diuresis, the 24 h urines were diluted to 4.8 l and an average final urine concentration of 3% was used in the test system. Patients as well as controls showed an excellent inhibitor activity without any significant differences between the 2 populations. The large distribution of values in the study A seems, therefore, to be mainly the effect of different states of diuresis. In a study with a similar fixed fluid intake, as used by Robertson et al. we found that the diuresis in individual urine

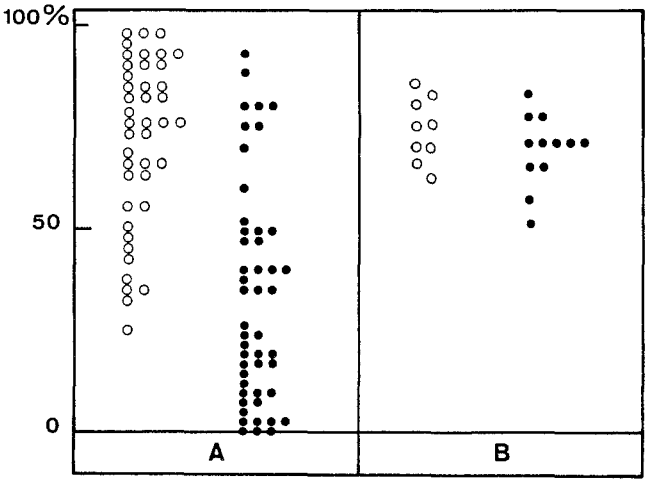

Fig. 1A, B. Inhibition of growth and aggregation of calcium oxalate crystals in healthy controls (○) and in calcium stone patients (●): **A** 1% of single urine portions (Robertson et al. [9]). **B** 3% of 24 h urine prediluted to 4.8 l (Baumann et al. [2])

portions varied from 20 to 160 ml/h. Individual measurements of urinary inhibitor activity are only comparable if they are obtained by similar states of diuresis and dilution in the test system. However, urine dilution brings further problems to urolithiasis research. Robertson et al. [10] have shown that inhibition of crystal aggregation is related to an increase of the negative zeta potential on crystal surfaces. This increase reaches its maximum in a urine diluted to about 5% and is almost lost in undiluted urine. Results obtained with diluted urine can therefore hardly reflect the situation in whole urine, where stone formation occurrs.

In 1968 we published a method for the measurement of formation products for the precipitation of calcium oxalate in whole urine [1], but only in the 1970s studying inhibitor containing solutions we became aware of the limits of this system. The method can be summarized as follows: Sodium oxalate is infused in calcium containing solutions or in urine by an infusion pump. The precipitation of calcium oxa-

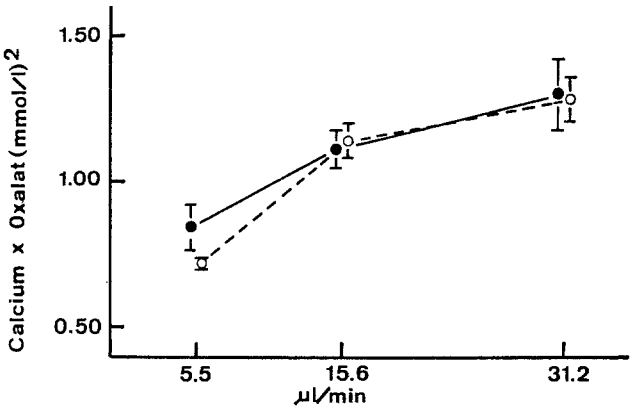

Fig. 2. Formation product (Ca) · (Ox) for precipitation of calcium oxalate in calcium containing (1.2 mmol/l) solution with (•) and without (○) 10^{-5} M Ethane-Dihydroxy-Biphosphonate plotted versus infusion rate of sodium oxalate (0.1 mol/l)

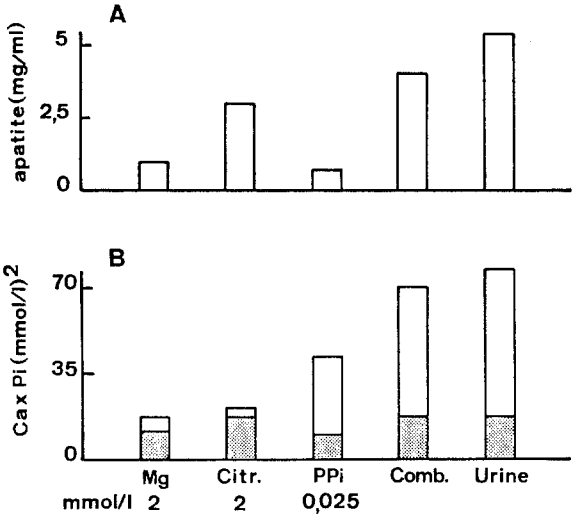

Fig. 3A, B. Inhibitor activity to calcium phosphate crystallization (▭) of micromolecular inhibitors and of urine: **A** Measured as critical crystal concentration at low and constant supersaturation (Bisaz et al. [5]). **B** Measured as critical (Ca) · (P) concentration product with a low and constant crystal concentration (Baumann et al.[4]). (▨) = apparent solubility product (Ca) · (P) for brushite

late is monitored by a nephelometer. From the critical oxalate addition necessary to induce precipitation, the formation product (Ca) · (Ox) can be calculated which is increased by crystallization inhibitors. Figure 2 shows that the formation product as well as its increase by an inhibitor are influenced by the infusion rate or the incubation time respectively. A potent inhibitor concentration of 10^{-5} M Ethane-Dihydroxy-Biphosphonate was only effective at low infusion rates and low supersaturation. Results of the measurement of formation products can therefore only be compared if they are determined at the same incubation time. However, this test system does not allow measurements at constant incubation times, because the volume of infused oxalate (v) and incubation time (t) are linked by the infusion rate (r), ($r = v \cdot t^{-1}$). Theoretically the problem could be solved by an extrapolation of results obtained from measurements at different infusion rates or by creating standard curves as introduced by Briellmann et al. [6]. Another approach is to prepare multiple samples with increasing oxalate addition and to look in which sample precipitation has occured after a constant incubation time. Pak and Holt [7] using this method have demonstrated that the formation products for calcium oxalate as well as for calcium phophate were often lower in urine than in control solution, thus indicating the presence of potent promoters of crystallization. Systems measuring crystallization in initially crystal-free urine can not differentiate between the individual effects of promoters and inhibitors.

To study urinary inhibitor activity, seed tests are preferred: Crystallization is initiated by the addition of small amounts of the same crystals as their growth will be studied. Such crystals are the most potent promoters and the effect of weaker promoters can therefore be neglected. In urine the growth of small amounts of calcium oxalate or calcium phsophate crystals is usually blocked by the action of crystallization inhibitors. Crystallization starts either when urinary supersaturation or when the crystal mass reaches critical values. Both parameters as well as growth and aggregation rates can be used to measure inhibitor activity. Figure 3 summarizes the results of 2 studies, where urinary inhibitor activity to apatite induced crystallization was measured and compared to the effects of low molecular inhibitors in calcium and phospate containing solutions. In the study of Bisaz et al. [5] (Fig. 3A) the critical hydroxyapatite concentration necessary for a 50% drop of the concentration product (Ca) · (P) was measured at low and fixed supersaturation (urine and solutions saturated with respect to brushite). 77% of urinary inhibitor activity could be attributed to urinary magnesium, citrate and pyrophosphate. Citrate was, with a contribution of 48%, the most important inhibitor. In our own study (Fig. 3B) the critical (Ca) · (P) concentration product for calcium phosphate crystallization induced by low concentration of hydroxyapatite (0.2 mg/ml) was measured. In order to eliminate the effect of chelators and of ionic strength the apparent concentration product for brushite solubility was also determined by equilibration experiments [4]. The low molecular inhibitors again accounted for about 85% of inhibitor activity found in urine of healthy men, but pyrophosphate was now, with a contribution of 52%, the most potent inhibitor.

The above mentioned examples show that results obtained by different methods can hardly be compared. Every test system has its own specification which has to be known for a correct interpretation of results and for the choice of adequate methods for research. In 1987 at the Harnsteinsymposium in Vienna the decision was made to organize a workshop on the measurement of crystallization conditions in urine. The following paper gives a report of this workshop.

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