Urol Res (1990) 18 [Suppl 1]:S25–S30

Urological Research

Physico-chemical aspects of calcium stone formation

J. M. Baumann

Department of Urology and Stone Research Laboratory, Biel Hospital Biel, Switzerland

In a recent study, the incidence of chemical abnormalities was shown in the urine of 3,473 stone-formers from 5 different regions of the USA [16]. With respect to stone metaphylaxis, it seems noteworthy that reduced urinary volume, which increases the concentration of stone-forming ions, was the most frequent disturbance (69%–78%). Further abnormalities included hypercalciuria (35%–43%), hypocitraturia (22%–29%), hyperoxaluria (18%–25%), hyperuricosuria (16%–19%) and hypomagnesiuria (9%–13%). The tendency to excrete larger crystals and crystal agglomerates in urine is another known characteristic of stone patients [26]. The question as to whether crystalluria directly promotes stone formation, as has been suggested in the work of Khan et al. [19], or only represents a sign of increased crystallization risk remains open. The aim of this paper is to show how chemical abnormalities are involved in calcium oxalate crystallization and why they should be corrected in stone metaphylaxis.

Crystallization processes can be divided into nucleation, growth, epitaxy and agglomeration. Spontaneous nucleation and agglomeration occur very rapidly whereas the other processes are time-consuming. Agglomeration does not require supersaturation.

Nucleation and growth

Nucleation and crystal growth are governed by the difference in the energies that are supplied by urinary supersaturation and consumed to build up crystal surfaces against surface tension (Fig. 1). The energy required for spontaneous nucleation of calcium oxalate is extremely high. Finlayson [11] has shown that it corresponds to at least an 80-fold supersaturation, which has never been found in urine or kidney tissue. Crystalluria and stone formation therefore seem to be the results of heterogeneous nucleation induced by promoters. Promoters probably present preformed surfaces that reduce the surface energy required for crystallization. For crystalliation to occur in a kink of a performed crystal surface, much less surface has to be created than that required for the formation of a free nucleus (*a* in Fig. 1).

Crystallization processes are modulated by chelators, inhibitors and promoters, which have a high affinity to stone-forming ions. The state of supersaturation with respect to a stone-forming salt is given by the activity product of the stone-forming ions. Ion activity is reduced by chelators that trap free ions and form soluble complexes (*b* in Fig. 1). Low-molecular-weight substances such as citrate and magnesium are chelators as well as inhibitors. An inhibitor remains active at very low concentrations at which no chelating effects can be measured. Inhibitors of growth and nucleation are supposed to block growth sites on crystal niduses, on preformed crystals and also on some promoters (*c* in Fig. 1). By polymerization [31] or immobilization on surfaces [21], macromolecular substances can convert from inhibitors to promoters. In this situation, they probably bind a serie of calcium ions in a constellation that is ideal for epitactical growth of calcium oxalate or calcium phosphate (*d* in Fig. 1). The pH influences the ionic dissociation of stone-forming compounds, chelators and inhibitors.

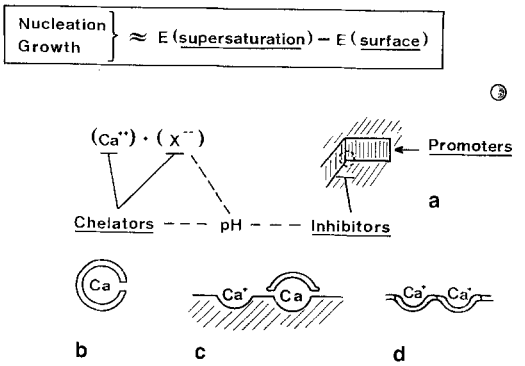

Fig. 1. Schema of the factors governing nucleation and growth. E = Energy. $(Ca^{++}) \cdot (X^{--})$ = activity product of calcium oxalate and calcium phosphate, respectively. *a–d* see text

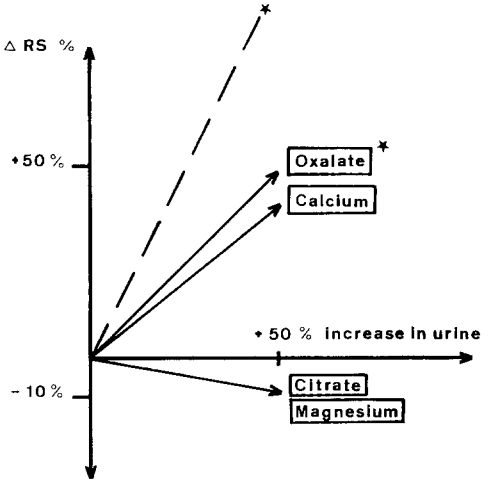

Fig. 2. The influence of a 50% increase in urinary compounds on urinary supersaturation with respect to calcium oxalate (in percent) as calculated by Werness et al. [36]. *RS* = Relative saturation; ———* = influence of oxalate extrapolated from equilibration experiments [1]

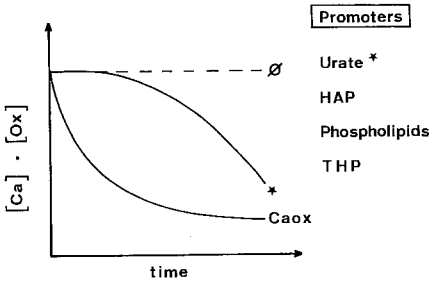

Fig. 3. Promoters destabilize metastably supersaturated urine or model solutions. $\emptyset$ = No addition; * = addition of urate; *CaOx* = addition of calcium oxalate seeds; *HAP* = hydroxyapatite; *THP* = Tamm Horsfall protein

Urinary supersaturation

Urinary supersaturation, the driving force for nucleation and growth, is now generally calculated by computer programs. In its latest version, this procedure requires an exact analysis of 23 components, from which 103 complexes are calculated [2]. Results are expressed as relative saturation (RS), which is the ratio between the calculated activity product and the thermodynamic solubility product of the stone-forming salt. Equilibration experiments use a simpler approach [22] whereby the state of saturation can be expressed as the ratio of the concentration products (CPR) of stone-forming ions before and after equilibration of urine with the stone-forming salt. Some reservations have been expressed concerning the attainment of true equilibrium using this method in inhibitor-containing solutions and in urine.

Figure 2 shows the effect of a 50% increase in the concentration of urinary calcium, oxalate, magnesium and citrate on the state of urinary saturation with respect to calcium oxalate, as calculated by Werness et al. [36]. Citrate and magnesium showed slight chelating effect, decreasing supersaturation by about 10%, but supersaturation was mainly governed by calcium and oxalate concentrations.

However, in equilibration experiments and studies of spontaneous and provoked crystalluria, the influence of calcium has been only minimal. In equilibration experiments the state of urinary saturation with calcium oxalate was exclusively governed by the urinary oxalate concentration [1]. Magnesium, citrate, calcium and pH had no significant influence in our study. Comparison of crystalluria with urinary calcium and oxalate concentrations revealed a high correlation between crystalluria and urinary oxalate but not calcium [24]. This was recently confirmed in evaporated urine by Hallson and Rose [15].

The difference between equilibration experiments and computer calculations is probably attributable to an overestimation of the free oxalate fraction in urine by the computer calculation. At the very low free oxalate concentration, which can be extrapolated from equilibration experiments, urinary supersaturation becomes extremely sensitive to changes in urinary oxalate [1].

Promoters of nucleation and growth

Promoters of nucleation and growth can be defined and measured by their destabilizing effect on metastably supersaturated solutions. This is schematically shown in Fig. 3. Without any addition, supersaturation and both calcium and oxalate concentrations remain stable during the whole observation period. When small amounts of calcium oxalate crystal are added, crystallization starts immediately and stops when the activity product in the solution has reached the solubility product. This also occurs in the presence of promoters, but with an induction time that is typical for heterogeneous nucleation and growth. Urate has proved to be a promoter of calcium oxalate crystallization in control solutions [34] as well as in urine [13]. Together with the nucleating effect, blocking of inhibitors in urine has also been described [27].

However, the presence of urate explains calcium oxalate crystalluria but not necessarily calcium stone formation. For stone formation to occur crystals and crystal agglomerates have to be trapped in the upper urinary tract. Fixed promoters such as hydroxyapatite in kidney calcifications or phospholipids in renal tubular membranes are therefore of extreme interest in stone research. Hydroxyapatite is an established promoter of calcium oxalate crystallization that is inhibited by citrate [7] and pyrophosphate [6]. Recently, Khan et al. [18, 20] showed that phospholipids in the stone matrix and in renal membranes are excellent promoters of calcium oxalate crystallization.

Inhibitors

Inhibitors have the following three effects: (1) they diminish nucleation and growth rates, (2) they increase the metastability of solutions and urine with respect to spontaneous as well as heterogeneous nucleation

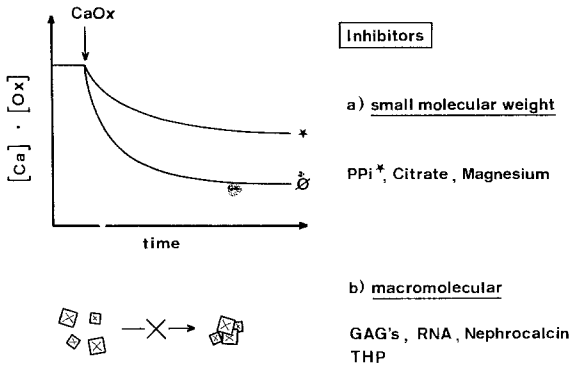

Fig. 4. Inhibitors decrease crystallization rates, increase metastability of urine or model solutions and diminish agglomeration. *CaOx* = calcium oxalate seeds; * = in presence of pyrophosphate; $\emptyset$ = without inhibitor; *PPI* = pyrophosphate; *GAG's* = glycosaminoglycans; *RNA* = ribonucleic acid; *THP* = Tamm Horsfall protein

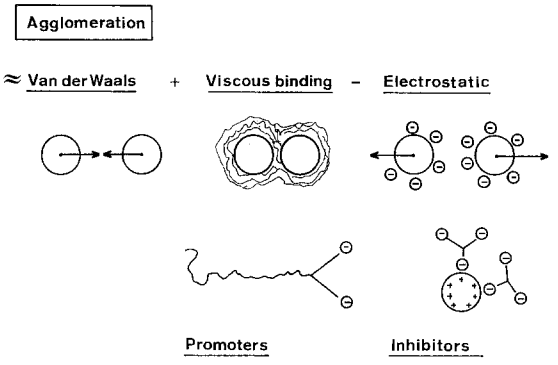

Fig. 5. Schema of the factors influencing agglomeration

Table 1. Effects of Tamm Horsfall protein in urine

1.	In artificial urine:
	Is a major inhibitor of agglomeration [17]
	causes agglomeration and crystal adhesion on gel surfaces [8]
2.	In diluted urine:
	with increasing concentration and ionic strength, interferes with other inhibitors and promotes agglomeration [31]
3.	In whole urine:
	with oxalate load is likely to act as an inhibitor of agglomeration [12]
4.	In evaporated urine:
	Is a promoter of crystal formation [14]

and growth, and (3) they reduce agglomeration. Figure 4 schematically shows the effect of pyrophosphate on the growth of calcium oxalate monohydrate seeds in urine or in a solution metastably supersaturated with calcium oxalate. In the presence of pyrophosphate, crystallization is retarded and stops before the solubility product is reached.

Pyrophosphate, citrate and magnesium are physiological small-molecular-weight inhibitors [29]. Among urinary macromolecules, glycosaminoglycans [27], RNA [25] and nephrocalcin, which was recently isolated by Coe et al. [10], have important inhibitory effects. Tamm Horsfall protein figures as an inhibitor as well as a promoter. Ryall et al. [29] have reported evidence that small-molecular-weight inhibitors in whole urine preferably influence nucleation and growth, whereas macromolecular compounds inhibit aggregation, a process that is attracting increasing interest in stone research.

Agglomeration

Finlayson [11] has shown that agglomeration of crystals is mainly governed by the attractive forces of Van der Waals, by viscous binding and by electrostatic repulsion. The polyanionic inhibitors of agglomeration that are absorbed on crystal surfaces increase electronegativity and, thus, crystal repulsion (Fig. 5). However, macromolecular compounds also have sticky forces that can increase viscous binding and thus promote agglomeration [30].

Much controversy exists about macromolecular inhibitors, especially Tamm Horsfall protein. Table 1 summarizes the results of five different studies performed in artificial, diluted, whole and evaporated urine. From their study in model solutions, Hess et al. [17] have concluded that Tamm Horsfall protein is an important urinary inhibitor of agglomeration. On the other hand, Bernstein and Achilles [8] showed that in a dynamic system, this protein caused agglomeration and crystal adhesion on gel surfaces. Scurr and Robertson [31] have demonstrated the dual effect of Tamm Horsfall protein, which changes with increasing concentration and ionic strength from an inhibitor to a promoter of agglomeration. Grover et al. [12] have studied the effect of Tamm Horsfall protein in whole urine, where crystallization was induced by a high oxalate load; their conclusion was that this protein may act as an inhibitor of agglomeration. However, in urine concentrated by evaporation, Hallson and Rose [14] showed that it is a strong promoter of crystal formation. All of these controversies demonstrate that results obtained in crystallization tests are essentially dependent on test conditions and on the medium in which the tests are performed. Experiments carried out in whole urine and under most natural conditions are therefore mandatory for stone research [3].

Inhibition of crystal growth in urine

Figure 6 summarizes the results of inhibitor measurements in whole urine and in inhibitor-containing solutions. Our method is based on the observation that in urine or in inhibitor-containing solutions, small crystal concentrations grow only at critical supersaturation [4]. Being determined by titration with oxalate and expressed as the concentration-product ratio, this critical supersaturation was taken as a measure of inhibitor activity.

In agreement with the observations of Smith [32], Ryall and Marshall [28] and Tiselius et al. [35], no significant difference could be found between stone-formers and healthy controls with respect to their capacity for urinary inhibition of calcium oxalate crystallization. Comparison

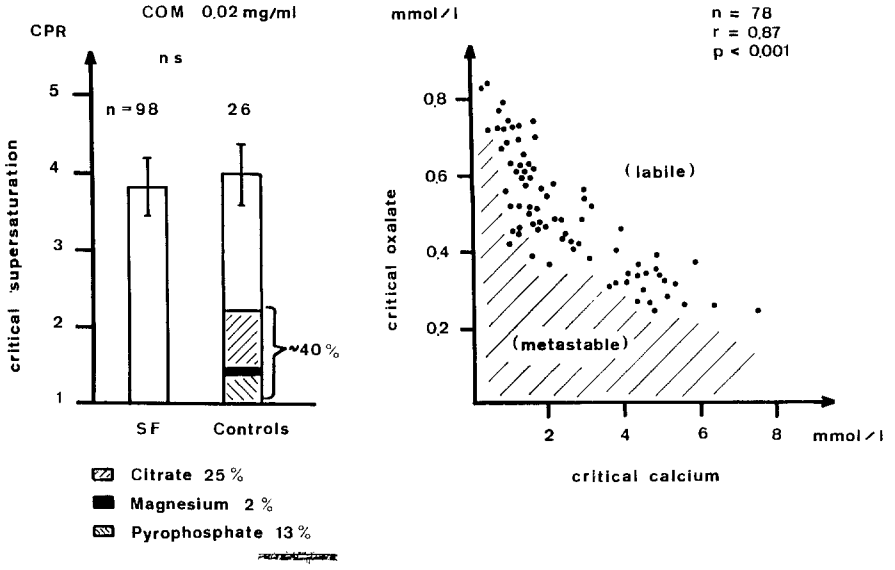

Fig. 6. Inhibition of calcium oxalate monohydrate (COM) growth in whole urine, measured as the critical concentration-product ratio (*CPR*). *SF* = Idiopathic calcium stone-formers; *NS* = not significant

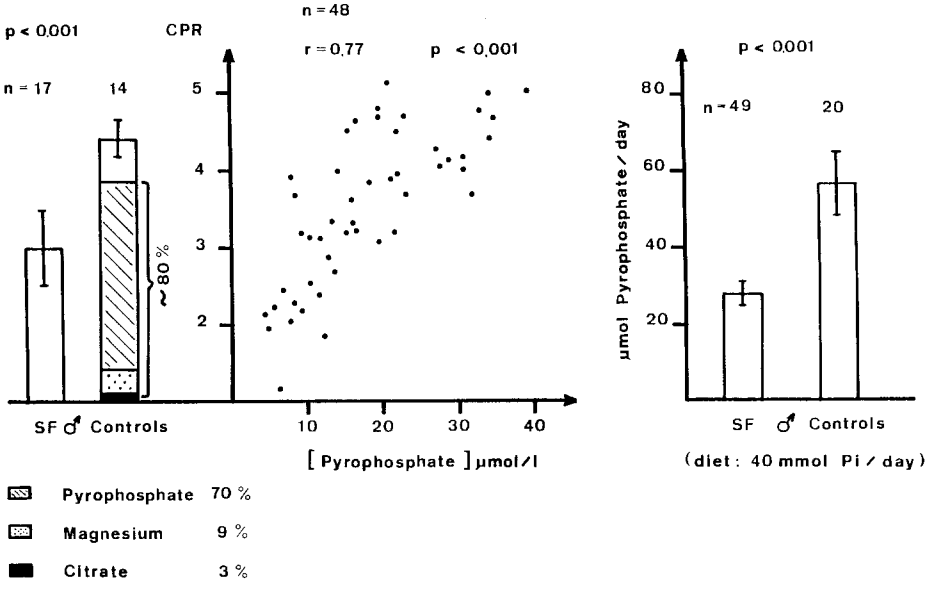

Fig. 7. Inhibition of hydroxyapatite (0.2 mg/ml) growth measured as the critical concentration-product ratio (*CPR*) and pyrophosphate excretion in urine. *SF* = Idiopathic calcium stone-formers; *NS* = not significant

of inhibition achieved in urine and in inhibitor-containing solutions showed that about 40% of the urinary inhibitor activity could be attributed to the small-molecular-weight inhibitors pyrophosphate, magnesium and citrate. Among these compounds, citrate was the most efficient inhibitor. Plotting of critical oxalate concentrations for crystal growth versus critical calcium concentrations revealed a strong negative correlation, which may explain the importance of hypercalciuria in calcium oxalate lithiasis, despite its low influence on concentration-product ratio and crystalluria. In the presence of hypercalciuria, the limit of metastability is reached already at very low oxalate concentrations (Fig. 6).

Figure 7 summarizes our measurements of the inhibition of hydroxyapatite growth in urine and in inhibitor-containing solutions. The critical supersaturation for hydroxyapatite growth was determined by calcium titration at pH 6.6, again expressed as the concentration-product ratio [5]. In agreement with the findings of Smith [32], inhibition was significantly decreased in men who were idiopathic stone-formers as compared with healthy men. In contrast to the results of Bisaz et al. [9], who used big crystal masses, pyrophosphate was the most important inhibitor, contributing about 70% of the inhibitor activity of whole urine. Urinary inhibitor activity strongly correlated with urinary pyrophosphate concentration. In stone-forming urine a significant decrease in pyrophosphate excretion was found when male patients and controls were examined under a constant dietary phosphate intake. However, further research should be carried out to determine whether calcium phosphate crystallization and hypopyrophosphaturia, the latter not being found by all authors, are important factors in calcium oxalate lithiasis.

Implications for stone metaphylaxis

From the current knowledge of crystallization conditions in urine, the following conclusions for stone metaphylaxis can be drawn:

1. Urinary supersaturation should be held as low as possible and should always be below the critical limit of heterogeneous nucleation and growth.
2. The metastable region for heterogeneous crystallization should be increased by enhancing urinary inhibitor activity.
3. Promoters destabilizing urinary metastability should be eliminated.

Theoretically, alkaline treatment provides all of these effects. It diminishes relative saturation of urine by increasing urinary citrate and, in some cases, by decreasing urinary calcium [23]. Tiselius [33] showed that alkalization increases urinary inhibitor activity, an effect that has recently been redemonstrated with respect to pyrophosphate [6] and Tamm Horsfall protein [17]. Alkalization of urine also increases the solubility of urate, which is thought to be an important promoter of calcium oxalate crystallization [13]. However, at pH values above 6.5 a sharp decrease in this solubility is observed, which, together with the poor solubility of calcium phosphate, may counteract the therapeutic effect of alkaline treatment. Efficient stone metaphylaxis therefore requires not only constant monitoring of patient compliance but also consequent control of the physico-chemical alterations that are produced in urine by the therapy.

Acknowledgements. We thank Dr. Zysset for his critical review, Mr. Affolter for drawing the figures and Mrs. Wirth for preparing the manuscript.

References

1. Ackermann D, Baumann JM (1987) Chemical factors governing the state of saturation towards brushite and whewellite in urine of calcium stone formers. Urol Res 15:63
2. Ackermann D, Brown CM, Purich DL (1989) Measurement of urinary supersaturation, creation of standard supersaturations. Urol Res 17:144
3. Baumann JM (1989) Problems related to the measurement of crystallization conditions in whole urine. Report from a workshop held on November 25, 1988, in Basle. Urol Res 17:143
4. Baumann JM, Wacker M (1980) The direct measurement of inhibitory capacity to crystal growth of calcium oxalate in undiluted urine and in other inhibitor containing solutions. Urol Res 8:171
5. Baumann JM, Lauber K, Lustenberger FX, Wacker M, Zingg EJ (1985) Crystallization conditions in urine of patients with idiopathic recurrent calcium nephrolithiasis and with hyperparathyroidism. Urol Res 13:169
6. Baumann JM, Ackermann D, Affolter B (1989) The influence of hydroxyapatite and pyrophosphate on the formation product of calcium oxalate at different pHs. Urol Res 17:153
7. Berg C, Tiselius HG (1989) The effects of citrate on hydroxyapatite induced calcium oxalate crystallization and on the formation of calcium phosphate crystals. Urol Res 17:167
8. Bernstein I, Achilles W (1990) Effects of Tamm Horsfall protein on the growth and adhesion of calcium oxalate crystals (abstract). Urol Res 18:62
9. Bisaz S, Felix R, Neumann WF, Fleisch H (1978) Quantitative determination of inhibitors of calcium phosphate precipitation in whole urine. Miner Electrolyte Metab 1:74
10. Coe FL, Parks JH, Nakagawa Y (1989) Macromolecular inhibitors. In: Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds) Urolithiasis. Plenum Press, New York London, p 97
11. Finlayson B (1978) Physicochemical aspects of urolithiasis. Kidney Int 13:344
12. Grover PK, Ryall RL, Marshall VR (1989) The effect of Tamm Horsfall mucoprotein on calcium oxalate crystallization in urine: two methods compared. In: Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds) Urolithiasis. Plenum Press, New York London, p 177
13. Grover PK, Ryall RL, Marshall VR (1989) The effect of increasing urate concentration on the precipitation of calcium oxalate from human urine. In: Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds) Urolithiasis. Plenum Press, New York London, p 461
14. Hallson PC, Rose GA (1979) Uromucoids and urinary stone formation. Lancet I:1000
15. Hallson PS, Rose GA (1989) Quantitative studies of calcium crystalluria. In: Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds) Urolithiasis. Plenum Press, New York London, p 51
16. Harvey JA, Hill KD, Pak CYC (1989) Epidemiology of stone disease in the united states as discerned from a stone-risk profile. In: Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds) Urolithiasis. Plenum Press, New York London, p 665
17. Hess B, Nakagawa Y, Coe FL (1989) Inhibition of calcium oxalate monohydrate crystal aggregation by urine proteins. Am J Physiol 257:99
18. Khan SR, Hackett RL (1989) Membrane-induced calcium-oxalate crystal nucleation. In: Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds) Urolithiasis. Plenum Press, New York London, p 121
19. Khan SR, Finlayson B, Hackett RL (1982) Experimental calcium oxalate nephrolithiasis in the rat. Am J Pathol 107:59
20. Khan SR, Shevock PN, Hackett RL (1988) In vitro precipitation of calcium oxalate in the presence of whole matrix or lipid components of the urinary stones. J Urol 139:418
21. Nancollas GH, Smesko SA, Campbell AA, Coyle-Rees M, Ebrahimpour A, Binette M, Binette JP (1989) Mineralization inhibitors and promoters. In: Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds) Urolithiasis. Plenum Press, New York London, p 83
22. Pak CYC, Hayashi Y, Finlayson B, Chu S (1977) Estimation of the state of saturation of brushite and calcium oxalate in urine: a comparison of three methods. J Lab Clin Med 89:891
23. Pak CYC, Fuller C, Sakhaee K, Preminger GM, Britton F (1985) Long-term treatment of calcium nephrolithiasis with potassium citrate. J Urol 134:11
24. Robertson WG, Peacock M (1980) The cause of idiopathic calcium stone disease: hypercalciuria or hyperoxaluria? Nephron 26:105
25. Robertson WG, Scurr DS (1986) Modifiers of calcium oxalate crystallization found in urine: I. Studies with a continuous crystallizer using an artificial urine. J Urol 135:1322
26. Robertson WG, Peacock M, Nordin BEC (1969) Calcium crystalluria in recurrent renal-stone formers. Lancet II:21
27. Robertson WG, Knowles F, Peacock M (1976) Urinary acid mucopolysaccharide inhibitors of calcium oxalate crystallization. In: Fleisch H, Robertson WG, Smith LH, Vahlensieck W (eds) Urolithiasis research. Plenum Press, New York, p 331
28. Ryall RL, Marshall VR (1981) The effect of normal and stone-forming urine on the growth and aggregation of calcium oxalate crystals in relation to urinary glycosaminoglycan and urate concentrations. In: Smith LH, Robertson WG, Finlayson B (eds) Urolithiasis, clinical and basic research. Plenum Press, New York London, p 611
29. Ryall RL, Grover PK, Harnett RM, Hibberd CM, Marshall VR (1989) Small-molecular-weight inhibitors. In: Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds) Urolithiasis. Plenum Press, New York London, p 91

30. Scurr DS, Robertson WG (1986) Modifiers of calcium oxalate crystallization found in urine: II. Studies on their mode of action in an artificial urine. J Urol 136:128
31. Scurr DS, Robertson WG (1986) Modifiers of calcium oxalate crystallization found in urine: III. Studies on the role of Tamm Horsfall mucoprotein and of ionic strength. J Urol 136:505
32. Smith LH (1986) Clinical relevance of inhibitors of crystal formation. In: Gasser G, Vahlensieck W (eds) Pathogenese und Klinik der Harnsteine XI. Steinkopff, Darmstadt, p 12
33. Tiselius HG (1981) The effect of pH on the urinary inhibition of calcium oxalate crystal growth. Br J Urol 53:470
34. Tiselius HG (1984) Effects of sodium urate and uric acid crystals on the crystallization of calcium oxalate. Urol Res 12:11
35. Tiselius HG, Bek-Jensen H, Fornander AM, Nilsson MA (1990) Calcium oxalate crystallization properties in urine samples from patients with calcium oxalate stone disease (abstract). Urol Res 18:65
36. Werness PG, Brown CM, Smith LH, Finlayson B (1985) Equil 2: a basic computer program for the calculation of urinary saturation. J Urol 134:1242

Prof. Dr. J-M Baumann
Urologische Klinik und Steinforschungslabor
Regionalspital Biel
CH-2500 Biel
Switzerland