Urol Res (1992) 20:7–11

Urological Research

Investigations on macromolecular precipitation inhibitors of calcium oxalate

P. Schnierle[1], F. Sialm[1], H. G. Seiler[1], F. Hering[2], and G. Rutishauser[2]

[1]Institute for Inorganic Chemistry, University of Basle, Basle, Switzerland
[2]Urological Clinic, Surgical Department, Canton Hospital Basle, Basle, Switzerland

Accepted: June 1, 1991

Summary. Certain important aspects of the urine oxalate tolerance test (OTT) have been revised. The stirring system has been changed and the test has been adapted to the kinetics of calcium oxalate precipitation. True equilibrium conditions are now ensured during the measurements. Furthermore, the endogenous oxalate concentration is determined and taken into consideration. As a result of these changes, the significance of the test results has greatly improved. The effects of the addition of small amounts of zinc on the precipitation of calcium oxalate have been used in a new variation of the OTT. This new test makes it possible to discriminate much faster and more simply between recurrent stone-formers and other subjects. Tamm-Horsfall protein (THP) has been tested for its effect on the precipitation of calcium oxalate by means of OTT. THP inhibits the precipitation of calcium oxalate, but THP of stone-formers has a diminished inhibitory activity. The inhibitory activity of this protein strongly depends on the method by which it is isolated.

Key words: Oxalate tolerance test – Calcium oxalate – Precipitation – Zinc – Tamm-Horsfall protein

The analysis of urinary electrolytes does not allow discrimination between calcium oxalate stone-formers and healthy persons. On the other hand, even measurements of calcium oxalate, and uric acid in 24-h urine samples or in random urine samples are not predictive of an individual's risk of forming stones. The fluctuations found here due to dietary influences or the time of day are too large to allow a reliable risk evaluation. Various formulas [3, 8, 9–11], which combine several parameters related to stone-forming, have also not been proven useful. Even sophisticated mathematical computer programs like Finlayson's EQUIL 2-FORTRAN [6] are unreliable, because organic macromolecular inhibitors are not taken into account. Different research groups have attempted to determine the crystallization behaviour in urine or in diluted urine and to correlate this to the individual's risk of forming stones [1, 2, 5, 7, 14]. The oxalate tolerance test (OTT), which we developed and have since improved, not only allows a distinction between stone-forming patients and healthy persons based on their different oxalate buffer capacity but also an individual risk evaluation based upon urine samples fractionated by gel permeation chromatography (GPC) to establish their inhibitory activity. Since the urinary electrolytes as well as the macromolecular organic inhibitors contribute to the oxalate tolerance of individual urine samples, this test system reflects physiological conditions. This paper describes our modification of the test system and the influence of the addition of small amounts of zinc to urine samples.

Materials and methods

The OTT system has been revised in certain important points. In urine or other test solutions the oxalate concentration required to start the precipitation of calcium oxalate can be obtained by titration with sodium oxalate and turbidimetric detection with a dive photometric cell at 700 nm (Fig. 1). To avoid friction failures the stirring system has been changed from a magnetic to an electric torch stirrer. The titration velocity of the oxalate solution has been optimized by taking into consideration the kinetics of calcium oxalate precipitation. In order to determine the optimal titration velocity, synthetic urine with different calcium concentrations was titrated at varying dose rates with sodium oxalate by means of a micro-burette allowing the addition of volumes of 2 µl. In a plot of dose rate versus oxalate concentration needed for a detectable precipitation, it could be seen that at a dose rate of 2 µl/12 s the curve reached approximately constant values (Fig. 2). In order to ensure real equilibrium conditions a dose rate of 2 µl/24 s was chosen. The endogenous oxalate concentration excreted in urine is now determined by ion-exchange chromatography. Urinary calcium is measured by FAAS (acetylene/N_2O). The total concentration (endogenous and exogenous) of each urine sample is plotted against the corresponding calcium concentration. By comparing this with a standard curve obtained by analogous titration of synthetic urine with varying calcium concentrations (Fig. 3), the individual stone-forming risk can be evaluated (Figs. 4–6). The region on or below the standard curve was defined as a zone which promotes stone formation because the oxalate tolerance values are comparable or smaller than those of the synthetic urine without macromolecular inhibitors. Values in the

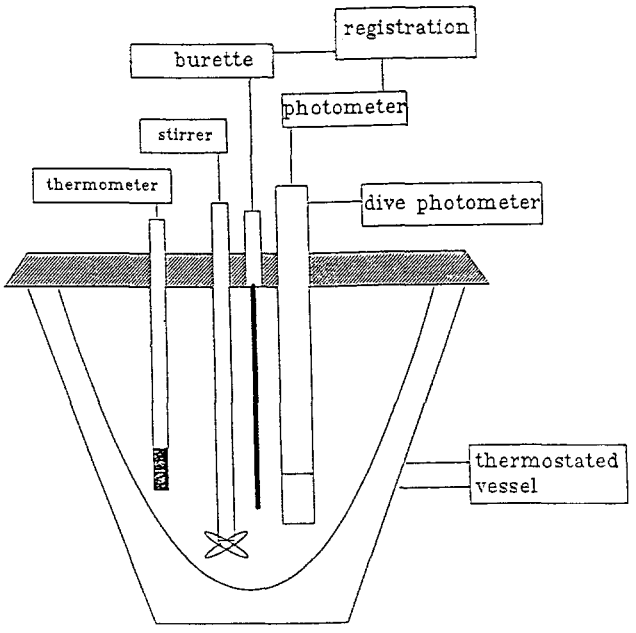

Fig. 1. Equipment used in the urine oxalate tolerance test

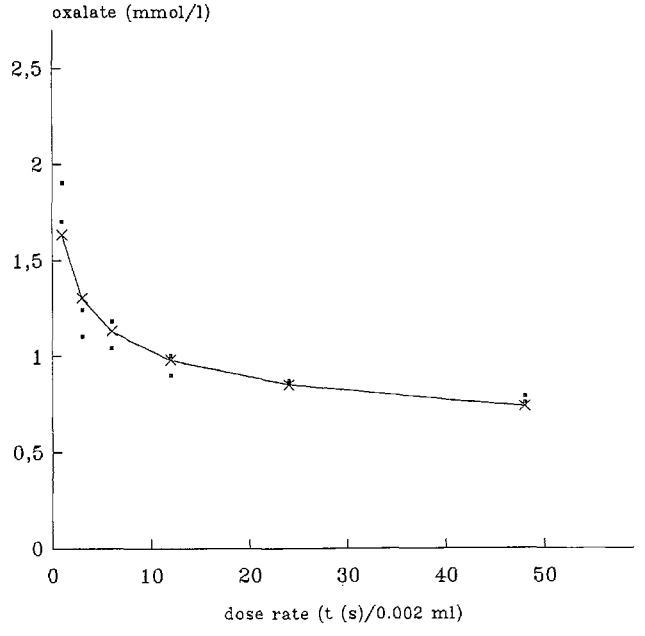

Fig. 2. Precipitation kinetics of calcium oxalate (calcium concentration 3.75 mmol/l); experimental curve; —×— calculated curve

region above the standard curve with high oxalate tolerance indicate, therefore, little risk of stone formation. In this region the calcium oxalate precipitation is inhibited compared with the standard curve. The precipitation behaviour of calcium oxalate has been studied in different salt solutions by means of the OTT. The oxalate precipitation was observed to be influenced by the addition of small amounts of zinc. Zinc increased the oxalate concentrations needed to obtain a detectable precipitation by enhancement of the induction period of the calcium oxalate precipitation (Fig. 7). The oxalate concentration required for a detectable precipitation of a urine sample with and without zinc is compared. An enhanced oxalate tolerance after the addition of zinc indicates an increased risk of recurrent stone-forming, because urine samples of recurrent stone-

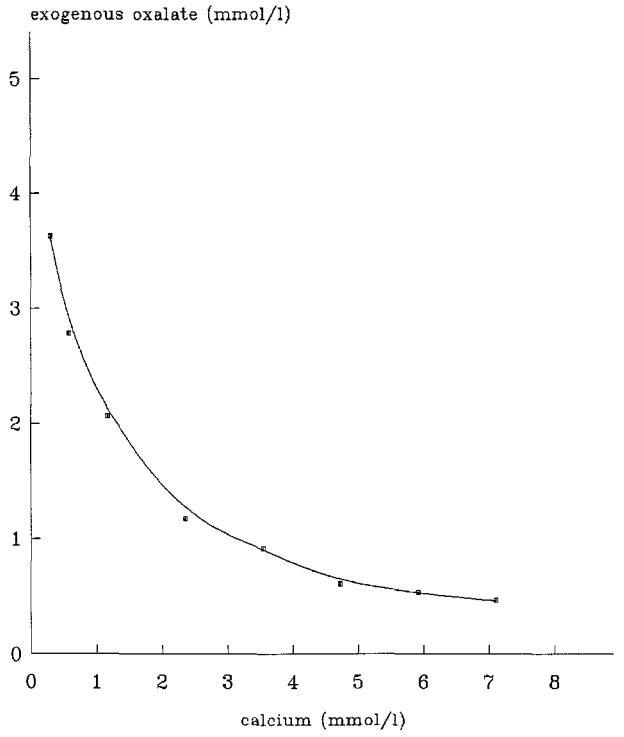

Fig. 3. Reference curve of the oxalate tolerance values

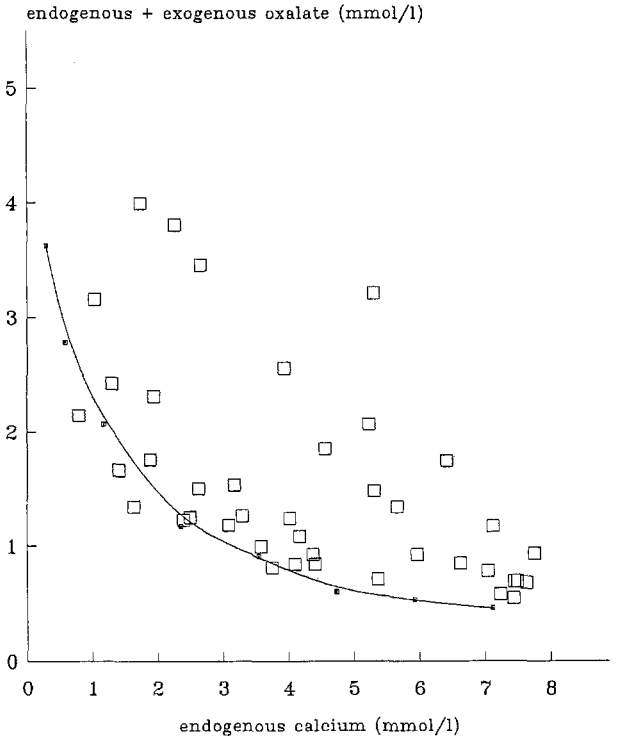

Fig. 4. Oxalate tolerance values of non-stone-formers

formers showed a higher oxalate tolerance after the addition of zinc (Fig. 8, 9), whereas healthy persons did not have better oxalate tolerance (Fig. 10). Urine of recurrent stone-formers behaves like a simple salt solution, whereas urine of healthy subjects behaves quite differently.

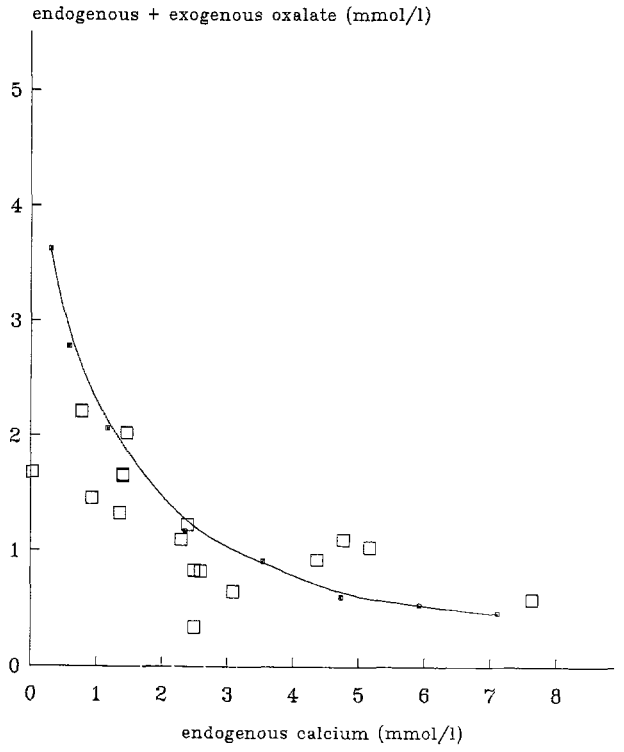

Fig. 5. Oxalate tolerance values of accidental stone-formers

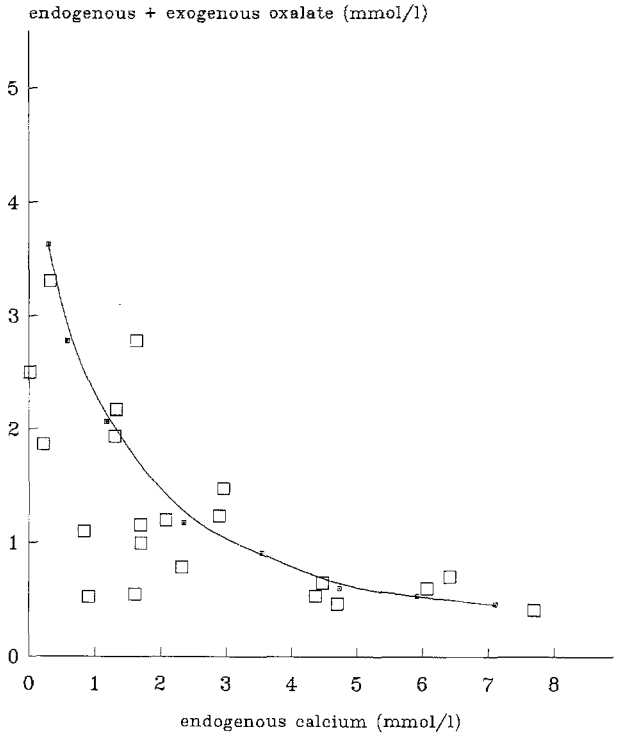

Fig. 6. Oxalate tolerance values of recurrent stone-formers

Urine of stone-formers and healthy subjects was dialyzed (Visking tool; Serva, Heidelberg, FRG) concentrated and subsequently fractionated by GPC (column 70×2.5 cm, Fractogel TSK HW 50 (S), Merck, Darmstadt, FRG). Tamm-Horsfall protein (THP) of stone-formers and healthy subjects was isolated according to the

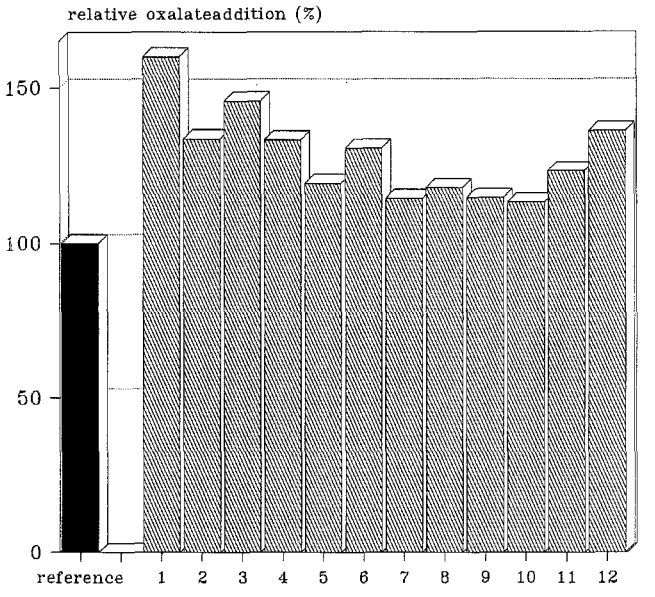

Fig. 7. Influence of zinc on the oxalate tolerance of different pure salt solutions; ■ pure salt solution; ▨ + zinc (0.15 mmol/l)

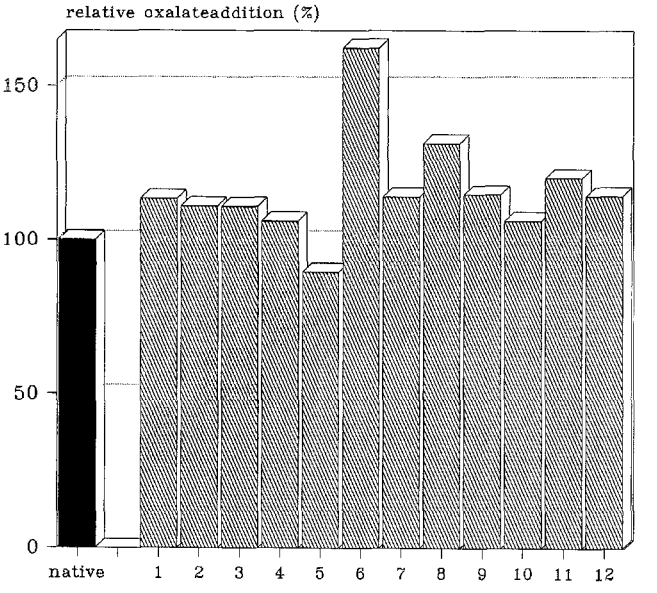

Fig. 8. Influence of zinc on the oxalate tolerance of recurrent stone-formers; ■ native (no zinc); ▨ + zinc (0.5 mmol/l)

method of Tamm and Horsfall by fractional salting out [15]. The different fractions of the GPC and the purified THP were tested for their effect on the precipitation of calcium oxalate by means of the OTT.

Results

A protein isolated by GPC from urine of healthy subjects and with a molecular weight of approximately 80 kDa showed an inhibitory effect of up to 30%, whereas a similar protein isolated from urine of stone-formers had a significantly diminished effect. THP (molecular weight

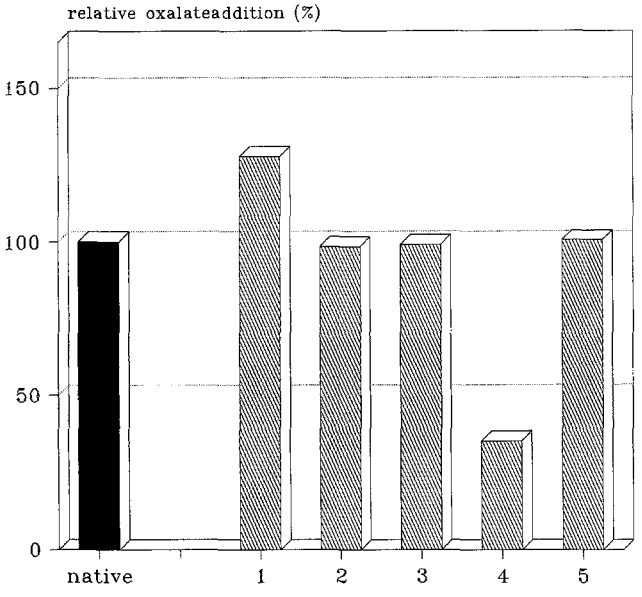

Fig. 9. Influence of zinc on the oxalate tolerance of accidental stone-formers; ■ native (no zinc); ▨ + zinc (0.15 mmol/l)

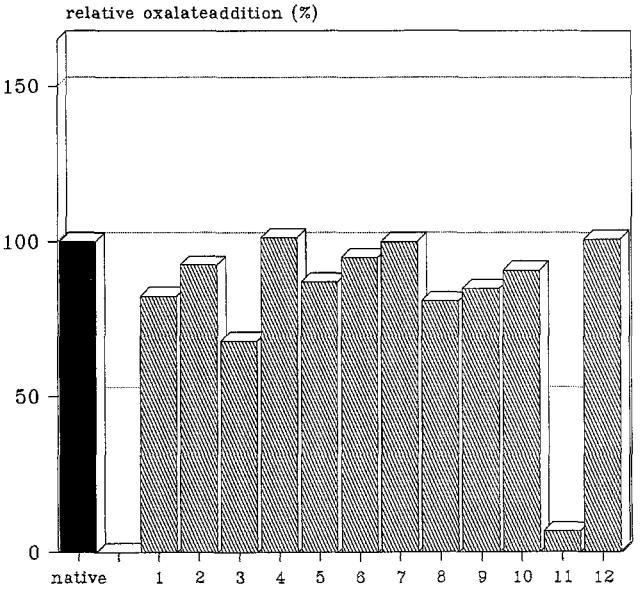

Fig. 10. Influence of zinc on the oxalate tolerance of non-stone-formers; ■ native (no zinc); ▨ + zinc (0.15 mmol/l)

approx. 80 kDa) isolated by salting out from urine of healthy subjects, and ston-formers acted as a promotor. Using electrophoresis no difference could be observed between the protein with the inhibitory effect and the salted-out THP. All patients suffering from recurrent calcium oxalate stones, with one exception, showed an increased oxalate tolerance after the addition of zinc. The buffering capacity of the urine samples for exogenous oxalate increased from 10% to 60%. Only one patient had a decreased tolerance value of approximately 10%. This suggested that the majority of the recurrent stone-formers displayed much better oxalate tolerance in their urine samples after the addition of zinc.

In contrast, oxalate tolerance values of three of five patients with an accidental stone episode were the same as without zinc. One patient had a decreased and one an increased oxalate tolerance of about 25%. In 3 of 12 healthy non-stone-forming controls urine oxalate tolerance was unaffected by the addition of zinc; in the remaining 9 controls a decrease in urine oxalate tolerance was observed.

Discussion

The adaption of the OTT to the precipitation kinetics of calcium oxalate determined in synthetic urine allows a considerably better discrimination between healthy persons, accidental and recurrent stone-formers. This has made it possible for the first time to evaluate the risk of developing recurrent stones. In addition, the effects of the addition of small amounts of zinc on tolerance have been used in a new variation of the OTT. Stone-formers showed an increased oxalate tolerance following the addition of zinc to their urine samples, while healthy persons and accidental stone-formers displayed the opposite effect, namely a decrease in oxalate tolerance.

In contrast to the mathematical formulae or computer programs published by various groups [6, 11, 13], which only considered the urinary electrolytes, the modified OTT also takes into consideration the inhibitory effect of organic macromolecular substances.

In the urine of healthy persons, zinc is complexed and cannot enhance the induction period of the calcium oxalate precipitation. This suggests that recurrent stone-formers have a disturbance in the high-affinity zinc-binding urinary glycoproteins. This hypothesis is further supported by the fact that neither the daily excretion rate nor the molar concentration of glycoproteins in healthy persons differs from those of stone-forming patients [4, 12]. The effectiveness of these inhibitors is also dependent on the method by which they are isolated. Salted-out glycoprotein from healthy persons initially has a reduced activity; it becomes active again after conditioning in synthetic urine at 37°C, probably through a change in the tertiary structure. A glycoprotein of approximately 80 kDa proved to be especially effective (THP). The dual function of THP (inhibiting as well as promoting effects on stone nucleation or aggregation), which has been reported by several research groups, can possibly be explained by a variably active structure, which may result from different isolation methods. The influence of the structure on different measurable effects (inhibitory/promoting) will be determined in further experiments.

References

1. Achilles W (1985) The gel crystallization method. Fortschr Urol Nephrol 23:252
2. Baumann JM, Lauber K, Lustenberger FX, Wacker M, Zingg EJ (1985) The direct measurement of inhibitor capacity in urine. Urol Res 13:169

3. Berg W, Schneider HJ, Brundig P, Bother C (1981) Verrechnungsmöglichkeiten und Patienten mit Kalziumoxalatlithiasis. Fortschr Urol Nephrol 20:21
4. Bichler KH, Kirchner CH, Ideler V (1976) Uromucoid excretion of normal individual and stone formers. Br J Urol 47:733
5. Briellmann T, Seiler H, Hering F, Rutishauser G (1985) The oxalate tolerance method. Urol Res 13:291
6. Finlayson B, Reid F (1978) Invest Urol 15:442
7. Hallson PC, Rose GA (1978) Rapid evaporation technique. Br J Urol 50:442
8. Hering F, Pyhel N, Ratajcak H, Friedrich R, Lutzeyer W (1981) Kalkulation der relativen Urinsättigung-Entscheidungshilfe bei der Therapieplanung. Fortsch Urol Nephrol 17:345
9. Ljunghall S, Danielson BG, Källsen R, Fritjofson A. Prediction of stone recurrence. In: Smith LH, Robertson WG, Finlayson B (eds) Urolithiasis. Plenum Press, New York, p 13
10. Pak CYC, Hayashi Y, Finlayson B, Chu S (1977) Estimation of state of saturation of brushite and calcium oxalate in urine and comparison of three methods. J Lab Clin Med 89:891
11. Robertson WG, Peacock M, Marshall RW, Marshall HD, Nordin BEC (1976) Saturation – inhibition index as a measure of the risk of calcium oxalate stone formation in the urinary tract. N Engl J Med 294:249
12. Sameull CT (1979) Uromucoid excretion in normal subjects, calcium stone formers and in patients with chronic renal failure. Urol Res 7:5
13. Tiselius HG, Almgard LE, Larson L, Sörba B (1978) A biochemical basis for grouping of patients with urolithiasis. Eur Urol 4:241
14. Will EJ, Bijvoot OLM, Blomen LJMJ, Vanderlinden H (1983) A seeded crystal growth method for measuring the effects of compounds or urine or the solubility, the growth and the agglomeration of calcium oxalate monohydrate crystals. J Crystal Growth 64:297
15. Tamm T, Horsfall FL (1950) Characterization and separation of an inhibitor of viral hemaglutination present in urine. Proc Soc Exp Med 74:108

Priv.-Doz. Dr. F. Hering
Urologische Klinik
Kantonsspital
CH-5404 Baden
Switzerland