[Frontiers in Bioscience 8, s326-332, May 1, 2003]

IS PRECIPITATION OF CALCIUM PHOSPHATE AN IMPORTANT FACTOR FOR THE DEVELOPMENT OF CALCIUM OXALATE STONES IN THE URINARY TRACT?

Hans-Göran Tiselius

Department of Urology, Huddinge University Hospital and Division of Urology, Center for Surgical Sciences, Karolinska Institutet, Stockholm. Sweden

TABLE OF CONTENTS

1. ABSTRACT

One commonly encountered problem in clinical urology is how to treat patients with calcium stone disease in a long-term perspective. At least ten per cent of the population in most parts of the world are afflicted by stone formation of which calcium stones undoubtedly dominate. The clinical problem comes from the fact that the disease is associated with a considerable recurrence rate. During a 10-year follow-up period recurrent stone formation can be expected in about half of the patients. In a Swedish epidemiological study it was shown that 30 per cent of patients who presented with their first stone had formed one or more new stones after 10 years. For those who had a history of more than one stone at the start of follow-up, the corresponding recurrence rate was 70 per cent (1). There is thus an obvious reason to provide some form of recurrence prevention at least to patients with the most severe course of the disease. Although several therapeutic alternatives today are available for such a purpose, their clinical efficacy is far from that desired. There are several explanations for our therapeutic shortcomings, but one important factor is our incomplete understanding of exactly how calcium stones form. Such knowledge is an absolute prerequisite for designing a rational effective and dynamic recurrence preventive treatment.

Of all calcium stones that form in the urinary tract the vast majority have calcium oxalate as the most common constituent (2-6). This salt is present either as calcium oxalate monohydrate (COM) or as calcium oxalate dihydrate (COD). Calcium oxalate trihydrate (COT) can be experimentally demonstrated, but is thermodynamically labile, and albeit this crystal phase probably is the one that precipitates first, it is not present in calcium stones, nor in the urinary sediment.

The dominance of calcium oxalate in the stones accordingly has led to the designation calcium oxalate stone disease. Nevertheless careful analysis of stone composition has disclosed that calcium phosphate (CaP) is a very frequent component of a large fraction of calcium stones, though often in small amounts only (5-10).

It is noteworthy that CaP was found in as many as 75 percent of those CaOx-rich stones that Leusmann analyzed (5). Moreover, Cifuentes-Delatte and coworkers reported that stones with an apparently papillary origin had a concave surface built up of CaP (11). The conclusion from these observations was that papillary crystals of CaP (i.e. Randalls plaque) were the focus of CaOx crystallization. Similarly CaP was demonstrated in 20.5 percent of the kidneys in an autopsy material. The crystals were located either interstitially or intratubularly (7).

Numerous CaP crystal phases are demonstrated in calcium stones or in urine. Hydroxyapatite (HAP) and carbonate apatite (CarbAp) are the most frequently encountered calcium phosphate salts, but brushite (Bru) octacalcium phosphate (OCP) and whitlockite also occur. There are, moreover, two amorphous types of CaP (ACP1 and ACP2) and it is assumed that ACP1 is the first form of CaP that precipitates in urine. The conversion of ACP1 to other CaP – salts is schematically shown in Figure 1.The regulation and clinical importance of this series of phase transformations will be further discussed below.

In terms of stone composition it needs to be emphasized that whereas CaOxCaP- stones and pure CaOx-stones occur in similar proportions in Europe and North America, pure CaP-stones are rare. This finding is

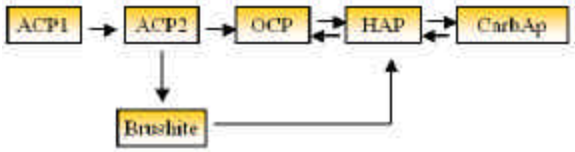

Figure 1. Interconversion between differentcrystal phases of calcium phosphate.

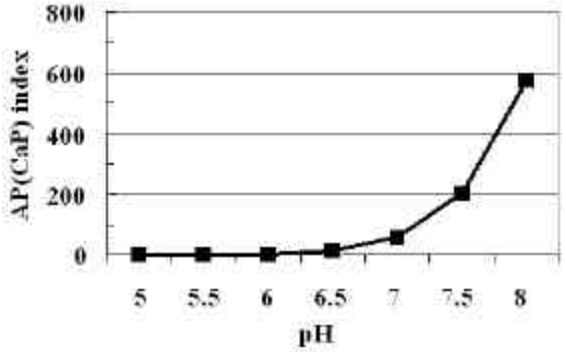

Figure 2. The effect of pH on AP(CaP) index in urine in which the 24h calcium is 6 mmol, phosphate 40 mmol and citrate 3 mmol. The volume was set to 1.5 L.

Table 1. Occurrence of salts in a Swedish population of patients subjected to stone removal with extracorporeal shock wave lithotripsy.

Crystal phase	Percent
Calcium oxalate monohydrate	89.3
Calcium oxalate dihydrate	68.2
Hydroxyapatite	69.8
Calcium oxalate + calcium phosphate	61.8
Brushite	3.4
Magnesium ammonium phosphate	4.6
Carbonate apatite	10.1
Uric acid /urate	4.2
Cystine	1.0

Table 2. Solubility (SP) and formation products (FP) of some CaP-salts (19, 23, 25, 26)

Salt	SP	FP
ACP	2.29×10^{-11} $(mol/L)^2$	-
OCP	8.30×10^{-48} $(mol/L)^8$	2.0×10^{-45} $(mol/L)^8$
HAP	2.35×10^{-59} $(mol/L)^9$	-
Brushite	1.87×10^{-7} $(mol/L)^2$	2.0×10^{-6} $(mol/L)^8$

surprising in view of the obvious fact that crystals of CaP very often are demonstrated in urine and that, moreover, urine in most people temporarily might be highly supersaturated with CaP (12-20). The present paper summarizes observations on the physical chemistry of CaP and its possible role in the development of calcium oxalate and calcium phosphate stones in the urinary tract.

2. OCCURRENCE OF CALCIUM PHOSPHATE IN CALCIUM STONES

Careful analysis of the composition of urinary tract calcium stones has disclosed the frequent occurrence of CaP. By wet chemical methods it was accordingly shown that stones with a weight of less than 20 mg had a surprisingly high content of CaP (21). It was, moreover, demonstrated that a high content of CaP in mixed CaOx/CaP stones was associated with a higher risk of recurrent stone formation than when the CaP content was low or CaP was absent (22) . As is evident from Table 1, mixtures of CaOx and CaP were the most common stone entity, whereas pure CaP stones were uncommon. These proportions of calcium stones most certainly vary form one geographical area to another, but differences in analytical routines and methods might give confounding results. Nevertheless it stands to reason that CaP is a common constituent of calcium oxalate stones and that it might be of etiologic importance.

3. PRECIPITATION OF CALCIUM PHOSPHATE

In order to form a solid CaP crystal phase the urine (or any other water solution) has to be sufficiently supersaturated with that CaP crystal phase. The ion-activity product of any CaP crystal phase is directly dependent on the free ion-concentrations of calcium and phosphate (6). By means of computerized iterative approximation it is possible to calculate the ion-activity products (AP) for the various CaP salts. In these calculations attention is paid to a number of complexes formed in urine and the procedure requires input of a large number of urine variables (23, 24).

The solubility products and formation products of the most common calcium phosphate crystal phases are summarised in Table 2 (19, 23, 25, 26). These products were derived from experiments in aqueous solutions. The most important determinants of the ion-activity products of CaP are the concentrations of calcium, phosphate and citrate as well as the pH. Based on these observations a simplified estimate (AP(CaP) index) of the ion-activity product of calcium phosphate (AP_{CaP}) can be derived and used to get approximate information on the level of supersaturation: (6).

AP(CaP) index (for a 24-hour urine collection) =

$$\frac{2.7 \times 10^{-3} \cdot \text{Calcium (mmol)}^{1.07} \times \text{Phosphate(mmol)}^{0.70} \times (\text{pH}-4.5)^{6,8}}{\text{Citrate (mmol)}^{0.20} \times \text{Volume (L)}^{1.31}}$$

It is evident that urine pH is by far the most important determinant. Figure 2 shows how AP(CaP) index changes in a 24-hour urine sample (in which the excertion of calcium, phosphate and citrate is 6.0, 40 and 3.0 mmol, respectively, and the volume 1500 mL), when the pH is increased form 5.0 to 8.0. When these data are expressed in terms of AP_{OCP} values it is evident that the FP_{OCP} is exceeded already at a pH between 6.5 and 7.

According to physical chemical laws the crystal phase with the highest level of SP is the one that most easily precipitates (6). As depicted in Figure 1 the first precipitate is ACP1 which is further converted to ACP2, OCP, HAP and CarbAp of which the latter two crystal

phases are thermodynamically most stable and accordingly most frequently observed in calcium stones (25, 27). Brushite has a higher solubility product than the other CaP crystal phases but does not precipitate until a sufficiently high concentration of $HPO4^{2-}$ is present. Although CarbAp is a common constituent of infection stones it is obvious that a conversion between HAP and CarpAp occurs also in the absence of infection. A conversion from ACP2 to brushite might be a common phase transformation, but brushite stones develop only rarely (28), probably because of the fact that brushite under normal conditions is thermodynamically labile. Magnesium is known to counteract both the conversion of ACP2 to brushite and of ACP2 to OCP and HAP (25, 27). It has accordingly been observed that a high calcium/magnesium ratio favors the formation of brushite.

Citrate apparently inhibits the transformation from OCP to HAP and stabilizes the amorphous calcium phosphate (29). The pH level is another important determinant of which crystal phase that will form when the concentration of calcium is increased and while HAP thus is the preferred crystal phase above pH 6.9, formation of brushite is favored by a pH less than 6.9 (29).

4. LEVELS OF CALCIUM SALT SUPERSATURATIONS IN THE NEPHRON

Calculations of the approximate ion-activity products in urine at different nephron levels clearly showed that, under physiological conditions, the supersaturation with CaOx at levels above the collecting duct was usually not high enough for CaOx crystal formation, notwithstanding this is a process of heterogeneous nucleation (30-31). In contrast urine both in the distal part of the distal tubule and in the loop of Henle might be highly supersaturated with CaP (17, 30, 32-34). Although it is reasonable to assume that the precipitation of calcium phosphate also is the result of heterogeneous nucleation, the ion-activity products might occasionally be at a level required for spontaneous crystal formation. Experimental data have shown that CaP might precipitate in urine with a composition corresponding to that in the loop of Henle as well as in the distal tubular urine (35).

5. CAN WE EXPLAIN CALCIUM OXALATE STONE FORMATION FROM AN ABNORMAL CRYSTALLISATION OF CALCIUM PHOSPHATE?

The dominance of CaOx in calcium stones formed in the urinary tract has resulted in a focus of almost all recent research on factors that are of importance for CaOx nucleation, growth and aggregation as well as on factors that regulate the interaction between CaOx crystals and cells. Much less attention has been paid to the CaP crystallization. Undoubtedly the precipitation of a CaOx crystal phase is of utmost importance for the stone-forming process, but the possible role of a CaP crystal phase needs serious consideration (36).

The fact that CaP and not CaOx, under the conditions prevailing in most stone-formers, can form a solid crystal phase at a nephron level above the collecting duct is an important feature (17, 30, 32). Over the years several authors have put our attention to an etiologic role of CaP (26, 37, 38). Pak and co-workers presented evidence that brushite served as a suitable surface for CaOx nucleation (39). The fact that brushite was an uncommon constituent of calcium stones was explained by a rapid transformation of brushite to HAP (37). Smith and Werness described calcium phosphate as “the forgotten crystal” and Baumann and co-workers proposed that CaP might act as a promoter for heterogeneous nucleation of calcium oxalate (37, 40, 41, 42).

Numerous experimental studies have also shown that calcium oxalate can be deposited on CaP crystals, whereas the reverse process does not easily occur (9, 17, 43, 44). Only calcium oxalate trihydrate seems able to induce CaP deposition and such a mechanism might explain the co-precipitation of CaOx and CaP that has been observed (45,13). Recently Achilles and his group showed in a gel flow crystallization system that granules of CaP induced precipitation of CaOx (46).

One conceptual problem for understanding the subsequent development of a CaOx stone is that the growth of CaOx or CaP is a relatively slow process. Stone formation might, however ensue when the CaP crystals for a sufficient period of time are retained in nephron urine that is critically supersaturated with CaOx.

In terms of solution chemistry it is important to note that the CaP phases precipitate in alkaline urine and dissolve in acid urine. Experiments have clearly shown that hydroxyapatite added to urine with pH 5.0, 5.25 and 5.5 dissolved during a two-hour period. This is probably one way by means of the kidney clears the nephron from small calcium phosphate crystals.

From a theoretical point of view small crystals can be eliminated either in this way or by internalization and subsequent dissolution by lysosomal enzymes (47, 48). Large crystal masses of CaP will probably move through the tubular system adherent to the wall and thus be subject to a pronounced retention. It is, moreover, possible that large crystal masses can destroy cells and become attached to the basement membrane (17). In this position and in urine that periodically has a low pH-level, dissolution of CaP might occur. During this process the release of calcium might establish high AP_{CaOx} -levels in the macromolecular environment that surrounds calcium salt crystals in urine. The combination of a large CaP crystal mass, a low pH and a peak CaOx supersaturation lays the ground for a heterogeneous nucleation of CaOx. This process might thus occur intratubularly or at the papillary tip following migration of crystalline material that following internalization has reached the interstitial tissue (17).

Conditions for a primary crystallization of CaOx is most certainly occasionally met in nephronic urine. Under these circumstances the net results will be stones composed of pure CaOx. It is, however, also possible that a pure CaOx stone is the end result of a heterogeneous

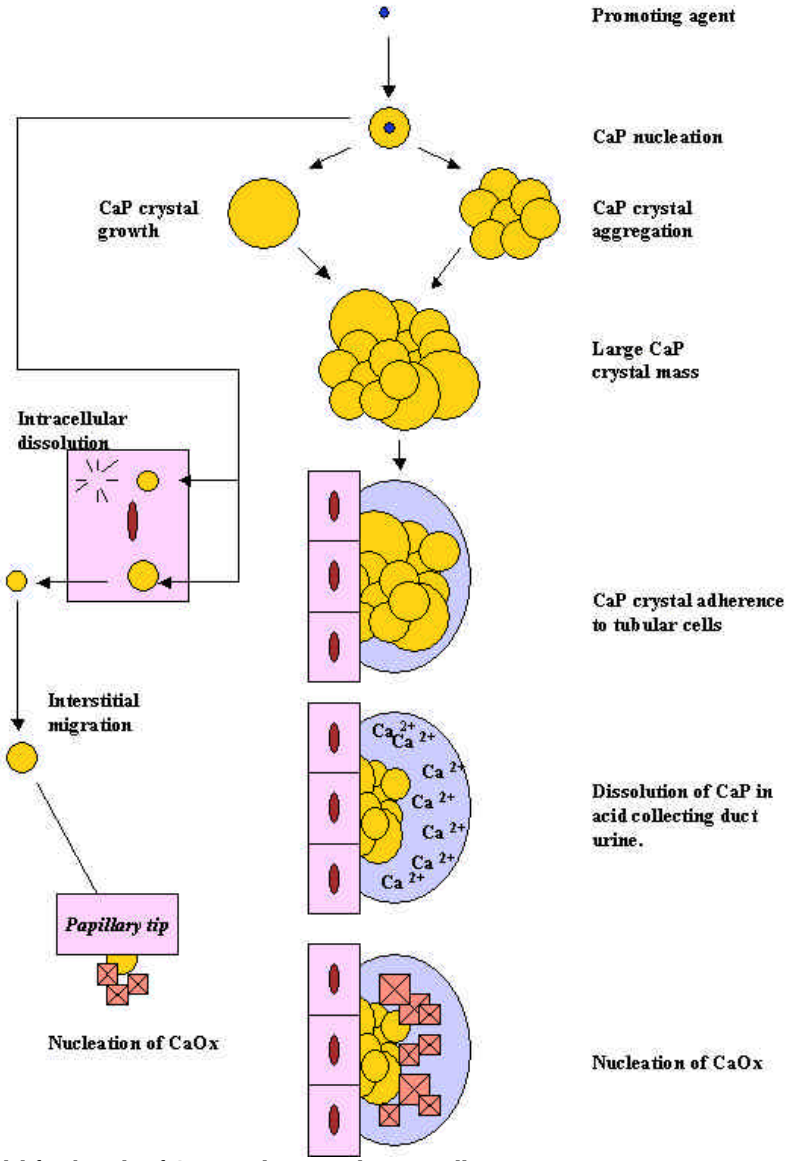

Figure 3. Tentative model for the role of CaP in calcium oxalate crystallisation.

nucleation of CaOx on CaP followed by a complete dissolution of the CaP – crystal phase.

When the intra-nephronic AP_{CaP} is high or when inhibitors of CaP crystal growth and CaP crystal aggregation are absent or present at low concentrations only, large crystal masses can form. Citrate, pyrophosphate and magnesium are small molecules with a powerful influence on CaP crystal growth. (49-51). Citrate and urinary macromolecules are powerful modifiers of crystal aggregation.

The crystal-cell interaction is most certainly of great interest in the stone forming process. Most of which has been described for CaOx crystals in this respect is also probably true for CaP, but a detailed discussion of this issue is beyond the scope of this review. A schematic view of the possible role of CaP in the stone forming process is presented in Figure 3.

The primary nucleation of CaP in the nephron is most certainly induced by a promoting agent. It is suggested that formation of free radicals at high nephron levels causes injury to the tubular cells with subsequent release of brush-border fragments and membrane lipids / phospholipids. Such urine constituents have been shown to induce nucleation of CaP as well as of CaOx. (17, 31, 52-56).

6. FORMATION OF CALCIUM PHOSPHATE STONES

An even better understanding of how calcium oxalate stones form can possibly be obtained by

considering the mechanism of pure CaP –stone formation. Stones composed of pure CaP are seen in patients with renal tubular acidosis (57, 58), in many patients with hyperparathyroidism (59, 60) and during treatment with carbanhydrase inhibitors (61, 62). These subgroups of patients have the common property of constant alkaline urine. For patient with RTA and for those treated with carbanhydrase inhibitors, there is also a low excretion of citrate.

In the presence of a CaP crystal: Why does not CaOx always precipitate? One possible explanation is that there is no dissolution of the CaP crystal phase. Other explanations are that the concentration of calcium is low because of extensive complex formation between calcium and phosphate or that the inhibiting power is augmented in the alkaline environment. Another interesting observation in this regard is that when cats were given an acidifying dietary formula with the aim of preventing struvite stone formation, the animals started to from CaOx stones (63).

All those observations taken together indicate that an acid pH of collecting duct urine or final urine plays an important role in CaOx stone formation. Why CaP stones do not regularly form during treatment with alkaline citrate or other alkalizing agents is probably due to the accompanying increased citrate excretion. Although more CaP crystals form in alkaline urine, they usually remain small (3).

7. THERAPEUTIC CONSIDERATIONS

In my view of an obviously central role of CaP in CaOx stone disease: Can we utilize this information to improve our preventive treatment of recurrent stone formers? It appears reasonable that any reduction of the supersaturation with CaP in urine in the loop of Henle and in the distal tubule should be beneficial . Such a step will decrease the risk of CaP nucleation as well as the growth rate of CaP crystals. A reduced excretion of calcium and phosphate together with a reduced pH and a high urine flow through these parts of the nephron might be used to achieve the goal of preventing CaP precipitation. It needs to be emphasized, however, that it is difficult to modify urine composition at high nephron levels.

Diclophenac sodium, eicosapentaenoic acid as well as thiazides might serve the purpose of reducing the excretion of calcium. Whether a reduced intake of phosphate significantly influences the ion-activity product of CaP at this level is not known. Although loop – diuretics like furosemide brings about a net increase in urinary calcium excretion, its diluting effect on urine in the ascending part of the loop of Henle and the distal tubules is an attractive therapeutic approach that has not been clinically studied.

Any attempt to counteract the formation of stable CaP crystal phases is probably worthwhile and a high intratubular magnesium/calcium –ratio might be beneficial in this respect. It is reasonable to assume that amorphous CaP more easily is eliminated from the tubular system and probably less prone to tubular cell adherence. A high concentration of citrate can most certainly be used to counteract CaP- nucleation, growth and aggregation.
Some of these therapeutic principles have a preventive power also on the CaOx – crystallization, whereas others have a specific effect on an abnormal CaP crystallization. There are, however, so far no clinical studies that have been designed with the aim of arresting the series of CaP crystallization events described above.

8. REFERENCES

1.Ahlstrand C & H-G. Tiselius: Recurrences during a 10-year follow-up after first renal stone episode. *Urol Res* 18, 397-399 (1990).

2. Brien G, G.Schubert & C. Bick: 10,000 anayses of urinary calculi using x-ray diffraction and polarizing microscopy. *Eur Urol* 8, 251-256 (1982)

3. Herring LC: Observations on the analysis of ten thousand urinary calculi. *J Urol* 88,545-562 (1962)

4. Leusmann D, H.Niggemann, S. Roth & H.von Ahlen: Recurrence rates and severity of urinary calculi. *Scand J Urol Nephrol* 29, 279-283 (1995)

5. Leusmann DB: Routine analysis of urinary calculi by scanning electron microscopy. *Scanning Microsc* 387-396 (1983)

6. Tiselius H-G: Solution chemistry of supersaturation. In: Kidney stones - Medical and surgical management. Eds: Coe FL, Favus MJ, Pak CYC, Parks JH, Preminger GM.. Lippincott-Raven publishers, Philadelphia. pp 33-64 (1966)

7. Cifuentes Delatte L, J.M Cifuentes & J.A: Medina A. Randall and his plaque. *Urology* 48, 343-346 (1996)

8. Grases F, J.G.March, A. Conte, A. Costa Bauzà: New aspects on the composition, structure and origin of calcium oxalate monohydrate calculi. *Eur Urol* 24, 811-386 (1993)

9. Khan SR & R. Hackett R: Role of organic matrix in urinary stone formation: An ultrastructural study of crystal matrix interface of calcium oxalate monohydrate stones. *J Urol* 150, 239-245 (1993)

10. Larsson L, B. Sörbo H-G. Tiselius H-G & S. Öhman: Wet chemical analysis of small (<20 mg) urinary calculi. In: Urinary stone. Eds: Ryall R, Brochis JG, Marshall V, Finlayson B. Churcill Livingstone, Melbourne. pp 339-342 (1984)

11. Cifuentes-Delatte L, J. Minon-Cifuentes & J.A. Medina: New studies on papillary calculi. *J Urol* 137,1024-1029 (1987)

12. Fan J & P.S. Chandoke: Examination of crystalluria in freshly voided urines of recurrent calcium stone formers and normal individuals using new filter technique. *J Urol* 161,1685-1688 (1999)

13. Hallson P.C & G.A. Rose: Seasonal variations in urinary crystals. *Br J Urol* 49,277-284 (1977)

14. Hallson P.C & G.A.Rose: Measurement of calcium phosphate crystalluria: Influence of pH and osmolality and invariable presence of oxalate. *Br J Urol* 64,458-462 (1989)

15. Herrman U, P.O. Schwille & P. Kuch : Crystalluria determined by polarization microscopy. Technique and results in healthy control subjects and patients with idiopathic calcium urolithiasis classified in accordance with calciuria. *Urol Res* 19,151-158 (1991)

16. Herrmann U & P.O. Schwille: Crystalluria in idiopathic recurrent calcium urolithiasis. Dependence on stone composition. *Urol Res* 20, 157-164 (1992)

17. Khan S.R: Calcium phosphate/calcium oxalate crystal association in urinary stones: implications for heterogeneous nucleation of calcium oxalate. *J Urol* 157,376-383 (1997)

18. Pak C.Y.C: Physicochemical basis for formation of renal stones of calcium phospahte origin. *J Clin Invest* 48,1914-1922 (1969)

19. Robertson W.G, M. Peacock & B.E.C Nordin: Activity products in stone-forming and non-stone-forming urine. *Clin Sci* 34,579-594 (1968)

20. Tiselius H-G.: Estimated levels of supersaturation with calcium phosphate and calcium oxalate in the distal tubule. *Urol Res* 25,153-159 (1997)

21. Öhman S, L. Larsson & H-G. Tiselius : Clinical significance of phosphate in calcium oxalate urinary calculi. *Ann Clin Biochem* 28,59-63 (1991)

22. Tiselius H-G & L. Larsson : Calcium phosphate - an important crystal phase in patients with recurrent calcium stone formation. *Urol Res* 21,175-180 (1993)

23. Marshall R.W & W.G. Robertson. Nomograms for estimation of the saturation of urine with calcium oxalate, calcium phospahte, magnesium ammonium phosphate, uric acid, sodium urate, ammonium urate and cystine. *Clin Chim Acta* 72,253-260 (1976)

24. Werness PG, C.M. Brown, L.H. Smith % B. Finlayson : EQUIL2: a basic computer program for the calculation of urine saturation. *J Urol* 134,1242-1244 (1985)

25. Christoffersen M.R, J. Christoffersen & W. Kibalczyc : Apparent solubilities of two amorphous calcium phosphates and octacalcium phosphate in the temperature range 30-42 ^{o} C. *J Crystal Growth* 106,349-354 (1990)

26. Koutsoukos P.G & G.H. Nancollas: Crystal growth of calcium phosphates - epitaxial considerations. *J Crystal Growth* 53,10-19 (1981)

27. Abbona F & M. Franchini-Angela: Crystallization of calcium and magnesium phosphate from solutions of low concentration. *J Crystal Growth* 104, 661-671 (1990)

28. Nancollas G.H: Crystallization theory relating to urinary stone formation. *World J Urol* 1,131-137 (1983)

29. Meyer J.L & A.H. Selinger: Effect of citrate on calcium phosphate phase transition. *Miner Electrolyte Metab* 3, 207-216 (1980)

30. Luptak J, H. Bek-Jensen H, A-M.Fornander, I. Höjgaard I & M-A.Nilsson: Crystallization of calcium oxalate and calcium phosphate at supersaturation levels corresponding to those in different parts of the nephron. *Scanning Microsc* 8, 47-62 (1994)

31. Khan S.R: Heterogeneous nucleation of calcium oxalate crystals in mammalian urine. *Scanning Microsc* 9,597-616 (1995)

32. Asplin J.R, N.S.Mandel & F.L. Coe: Evidence for calcium phosphate supersaturation in the loop of Henle. *Am J Physiol* 270,F604-F613 (1996)

33. Kok D.J: Intratubular crystallization events. *World J Urol* 15,219-228 (1997)

34. Kok D.J & S.R.Khan: Chances for a free and fixed particle mechanism. In: Proceedings of the Fifth European Urolithiasis Symposium. Eds: Rao P.N, Kvanagh J.P. pp 431. (1995)

35. Tiselius H-G & I Höjgaard: Some aspects on the intratubular creystallisation of calcium salts. *J Am Soc Nephrol* 10, S371-S375 (1999)

36. Tiselius H-G: Is calcium phosphate the primary product of crystallisation. *Scanning Microsc* 18,110 (1997)

37. Baumann J.M: Can the formation of calcium oxalate stones be explained by crystallization processes in urine? *Urol Res* 13, 267-270 (1985)

38. Hering F, G. Lueoend G, T.Briellmann, H. Seiler, H. Guggenheim & G. Rutishauser: Stone formation in the human kidney. In: Urolithiasis. Eds:Walker V.R, Sutton R.A.L, Cameron E.C.B, Pak C.Y.C, Robertson W.G. Plenum Press, New York. pp 73-74 (1989)

39. Pak C.Y.C: Potential etiologic role of brushite in the formation of calcium (renal) stones. *J Crystal Growth* 53,202-208 (1981)

40. Smith L.H & P.G. Werness: Hydroxyapatite - the forgotten crystal in calcium urolithiasis. *Transactions of the American Clinical Chemistry Association* 95,183-190 (1982)

41. Baumann J, K. Lauber,F.X.Lustenberger, M. Wacker & E.J. Zingg EJ: Crystallization conditions in urine of patients

with idiopathic recurrent calcium nephrolithiasis and with hyperparathyroidism. *Urol Res* 13,169-174 (1985).

42. Baumann J.M, B. Affolter B, U.Caprez, U. Henze, D.Lauper & F. Maier: Hydroxyapatite induction and secondary aggregation of calcium oxalate, two important processes in calcium stone formation. *Urol Res* 29, 417-422 (2001)

43. Baumann J.M, D.Ackermann & B.Affolter : The influence of hydroxyapatite and pyrophosphate on the formation product of calcium oxalate at different pHs. *Urol Res* 17,153-155 (1989)

44. Tiselius H-G, I, Höjgaard, A-M. Fornander, M-A.Nilsson: Is calcium phosphate the natural promoter of calcium oxalate crystallization? In: Urolithiasis 1996. Eds: Pak C.Y.C, Resnick M, Preminger G.M. Millet the Printer Inc, Dallas. pp 238-239 (1996)

45. Markovic M & H. Füredi-Milhofer: Precipitation of calcium oxalate from high-ionic-strength solutions. *Journal of the Chemical Society* 84,1301-1310 (1988)

46. Achilles W, U. Jöckel, A.Schaper, M. Burk & H.Riedmiller: In vitro formation of "urinary stones": Generation of spherulites of calcium phosphate in gel and overgrowth with calcium oxalat using a new flow model of crystallization. *Scanning Microsc* 9,577-586 (1995)

47. Asplin J R, J.H. Parks, M.S.Chen, J.C. Lieske, F.G.Toback, S.N.Pillay Y.Nakagawa & Coe FL: Reduced crystallization inhibition by urine from men with nephrrolithiasis. *Kidney Int* 56,1505-1516 (1999)

48. Lieske J.C & F.G. Toback: Renal cell-urinary crystal interactions. *Curr Opin Nephrol Hypertens* 9,349-355 (2000)

49. Berg C & H-G. Tiselius: The effects of citrate on hydroxyapatite induced calcium oxalate crystallization and on the formation of calcium phosphate. *Urol Res* 17,:167-172 (1989)

50. Hallson P.C, G.A.Rose & S.Sulaiman: Raising urinary citrate lowers calcium oxalate and calcium phosphate crystal formation in whole urine. *Urol Int* 38,179-181 (1983)

51. Meyer J.L & W.C.Jr. Thomas: Trace metal-citric acid complexes as inhibitors of calcification and crystal growth. I. Effects of Fe(III), Cr(III) and Al(III) complexes on calcium phosphate crystal growth. *J Urol* 128, 1372-1375 (1982)

52. Fasano J.M & S.R. Khan: Intratubular crystallization of calcium oxalate in the presence of membrane vesicles: an in vitro study. *Kidney Int* 59,169-178 (2001)

53. Khan S.R, F. Atmani, P. Glenton, Z.Hou, D.R. Talham & M. Khurshid: Lipids and membranes in the organic matrix of urinary calcific crystals and stones. *Calcif Tissue Int* 59, 357-365 (1996)

54. Khan S.R & P.A. Glenton : Deposition of calcium phosphate and calcium oxalate crystals in the kidneys. *J Urol* 153, 811-817 (1995)

55. Khan S.R, P.N. Shevock & R.L. Hackett: Presence of lipids in urinary stones: Results of preliminary studies. *Calcif Tissue Int* 41,91-96 (1988)

56. Kim K.M: Calcification of liposomes and red cell ghosts in vitro and in vivo. *Scanning Microsc* 3, 293-304 (1993)

57. Backman U, B.G. Danielson, G. Johansson , S. Ljunghall & B.Wikström: Incidence and clinical importance of renal tubular defects in recurrent stone formers. *Nephron* 25, 96-101 (1980)

58. Penney M.D & D.A.Oleesky: Renal tubular acidosis. *Ann Clin Biochem* 36, 408-422 (1999)

59. Rodman J.S & R.J. Mahler: Kidney stones as a manifestation of hypercalcemic disorder. Hyperparathyroidism ans sarcoidosis. U*rol Clin North Am* 27, 275-285 (2000)

60. Silverberg S.J, E. Shane, T.P. Jacobs,E.S. Siris, F.Gartenberg, D. Seldin, T.L. Clemens & J.P Bilezikian: Nephrolithiasis and bone involvement in primary hyperparathyroidism. *Am J Med* 89,327-334 (1990)

61. Ahlstrand C & H-G.Tiselius: Urin composition and stone formation during treatment with acetazolamide. *Scand J Urol Nephrol* 21,225-228 (1987)

62. Kass M.A, A.E. Kolker, M. Gordon, I. Goldberg, D.K. Gieser, T. Krupin & B.Becker: Acetazolamide and urolithiasis. *American Academy of Ophtalmology* 88,261-265 (1981)

63. Buffington C.A & D.J. Chew: Calcium oxalate urolithiasis in cats. *J Endourol* 13, 659-663 (1999)

Key Words: Calcium Phosphate; Calcium Oxalate; Kidney Calculi; Nephron; Urine; Urolithiasis, Review

Send correspondence to: Dr Hans-Göran Tiselius, Department of Urology, Huddinge University Hospital, SE-141 86 Stockholm, Sweden, Tel: +46 8 58 58 77 62, Fax: +46 8 58 58 77 60, E-mail: hans.tiselius@hs.se