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INVITED REVIEW

A hypothesis of calcium stone formation: an interpretation of stone research during the past decades

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Abstract An interpretation of previous and recent observation on calcium salt crystallization and calcium stone formation provide the basis for formulation of a hypothetical series of events leading to calcium oxalate (CaOx) stone formation in the urinary tract. The various steps comprise a primary precipitation of calcium phosphate (CaP) at high nephron levels, establishment of large intratubular and/or interstitial (sub-epithelial) aggregates of CaP. These crystal masses subsequently might be dissolved during periods with low urine pH. On the denuded surface of subepithelial or intratubularly trapped CaP, release of calcium ions can result in very high ion-activity products of CaOx, particularly during simultaneous periods with peaks of CaOx supersaturation. Crystals of CaOx may result from nucleation in the macromolecular environment surrounding the apatite crystal phase. In the presence of low pH, low citrate and high ion-strength of urine, formation of large CaOx crystal masses can be accomplished by self-aggregation of Tamm–Horsfall mucoprotein. Following dislodgment of the initially fixed CaOx stone embryo, the further development into to clinically relevant stone is accomplished by CaOx crystal growth and CaOx crystal aggregation of the retained stone material. The latter process is modified by a number of inhibitors and promoters present in urine. The retention of the stone is a consequence of anatomical as well as hydrodynamic factors.

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Introduction

It is obvious that effective prevention of recurrent stone formation requires understanding of the underlying pathology. The mechanisms of development of uric acid, infection and cystine stones are reasonably well clarified and the necessary therapeutic regimens rather straightforward. For the most common type of stones—calcium stones—there are, however, several shortcomings in this regard. Although several of risk-factors for the formation of calcium oxalate (CaOx) and/or calcium phosphate (CaP) stones have been identified, a full understanding of the basic pathology is lacking [1–3].

The major, and by far dominating, problem has been to get an explanation for how CaOx stone formation is initiated and how clinically important concretions can develop in the urinary tract. It seems as if we have been able to deal with the smoke, but without detecting the fire. The importance of this mystery is emphasized by the uncontested dominance of CaOx in analyzed stones [1, 4]. With few exceptions and for obvious reasons calcium stone research, during the past three to four decades, accordingly has had its focus on CaOx [4, 5] and this research has given us an impressive knowledge of various aspects of CaOx crystallization.

Analysis of urine composition in stone forming patients and normal subjects has been carried out with the aim of detecting any differences in levels of supersaturation and

concentration or activity of inhibitors and/or promoters of crystal nucleation, growth and aggregation [6–10]. Moreover, various mechanisms of interaction between crystals and cells have been experimentally illustrated [11–13].

Recent improvements of video and imaging technology have enabled detailed inspection of the renal papilla and attached crystalline material [14, 15]. In addition, microscopic examination of tissues and stones has shown a close association between crystalline material and the papillary tissue. Such a relationship was suggested already by Randall et al. [16], but the contribution of sub-epithelial calcifications to the stone forming process more or less was neglected during the past almost 75 years.

The formation of a CaOx stone—which is difficult to accomplish in vitro—most certainly is the result of a concert action between several factors and it is tempting to put together previous and recent observations in an attempt to get a unifying concept of how various mechanisms might contribute to result in a stone dominated by CaOx.

It is not the intention of this review to make a complete summary of all that has been published in this area, but rather to make a personal interpretation of the most relevant and interesting findings in previous and recent clinical as well as experimental research and observations during the past 3–4 decades. Neither has it been possible to find satisfactory evidence to support all conclusions and assumptions made, but in those cases indirect clues have been used as a guide.

Stone composition

It is fundamental to start this overview by looking at the chemical composition of calcium stones. As shown in Table 1, the dominance of CaOx is obvious and the vast majority of concrements undoubtedly was classified as CaOx stones [17–20]. On the other hand, the analytical result also emphasizes that CaP in small quantities was surprisingly common at least in renal and ureteral stones formed by patients in the author's geographical area and subjected to active stone removal. Whereas, in the author's experience pure CaOx thus accounted for approximately 20–30 percent of all calcium stones, those only built up of CaP were much less common. Stone analytical results from other parts of the world have shown similar results [1, 21], although local geographical variations have been observed. It is thus of note that in certain regions, such as in the Balkan area and in the Arabic countries, CaOx stones without CaP seem to be more common than stones composed of a mixture of CaOx and CaP [personal observation]. Differences in analytical techniques as well as in dietary, climate and living conditions might explain variations in calcium stone composition [1, 19]. The bottom-line of all these reports is, however, that CaOx always is the dominating stone constituent of calcium stones.

Table 1 Chemical composition of 3,906 actively removed calcium stones

Stone component	Percentage
CaOx	34.6
CaOx + ≤ 25% CaP	52.9
CaOx + > 25 ≤ 75% CaP	7.8
CaOx + > 75 % CaP	2.5
CaP	2.1

Morphological studies also have shown that numerous stones have a concave shape reflecting a previous attachment to a papilla [22, 23]. Moreover, small amounts of CaP were detected in the assumed attachment part of the stone [22, 24–27]. These basic observations suggest and agree very well with the assumption that CaOx stones have their origin on a CaP precipitate and that the early stage of these stones are attached to or in any way associated with the renal papilla [15, 24]. Subsequent video-endoscopic observations have shown that stones initially are fixed to the papillary surface [27].

Steps in calcium stone formation

A schematic summary of the important steps that might lead to formation of a CaOx stone is shown in Fig. 1. The scientific support and theoretical considerations for the various steps in this hypothetical sequence of events are discussed below.

The initial crystal formation

It is axiomatic that without a crystallization process no stones will form [28, 29]. Neither will crystallization take place unless the urine is sufficiently supersaturated with the crystal salt [1, 30, 31]. Inasmuch as a majority of people do not form stones, it is highly likely that the CaOx stone formation is the result of an abnormal and pathological crystallization propensity [1, 6, 30, 32]. One important observation in this regard was that stone forming patients have both more and larger crystals in their urine than is the case in subjects without stone disease [33–35].

There is accordingly today a lot of evidence that the initial crystal formation starts in a proximal nephron segment [29, 32, 36–38]. Although high levels of supersaturation with CaOx can be encountered in final (caliceal) urine as well as in collecting duct urine (probably most likely in the distal part of that segment) [30, 32, 37, 39, 40],

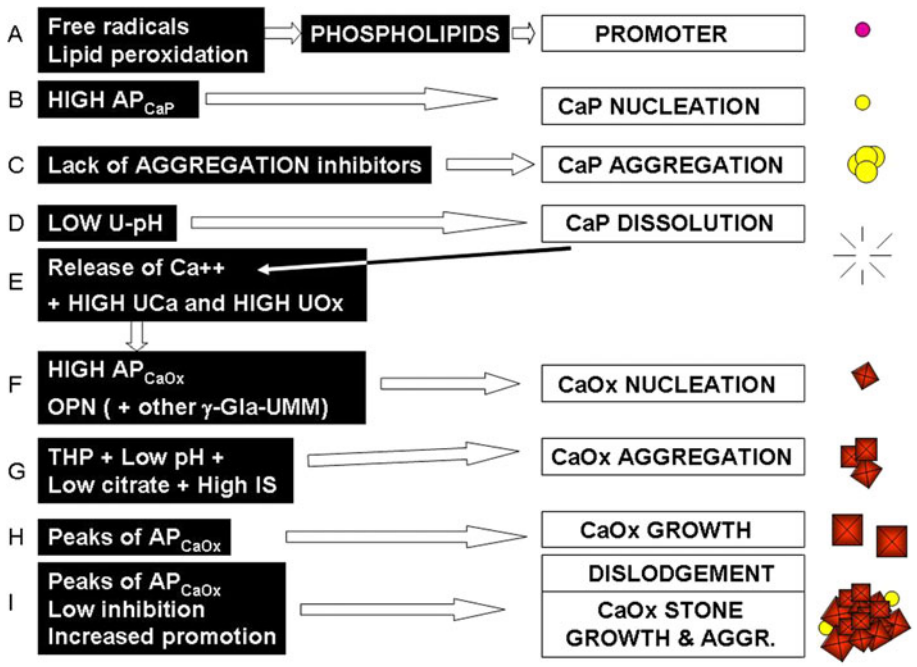

Fig. 1 A simplified summary of the various steps resulting in a CaOx renal stone. For further explanation the reader is referred to the text

there is apparently no driving force for CaOx precipitation at nephron levels above the collecting duct. In contrast, high levels of supersaturation with CaP can be expected in nephron segments with a high calcium concentration and a high pH [38, 39, 41–48]. Such a prerequisite seems to be met in the ascending loop of Henle and possibly also in the distal part of the distal tubule [36–40, 43, 45, 49–52]. In several reports based on SEM examinations, authors have found crystals in the lumen of the loop of Henle [17, 36, 38, 43, 53–56]. Although the chemical composition of these particles has not been identified in all these cases, their spherical shape and morphology are strongly suggestive of CaP nuclei [17, 54, 56, 57].

It is generally considered from the understanding of physical chemistry that the ion-activity product of CaOx (AP_{CaOx}) in urine never exceeds the labile level of supersaturation required for homogeneous nucleation (that is the formation product of CaOx) and therefore a spontaneous nucleation of CaOx always has been considered impossible [1]. This is indisputable at least if we consider urine composition as we usually can measure it. For CaP, on the other hand, it is at least theoretically possible that a spontaneous precipitation will occur at high pH levels, but if such a process can be expected in vivo is an unresolved issue. Nevertheless, the levels of AP_{CaP} might be sufficiently high to result in heterogeneous CaP nucleation also at high nephron levels [38, 47]. It is of note that the presence of amorphous CaP is a common and apparently normal finding in urinary sediments and several authors have shown that CaP is the most commonly encountered crystal material in urine [17, 58].

The most likely explanation is that the primary nucleation of CaP is facilitated by an appropriate promoter [53, 59–62]. A number of promoting agents has been described, such as phospholipids, lipids, cell debris and tubular cell membrane material possibly released from the brush border of the proximal tubules [59, 61, 63]. Damage to the brush border has been assumed the result of lipid per-oxidation caused by free oxygen radicals [63]. There is no definite proof of such a mechanism, but it is of interest that Achilles and coworkers [57] in a gel crystallization system observed that the nucleation of CaP spherolites was promoted by the phospholipid phosphatidylcholine in a way similar to the nucleation of CaOx that Khan and coworkers observed on monolayers of phospholipids [64]. Complex formation also has been demonstrated between CaP and phosphatidyl serine [65]. Alternatively, the macromolecular layer covering the cells of the tubular system might serve as a nucleation promoter. Theoretically, condensations of negative binding sites might accumulate calcium ions and cause temporary very high local AP_{CaP}-levels with ensuing CaP nucleation [12, 62, 66–68]. The γ-carboxyglutamic acid residues (Gla) in urinary maromolecules (UMM) constitute the main binding sites for calcium. Because Gla has a very high affinity for calcium ions, UMM thus can bind both free calcium ions in solution and calcium appearing on the surface of crystals. In this way, UMMs can exert not only inhibition of crystal growth and aggregation, but also promotion of nucleation. It thus is possible that in urine with a high content of calcium and a rich supply of UMM calcium binding sites, the local accumulation of calcium at each site will be lower and the crystals

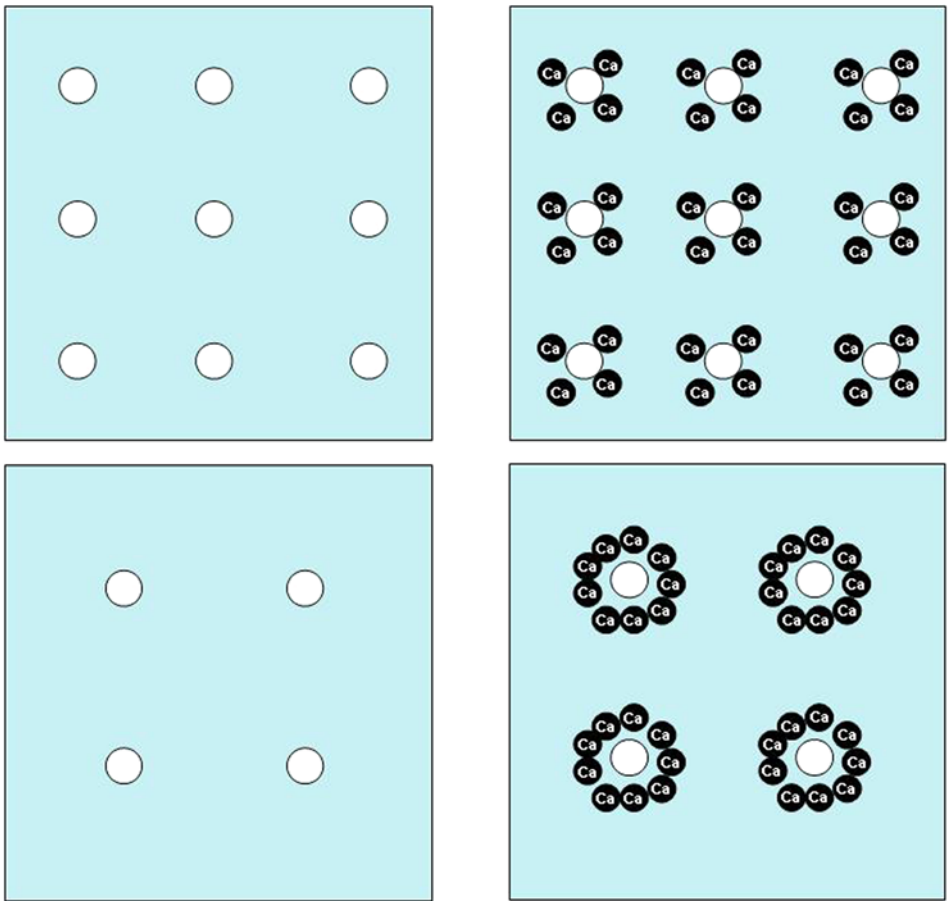

Fig. 2 A schematic description of how macromolecules with many nucleation sites (*above*) give relatively lower focal calcium concentrations, than is the case for macromolecules with few nucleation sites (*below*)

smaller than will be the case with UMM containing fewer binding (Fig. 2). Normal concentrations and structures UMM might thus serve as a defense against formation of large intratubular precipitates of calcium salts. In this respect, it is of note that less Gla residues were found in nephrocalcin and urinary prothrombin fragment 1 isolated from stone formers' urine [69, 70]. In alkaline urine, Tamm–Horsfall protein (THP) has been shown to inhibit CaOx crystal aggregation, but also to increase the volume of deposited material [71]. Whether THP thereby can act as a promoter of nucleation is a matter of debate, but both THP and nephrocalcin are excreted in the loop of Henle [70].

It is thus attractive to assume that mechanisms of calcium binding and focal accumulation of calcium ions modify the formation of CaP in tubular urine as well as that of CaOx in collecting duct/caliceal urine. That issue is further discussed below. The primary step in the abnormal crystallization can thus at least partly be explained by an increased nucleation promotion, a greater propensity to form CaP crystals/crystal aggregates due to high intratubular levels of $AP_{CaP,}$ a deficient inhibition of crystal growth/aggregation or a concert action of these factors.

The further fate of CaP

There are essentially two possible routes for the subsequent fate of the precipitated CaP, graphically depicted in Fig. 3. Either is CaP precipitates transported downwards with the urine stream in direction towards the collecting duct and the caliceal space as shown in Fig. 3b or is the CaP material internalized by tubular cells as indicated in Fig. 3a.

Internalization has been extensively observed and studied for CaOx crystals, but very little for CaP [12, 72, 73]. It is, however, likely that what in this regard has been demonstrated for CaOx also is valid for CaP. Internalization seems to be another defense mechanism by means of which the nephron cells eliminate intratubular precipitates in order to prevent obstruction of tubules [13]. Lysosomes in tubular cells as well as in interstitially located macrophages might thus dissolve both CaP and CaOx [7].

Microscopic examination of tissue samples from stone formers has shown CaP in the basement membrane of the cells lining the loops of Henle [14, 49, 74]. If these crystals are the result of nucleation because of a high AP_{CaP} level in the alkaline interstitial tissue following absorption of calcium from loop urine is unknown [75] and the details of

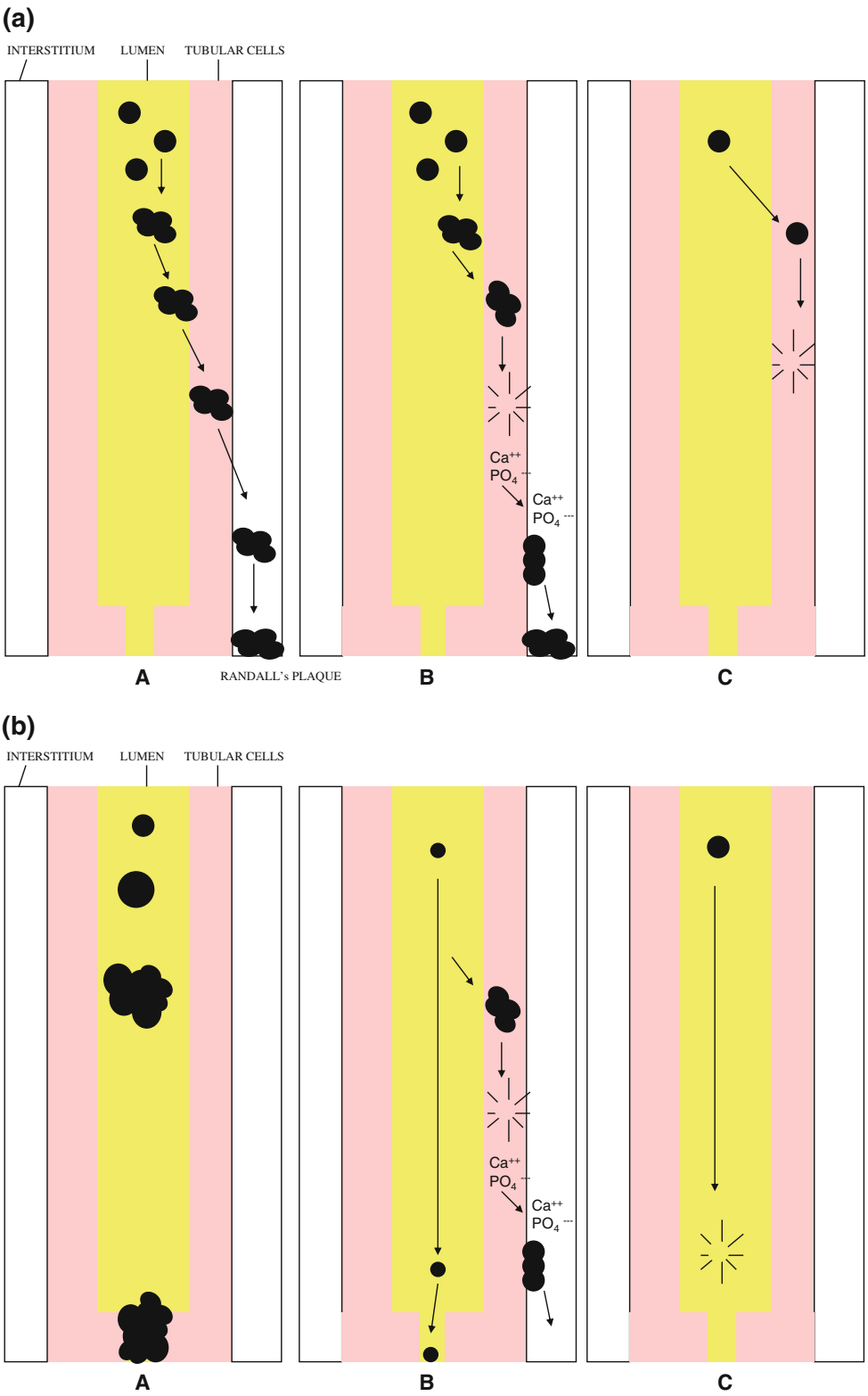

Fig. 3 **a** Interstitial route for CaP. Intratubular precipitation and aggregation of CaP (*black dots*) with subsequent translocation to the interstitial tissue and formation of a subepithelial plaque (*A*), internalization, dissolution and re-precipitation of CaP in the basement membrane of the loop of Henle with subsequent formation of a subepitheial plaque (*B*), or internalization and dissolution of CaP with elimination of calcium and phosphate without further precipitation (*C*). **b** Intratubular route for CaP. Precipitation and aggregation of CaP with slow transport down the nephron to the narrow opening if the collecting duct at the papilla (*A*), precipitation of CaP that either remains small and is expelled or is internalized at a lower nephron level with intracellular dissolution and formation of a subepithelial plaque (*B*) or precipitation of CaP that remains small and is dissolved intratubularly during periods of low pH (*C*)

calcium absorption in the loops of Henle are so far incompletely clarified. It is of note, however, that CaP crystalline material frequently has been demonstrated in the lumen [13, 76] and theoretically, a possible course of events is that CaP spherulites either are translocated to the basement membrane/interstitial tissue through the cells or between the cells (Fig. 3a:A). It has been shown that large crystals or crystal masses might be covered by epithelial cells. Endocytosis associated with cell proliferation has been described for both CaOx monohydrate and

hydroxyapatite [11]. Crystal of CaP or anyother crystals can be overgrown by tubular cells by this exotubulosis, which apparently is a possible mechanism by means of which intratubularly formed crystals can be translocated to the interstitial tissue [13]. The crystal material might also be internalized and dissolved by lysosomal enzymes (Fig. 3a:B, a:C) [7] after which the ensuing calcium and phosphate ions are eliminated from the cells to the surrounding interstitial tissue where CaP re-crystallizes if the local AP_{CaP} becomes sufficiently high (Fig. 3a:B). Such mechanisms may explain why accumulated CaP is found in the basement membrane. Subsequent elimination of crystalline material from the interstitial tissue by endocytosis and dissolution [7] is one possible explanation for the lack of subepithelial apatite deposits in normal subjects [75].

It is the CaP crystals or CaP deposits adjacent to the long loops of Henle that apparently constitute the subepithelial calcifications that we recognize as Randall's plaque [15, 24, 77]. Only the long loops of Henle that are found close to the papillary surface are thought to have sufficiently high intratubular concentrations of calcium and phosphate to enable CaP nucleation [75, 78].

Small CaP crystals (spherulites) may remain unattached to the tubular walls because of repulsive forces between the negatively charged UMM surrounding the crystal material and that covering the surface of tubular cells. In the presence of inhibitors, the crystals are thus likely to remain small and less innocuous than the large. In contrast, large crystals and crystal aggregates because of their size and mass (weight) logically might be prone to adhere to the tubular cells whereby the internalization is facilitated (Fig. 3a). Alternatively, CaP moves down the nephron at a slower rate than small crystal (Fig. 3b) [12, 72, 73]. Factors that determine which route (interstitial or intraluminal) that the CaP precipitate will take is unknown, but it can be speculated that if the size of the CaP aggregate is too large to be handled by the tubular cells it remains intratubularly, otherwise the interstitial route is preferred. This assumption is in agreement with the observation that in most patients with idiopathic calcium stone disease, the origin of the stones was a Randall's plaque [27, 74, 75, 77–79].

Large intratubular crystals and crystal masses are the result of rapid growth and aggregation, most probably determined either by insufficient concentrations of inhibitors or by structurally abnormal inhibitors [80]. Any formation of calcium salts in urine are modified by inhibiting substances and it has been known since long that citrate, magnesium, and pyrophosphate—also in very low concentrations—counteract growth of CaP crystals [81, 82]. Low free as well as total urinary concentrations of citrate are common findings in recurrent stone forming patients [83]. It has been demonstrated that citrate also is a powerful inhibitor of crystal aggregation [84, 85]. From a therapeutic point of view urinary citrate can be increased by administration of alkali, and although AP_{CaP} is increased by the increased pH, the CaP crystals apparently remain small [86].

Various UMMs have a pronounced inhibitory influence on growth as well as on aggregation of CaP and CaOx, an effect that is accomplished by binding to the crystal surface [87–91]. Most of this knowledge has been obtained from studies in systems with CaOx crystals, but inasmuch as the inhibitory properties are related to the binding of calcium to Gla-units in the molecules, it can be assumed that most of the findings for CaOx also are valid for CaP. Accordingly, both osteopontin (OPN) and polyaspartic acid are potent inhibitors of hydroxyapatite formation [82]. Moreover, extraction of macromolecules from CaOx and CaP disclosed a similar pattern of proteins with albumin, inter-α-inhibitor, OPN, prothromin related proteins, and THP [92].

The role of Randall's plaque

That Randall's plaques were of importance for calcium stone formation was suggested already when these subepithelial calcifications first were described [16]. When other important factors for CaOx stone formation were discovered the role of these common sub-epithelial calcifications was forgotten or neglected and considered unimportant for the stone formation. Nevertheless, several reports during the past three decades have suggested an important link between CaP and CaOx [1, 17, 18, 20, 76, 93, 94]. The recent revival of the role of Randall's plaque definitely opens a window to a better understanding of stone formation and helps to explain several so far obscure steps in the formation of CaOx stones [14, 27, 75, 78, 79]. Whether the sub-epithelial accumulation of CaP has a physiological role is not known and as long as the epithelial coverage at the papilla is intact there is apparently no pathological role either. It was early observed, however, that Randall's plaques were more common in patients with stone disease than in those without [16].

The mechanism of erosion of the surface epithelium—of obvious importance for the further crystallization process—is not understood, but might be a result of the toxic or mechanical effects by the sub-epithelial apatite mass [14, 95]. Following erosion of the papillary epithelial cells, the denuded apatite surface will be covered by macromolecules [66, 96]. Both OPN and THP were found in close association with the apatite plaque [66, 97].

Extensive studies on the crystal pathology in humans with stone disease and in normal subjects [14, 27, 75] made it possible to make a distinction between sub-epithelial plaques and intratubular deposits of crystalline material. It is of note that no plaques were found in normal subjects, whereas

patients with idiopathic calcium stone disease, patients with brushite stones and those with hyperparathyroidism, small bowel resection and ileostomies had plaques with various extensions. In the studied patients, no intratubular deposits were found in idiopathic stone formers.

In the studies referred to above [14, 27, 75] development of sub-epithelial apatite plaques appeared to be associated with a high urinary excretion of calcium, low urine pH and small urine volumes. Although this relationship is striking, it is not proven that these factors reflect the formation of interstitial plaques. These patients also had a stone disease and the urine abnormalities listed might be parallel findings that in addition to the presence of plaques provide an explanation for CaOx precipitation and stone formation.

The crucial role of pH

One of the most powerful risk factors of CaP precipitation at high nephron levels is the pH. Without sufficiently alkaline urine the prerequisite for CaP precipitation (Fig. 1B) is lacking [39, 41, 55, 98]. In this regard, it has been shown that the concentration of citrate is an important modifier of the further crystal morphology (Fig. 1C) [1].

In addition, for the further course of the stone forming process, pH seems to be of fundamental importance. If we consider the CaP crystals that reach the collecting duct, they are exposed to increasing levels of AP_{CaOx} as a result of nephron handling of urine. In addition to the supersaturation with CaOx, low pH levels cause CaP crystal dissolution (Fig. 1D) [45, 93, 99, 100] with release of Ca^{++} and PO_4^- ions. The calcium concentration surrounding the dissolving CaP might locally be very high and accordingly—when added to the calcium concentration already present in urine—further increase AP_{CaOx} and the risk of CaOx crystal formation (Fig. 1E, F). This risk of CaOx precipitation is apparently particularly high if the CaP crystal material temporarily is attached to or adherent to the tubular wall or trapped in the ducts of Bellini (Fig. 3).

Small intratubular CaP crystals will probably dissolve completely or remain so small that they are expelled without further complications (Fig. 3a, b). In contrast large CaP crystals or crystal aggregates can provide a base for CaOx stone formation, whereby the ensuing stones initially are fixed to the papillary tip at the narrow opening of the collecting duct (Fig. 3b) [26]. Although intratubular CaOx crystals theoretically might reach a critical size without fixation [32], crystal retention caused by attachment or fixation of initially formed crystalline material has been considered important for the subsequent stone forming process [101].

Of great interest is to consider what—in this regard—happens to the Randall's plaque. With an intact epithelium covering the plaques nothing happens and very little is known about the long-term course of Randall's plaque under these circumstances. When the epithelium for some reason is eroded [27], however, the apatite crystal mass is exposed to urine. There are no clinical observations that CaP will grow on the apatite surface and the reason for that is probably that the surface is well covered and protected by UMMs, serving as efficient inhibitors of growth and aggregation.

In most studies on urine composition, the pH is measured in complete 24 h collections. Although some reports thus have demonstrated lower pH levels in urine from CaOx stone formers than in normal subjects [102, 103], as well as in patients with COM stones compared with those forming COD stones [104]. This way of measurement, however, does in no way disclose periods with critically low pH-levels. There is a considerable variation in urinary pH during the day [105] and as shown in the example of measurements in short term urine fractions during a 24-h period (Fig. 4) there was a range between 5.65 and 7.25. There are also seasonal variations [106].

During periods with decreased urine pH (peaks of hydrogen concentration), the acidity might affect the apatite and cause CaP dissolution with accumulation of high concentrations of calcium ions in the macromolecular environment immediately adjacent to the apatite crystal surface (Fig. 1D) [96, 107]. Albeit also phosphate is released from the apatite crystals, the accumulation of calcium to specific binding sites on UMM, probably can result in a supersaturation level sufficiently high for CaOx nucleation (Fig. 1F). This risk is apparent inasmuch as high CaOx supersaturation levels very often occur simultaneously with periods of low pH [39, 45, 93, 98].

Dissolution of CaP was demonstrated in solutions and urine with a pH of 5.8 or lower [45]. Moreover, in experimental studies a suspension of CaP supersaturated with CaOx did not result in CaOx crystallization until the sample was acidified [100]. Figure 5 shows a concentration adjusted and simplified interpretation of the outcome of such experiments. It is likely that UMMs decrease the rate of CaP dissolution, but at the same time the diffusion of

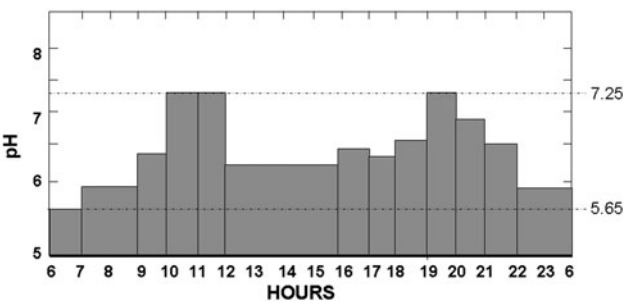

Fig. 4 Example of urinary pH variation during a 24 h period

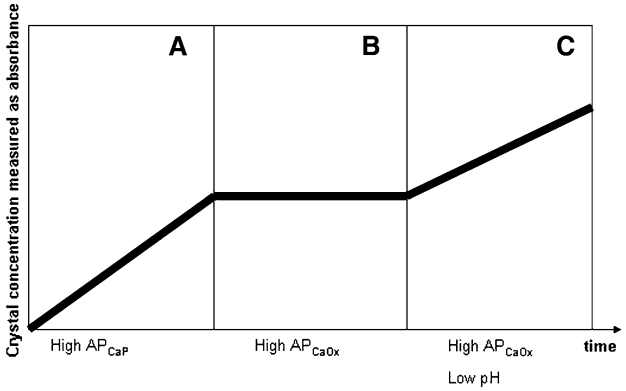

Fig. 5 Simplified and dilution corrected interpretation of experiments in which urine first was supersaturated with CaP in order to get crystals of CaP (*A*), subsequently supersaturated with CaOx by addition of oxalate (*B*) and finally acidified to a pH of approximately 5.0 (*C*). The crystal volume was measured as absorbance. During period C the CaP crystals disappeared and CaOx crystals were formed [100]

released ions is counteracted and that might contribute to establishment of the very high local concentrations of calcium (Fig. 1E) as discussed above.

Although dissolution of apatite in acid environment causes release of a substantial number of calcium ions, it is difficult to calculate the exact effect that this calcium supplement has on AP_{CaOx} because of the simultaneous increment in phosphate ion concentration. Moreover, the available iterative approximation computer programs are not constructed to deal with very high concentrations of both calcium and phosphate.

Theoretical calculations on apatite dissolution indicate that—provided no immediate diffusion occurs—dissolution of only 1–5 percent of a sphere of hydroxyapatite with a diameter of 1 μm enclosed in a cube with a volume of 1 μm^3 may cause extremely high concentrations of calcium (50–250 mmol/L) in the surrounding fluid space. These calculations were based on the assumption that the density of hydroxyapatite was 3.16 g/cm^3. From these observations, it seems reasonable to assume that what happens in the macromolecular layer covering the apatite surface is nucleation and rather than crystal overgrowth.

It has long been puzzling that although CaOx stones are so common, AP_{CaOx} levels in urine never seem to reach the sate of labile supersaturation necessary for nucleation [1]. Dissolution of CaP either in Randalls's plaques (Fig. 3a) or in fixed intratubular CaP masses (Fig. 3b), provide a possible explanation for establishing sufficiently high AP_{CaOx} levels resulting in nucleation. Further studies should be designed in order to further elucidate such a possibility.

In case of constantly alkaline urine, no CaP dissolution occurs and the end product will be pure CaP. Accordingly, such stones are found in patients with renal tubular acidosis [48, 98] and following treatment with acetazolamide [108]. Both these clinical conditions have an association between high pH and low citrate. With the exception of brushite—for which the mechanisms of formation are less well understood—there are few other medical conditions in which pure CaP stones are encountered.

It seems reasonable to assume, however, that macromolecules play an important role in the crystallization process by controlling the nucleation. It is well recognized that macromolecules provide an important template for CaOx crystal development [59, 62, 67, 107, 109–111].

Accumulation of calcium ions at Gla-foci might thus result in CaOx precipitation (Fig. 1F) in the same was as discussed above for CaP [8, 112]. Under certain conditions and in presence of a sufficient supply of oxalate ions, the driving force of AP_{CaOx} results in clusters of CaOx molecules exceeding a critical size and nucleation occurs. It can moreover be speculated that with a rich supply of calcium binding sites in UMM the corresponding local supersaturation levels do not reach the level necessary for critical nucleus formation. In such circumstances, calcium and oxalate remain in solution without crystallization [1]. At moderately increase levels of AP_{CaOx} the nuclei formed are likely to remain so small that they easily can be prevented from further growth an aggregation. Accordingly, macromolecules seem to have a dual role as being both inhibitors and promoters of the CaOx crystallization [12, 96], it is just the circumstances that are decisive. Calcium ions on the surface of crystals attract macromolecules because of the calcium binding sites and in this way, the macromolecules on the crystal surface counteract crystal growth and crystal aggregation. Similarly, the same free binding sites might attract calcium ions and enable nucleation.

In the analysis of crystallization properties of urine a low concentration of UMM calcium-binding sites most certainly will be reflected in a low inhibitory activity of crystal growth and crystal aggregation. In numerous reports, lower inhibitory activities of these two growth processes have been demonstrated in stone formers than in normal subjects. Several attempts to combine AP_{CaOx} levels and inhibitors have almost invariably disclosed significant differences between stone formers and normal subjects even though most of these measurements were carried out in 24 h or other long-term urine samples [113–115].

It is well recognized that the initial nucleation process has been much more difficult to measure experimentally in a standardized way than the subsequent crystal growth and crystal aggregation [116].

The further development of CaOx crystals

It is highly interesting to note that THP was found among the macromolecules associated with Randall's plaque

[14, 27]. Moreover, previous studies have shown that at low pH, high ion strength, and high concentrations of calcium, THP self-aggregates [97]. Periods with low urine pH with CaP dissolution occurs when urine is highly concentrated (that is has a high ion strength) and together with CaOx nucleation induced by CaP it is highly likely that THP brings about a pronounced aggregation of crystals (Fig. 1G). In contrast during periods with high diuresis and alkaline urine (or at least higher pH levels) the low ion-strength and the low concentration of calcium ions, THP has an inhibitory effect [56] and that inhibitory power also counteracts both CaP crystal growth and aggregation and accordingly helps to explain why pure CaP stones are so uncommon.

Whether aggregated THP molecules also promote CaOx nucleation is not known and I am not aware of any such studies [60]. A high concentration of citrate reduces self-aggregation of THP, and the highest citrate excretion and citrate concentrations in urine is seen when the pH is high [117].

Interestingly, Achilles and co-workers observed in a gel system at pH 6.8–6.9, that a metastable solution of CaP resulted in formation of CaP spherulites [4]. Nucleation of CaOx subsequently occurred at pH 6.0 when a solution metastably supersaturated with CaOx was allowed to flow over the same system. The recorded nucleation of CaOx in a gel system might very well reflect what happens in the macromolecular environment covering the Randall's plaques following erosion of the epithelium or in the macromolecular cover of CaP crystal masses with an intratubular position.

Formation of the clinically relevant CaOx stone

Once a CaOx stone embryo has been initiated at the surface of Randall's plaque or at a CaP plug in the ducts of Bellini, they can increase in size by growth as long as AP_{CaOx} exceeds the solubility product. The protection by inhibitors of crystal growth counteracts that process and a clinically important growth is likely only during periods with peaks of AP_{CaOx} and low inhibitory activity (Fig. 1H).

The CaOx stone embryo will subsequently be dislodged for unknown reasons. Probably the size plays a major role but destruction of then surrounding tissues caused by the toxic effects of CaOx itself might contribute to this course. As a free crystal mass and retained by anatomical or hydrodynamic factors, the stone material might increase in size by further growth and attraction of aggregates of CaOx (Fig. 1I). Some of the stones will be completely free of CaP, whereas others will have small remnants of CaP. Secondary growth of CaP on CaOx does not occur [6].

Possible outcomes of the initial CaP precipitation

Four principally different outcomes can be expected from the CaP that has been produced in the nephron and these are shown in Fig. 6.

With a low pH but without a sufficiently high AP_{CaOx}, CaP will dissolve without pathological or clinical consequences. The real risk appears when a low pH coincides with high AP_{CaOx} and that is the possible basis for CaOx stone formation discussed in detail above. With high levels of AP_{CaOx} but without a pH that causes CaP dissolution it is unlikely that a sufficiently rapid CaOx crystallization is induced. It has been demonstrated that CaOx can grow on the surface of CaP and that might take place, particularly in the absence of sufficient inhibition, but such a growth process will be slow and in most situations probably without clinical importance. The CaP crystals with or

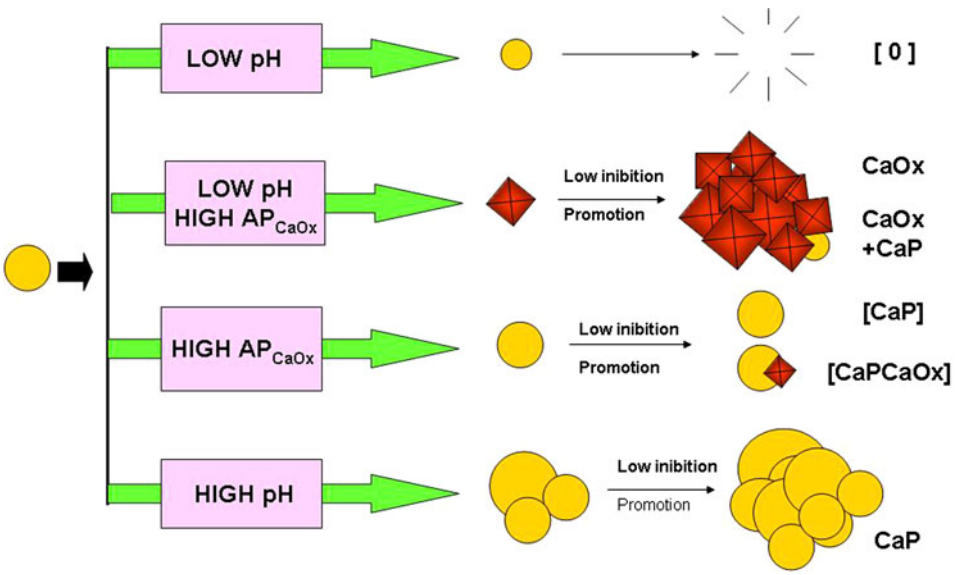

Fig. 6 Four possible results of a primary CaP precipitation (*yellow dot*). CaOx is shown as *red squares*. For further explanation, please refer to the text

without small amounts of CaOx on the surface are excreted. Finally, constantly alkaline urine enables further growth and precipitation of CaP and such conditions result in the pure and relatively rare CaP stones.

Other important factors, such as inhibitors of growth and aggregation modify the crystallization steps at all levels and play an important role in the development of a stone both in its attached and released state [8].

It is highly likely that rational recurrence prevention needs to take into account the dynamic variation that takes place during the 24 h period and that a much more elaborate technique for studying urine composition should be applied in the clinical work-up of patients with recurrent calcium stone disease.

Can CaOx stones form in the absence of a CaP crystal phase?

It is tempting to assume that formation of a CaP crystal phase always precedes the formation of CaOx [27]. In conditions with very high concentrations of oxalate as seen in patients with primary and enteric hyperoxaluria and experimentally following administration of ethylene glycol to animals, it seems reasonable that in the presence of suitable promoters a primary heterogeneous nucleation of CaOx might occur also at a higher level in the nephron than what is usually the case in other stone formers. The explanations for that outcome most certainly are the very high AP_{CaOx} peak levels that can be established in the nephron due to exceptionally large excretion of oxalate. Under normal conditions and in the average stone forming patients high AP_{CaOx} levels seems to be encountered only in distal collecting duct and caliceal urine.

Indirect evidence for the hypothesis presented above

There are indeed a number of observations that fit into the model of stone formation suggested and discussed in this review and which accordingly give support to the hypothesis. The most powerful pharmacological agents given to patients with recurrent CaOx stone disease are thiazides and potassium citrate [118]. Administration of thiazides results in a reduced *calcium* excretion whereas potassium citrate increases both urinary *pH* and the excretion of *citrate* [119].

Under conditions with very concentrated urine caused by losses of water in a warm climate together with an extra load of oxalate, stones of pure CaOx develop. This is probably the reason for the massive CaOx stone formation seen for instance in the Arabic population, where even CaOx staghorn stones—seldom encountered in Northern Europe—can develop.

Although CaP stones are found in a relatively low frequency, the appearance of amorphous CaP in urine is very common. The reason for that might be that with normal levels of citrate the aggregation of CaP does not occur despite peaks with alkaline urine and the crystalline material is easily eliminated [86]. Low citrate and high calcium concentrations is a general feature of urine composition in numerous studies.

Another and highly interesting observation was that cats given acidifying agents, in order to counteract struvite stone formation, instead started to form CaOx stones [120].

Conclusions

The various steps in the stone forming process reviewed and discussed in this article rise a number of considerations of importance for the evaluation and recurrence preventive treatment. Although efforts to counteract the stone forming propensity at all levels of the suggested series of events might be more or less rewarding, it seems essential to stop the primary precipitation of CaP.

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