Chapter 9

The Way from Renal Calcifications and Urinary Crystals to Kidney Stones: An Important Aspect in the Pathogenesis of Calcium Nephrolithiasis

Johannes M. Baumann

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.70598

Abstract

The formation of calcium (Ca) stones occurs in an initial phase by fixed growth on kidney calcifications consisting either of intratubular crystal accumulations protruding in renal calices (Randall's plugs) or of interstitial hydroxyapatite deposits (Randall's plaques) broken through the covering epithelial layers. Crystal aggregation (AGN) seems to be responsible for stone growth during crystalluria. This chapter reports on new aspects of the AGN of calcium oxalate being the most frequent stone compound and tries to explain why despite the widespread occurrence of kidney calcifications and crystalluria not everybody forms stones. Urinary crystals normally are protected from AGN by coats of urinary macromolecules (UMs) which by their identical electronegative charge create zones of electrostatic repulsion. At high urinary concentration or ionic strength respectively, these zones are compressed and can be bridged by self-aggregated UMs. Self-AGN occurs in concentrated urine by the adsorption of UMs on free surfaces like Randall's plugs or plaques. High oxalate excretion and high urine concentration favoring intratubular crystal accumulation, breaking of epithelial layers on Randall's plaques and self-AGN of UMs are most deleterious factors in Ca stone formation and have to be avoided by stone metaphylaxis.

Keywords: calcium nephrolithiasis, crystalluria, Randall's plaques and plugs, urinary macromolecules, calcium oxalate aggregation

1. Introduction

The pathogenesis of kidney stones that often are accompanied by very painful colic and can lead to renal failure and even to the loss of a kidney is far from being clear. In Western

populations, nephrolithiasis has reached a prevalence up to 10% [1]. Kidney stones are composed of crystal aggregates within an organic matrix. Long times stone formation mainly was ascribed to a pathological excretion of substances being involved in crystal formation. Fourier transform infrared spectroscopy being now commonplace for stone analysis shows that calcium oxalate (CaOx) is the most frequent stone compound. For calcium nephrolithiasis, the most frequent stone disease and the topic of this chapter, the important substances are calcium, oxalic and citric acid the latter being a calcium chelator [2]. However, not all stone formers show a pathological excretion of these compounds, and some anomalies also are found in urine of people without stone formation. Later on, stone research was focused on urinary macromolecules (UMs, mainly proteins and some glycosaminoglycans) being an integral part of the stone matrix and some of them promoting or inhibiting the crystallization of stone minerals [3]. In the meantime, more than 70 of such substances were isolated [4, 5], and 11 of them containing anionic residues like carboxyglutamic acid, phosphate and sialic acid are thought to be relevant for stone formation [6]. But anomalies with respect to excretion or composition of these UMs were exclusively found in some small groups of patients [7].

Modern urological endoscopy which allows the inspection of the whole renal cavity showed that calcium oxalate and phosphate stones comprising about 80% of all concretions [8] generally start by a fixed growth on papillary calcifications, which were already described as potential source of nephrolithiasis in 1937 by Randall [9]. Recently much work was done to elucidate the pathogenesis of these calcifications being present either as interstitial deposits of calcium phosphate (Randall's plaques) or as intratubular accumulation of calcium oxalate crystals (Randall's plugs) [10–12]. These calcifications when protruding into renal calyces can induce stone formation either by heterogeneous nucleation of new crystals or more probably by the aggregation (AGN) of crystals during crystalluria. The initial fixation on kidney calcifications allows stones to grow to a critical size where they cannot be washed out anymore by the urine flow and where they become symptomatic. However, the finding of kidney calcifications and crystalluria is much more frequent than stone disease [3], and Randall's plaques can persist during some decades without or with only minimal stone formation [13]. Therefore, the question raises whether special crystallization conditions in urine might be responsible for stone formation by the apposition of new crystals on Randall's plaques and plugs. This question stimulated us to an intensive study of the formation and especially the AGN of calcium oxalate crystals being with 60% the most frequent stone compound [8]. Experiments were directly performed in urine where like in other biological mediums, crystals as well as Randall's plaques and plugs always are coated by proteins [14]. Instead of the study of individual compounds thought to be involved in stone formation, the overall effect of UMs was compared to that one of urine and urinary ultrafiltrate. UMs were isolated either by a hemofiltration procedure or by temporary adsorption on Ca phosphate to which urinary proteins have a high affinity.

2. Measurement of calcium oxalate (CaOx) crystallization in urine

An approved test system which uses an increase of the rate of particle sedimentation as measure for crystal AGN was modified [15]. Contrary to current crystal production in standardized and

http://dx.doi.org/10.5772/intechopen.70598

protein-free solutions and with a long period of crystal ripening, CaOx was directly produced in urine by an oxalate titration [16]. Crystallization was followed monitoring optical density (OD) by a spectrophotometer. Typical crystallization curves are shown in **Figure 1A** and **C**. During a titration period of 15 min with the addition of 0.1 mM/min of sodium oxalate, a rapid increase of OD indicating crystal formation is observed. From the time lag of this increase, the critical oxalate addition to induce crystallization can be calculated and used as a measure for the metastability of urine with respect to CaOx crystallization. At the end of titration, maximal OD reflecting particle concentration in solutions [15] is determined, and magnetic stirring is stopped. Following the further course during at least 30 min, two different types of curves are observed. One type (**Figure 1A**) is characterized by a continuous slow OD decrease, which by scanning electron microscopy (SEM) of the sediment (**Figure 1B**) mainly can be attributed to the sedimentation of single crystals of CaOx monohydrate. From this low −mdOD/dt, the sedimentation rate of single crystals and an average particle size can be calculated [17]. The other type of curve is represented in **Figure 1C**. After an initial phase of slow OD decrease varying from 7 to 35 min and called suspensions stability, a rapid decline of OD is observed which by SEM of the sediment (**Figure 1D**) can be attributed to crystal AGN. Since OD mainly reflects particle concentration, the rapid OD decrease can be explained by an increased particle clearing in the observation field of the spectrophotometer. This high clearing bases on the one hand on an accelerated sedimentation of crystal aggregates (the sedimentation rate increases with particle diameter in square) and on the other hand on the diminution of particle concentration by the integration of a lot of single crystals into few large aggregates. Interestingly, the rapid

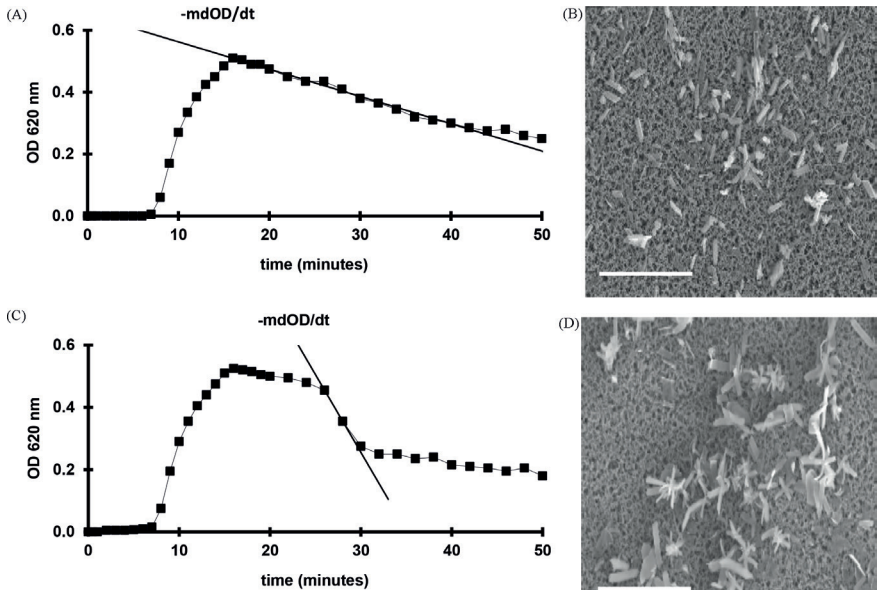

Figure 1. Crystallization curves (CC) of urine and scanning electron microscopy (SEM) of sediments: (A) urine with inhibition of CaOx AGN in CC (low maximal rate of OD decrease, −mdOD/dt) and (B) mainly with single crystals of CaOx monohydrate and only a few small aggregates in SEM, (C) urine with intensive AGN (high −mdOD/dt) in CC, and (D) large aggregates predominating in SEM. Bars on SEM indicating 20 μm.

OD decrease reflecting AGN stops after on average 30% of OD has disappeared by AGN [16]. Therefore, AGN in crystal suspensions seems to be limited to a critical OD or particle concentration. The maximal OD decrease observed in our experiments is expressed as maximal OD change per minute (−mdOD/dt).

UM solutions and urinary ultrafiltrate (UF) were obtained using a hemofilter the excluding limit of the dialysis membrane being 5 kDa [18]. To gain UF urine was placed on one side of the membrane and the filtrate collected on the other side. UMs were isolated by dialyzing urine against bi distilled water. This procedure showed a volume recovery of 96% and thus allowed the isolation of UMs in their almost original concentration. UM solutions also were prepared by Ca phosphate precipitation in urine, which was induced by the addition of sodium hydrogen phosphate at pH 7.0 [19]. After centrifugation and discharge of the supernatant, the precipitate was dissolved in distilled water buffered to pH 5.0 and with a volume corresponding to the urine volume used for precipitation. To obtain comparable results experiments in urine, ultrafiltrate and UM solutions were performed at identical pH, Na, and Ca concentration.

3. Crystallization conditions of CaOx in urine and within the kidney

CaOx crystallization in urine is a complex process. It occurs when the product of ion activities of Ca and oxalic acid ($Ca_a \times Ox_a$) exceeds a critical value called formation product (FP). In filtered urine, this FP was found to be very high. It exceeded about 14 times the product of ion activities ($Ca_e \times Ox_e$) observed in urine after equilibration with CaOx crystals in excess, the ratio $Ca_a \times Ox_a/Ca_e \times Ox_e$ being a measure for the state of saturation [2]. Ion activities and thus crystallization tendency of calcium and oxalic acid are reduced by the formation of highly soluble complexes of Ca with citric acid and of Ox with magnesium. These complexes reduce the concentrations of Ca and Ox being disposable for crystallization. The influence of stone forming substances, chelators, and pH on the state of urinary saturation with respect to CaOx can be calculated by special computer programs [20] or experimentally be tested [21]. Equilibration experiments performed with CaOx crystals in 76 urines from recurrent calcium stone formers demonstrated that supersaturation with respect to CaOx was only significantly ($p < 0.001$) correlated with urinary Ox concentration. The same experiments performed with brushite on the other hand showed that Ca phosphate saturation mainly is governed by Ca concentration and pH. Also clinical observations demonstrated that hyperoxaluria generally is much more important for the genesis of CaOx stones than hypercalciuria [22]. In 60 urines, an average addition of 0.64 mM Ox without a significant difference between stone patients and controls was necessary to induce CaOx crystallization [16]. Such high urinary Ox concentrations are apart from rare cases of primary hyperoxaluria exclusively observed after excessive Ox ingestion [23]. Nevertheless, crystalluria is a frequent finding. Crystals are found in 9–48% urines of stone patients and in 2–26% of healthy controls [24]. The discrepancy between clinical and experimental observations can be explained by heterogeneous crystal nucleation where preformed surfaces of Ca phosphate crystals, damaged renal tubular cells, and cellular debris in urine [3] allow the formation of CaOx far below the high FP necessary for spontaneous nucleation. Cellular debris comprises about 50% of urinary deposits [25].

http://dx.doi.org/10.5772/intechopen.70598

For stone formation, crystals have to be retained in the kidney. This seems for single crystals hardly to be possible. In the nephron, Ox normally reaches a sufficient concentration for CaOx crystallization at the end of collecting ducts (CD), but at extremely high Ox concentrations, crystallization already can occur in the descending loop of Henle (DLH) [26]. Both situations are schematically indicated in **Figure 2**. Even at the high Ox concentrations necessary to induce crystal formation in DLH crystallization is a relative slow process compared to urinary transit time (UT) through the nephron. Following CaOx crystallization in urine by repeated measurement of the ionic Ca decay by a ion selective electrode shows that even after an extreme Ox addition of 1 mM crystallization reaches during an average transit time through the nephron of 3 min only about half of its final value (**Figure 3**). The figure demonstrates that the study of crystals in urine which previously has remained several times in the bladder hardly can be representative for the situation in the kidney. For the end of the nephron when crystals have passed inner tubular diameters of minimal 15–60 μm, maximal crystal diameters of 4 μm were calculated [27]. The discrepancy of crystals size and tubular dimensions is even more pronounced when crystallization starts at the end of collecting ducts where crystals only can grow during a few seconds until they reach the renal papilla (**Figure 2**). Therefore, for the formation of obstructing plugs, crystals have to aggregate as demonstrated

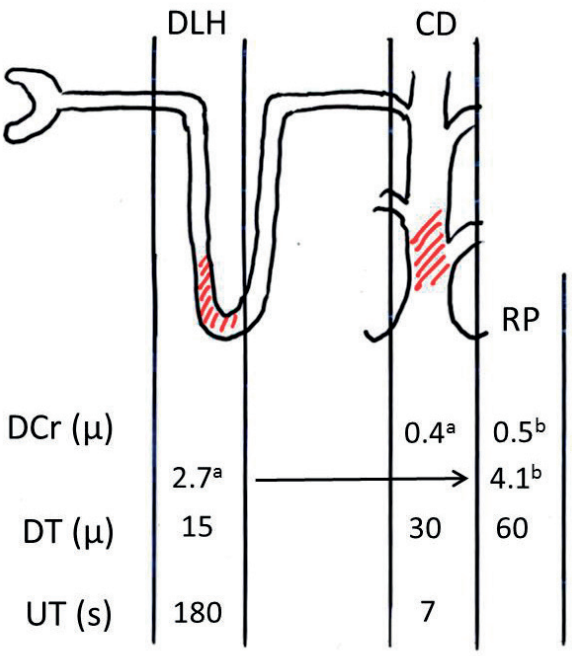

Figure 2. Localization of CaOx crystallization in the nephron (hatched areas): descending loop of Henle (DLH), collecting duct (CD), renal papilla (RP), maximal crystal diameter (DCr) expected at nucleation site ([a]), at RP ([b]), minimal inner tubular diameter (DT), urinary transit time (UT) to RP.

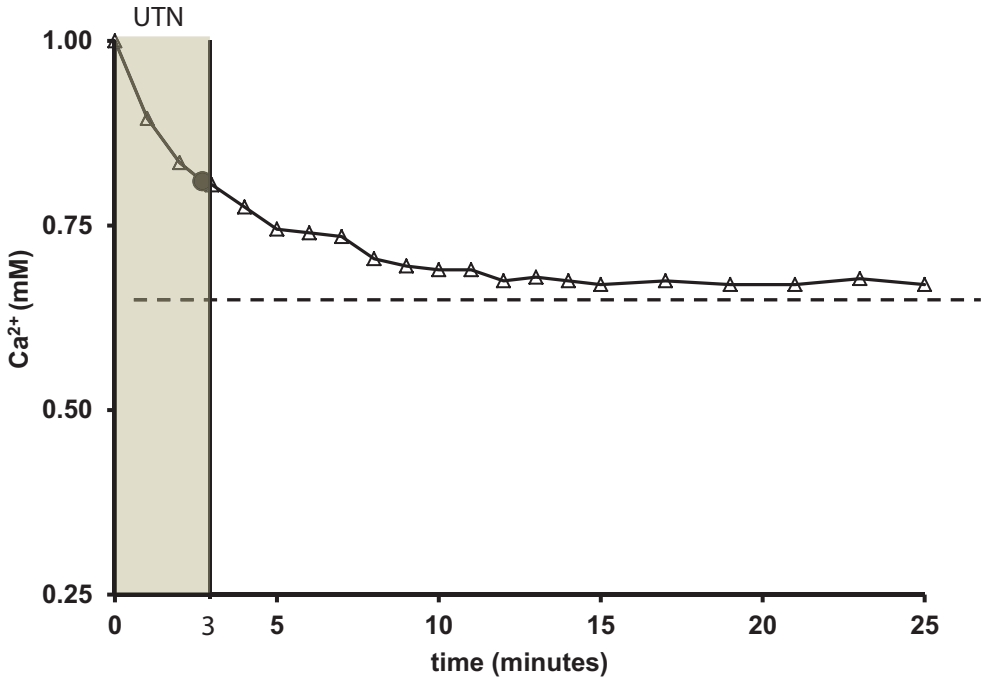

Figure 3. Ionic Ca (Ca^{2+}) decay after addition of 1 mM Ox to urine: half time (•) and endpoint of crystallization (dashed line), average urinary transit time through the nephron (UTN).

by the finding of large crystal aggregates on micro photographs of Randall's plugs [10]. AGN also seems to be responsible for the acquisition of CaOx on Randall's plaques and generally for the growth of calcium stones [28].

4. Crystal aggregation (AGN) in urine and within the kidney

Already in 1969, crystal AGN was found to be an important element in stone formation. In a study of six patients with short-term stone recurrence and six healthy controls, patients showed contrary to the controls 3 h after Ox ingestion large CaOx aggregates with diameters up to 200 μm in their urine [29]. However, in our crystallization test, AGN could also be produced by high Ox addition in 11 urines of 30 healthy people but less frequent than in urine of unselected stone patients (20/30, $p < 0.05$) [16]. Interestingly, this AGN started with a delay of 15–35 min being not only beyond the average urinary transit time (UT) of 3 min in the nephron but also beyond an average UT in the renal pelvis of 12 min [30]. Crystal AGN seems thus to depend on urinary Ox or crystal concentration respectively and on an induction time also called suspensions stability.

For AGN, particles have to collide. Under physiological conditions (without stirring or shaking), this collision occurs by particle sedimentation or Brownian motion (diffusion). At maximal crystal concentrations of 24,000 crystals/ml being observed during crystalluria [27], only 2.5×10^{-5} collisions per minute can be expected by diffusion within the short UT through the nephron [17]. Sedimentation on the other hand can accumulate under these conditions on a tubular wall being in horizontal position 1.3 crystals/min and on kidney calcifications or stone surfaces 624 crystals/cm^2 and minute. These accumulation rates are positively correlated with particle diameter in square and particle concentration whereas collision rates increase by particle concentration in square [17]. To get some insight in AGN processes within an appropriate observation time, studies have to be performed in highly concentrated crystal suspensions where AGN mainly occurs on the basis of diffusion. For the measurement of CaOx AGN in urine, Ox additions of 1.5 mM were necessary. Even under these extreme conditions, urine showed a high inhibitory activity with respect to CaOx AGN. AGN only was observed in 31 of 60 urines and always with some delay [16].

Inhibition of AGN can be ascribed to an electrical surface potential which allows identically charged particles only to near to critical distance where diffusion or sedimentation is compensated by electrostatic repulsion [31]. Also lower surface potentials act as intermediate barrier, which slows down AGN by the fact that numerous attempts are necessary to overcome electrostatic repulsion. This may explain the delay of AGN which was always observed in urine. In urine, crystals are coated by urinary macromolecules (UMs) which provide by their anionic residues an electronegative surface potential in the order of −15 mV [18]. Scanning electron microscopic pictures of CaOx crystals being incubated in albumin or globulin solutions showed protein coats on the crystals with diameters of 10–20 nm [14]. UM coats can be composed as mentioned in the Introduction by more than 70 different proteins [5]. Confronted with such multiple substances often with an unknown influence on crystallization processes, we decided to center our studies on whole urine, on UM solutions which were directly isolated from urine and on albumin being a frequent compound of urinary crystals and stones [3].

Although the adsorption of proteins like prothrombin fragment 1 and albumin was ascribed to a face-specific interaction between Ca in the lattice of CaOx crystals and selected anionic groups in the proteins [32], the coating of surfaces by UMs can also be a rather unspecific process. As **Figure 4** shows, not only crystals but also latex beads can be easily coated by UMs. This coating bases on a hydrophobic effect between proteins and latex. By Zetasizer analysis, latex beads showed after the incubation in UM solutions, an increase of particle diameters by 40 μm corresponding to an UM coat of 20 μm. A pH change in the UM solution from 6.0 to 5.0 diminished the negative surface potentials of the coated beads from −34 to −24 mV and produced a massive increase of particle diameters or AGN, respectively. This pH effect which bases on a reduced ionization of the anionic protein residues by an increased H^+ concentration demonstrates that surface potentials are essential for the inhibition of AGN. Further experiments which were performed with latex beads in almost electrolyte and especially Ca-free albumin solutions showed that the AGN of UM-coated particles too can base on a hydrophobic effect [19].

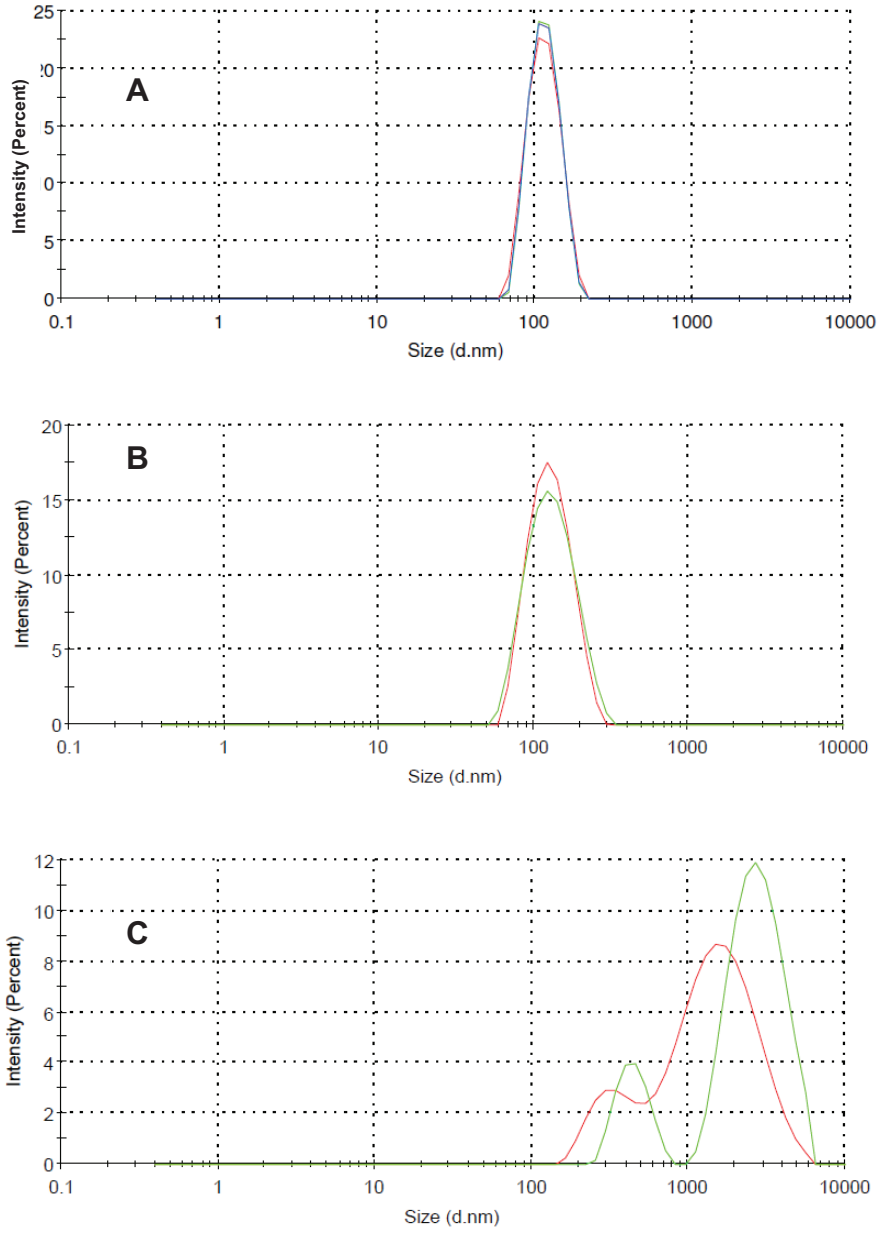

Figure 4. Affinity of urinary macromolecules (UMs) to latex beads (LBs) measured by particle size distribution (PSD) within a 2-min interval: (A) PSD of LBs before incubation in UM solution, (B) after incubation, (C) pH change from 6.0 to 5.0 in suspension of latex in UM solution showing from the left to the right curve a progressive increase of particle size due to AGN of the coated latex beads.

http://dx.doi.org/10.5772/intechopen.70598

Pathological UMs with reduced anionic groups are found to be responsible for crystal AGN in urine [33–35] and were observed in urine of some stone patients [36–38]. However, also dialysis in a hemofilter abolishes the inhibitory activity of UMs [16]. All UM solutions obtained by dialysis from 29 urines with previously low maximal sedimentation rates showed a massive increase of −mdOD/dt indicating AGN (**Figure 5**). In order to find low molecular weight substances which might be responsible for the inhibition of AGN, crystallization tests were repeated in urinary ultrafiltrate (UF) with an identical result as obtained in the UM solution. Also a loss of important substances by the adsorption on the large surface of the hemofilter could be excluded by repeating crystallization tests in UM solutions where UMs were isolated only by temporary adsorption on Ca phosphate and consecutive dilution as described above. Further experiments performed with albumin brought an explanation for this peculiar loss of AGN inhibition after temporary contact with large surfaces. Albumin at high concentration has a tendency to self-AGN [39], which is demonstrated in **Figure 6A**. By the measurement of particle size distribution (PSD) immediately after the preparation of an albumin solution (AS) in a high urinary concentration of 20 mg/l, a main peak at 10 nm being typical for albumin and a lower second peak around 1000 nm were found. Two minutes later, the main peak was somewhat diminished and peaks around 350 and 4100 nm became visible indicating progressive self-AGN of albumin. However, in the CaOx crystallization test performed in AS, only a minimal acceleration of sedimentation was observed (**Figure 7A**). The diluted Ca phosphate precipitate of AS on the other hand showed in PSD that all extracted albumin was

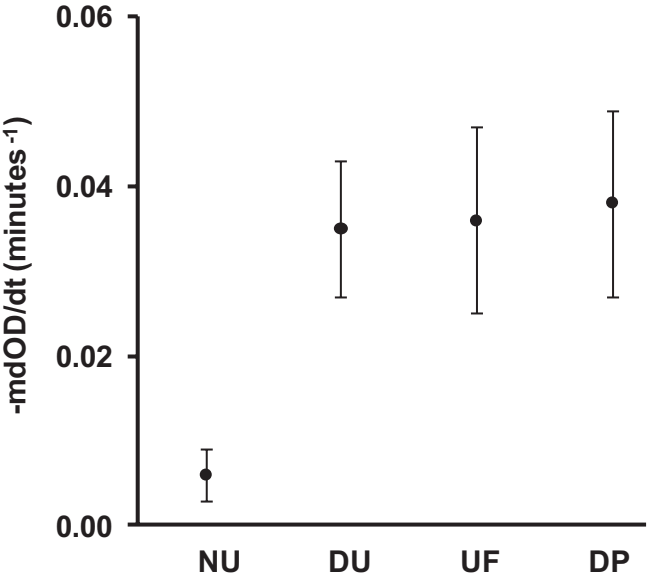

Figure 5. Effect of UM isolation on inhibition of CaOx AGN (low −mdOD/dt): native urine (NU), dialysed urine (DU), ultrafiltrate (UF), and dissolved Ca phosphate precipitates of urine (DP) ($n = 29$, $x \pm$ SD, NU vs. DU, UF and DP $p < 0.001$).

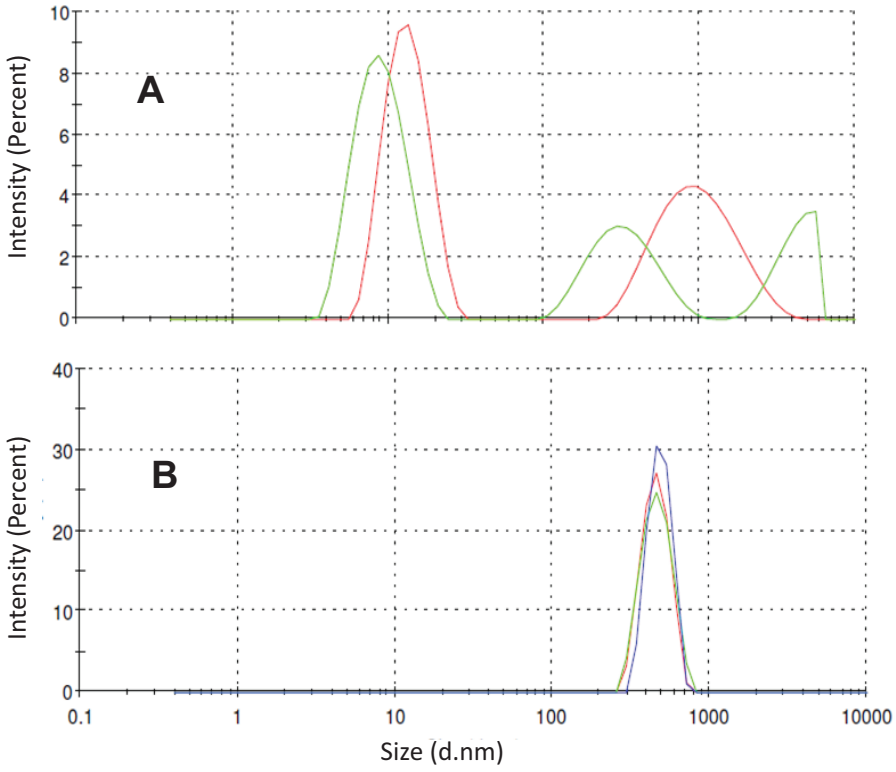

Figure 6. Particle size distribution (PSD) of albumin: (A) PSD in albumin solution in high urinary concentration (AS, 20mg/l) and (B) PSD in dissolved Ca phosphate precipitate of AS (DPA).

aggregated into a high single peak at 480 nm (**Figure 6B**) and in the crystallization test, a rapid OD decrease being characteristic for intensive crystal AGN was found (**Figure 7B**). These observations demonstrate that crystal AGN also occurs in the presence of normal but self-aggregated UMs. UM aggregates with increasing size like aggregated albumin obviously can bridge critical distances of electrostatic repulsion and connect crystals probably by hydrophobic binding to their UM coats. Scanning electron microscopy of Ca Ox crystals being incubated in gamma globulin solutions showed at crystal convergence of some aggregates large amounts of amorphous material suggesting a crystal binding by aggregated proteins [14].

The formation of Randall's plugs seems to be a product of crystal AGN. Randall's plugs are observed as origin of kidney stones at states of massive chronic supersaturation of urine either with respect to CaOx as found in primary hyperoxaluria or with respect to Ca phosphate in primary hyperparathyroidism [40]. Under these conditions, crystallization as mentioned

http://dx.doi.org/10.5772/intechopen.70598

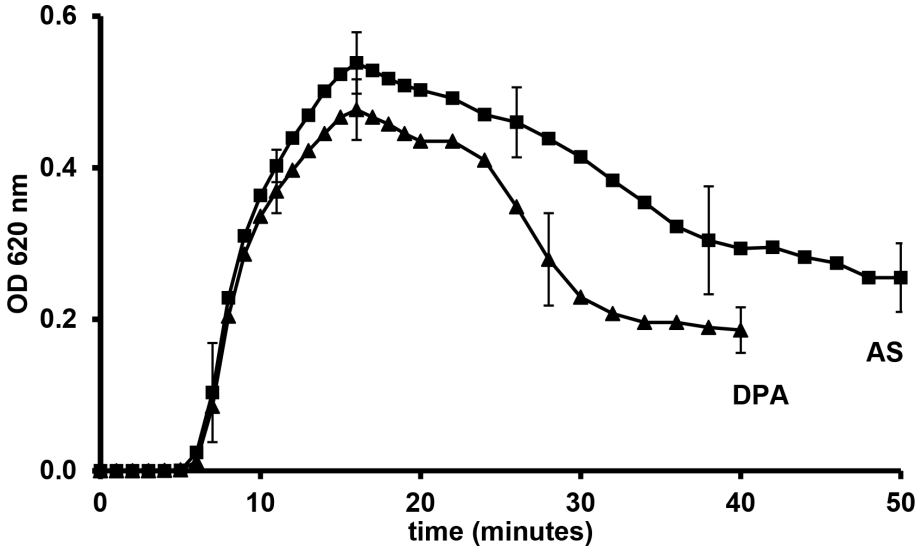

Figure 7. Crystallization curve of AS and DPA: AS shows with a relative low −mdOD/dt inhibition of CaOx AGN, and a high −mdDO/dt in DPA indicates massive AGN ($n = 5$, $x \pm SD$, $p < 0.01$).

above already can start in DLH. During their way through the nephron, crystals can drop by sedimentation on tubular walls where they stick and together with following crystals can form large crystal accumulations. Such accumulations probably are an ideal platform for the adsorption and self-AGN of UMs. Massive crystalluria and even hyperoxaluria are known to damage tubular cells and thus to favor the sticking of crystals on tubular walls [41]. Damaged tubular cells produce pathological proteins which also favor this sticking and the AGN of the crystals [3]. Furthermore, basement membrane denuded by its epithelial layer can induce heterogeneous nucleation of CaOx [42]. These mechanisms can provoke massive crystal aggregates which protruding from ducts of Bellini into renal calices can raise stone formation in the kidney as observed by urological endoscopy [40].

5. CaOx AGN in the presence of hydroxyapatite (HAP), a possible model for stone formation on Randall's plaques (RPLs)

Idiopathic Ca nephrolithiasis where by definition no metabolic or endocrinologic disorders like primary hyperoxaluria or hyperparathyroidism are found is the most frequent stone disease and generally starts by stone growth on RPLs [10–12, 40]. RPLs are subepithelial deposits of Ca phosphate in the renal papilla which by disruption of the covering epithelial layer can come in contact with urine and give raise to stone growth. The Ca phosphate deposits, mainly HAP, are a consequence of the transformation of epithelial cells into an osteoplastic phenotype with an increased production of bone-specific proteins

like osteopontin (OP) favoring tissue mineralization [12]. Histological analysis with immunohistochemistry or infrared spectroscopy of RPLs with an adherent stone showed that the RPLs consisted of an OP matrix with HAP deposits, whereas the adherent stone in addition to OP and HAP contained Tamm-Horsfall glycoprotein (THG) and CaOx crystals [40]. Stone formation on RPLs thus seems to occur at the interface of HAP and CaOx crystals being embedded in proteins like OP and THG which like albumin have a tendency to self-AGN [3]. The osteoplastic transformation of epithelial cells which mainly occurs in the loop of Henle was ascribed to vascular or metabolic disorders since in epidemiological studies, stone disease was found to be associated with hypertension, myocardial infarction, diabetes, or metabolic syndrome [12]. However, RPLs seem to be very frequent and not always associated with stone disease. High-resolution radiography of 50 consecutive sets of cadaveric kidneys showed in 57% radiographic evidence consistent with RPLs [43]. In an older study, in all kidneys of 100 randomly selected autopsies, some papillary calcifications were found but only in seven cases of kidney stones [44]. Urological endoscopy revealed RPLs even in 43% of cases not being related to stone disease [45]. The mechanisms involved in the progression of a RPL to a kidney stone are far from being clear. One of these processes is breaking of epithelial layers, which brings the calcifications of RPLs in contact with pelvic urine [12]. A further unknown factor is the formation of CaOx crystals on the denuded and protein-coated HAP of RPLs. Therefore, we tried to mimic this formation by the study of CaOx crystallization in the presence UM-coated HAP (cHAP). Contrary to previous studies performed immediately after thawing of frozen urine, 15 freshly voided urines of five healthy men were examined.

To prepare cHAP, commercially available HAP crystals (0.05 mg/ml) were incubated at 37° during at least 30 min in one portion each of 15 urines from healthy persons [46]. After centrifugation and discharge of the supernatant, the crystals were suspended in a second portion of the same urine. Ca and pH were adapted to the standard values of 2 mM and 6.0, respectively, used in the tests. CaOx crystallization tests as described above were performed in urine with and without the cHAP suspension. By this procedure, a high −mdOD/dt indicating AGN was observed in 8 of 15 urines performed with cHAP, whereas in the test performed without cHAP, only one of the 15 urines showed a high −mdOD/dt. Interestingly, urines with and without cHAP-induced AGN significantly ($p < 0.01$) could be distinguished by their sodium concentration (**Figure 8**) being an indicator for urinary ionic strength [47]. A high ionic strength seems to favor AGN, whereas urine dilution abolished the cHAP-induced AGN. All urines with an initially high −mdOd/dt showed after dilution and adaption of Ca and pH to the initial values only a slow OD decrease being typical for the sedimentation of single crystals without AGN. Urinary dilution down to 33% did not disturb urinary inhibitory activity with respect to not cHAP-related CaOx AGN, which is in agreement with the finding that urine even diluted to 20% is still an excellent inhibitor of the AGN of CaOx crystals [48]. The promotion of AGN by a high ionic strength can be explained by its influence on the extension on electrostatic surface potentials (ESPs). In electrolyte-containing solutions, ESP exponentially decreases with increasing distance from negatively charged particles by a cation accumulation (in our experiment of sodium) [31]. In concentrated urine with a high

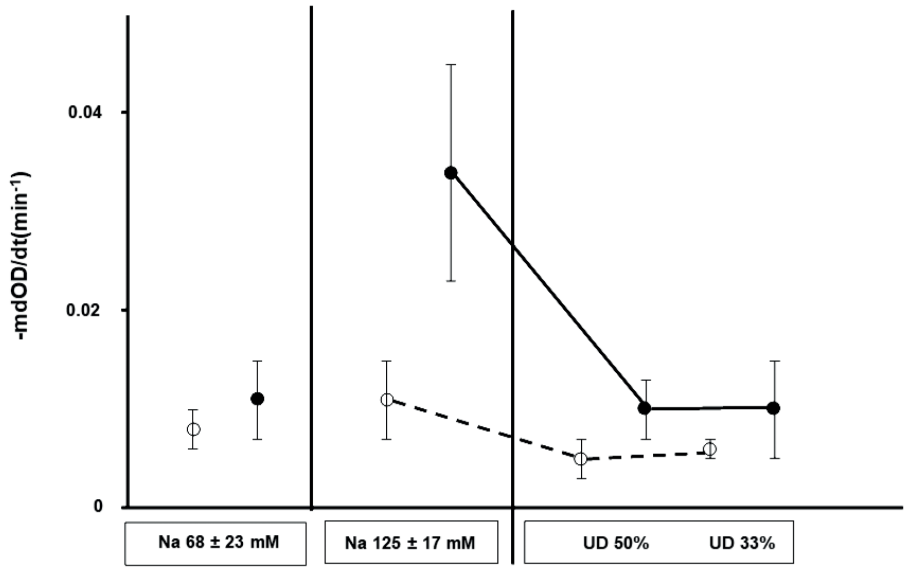

Figure 8. CaOx AGN (increase of −mdOD/dt) induced by UM-coated hydroxyapatite (cHAP) in urine: crystallization test without (o) and with the addition of 0.05 mg/ml cHAP (•) at low and at high sodium concentrations (Na) and after urinary dilution (UD) ($n = 8$, $x \pm SD$).

ionic strength, ESP is compressed to a few nanometers where crystals can approach to distances where self-aggregated UMs can take over their bridging effect.

To get more information about CaOx AGN being induced by cHAP, supplementary experiments were performed in solutions of albumin in a high urinary concentration (AS, 20 mg/l). The comparison of experiments performed in AS and in concentrated urine showed almost identical results (**Figure 9**). A high inhibitory activity with respect to CaOx AGN of AS and urine was abolished by the cHAP addition and also in the dissolved Ca phosphate precipitates (DPs) of urine and AS. Under the special conditions of our experiments, albumin seems to be an ideal substance to mimic UM effects. Adsorption on surfaces favors self-AGN of UMs as demonstrated with respect to albumin and thus can turn inhibitors to promoters of AGN [46]. Ca phosphates are excellent substrates for UM adsorption [5]. HAP of RPLs being denuded from their epithelial cells is therefore an ideal platform to initiate stone formation by the adsorption and self-AGN of UMs. Since the effect of UMs or albumin on CaOx AGN was identical when adsorbed on HAP or when being in solution after temporary adsorption, HAP of RPLs is more likely to act as a mediator of crystal AGN than as a heterogeneous nucleator for CaOx formation as suggested by others [12]. Indeed, scanning electron microscopy of urinary sediments after HAP-induced CaOx AGN showed in agreement with findings on RPLs large CaOx aggregates which were in the surroundings but not in direct contact with the HAP crystals [49].

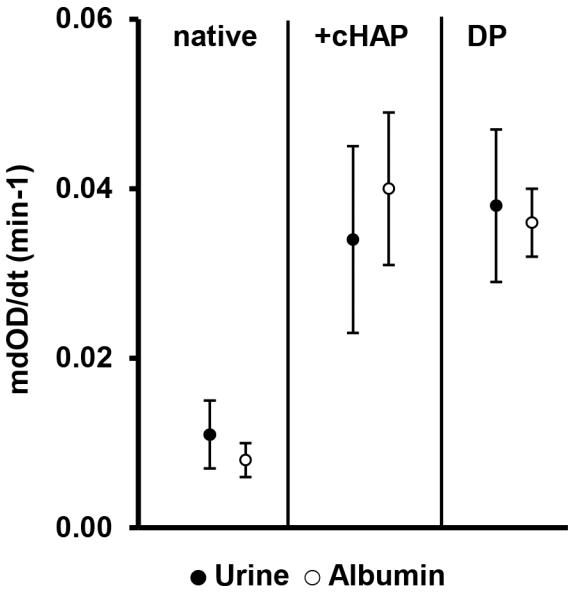

Figure 9. Almost identical results obtained in crystallization test (CT) performed with concentrated urine (•) and albumin solution (AS, 20 mg/l (o): CT performed in native urine or AS (native), in urine or AS with UM or albumin coated HAP (+cHAP) and in dissolved Ca phosphate precipitates of urine or AS (DP) ($n = 8$, $x \pm SD$).

6. Summary and conclusions

The formation of Ca stones in the kidney during crystalluria seems to base on crystal AGN either on Randall's plugs or on plaques. Urine has, as demonstrated by our study, a high inhibitor capacity with respect to CaOx AGN. Coating of crystals by electronegative charged UMs creates zones of electrostatic repulsion between the crystals which under normal conditions and within the short urinary transit time in the kidney hardly can be overwhelmed by diffusion or sedimentation being responsible for particle collision and thus AGN. Zones of electrostatic repulsion are reduced in the presence of pathological UMs with a lack of anionic residues or in concentrated urine with a high ionic strength. UMs with a lack of negatively charged anionic groups create an insufficient surface potential on UM-coated crystals. At high ionic strength, the extent of surface potentials is compressed by an increased accumulation of cations. Under these conditions, zones of electrostatic repulsion probably can be bridged by normal but self-aggregated UMs. Self-AGN occurs by the adsorption of UMs on surfaces especially of HAP with its high affinity to UMs. The AGN of UM-coated particles like urinary crystals or latex beads probably mainly bases on a hydrophobic effect between the large hydrophobic protein segments. This effect can occur either directly between pathological UM coats or is mediated by self-aggregated but normal UMs.

Stone formation on Randall's plugs mainly occurs at high urinary supersaturation with respect to Ca salts as observed in primary hyperoxaluria or in hyperparathyroidism both with

high recurrence rates of stone formation. At such states of chronic urinary supersaturation, crystallization already can start in the descending loop of Henle and crystals during their way through the nephron can stick to tubular walls and by sedimentation can accumulate further crystals. Tubular cells damaged by massive crystalluria favor crystal sticking and produce pathological UMs, which enhance crystal AGN. Crystal accumulates are ideal platforms for the adsorption of UMs and their self-AGN and thus for crystal AGN. By such mechanisms, large crystal plugs can be formed which when protruding out from ducts of Bellini into the renal calices may give raise to stone formation. Whereas in hyperparathyroidism nephrolithiasis can be cured by parathyroidectomy, the treatment of primary hyperoxaluria is extremely difficult but not the topic of this chapter. However, in stone metaphylaxis, a high fluid intake is essential which apart from urinary supersaturation also reduces ionic strength and urinary transit time through the collecting ducts and the renal pelvis.

The formation of Randall's plaques (RPLs) being the origin of most idiopathic Ca stones seems to be a complex process of biomineralization. RPLs are frequent but not always connected with stone disease. Even in the case of nephrolithiasis, stone formation on a RPL can take decades, whereas other RPLs in the same kidney can remain stone free [13]. Idiopathic Ca nephrolithiasis often is characterized by long stone free intervals [50]. In these cases, stone formation seems more to be the result of a coincidence of noisy factors than a real disease. A most dangerous constellation is an excessive ingestion of Ox rich food (e.g., chocolate) in combination with a poor fluid intake. After excessive Ox intake, a threefold increase of urinary Ox was observed [23]. High urinary Ox and consecutive crystal concentrations can, as mentioned above, induce the secretion of pathological UMs and destroy epithelial layers of RPLs. The consequence is that HAP deposits come in contact with crystals in a concentrated urine where self-AGN and AGN are enhanced by a high ionic strength and a high UM concentration. Therefore, for idiopathic Ca stone patients, dietary Ox restriction and a high fluid intake are mandatory. However, only the last measure is evidence based [51]; since in studies of stone metaphylaxis, the compliance of patients especially of those with long stone-free intervals often is rather poor.

Author details

Johannes M. Baumann

Address all correspondence to: johannes.denise.baumann@bluewin.ch

Stone Research Center Biel, Biel, Switzerland

References

[1] Romero V, Akpinar H, Assimos DG. Kidney stones: A global picture of prevalence, incidence and associated risk factors. Reviews in Urology. 2010;**12**(2-3):e86-e96

[2] Baumann JM, Affolter B. From crystalluria to kidney stones, some physicochemical aspects of calcium nephrolithiasis. World Journal of Nephrology. 2014;**3**(4):256-267

[3] Khan SR, Kok DJ. Modulators of urinary stone formation. Frontiers in Bioscience. 2004;**9**:1450-1482

[4] Thurgood LA, Wang T, Chataway TK, Ryall RL. Comparison of the specific incorporation of intracrystalline proteins into urinary calcium oxalate monohydrate and dehydrate crystals. Journal of Proteome Research. 2010;**9**(9):4745-4757

[5] Thurgood LA, Ryall RL. Proteomic analysis of proteins selectively associated with hydroxyapatite, brushite, and uric acid crystals precipitated from human urine. Journal of Proteome Research. 2010;**9**(10):5402-5412

[6] Robertson WG. Do "inhibitors of crystallization" play any role in the prevention of kidney stones? A critique. Urolithiasis. 2017;**45**:53-56

[7] Jaggi M, Nakagawa Y, Zipperle L, Hess B. Tamm-Horsfall protein in recurrent calcium kidney stone formers with positive family history: Abnormalities in urinary excretion, molecular structure and function. Urological Research. 2007;**35**:55-62

[8] Mandel NS, Mandel IC, Kolbach-Mandel AM. Accurate stone analysis: The impact on disease diagnosis and treatment. Urolithiasis. 2017;**45**:3-9

[9] Randall A. The origin and growth of renal calculi. Annals of Surgery. 1937;**105**:1009-1027

[10] Daudon M, Bazin D, Letavernier E. Randall's plaques as the origin of calcium oxalate kidney stones. Urolithiasis. 2015;**43**(Suppl 1):5-11

[11] Evan AP, Worcester EM, Coe FL, Williams J Jr, Lingemann JE. Mechanisms of human kidney stone formation. Urolithiasis. 2015;**43**(Suppl 1):19-32.

[12] Khan RS, Canales BK. Unified theory on the pathogenesis of Randall's plaques and plugs. Urolithiasis. 2015;**43**(Suppl 1):109-123

[13] Kok DJ, Boellaard W, Ridwan Y, Levchenko VA. Timeless of the "free-particle" and "fixed-particle" models of stone-formation: Theoretical and experimental investigations. Urolithiasis. 2017;**45**(1):33-41

[14] Khan SR, Finlayson B, Hackett RL. Stone matrix as proteins adsorbed on crystal surfaces: A microscopic study. Scanning Electron Microscope. 1983;**1**:379-385

[15] Hess B, Nakagawa Y, Coe FL. Inhibition of calcium oxalate monohydrate crystal aggregation by urine proteins. The American Journal of Physiology. 1989;**257**:F99-F106

[16] Baumann JM, Affolter B, Casella R. Aggregation of freshly precipitated calcium oxalate crystals in urine of calcium stone patients and controls. Urological Research. 2011;**6**:421-427

[17] Baumann JM, Affolter B, Meyer R. Crystal sedimentation and stone formation. Urological Research. 2010;**38**:21-27

[18] Baumann JM, Affolter B, Caprez U, Clivaz C, Glück Z, Weber R. Stabilization of calcium oxalate suspension by urinary macromolecules, probably an efficient protection from stone formation. Urologia Internationalis. 2007;**79**:267-272

[19] Baumann JM, Affolter B, von Arx U, Noël M. Alteration of urinary macromolecules by adsorption on surfaces, probably an important factor in urolithiasis. Urolithiasis. 2013;**41**(6):467-474.

[20] Brown CM, Ackermann DK, Purich DL. EQUIL93: A tool for experimental and clinical urolithiasis. Urological Research. 1994;**22**:119-126

[21] Ackermann D, Baumann JM. Chemical factors governing the state of saturation towards brushite and whewellite in urine of calcium stone formers. Urological Research. 1987;**15**:63-65

[22] Robertson WG, Peacock M. The cause of idiopathic calcium stone disease: Hypercalciuria or hyperoxaluria? Nephron. 1980;**26**(3):105-110

[23] Balcke P, Zazgornik J, Sunder-Plassmann G, Kiss A, Hauser AC, Gremmel F, Derfler K, Stockenhuber F, Schmidt P. Transient hyperoxaluria after ingestion of chocolate as a high risk factor for calcium oxalate calculi. Nephron. 1989;**51**:32-34

[24] Fogazzi GB. Crystalluria: A neglected aspect of urinary sediment analysis. Nephrology Dialysis Transplantation. 1996;**11**:379-387

[25] Marickar YM, Salim A. Photomicrography of urinary deposits in stone clinic. Urological Research. 2009;**37**:359-368

[26] Robertson WG. Kidney models of calcium oxalate stone formation. Nephron Physiology. 2004;**98**:21-30

[27] Kok DJ, Khan SR. Calcium oxalate nephrolithiasis, a free or fixed particle disease. Kidney International. 1994;**46**:847-854

[28] Saw NK, Rao PN, Kavanagh JPA. Nidus, crystalluria and aggregation: Key ingredients for stone enlargement. Urological Research. 2008;**36**:11-15

[29] Robertson WG, Peacock M, Nordin BE. Calcium crystalluria in recurrent renal-stone formers. Lancet. 1969;**2**:21-24

[30] Finlayson B, Reid F. The expectation of free and fixed particles in urinary stone disease. Investigative Urology. 1978;**15**:442-448

[31] Müller RH. Zetapotential und Partikelladung in der Laborpraxis. Stuttgart: Wissenschaftliche Verlagsgesellschaft; 1996

[32] Cook AF, Grover PK, Ryall RL. Face-specific binding of prothrombin fragment 1 and human serum albumin to inorganic and urinary calcium oxalate monohydrate crystals. BJU International. 2009;**103**:826-835

[33] Webber D, Rodgers AL, Sturrock ED. Glycosylation of prothrombin fragment 1 governs calcium oxalate crystal nucleation and aggregation, but not crystal growth. Urological Research. 2007;**35**:277-285

[34] Wang L, Guan X, Tang R, Hoyer JR, Wierzbicki A, De Yoreo JJ, Nancollas GH. Phosphorylation of osteopontin is required for inhibition of calcium oxalate crystallization. Journal of Physical Chemistry B. 2008;**112**:9151-9157

[35] Viswanathan P, Rimer JD, Kolbach AM, Ward MD, Kleinman JG, Wesson JA. Calcium oxalate monohydrate aggregation induced by aggregation of desialylated Tamm-Horsfall protein. Urological Research. 2011;**39**:269-282

[36] Coe FL, Nakagawa Y, Asplin J, Parks JH. Role of nephrocalcin in inhibition of calcium oxalate crystallization and nephrolithiasis. Mineral and Electrolyte Metabolism. 1994;**20**(6):378-384

[37] Webber D, Radcliffe CM, Royle L, Tobiasen G, Merry AH, Rodgers AL, Sturrock ED, Wormald MR, Harvey DJ, Dwek RA, Rudd PM. Sialylation of urinary prothrombin fragment 1 is implicated as a contributory factor in the risk of calcium oxalate kidney stone formation. FEBS Journal. 2006;**273**:3024-3037

[38] Kolbach AM, Afzal O, Halligan B, Sorokina E, Kleinman JG, Wesson JA. Relative deficiency of acidic isoforms of osteopontin from stone former urine. Urological Research. 2012;**40**:447-454

[39] Cerini C, Geider S, Dussol B, Hennequin C, Daudon M, Veesler S, Nitsche S, Boistelle R, Berthézène P, Dupuy P, Vazi A, Berland Y, Dagorn JC, Verdier JM. Nucleation of calcium oxalate crystals by albumin: Involvement in the prevention of stone formation. Kidney International. 1999;**55**:1776-1786

[40] Coe FL, Evan AP, Worcester EM, Lingeman JE. Three pathways for human kidney stone formation. Urological Research. 2010;**38**:147-160

[41] Kahn SR. Calcium oxalate crystal interaction with renal tubular epithelium, mechanism of crystal adhesion and its impact on stone development. Urological Research. 1995;**23**:71-79

[42] Khan SR, Masiamini SA, Atmani F, Glenton PA, Opalko FJ, Thamilselvan S, Hammett-Stabler C. Membranes and their constituents as promoters of calcium oxalate crystal formation in human urine. Calcified Tissue International. 2000;**66**(2):90-96

[43] Stoller ML, Low RK, Shami GS, McCormick VD, Kerschmann RL. High resolution radiography of cadaveric kidneys: Unraveling the mystery of Randall's plaque formation. Journal of Urology. 1996;**156**(4):1263-1266

[44] Haggitt RC, Pitcock JA. Renal medullary calcifications: A light and electron microscopic study. Journal of Urology. 1971;**106**:342-347

[45] Low RK, Stoller ML. Endoscopic mapping of renal papillae for Randall's plaques in patients with urinary stone disease. Journal of Urology. 1997;**158**(6):2062-2064

[46] Baumann JM, Affolter B. The paradoxical role of urinary macromolecules in the aggregation of calcium oxalate: A further plea to increase diuresis in stone metaphylaxis. Urolithiasis. 2016;**44**(4):311-317

[47] Guerra A, Allegri F, Meschi T, Adorni G, Prati B, Nouvenne A, Novarini A, Maggiore U, Fiaccadori E, Borghi L. Effects of urine dilution on quantity, size and aggregation of calcium oxalate crystals induced in vitro by an oxalate load. Clinical Chemistry and Laboratory Medicine. 2005;**43**:585-589

[48] Kok DJ, Papapoulos SE, Bijvoet OL. Crystal agglomeration is a major element in calcium oxalate urinary stone formation. Kidney International. 1990;**37**:51-56

[49] Baumann JM, Affolter B, Caprez U, Henze U, Lauper D, Maier F. Hydroxyapatite induction and secondary aggregation of calcium oxalate, two important processes in calcium stone formation. Urological Research. 2001;**29**:417-422

[50] Strohmaier WL. Course of calcium stone disease without treatment. What can we expect? European Urology. 2000;**37**:339-344

[51] Fink HA, Akornor JW, Garimella PS, MacDonald R, Cutting A, Rutks IR, Monga M, Wilt TJ. Diet, fluid, or supplements for secondary prevention of nephrolithiasis: A systematic review and meta-analysis of randomized trials. European Urology. 2009;**56**:72-80