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Calcium oxalate crystal interaction with renal tubular epithelium, mechanism of crystal adhesion and its impact on stone development

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Abstract The interaction between renal epithelial cells and calcium oxalate (CaOx) crystals and/or oxalate ions plays a critical role in the formation of urinary stones. Epithelial cells respond to hyperoxaluria and the presence of CaOx crystals in the kidneys by increased enzymuria and internalization of the crystals. Crystal cell interaction results in movement of crystals from the luminal to the basolateral side between the cells and the basement membrane. Once beneath the epithelium, crystals adhere to the basement membrane and become anchored inside the kidneys. Crystals anchored to basement membrane of the peripheral collecting duct aggregate with other crystals and move through an eroding epithelium to the papillary surface, furnishing an encrustation platform or a nidus for future development of a kidney stone. Thus interaction between renal epithelial cells and CaOx crystals and/or oxalate ions is an essential element in the development of urinary stone disease.

Key words Calcium oxalate · Nephrolithiasis · Basement membrane · Kidney stone · Crystallization · Hyperoxaluria

Finlayson and Reid suggested that the process of urinary stone formation cannot be properly understood without making a distinction between free and fixed particle mechanisms of crystal retention [9]. They calculated the possibility of stone development within renal tubules, renal pelvis and urinary bladder from free or fixed calcium oxalate (CaOx) crystals. It was concluded that there was no likelihood of single crystals growing large enough to be held within the renal tubules or renal pelvis by a free particle mechanism and thus crystal attachment was deemed necessary for the initiation of stone formation. Hautmann and associates agreed that crystal retention was necessary for stone formation but suggested that it starts with the formation of crystals within the renal papillary interstitium and not from crystalluria particles [18]. In order to understand the mechanisms involved in crystal retention within the kidneys, it is necessary to identify the initial site of crystal deposition within the kidneys and understand the interaction between crystals and the surrounding cells. This aspect of stone formation was investigated in a rat model. I will present results of the study, review current literature and discuss the importance of crystal cell interaction in stone formation.

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Materials and methods

Hyperoxaluria is the main risk factor for human idiopathic CaOx stone formation [44], and induction of hyperoxaluria is essential for the development of CaOx urolithiasis in rats [21]. We used male Sprague-Dawley rats weighing between 200 and 250 g, and examined the process of crystal deposition in the kidneys following chronic hyperoxaluria.

Hyperoxaluria was induced by administration of 0.75% ethylene glycol (EG) alone or with 2% ammonium chloride (AC), through drinking water. There were five rats in the ethylene glycol only group and four rats in the ethylene glycol plus ammonium chloride group. Urine was collected and rats were sacrificed at regular intervals. Experiments were terminated after 1 week on EG+AC treatment and after 8 weeks on EG treatment. Bladder urine was collected and kidneys harvested at the time of sacrifice. Methodological details of hyperoxaluria induction, urine collection and analysis for crystalluria, and processing of kidneys for light and electron microscopic examinations are available in earlier publications [21–23, 26, 27]. In brief, at the time of sacrifice, all animals were anesthetized with i.p. sodium pentobarbital. Kidneys were fixed by retrograde perfusion through the aorta with a fixative containing a formaldehyde-glutaraldehyde mixture. Following the perfusion fixation kidneys were removed. A median slice of the kidney was taken for light microscopic examination. The rest of the kidney was divided into cortical and papillary sections and cut into small pieces, which were then further fixed by immersion in the same fixative for scanning electron microscopic (SEM) examinations and in a mixture containing ethylenediaminetetra-acetic acid (EDTA) and the fixative for transmission electron microscopic (TEM) examination. Renal papillae with large stones on their tips or their bases were first examined by SEM and then rehydrated and processed for TEM analysis.

Crystals were identified on the basis of their morphology and elemental composition. Morphological examination was undertaken by scanning electron microscopy and elemental composition was discerned by energy dispersive X-ray microanalysis [22].

Results

Both the hyperoxaluric challenges, one with and the other without, AC resulted in crystalluria, i.e., excretion of crystals in the urine, and nephrolithiasis, i.e., crystal deposition in the kidneys, but at different times during the treatment. EG administration with AC resulted in the development of persistent crystalluria in all rats by day 3 and nephrolithiasis by day 7. However, on EG administration alone, it took about 12 days for all rats to show persistent crystalluria and more than 3 weeks for nephrolithiasis to occur. The contrasting effects of the two treatments can be better appreciated by examination of Figs. 1 and 2a. Lesions in the two illustrations are similar in appearance and location but whereas Fig. 1 illustrates a renal papilla after only 1 week on EG+AC Fig. 2a shows a renal papilla after 8 weeks of EG only administration.

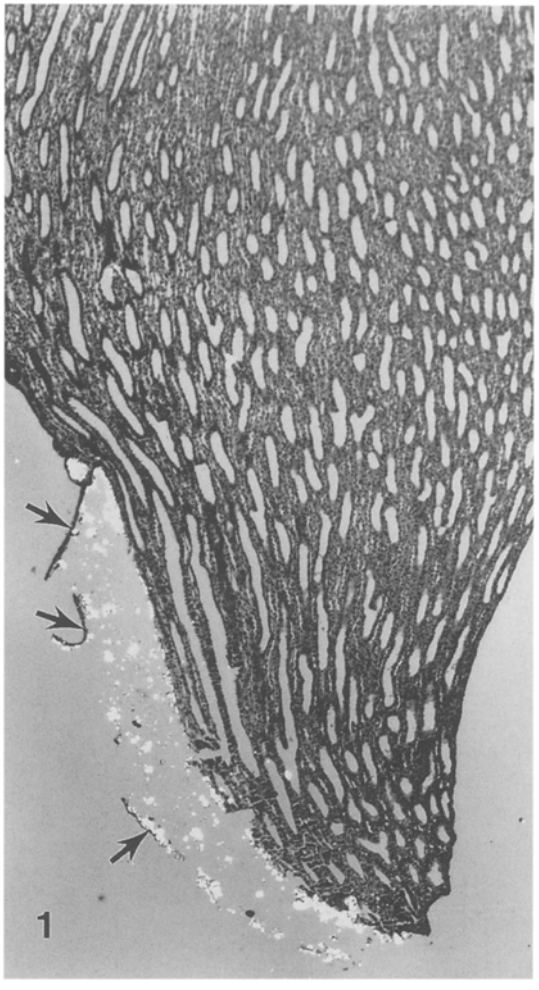


Fig. 1 Light microscopic illustration of H&E stained paraffin section through the renal papilla of a rat, 7 days after EG+AC treatment. A CaOx stone consisting of birefringent COM crystals is present at the papillary surface. *Arrows* indicate the eroding surface epithelium, ×50

Initially smaller single dipyramidal CaOx dihydrate (COD) crystals were seen in the urine. Later most of the crystalluria particles were large aggregates of dumbbell-shaped CaOx monohydrate (COM) crystals and twinned COD crystals. Tubular crystalline casts up to 200 μm long as well as spherulitic ministones about 75 μm in diameter were often found in the bladder urine collected at the time of sacrifice. Primary constituents of both the casts and stones were COM crystals with occasional COD crystals.

At the time of sacrifice all rats showed nephrolithiasis, rats on EG+AC showing more crystals in their kidneys than the rats on EG alone. Crystals were generally restricted to the renal medulla where they were randomly distributed in the tubules and were present as aggregates of COM crystals mixed with a small number of CODs. Few scattered crystals were also seen in the cortical tubules. All rats receiving both EG and AC had crystal deposits or ministones on the renal papillary surfaces (Fig. 1). In contrast only two of five rats receiving EG alone had crystal deposits on the papillary surfaces (Fig. 2) and this was after 8 weeks of the treatment. Large stones were generally located at the papillary tips or sides (Figs. 1, 2) protruding into the calyx, and smaller ones at the papillary base, extending into the fornix.

Crystals were observed in the tubular lumen, intercellular spaces of the tubular epithelium and inside the epithelial cells and the interstitium. Most crystals were, however, located inside the tubular lumen. Demineralization during processing for TEM examination of crystals resulted in the formation of crystal ghosts which represented the EDTA-insoluble organic matrix. All crystals contained abundant organic matrix which was highly organized. Intraluminal crystals were associated with cellular degradation products consisting mostly of membranous vesicles. Intracellular crystals were seen lying free in the cytoplasm (Fig. 3) and not inside a membrane bound vesicular entity. This would suggest that their intracellular position was not a product of endocytosis. Many of the crystals were seen attached to the epithelial basement membrane (Fig. 4). Epithelial cells of crystal-containing tubules appeared injured, showing intracellular edema and blebbing of the microvilli.

Papillary surface deposits or ministones consisted largely of COM crystals (Figs. 1, 2c) and mostly appeared as ulcerations (Figs. 1, 2a) which started inside the superficial collecting ducts of the renal papillae. The surface epithelium surrounding the crystal deposits appeared stretched (Fig. 2b). Removal of the deposits revealed fibrous bases. The nucleus of the deposits was located inside the papillary collecting ducts and contained random aggregates of crystals. The outer segment of the deposits was well organized and appeared striated (Fig. 2d), and was similar in morphology to the outer layers of human CaOx stones. TEM examination of these deposits demonstrated abundant matrix (Fig. 5) which also appeared strat-

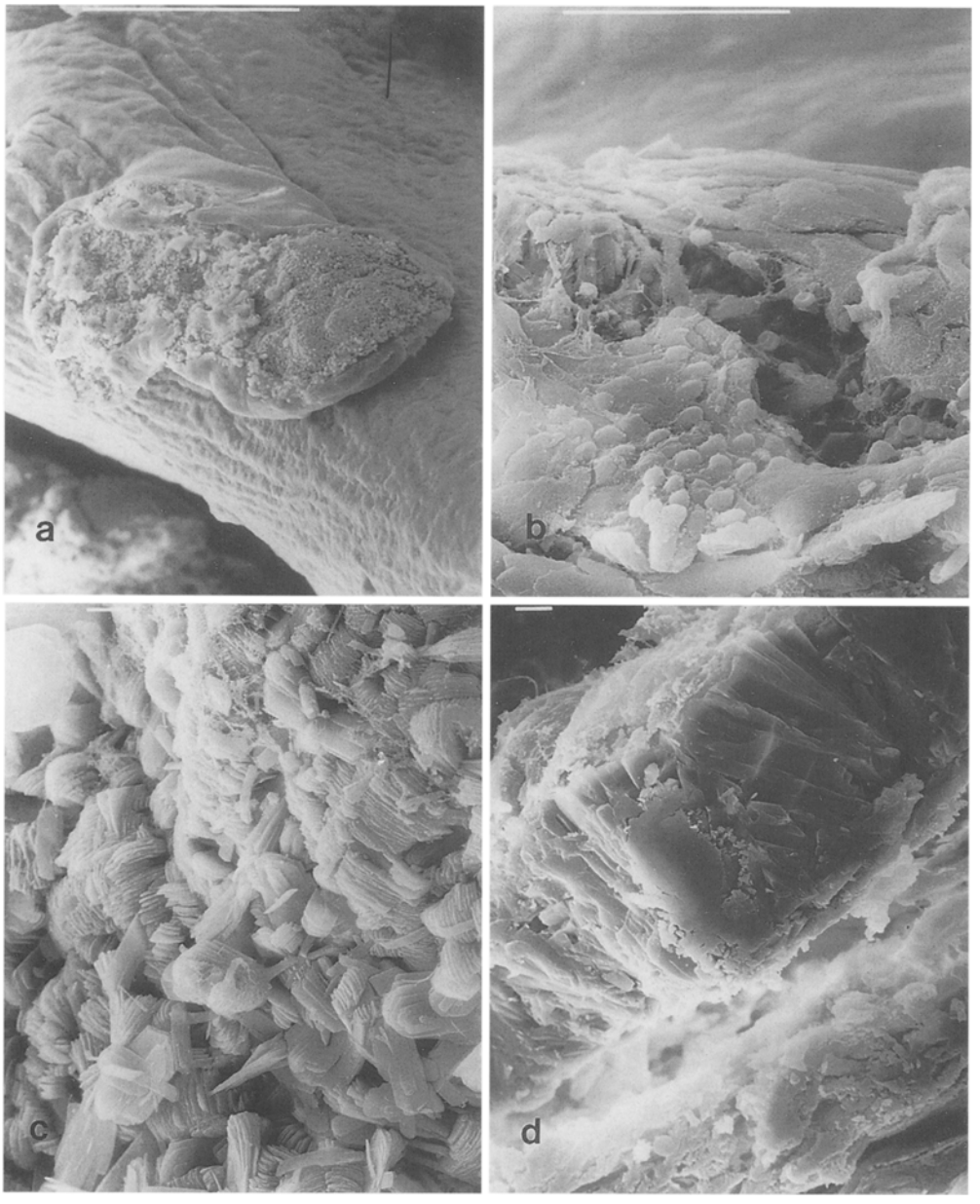


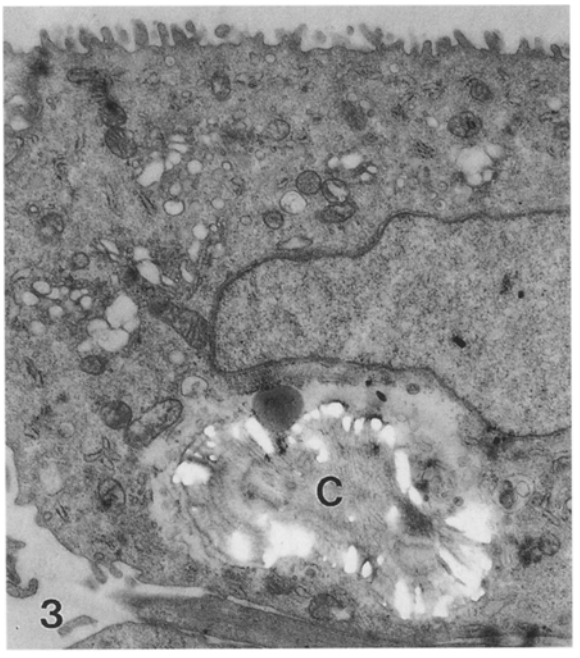
Fig. 2a–d Scanning electron micrograph of a ministone on the renal papillary surface of a rat after 8 weeks on ethylene glycol only treatment. **a** Stone appears as an ulceration on the papillary surface. *Bar*: 500 μm. **b** Higher magnification showing stretching of the eroding papillary surface epithelium. *Bar*: 50 μm. **c** Surface view of the stone showing plate-like COM crystals. *Bar*: 5 μm. **d** Fractured outer surface of the stone showing radially arranged COM crystals. *Bar*: 5 μm

ified and organized. This arrangement of the organic matrix is similar in appearance to the matrix of human idiopathic COM stones [25]. Similar to the idiopathic stones, the matrix of rat ministones also contained necrosing cells and membranous cellular degradation products.

Inner layers of the ministones were in close contact with the tubular epithelium (Fig. 5). Many crystals were seen attached to the epithelial basement membrane. Attachment appeared to be arranged by integration of epithelial basement membrane with the organic matrix of the CaOx crystals (Figs. 4, 5). Basement membrane was often observed going through the matrix of well-defined crystals, apparently threading the crystals together like beads on a necklace.

Discussion

The results of both this and earlier studies [20] indicate that nephrolithiasis in rats is preceded by crystalluria. In addition, the present study has also shown that crystal deposition in the kidneys started with the appearance of large twinned crystals and crystal aggregates in the urine. Crystalluria is an indicator of urinary supersaturation and is quite common in human stone formers [49]. However, non-stone-formers also excrete crystals in their urine. The difference between the crystalluria of stone formers and non-stone-formers is excretion of large and aggregated crystals by the stone formers [45]. Unattached large and aggregated



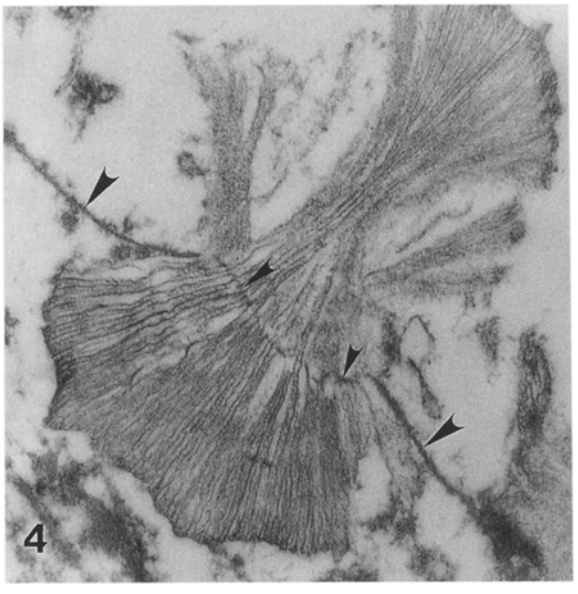


Fig. 3 Section through an epithelial cell with a COM ghost (*C*) lying free in the basal cytoplasm of the cell. Crystal ghost contains both radially and concentrically organized organic matrix, ×12 000

Fig. 4 A COM ghost attached to the basement membrane (*arrowheads*). The membrane appears to go through the radially organized organic matrix of the crystal, ×9 000

crystals have a better chance of retention within the kidneys and initiating the stone formation than unattached single, small crystals [9, 24, 32].

Crystallization in the urine is modulated by a number of ions and macromolecules [44]. These modulators can influence morphology of the crystals, their aggregation behavior and their chances of adhering to the renal epithelial cells. Production of twinned and aggregated crystals by human stone formers as well as stone forming rats as shown here indicates changes in the urinary crystallization modulators. Many of the macromolecular modulators are specifically produced by the renal epithelial cells [6] lining the proximal tubules and the loops of Henle. A challenge to these epithelial cells can interfere with the production of macromolecular crystallization modulators. It has already been reported that urine of rats treated with gentamicin sulfate, which is specifically toxic to the proximal tubular epithelial cells, is less inhibitory to CaOx crystallization [10]. Inhibition of seeded CaOx monohydrate crystal growth is significantly reduced by whole urine or < 10 000 mol. wt. urinary compounds obtained from male Sprague-Dawley rats treated with gentamicin sulfate [15].

In this study, deposition of crystals in the renal tubules was associated with injury to the tubular epithelium. Earlier studies have also shown that CaOx crystal formation and deposition in the renal tubules is associated with renal epithelial cell injury [20, 21, 26, 27]. Epithelial cells lining the crystal-containing tubules suffered damage. The first noticeable changes occurred in the proximal tubules where the brush border was distorted by clubbing of the microvilli, formation of blebs and focal loss of the brush border. Progressive changes in the cells resulted in their ultimate death and detachment from the basement membrane. Numerous dividing cells were found in the epithelial lining of the tubules. Initially crystals were present in the tubular lumina. Many of them were seen in close proximity to the cell surface and in association with the microvilli. Some were found attached to the injured cells. Later, many crystals were found in the intercellular spaces and also attached to the denuded basement membrane. Hyperoxaluria in the absence of CaOx crystal deposition in the kidneys also appears injurious to renal epithelial cells as suggested by an increase in urinary excretion of enzymes, γ-glutamyl transpeptidase, alkaline phosphatase and *N*-acetyl-β-glucosaminidase, which are indicative of cell injury [28, 29].

Tissue culture studies by Hackett et. al. [13, 14] have also provided evidence that both CaOx crystals and oxalate ions are injurious to renal epithelial cells. Exposure of MDCK cells in monolayer to oxalate or CaOx crystals resulted in detachment and shedding of cells from the substrate. Levels of γ-glutamyl transpetidase, leucine aminopeptidase, lactate dehydrogenase and *N*-acetyl-β-glucosaminidase significantly increased in the culture media. Cells remaining in the monolayer appeared injured, showing blebbing and swelling of the microvilli, with their eventual loss. Cell-to-cell contacts appeared stretched and intercellular spaces widened. Trypan blue exclusion by the cells was significantly decreased. Crystals became intimately involved with the cells and their microvilli. Some of the crystals were endocytosed by cells and others were seen in the intercellular spaces. Still others were present underneath the cells. When monolayers were exposed simultaneously to oxalate and CaOx crystals, the effects appeared slightly additive. Exposure of another kidney cell line, LLC-PK1 cells, to oxalate produced concentration dependent changes in cell morphology including vacuolization, disruption of the monolayer and decreased viability as ev-

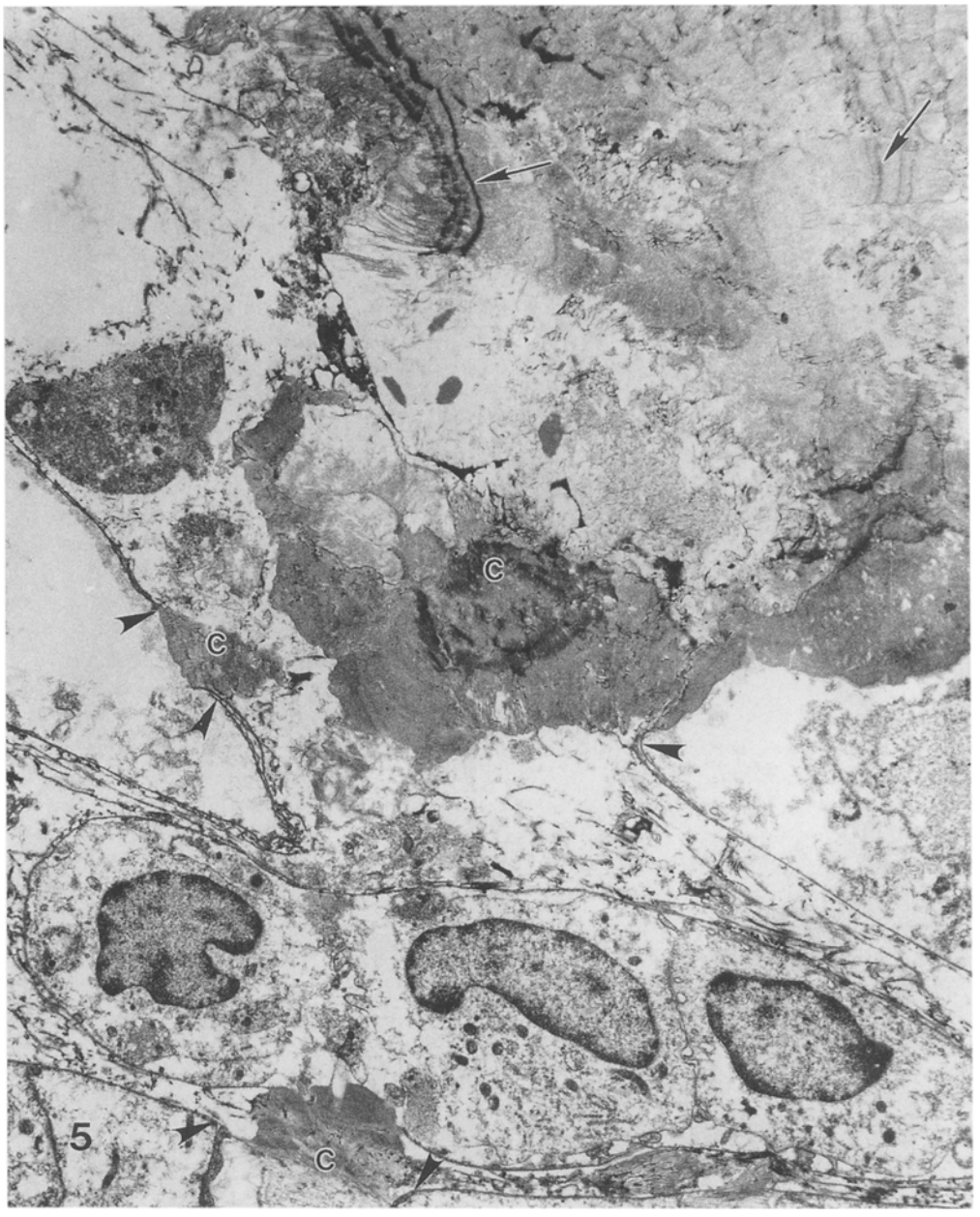


Fig. 5 A section through the ministone showing stone on the outside (*top*) and papillary tissue on the inside (*bottom*). Stone contains crystal ghosts (*C*) with well-organized organic matrix and is anchored to the basement membrane (*arrows*) of the papillary epithelial cells. ×6 000

idenced by trypan blue exclusion [39]. Oxalate was also shown to have a mitogenic effect on the LLC-PK1 cells. It is interesting to note here that changes seen in renal epithelial cells in culture on exposure to oxalate and CaOx crystals are similar to alterations observed in renal tubular epithelium after hyperoxaluric challenges as discussed in an earlier paragraph.

Lieske et. al. demonstrated that monkey kidney epithelial cells (BSC-1) in culture can also internalize CaOx crystals and undergo proliferation in the presence of the crystals [34, 35, 37]. First, crystals adhered to microvilli of the epithelial cells and were then internalized. Inside the cells, there was a generalized reorganization of the intermediate filament network and concentration of F-actin at the sites of crystal contact. Uptake of crystals, however, did not adversely affect renal epithelial cell growth. Internalized crystals were apparently distributed to daughter cells during division. In this respect, results obtained by Lieske et. al. are at variance with those obtained by Hackett et. al. and Menon et. al. The differences may be a result of dissimilarities in experimental conditions and the cell lines used. It has recently been shown [17] that CaOx crystals induce expression of immediate early genes c-*myc*, c-*jun*, *EGR-1* and *NUR-77* and genes encoding plasminogen activator inhibitor (PAI-1) and platelet-derived growth factor (PDGF)-A in BSC-1 kidney epithelial cells in culture. It was suggested that the results can explain the appearance of interstitial fibrosis in the kidneys of patients with nephrolithiasis. Administration of folic acid or mercuric chloride to CF-1 mice also results in expression of c-*myc*

in the kidneys [5]. Activation of c-*myc* and c-*jun* has also been demonstrated in primary cultures of rat proximal tubular epithelium exposed to oxidative stress [38].

The above mentioned studies indicate that oxalate as well as CaOx crystals generate a concentration-dependent response by renal epithelial cells and may cause overt or covert cellular injury and provoke an inflammatory response. Even mild hyperoxaluria without crystal deposition can cause injury to the renal epithelium as indicated by increased excretion of enzymes of epithelial and membrane origin [28]. Crystal formation and deposition may further exacerbate this situation resulting in cell necrosis and cell detachment from the basement membrane. Damage to the plasma membrane may also impair various transport mechanisms involved in calcium homeostasis resulting in an influx of calcium into the cells and formation of CaOx crystals inside the cells as demonstrated here. Intracellular CaOx crystals have previously been shown after 8 days of EG+AC administration [2], long enough time for cellular plasma membrane to become damaged. It has also been shown that oxalate uptake is significantly increased in renal papillary cells from stone forming rats [46] and that oxalate forms complexes with intracellular calcium, suggesting that crystals may be formed inside the cells through this mechanism.

Total necrosis of the cells results in detachment from their basement membrane. This event can have far reaching repercussions. Membranous cellular degradation products (CDPs) formed by these cells may induce heterogeneous nucleation of crystals [30] and thus formation of crystals at much lower supersaturation. Entanglement of crystals with the CDP may result in crystal aggregation and retention within the nephron [23]. A concomitant result is the exposure of basement membrane [21, 27], a phenomenon which is important in stone genesis since it appears that some of the basement membrane components have a high affinity for CaOx crystals or crystal-associated matrix material and these molecules may help promote crystal adhesion [31]. Light microscopic examination of crystal deposits in both human and rat kidneys has shown association of crystals with the epithelial basement membrane [40, 41, 50]. Our TEM studies described here and in an earlier publication [27] have clearly demonstrated CaOx crystal attachment to basement membrane of the renal tubules.

Proliferative response to oxalate and/or CaOx crystals shown by renal epithelial cells in culture and in the rat renal tubules suggests that hyperoxaluria and nephrolithiasis would result in active cell division and the appearance of many immature cells in the renal tubular epithelium. Less-differentiated immature MDCK cells bind and/or endocytose significantly more CaOx crystals than older, more mature and differentiated cells [47]. In addition, cell division loosens cell attachment to the basement membrane which increases the possibility of detachment and subsequent sloughing of cells exposing the epithelial basement membrane [35].

So far we have discussed the changes in renal epithelium caused by their exposure to oxalate and CaOx crystals. Other factors may also induce changes in the renal epithelium that can promote development of nephrolithiasis. Riese et al. [42] showed that when rat renal papillary collecting duct cells in primary culture are exposed to crystals of CaOx, uric acid or hydroxyapatite, crystals adhere to a specific population of cells which grow in clumps. Cells in clumps are viable epithelial cells with impaired intercellular tight junctions which cause a movement of basolateral components to the apical cell surface. It is this basolateral component which is proposed to be involved in crystal binding.

Glycosaminoglycans (GAGs) may also be involved in crystal adherence to epithelial surfaces [3]. GAGs maintain a protective coating on the surface of bladder urothelium. Gill et al. [11] demonstrated that removal of this coating resulted in a marked increase in the adherence of CaOx crystals to the urothelium. Heparin treatment restored the original anti-adherence properties of the urothelium. Recently it has been shown that CaOx crystal adhesion to BSC-1 cells in monolayer culture is also inhibited by various GAGs including heparin, dextran sulfate, chondroitin sulfate A and B, and heparan sulfate [36]. Crystal adhesion to MDCK cells was also reduced by GAG treatment [47]. Prior exposure of crystals, but not of cells, to the GAGs impeded crystal adhesion suggesting that these molecules exert their influence through interaction with the crystal surfaces. GAGs such as heparin and chondroitin sulfate are well known to have high affinity for CaOx crystals [33]. Endocytosis of CaOx crystals by BSC-1 and MDCK cells in culture was, however, inhibited by prior exposure of cells to heparin [34].

Urinary glycoproteins have also been suggested to affect crystal adhesion to the renal tubular epithelial cells. Nephrocalcin and osteopontin but not Tamm-Horsfall protein (THP) inhibit crystal binding to the BSC-1 cells in culture [36] and like GAGs they influence crystal adhesion through interaction with the CaOx crystal surfaces. THP has, however, been shown to reduce crystal endocytosis by interacting with epithelial cells. Both osteopontin and THP are found to be closely associated with crystal deposits in the kidneys [12].

The present study has demonstrated that CaOx crystal formation in the kidneys begins in the tubular lumen. Earlier studies of nephrolithiasis in rat have also shown that in both acute and chronic hyperoxaluria initial crystals are formed in the renal tubular lumen [21, 26, 27]. After a 3-mg/100 g rat body weight dose of sodium oxalate administered by i.p. injection, CaOx crystals were seen in cortical tubules within 15 min, in both cortical and medullary tubules within 30 min and in medullary segments only within 1 h; kidneys were clear of crystals within 3 h. Such a rapid movement can only be possible when crystals are present in the tubular lumen and are moving with the urine. A similar pattern was seen at higher doses but crystals stayed in the kidneys for a longer duration. Higher doses resulted in larger crystals and crystal aggregates, much larger in size than the diameter of the tubular lumen. These crystal aggregates were therefore unable to transit the nephron. Of interest are the many examples of crystals preferentially depositing at locations within the kidneys

Table 1 Renal cellular injuries and their impact on caox nephrolithiasis

Altered elements	Result	Pathological consequence
Epithelial cell surface GAGs	Crystal binding to epithelial cell surface	Crystal retention
Epithelial cell plasma membrane	Alteration in cellular oxalate transport	Increased oxalate in tubular fluid; higher CaOx RSS; crystal nucleation
Epithelial cell plasma membrane	Movement of calcium into cells	Crystal formation inside cells; crystal retention
Epithelial cell plasma membrane	Crystal endocytosis	Crystal exocytosis to basolateral side and anchoring to basement membrane
Epithelial tight junctions	Movement of basolateral components to luminal surface	Crystal binding to epithelial cell surface; crystal retention
Epithelial brush border	Sloughing of brush border; membranous vesicles in tubular fluid and urine	Heterogeneous nucleation at lower RSS
Entire cells	Cellular degradation products in tubular fluid and urine	Heterogeneous nucleation at lower RSS; crystal aggregation and retention
Entire cells	Cell sloughing, cell proliferation	Exposure of basement membrane; crystal attachment, retention and anchoring to basement membrane
Epithelial cells of proximal tubule and loop of Henle	Impaired production of crystallization modulators	Reduced crystallization inhibitory activity; increased crystal aggregation and binding to cells

where movement through the lumen is disrupted [24]. For example, crystals deposit at the corticomedullary junction, where there is a narrowing of the tubular diameter from the proximal tubule to the loop of Henle. Crystal deposition at the base of the papilla may be aided by "kinks" in the renal tubules. Again these examples illustrate the luminal nature of the crystals.

It is the opinion of several researchers that intraluminal calcium crystals can evolve to an interstitial position [3, 8, 43]. The results of our studies with acute hyperoxaluria have shown that, after a single i.p. injection of sodium oxalate, CaOx crystals appeared first in the tubular lumen and later in the interstitium, establishing the direction of crystal movement. We found the same trend in chronic hyperoxaluria [20].

Many reasons have been given to support the intention that the crystals that induce stone formation are formed in papillary interstitium rather than the tubular lumen [18]: (1) Urine passes through the renal tubules too fast for crystals to attain the critical size necessary for blocking the renal tubules while crystals in the interstitium with unlimited time to grow would face no such problem for retention within the kidneys. It has been shown here and is now generally recognized that most crystals deposit in the kidneys as aggregates [24, 32]. Single crystals do not have to grow to a certain size for retention within the tubules. They can accumulate with each other and accrue mass through conglomeration to a size large enough to block the renal tubules. (2) The renal papilla has the highest concentration of calcium and oxalate than any other segment of the kidneys and elevated levels of calcium and oxalate in the renal papillae reflect high concentrations in the interstitium. High concentrations of calcium and oxalate in the renal papillae may be important in papillae, being the most common site of stone formation. But there is no direct proof for calcium and oxalate concentrations being higher in the interstitium than in the renal tubules.

de Bruin et al. recently studied CaOx crystal fixation in rat kidneys [7]. They produced nephrolithiasis in rats by induction of mild chronic hyperoxaluria. Once crystals had deposited in the kidneys, lithogenic challenge was withdrawn for 2 days to clear the system of any free crystals. Afterwards rats were challenged with low grade chronic hyperoxaluria for up to 30 days. It was expected that retained crystals would grow into stones. The initial mild challenge resulted in the luminal deposition of crystals. Retained crystals appeared (1) as aggregates blocking the renal tubules, (2) as crystals attached to the epithelial cells, (3) present in the intercellular spaces, (4) under the epithelial cells or (5) in the interstitium. After 30 days of low-grade hyperoxaluria crystals were seen only in the interstitium. None of the interstitial deposits developed into stones. This study clearly demonstrates the movement of crystals from lumen to the interstitium and confirms our earlier findings. It also demonstrates that interstitial crystals do not necessarily develop into stones even in the presence of chronic hyperoxaluria.

The results of the de Bruijn study are at variance with an earlier study by Vermeulen [48], who first challenged rats with a high dose of lithogen or what he termed as a triggering dose. Soon afterwards, lithogenic challenge was reduced to a quarter or a maintenance dose. This treatment resulted in severe stone disease. The difference between Vermeulen's study and that of de Bruijn et al. is that Vermeulen did not allow the luminal crystals to clear the kidneys while de Bruijn et. al. did. These data strongly suggest that intraluminal crystals are critical to stone formation. It should be mentioned here that interstitial calcific deposits are quite common in human kidneys [1, 4, 16, 40, 41] and degree of calcification increases with age. In many

studies almost all kidneys examined had some interstitial calcific deposits [1, 16]. On the other hand, nephrolithiasis is not a common occurrence and its incidence actually decreases with age. Therefore the presence of interstitial calcific deposits does not imply that stones will form.

In this study ministones developed on the surface of the renal papillae at two anatomic locations, the neck region of the calyx and the fornix. The nucleus of the stone consisting primarily of aggregated crystals was located in the superficial collecting ducts and implied that crystal retention within the tubules started with aggregation. Apparently, as aggregates increased in size they could not move with the same velocity as urine and stayed in the tubules. Associated with the retention was the appearance of crystals in the intercellular spaces, inside the cells as well as in the interstitium. This would indicate that either the intraluminal crystals secondarily become intracellular and interstitial, and that crystal presence in the intercellular spaces represents a stage in their movement or that crystals actually form inside the cells and in the interstitium. Whichever pathway predominates, the sequence of events appears as follows. Hyperoxaluria results in the formation of intraluminal CaOx crystals which are initially small. Most of them clear the renal tubules via urinary flow. Under the influence of some renal factors, most probably crystallization modifiers, crystals are formed which are larger and/or aggregated. Many of these crystals do not clear the kidneys because of their size and location in the kidneys and remain in the tubules. Persistent hyperoxaluria and crystalluria causes renal tubular epithelial injury. Necrotic cells are sloughed exposing the basement membrane which provides an attachment platform for the CaOx crystals. Alternatively crystals adhere to the epithelial cell surface first and are then internalized followed by exocytosis on the basolateral side. Crystals are subsequently lodged under the cells, bind to the basement membrane and become anchored in the kidneys.

The fate of the retained crystals appears to depend on their location inside the papilla. Crystals present in the inner tubules of the papilla can only move into the interstitium. Once in the interstitium there is no contact with the urine. On the other hand deposits present in the superficial papillary collecting ducts or ducts near the openings of the ducts of Bellini can migrate to the surface. Once crystal deposits appear on the papillary or forniceal surface they are exposed to a slow moving urine of the renal pelvis and calices from where they receive their nourishment of calcium and oxalate ions. They can now grow even in the absence of hyperoxaluria since normal mammalian urine is generally supersaturated with respect to the CaOx. Thus the crystal deposits present in the superficial collecting ducts of the renal papillae develop into the stones.

Concluding remarks

Calcium oxalate nephrolithiasis starts with hyperoxaluria which results in increased urinary CaOx supersaturation and formation of CaOx crystals within the renal tubular lumen. Both CaOx crystals and oxalate ions provoke a response from renal tubular epithelial cells in culture as well as in the kidneys. Cells release cytoplasmic, lysosomal and membrane-associated enzymes and endocytose luminal crystals present in the vicinity. Epithelial cells often undergo necrosis and are sloughed, exposing the basement membrane. Sloughed cellular membranes can induce nucleation of CaOx crystals at lower supersaturation and can also be involved in crystal aggregation and retention within the renal tubules. Damage to the cells of proximal tubules and loop of Henle may disrupt production of crystallization modulators. Crystal retention occurs by adherence to the cells or aggregation. Tubular epithelial cells endocytose the crystals on the luminal side and exocytose them on the basolateral side. Consequently, crystals move from the lumen to the interstitial location in the kidney. In the process crystals come into contact with the basement membrane, to which they bind, and are thus anchored in the kidneys. Thus the cellular response to oxalate and CaOx crystals (Table 1) plays an important role in crystal retention within the renal tubules, their fixation within the kidneys and evolution into the urinary stone.

The mechanisms involved in stone formation as described above are based on (1) the observations in rats where crystalluria and nephrolithiasis were induced by producing hyperoxaluria and (2) investigations of the response of renal epithelial cells in culture to oxalate and CaOx crystals in the presence or absence of crystallization modifiers. Results of these studies indicate that hyperoxaluria alone can induce structural and functional changes in the tubular epithelial cells which can induce nephrolithiasis. It should, however, be pointed out that many other type of challenges, toxic, inflammatory or immunologic, as well as abnormalities of renal structure and function can promote nephrolithiasis in the presence of supersaturated urine [19].

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