Scanning Microscopy

Volume 5 | Number 3 Article 13

7-11-1991

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Scanning Microscopy, Vol. 5, No. 3, 1991 (Pages 707-712)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/91$3.00+.00

RETENTION OF CALCIUM OXALATE CRYSTALS IN RENAL TUBULES

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(Received for publication March 19, 1991, and in revised form July 11, 1991)

Abstract:

Crystal retention within the renal tubules is essential for nephrolithiasis and the development of urinary stone disease. We studied the mechanisms involved in this process by inducing calcium oxalate crystal deposition within the rat renal tubules and examining them using various microscopic techniques. Crystals appeared to be retained either by attachment to the tubular epithelium or by aggregating with other crystals thus becoming large enough to be retained by their collective size.

Key Words: Crystal attachment, crystal retention, calcium oxalate, urolithiasis, nephrolithiasis, urinary stone formation, urinary stone disease, crystal aggregation.

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Introduction:

It is generally agreed that retention of crystals in the urinary tract is essential for nephrolithiasis and the development of urinary stone disease (Smith, 1987). But there is no agreement as to the mechanisms involved in crystal retention (Robertson and Peacock, 1985). Crystals may be retained either through a fixed particle mechanism by becoming attached to the tubular epithelium (Finlayson and Reid, 1978), or through a free particle mechanism by growing to a size sufficient for them to be trapped within the tubules (Vermeulen et. al., 1967). Finlayson calculated the expectation of free and fixed particle mechanisms in urinary stone formation and concluded that there was no likelihood of single crystals growing large enough to be held in the renal tubules within the urinary transit time through them (Finlayson and Reid, 1978). Our studies have shown that crystals retained at the renal papillary tip following acute hyperoxaluria, are found attached to the injured epithelium lining the ducts of Bellini and that basement membrane is involved in this process (Khan et. al., 1979, 1982). Mandel and associates (1991) have also proposed a role for injured epithelial cells in crystal retention in the ducts of Bellini. The purpose of the current study was to examine crystal deposits in renal tubules of rats with experimentally induced hyperoxaluria and determine the nature of crystal retention in cortical portions of the nephron.

Materials and Methods:

Acute hyperoxaluria was induced in male Sprague-Dawley rats by single intraperitoneal injections of sodium oxalate at a dose of 7mg/100g of rat body weight. After 15 or 30 minutes or 1, 3, 6, 12, 24, or 72 hours or 7 days, rat kidneys were fixed by immersion or perfusion and processed for light microscopic (LM) and scanning (SEM) and transmission (TEM) electron microscopic examinations. For LM examination small pieces of tissues were immersion fixed in formalin and embedded in paraffin. Sections of paraffin embedded tissue were stained with hematoxylin and eosin and examined with

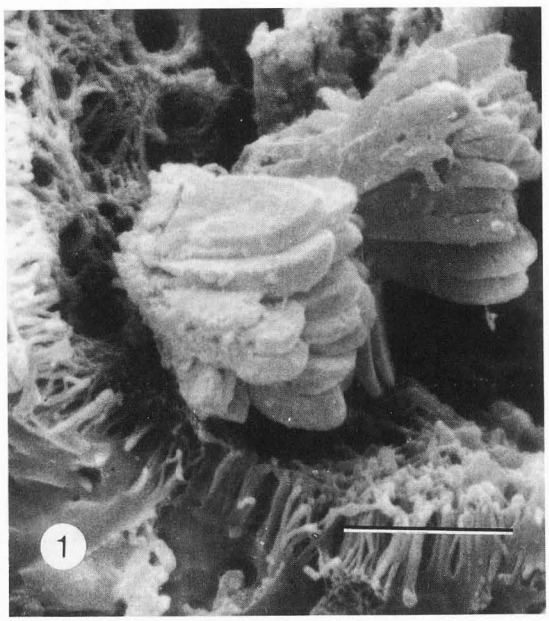


Fig. 1. Calcium oxalate monohydrate crystals in the lumen of a proximal tubule 1 hour after the oxalate challenge. In the foreground a unit consisting of wedge-shaped crystallites fused at one end and free at the other. In the background there is a dumbbell-shaped crystal consisting of interpenetrating crystallites. Bar = 5μm.

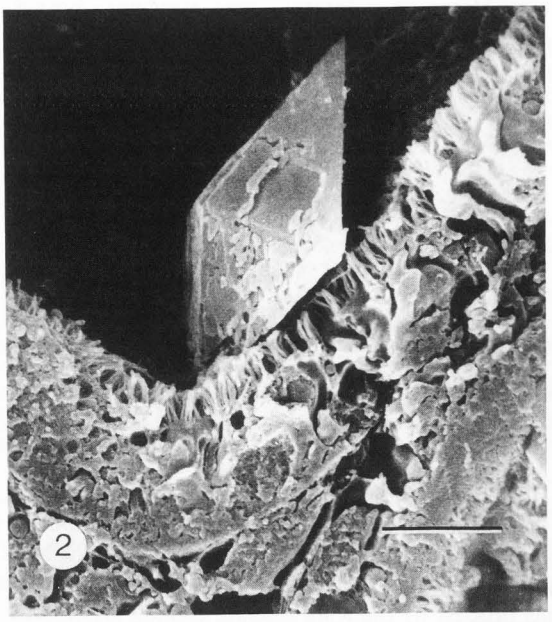


Fig. 2. A single pyramidal calcium oxalate dihydrate crystal present in the tubular lumen 1 hour after the oxalate challenge. Bar = 5μm.

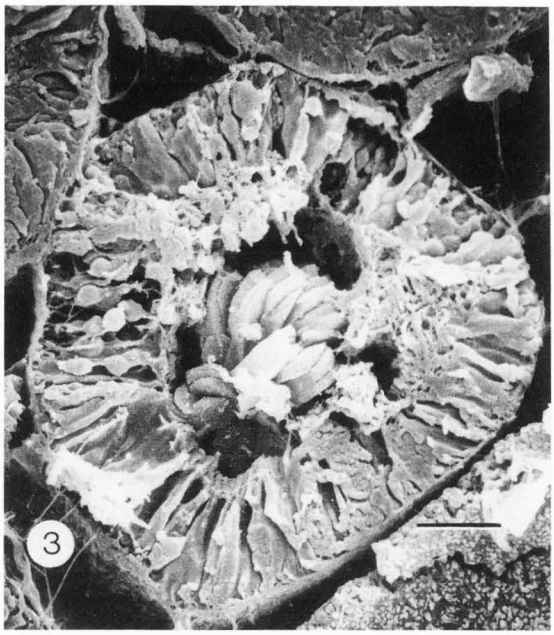
bright field or polarized optics (Khan et. al., 1979). For SEM and TEM examinations kidneys were fixed by retrograde perfusion through the aorta with a formaldehyde-glutaraldehyde mixture (Khan et. al., 1982). Small tissue pieces were then processed using standard procedures. For SEM analysis processed tissue samples were sputter-coated with silver or gold-palladium. For TEM examination tissue samples were embedded in Spurr's plastic. Thin sections were examined after staining with uranyl acetate followed by lead citrate.

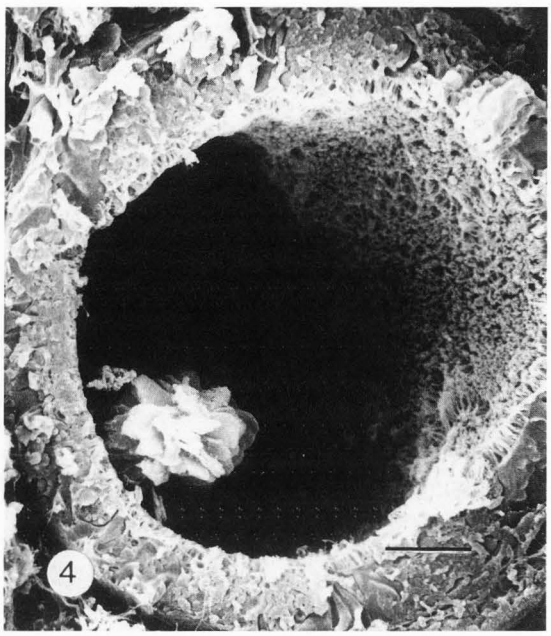
Results:

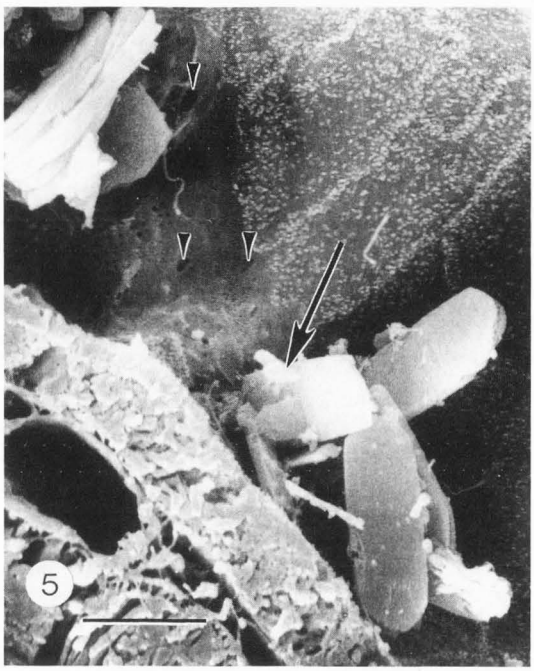
As expected, calcium oxalate (CaOx) crystals were found in all the kidneys examined. When analyzed by SEM, calcium oxalate monohydrate (COM) crystals were found as rosettes or dumbbells or spherolites of monoclinic tabular crystallites (Fig. 1) while calcium oxalate dihydrate (COD) crystals were present as single dipyramids (Fig. 2). Dumbbells and spherulites appeared to have formed by interpenetrant twinning of the COM crystallites. Rosettes consisted of radially arranged wedge-shaped crystallites joined at one end and free at the other. Such rosettes were sometimes observed lying free in the tubular lumen (Fig.1). Other times, they were seen pulling away from the luminal brush border of the tubular epithelium. Individual crystals of COD ranged in size from 10 μm to 15 μm while that of COM from 6 μm to 15 μm. Crystallites of a rosette shaped COM were often smaller than 5μm. Although both COM and COD were seen at all time intervals and dosages, at any given time there were more COM crystals than COD crystals. For the first hour after injection, crystals were present either as individual CODs or small rosettes or rosettes of a small number of tabular COM crystallites. Even single plates of COM were observed. At later time periods however, crystals were aggregated and the individual rosettes and spherolites appeared compact with more crystallites.

Although crystals were seen in both cortex and medulla for all time periods studied here, location of crystals changed with time. After 1 hour of the intraperitoneal oxalate challenge, more crystals were seen in the cortex then in the medulla but after 12 hours they appeared to be equally distributed in the cortex and medulla. After 3 days crystals appeared to concentrate in the outer medulla and the papillary tip. After 7 days kidneys were clear of crystals.

Only a limited number of tubules contained crystals but the number of involved tubules increased with time until 6 hours when largest number of tubules appeared to have crystals. Crystal containing portions of the renal tubules were dilated and appeared necrotic. Crystals were present individually or mixed in with other crystals. They were associated with filamentous and vesicular material which appeared eosinophilic when examined with light microscope in paraffin embedded sections. Transmission electron microscopy revealed this crystal associated material as cellular degradation products.







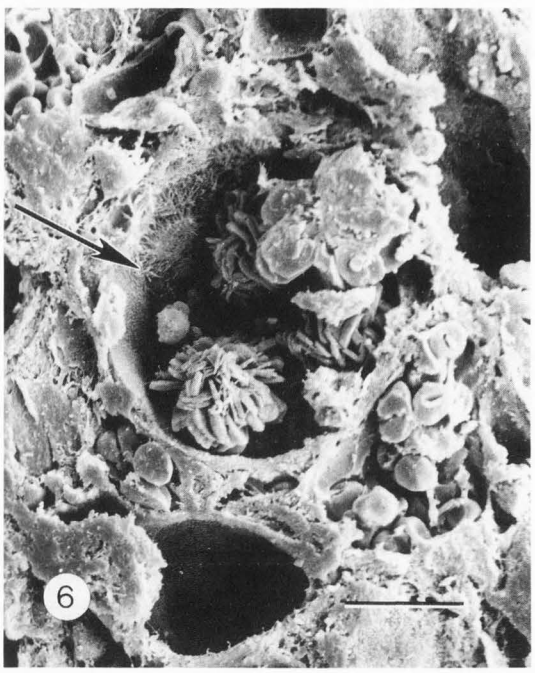


Fig. 3. A cross fractured proximal tubule with total luminal obstruction by calcium oxalate monohydrate crystal aggregates 1 hour after the oxalate challenge. Bar = 5μm.

Fig. 4. A spherulitic crystal aggregate of calcium oxalate monohydrate present in the wide open proximal tubular lumen 1 hour after the oxalate challenge. Bar = 5μm.

Fig. 5. Single plate-like crystals of calcium oxalate monohydrate present in the ascending limb of the loop of Henle 1 hour after the oxalate challenge. One of the crystals (arrow) is attached to an epithelial cell which appears damaged displaying discontinuities (arrowheads) in its luminal plasma membrane. The other cells appear normal. Bar = 5 μm.

Fig. 6. Cross fractured tubules at the cortico-medullary junction. A number of spherulitic calcium oxalate monohydrate crystals are aggregating in one of the tubules 6 hours after the oxalate challenge. Arrow points at the zone of transition from proximal tubule to the loop of Henle. Bar = 10 μm.

In the first hour of the sodium oxalate challenge, crystals were small, located individually within the proximal tubular lumen and appeared to lie free next to the luminal surface of the epithelial membrane. One hour after the injection crystals started to aggregate. Some of the aggregates were large enough to block the proximal tubules (Fig. 3) while most were still smaller than the lumen of the tubule they were in (Figs. 1,2,4). Individual plates of COM crystals were sometimes seen attached to the luminal surface of the epithelium (Fig. 5). Cells involved in attachment appeared damaged, displaying uneven luminal surface, bleb formation and discontinuities in their luminal membrane. Six hours after the oxalate challenge only large COD crystals approximately 20-25μm across or aggregates of spherulitic COM were seen in the cortical renal tubules. After 3 days CaOx crystal deposits were found mainly in the outer medulla in the zone of cortico-medullary junction and consisted of approximately 20 μm across crystals aggregating at the zone of transition from proximal tubule to the thin loop of Henle (Fig. 6).

Discussion:

Sodium oxalate administration via intraperitoneal injection results in a rapid development of hyperoxaluria whose length and intensity depends on the severity of the challenge (Khan, 1991). The largest amount is excreted within the first 6 hours after the injection. At a dose of 7mg/100g, urinary oxalate can reach 4.87$\pm$1.36 mmol/l during this period. Such an acute oxalate challenge almost instantaneously produces calcium oxalate crystals in the kidneys. Up to 7 μm crystals can be seen in rat kidneys within 1 minute after intravenous infusion (Blumenfrucht et. al., 1986) and in less than 15 minutes after intraperitoneal administration of oxalate (Khan et. al., 1979) demonstrating the efficiency of oxalate processing and transport to the kidneys. Obviously oxalate challenge produces a surge in oxalate concentration of the urine in proximal tubules resulting in increased CaOx supersaturation and crystallization of CaOx. Rapidity with which crystals appear in the kidneys also indicates that under favorable circumstances calcium oxalate crystals can nucleate and grow very quickly in the renal tubules.

Crystals may have nucleated free in the tubular lumen or on the epithelial surface. Individual plates of crystals of COM were found attached to the luminal membrane of the epithelial cells while similar plate-like crystals were lying free in the close vicinity indicating that fully formed crystals may have become attached to the epithelial surface. The morphology of rosettes with radially arranged wedge-shaped crystallites joined at one end and free at the other indicates that such crystals were formed attached to a substrate. The presence of such crystals lying free in the tubular lumen and sometimes seen pulling away from the brush border with associated organic material indicates that these crystals may have once been attached to the luminal surface of the epithelium but then tore away carrying with it part of the brush border.

In an earlier study (Khan et. al., 1979) we have shown that an acute oxalate challenge through intraperitoneal administration of sodium oxalate at a low dose of 3mg/100g, results in the formation of a small number of CaOx crystals which appeared first in the renal cortex and then in the medulla. These crystals cleared the cortex first and then the medulla and the whole process appeared to be over within an hour. No overt injury to the renal tubular epithelium was observed and crystals were observed lying free in the tubular lumens. Apparently at that low a dose, crystals formed in the proximal tubules, moved down the nephron and were quickly excreted with the urine. At higher doses of sodium oxalate i.e. 5, 7, or 9mg/100g, which result in higher urinary oxalate (Khan, 1991) however, more CaOx crystals were formed. They were retained in the nephron as large deposits for a much longer period of time indicating that crystal retention is dependent upon the amount of urinary oxalate, the number of crystals formed and their tendency for aggregation.

Finlayson proposed that crystals travelling through the nephron would not have sufficient transit time to grow large enough to be trapped because of their size (Finlayson and Reid, 1978). We found that most individual CaOx crystals deposited in the renal tubules were 12 μm to 15 μm across, much smaller than the tubular lumen they occupied. Thus even though crystals were retained in the renal tubules they were not there because of their size. Moreover the renal tubules were almost always dilated at the site of crystal retention. Thus results of experimental studies appear to concur with Finlayson's hypothesis about crystal growth and its influence on their retention. He had further suggested that crystal retention within the tubules was accomplished by fixation to the tubular epithelium. We saw a number of crystals that were either attached to the luminal surface of the tubular epithelium or had undoubtedly grown attached to a surface. But we also saw crystals that aggregated with other crystals and blocked the renal tubules because of their aggregated mass. Such aggregates were common at the junction of proximal tubule and the loop of Henle and were retained for the longest time. There is a decrease in diameter of tubular lumen from approximately 35μm in the proximal tubule to as small as 15μm in the thin loop of Henle (Kaissling and Kriz, 1979). The crystals and their aggregates that could easily pass through one segment of the nephron because of its larger luminal diameter were stopped at the narrower entrance to the other segment of the nephron. Thus in addition to fixed particle mechanism as proposed by Finlayson, crystals may also be retained by increasing their collective size through aggregation and thus be trapped in the renal tubules because of their aggregated mass. Thus crystal aggregation may play a major role in urinary stone formation (Robertson and Peacock, 1985; Kok et. al., 1990).

A number of processes may be involved in crystal aggregation. Crystals travelling freely through the renal tubule may come in contact with each other at the site of the narrowing of the tubular lumen. At this point cellular degradation products (CDP) which are almost always seen associated with the crystals in experimental urolithiasis (Khan et. al., 1982) and have also been observed inside the urinary stones (Finlayson et. al., 1984) may trap the crystals together thereby assisting in their aggregation. Entrapment of calcium oxalate crystals by CDP's resulting from the experimentally induced urothelial injury has been demonstrated to assist in crystal retention (Khan et. al., 1984). The CDP's may originate from normal sloughing of the dead tubular epithelial cells or from cells injured by their interaction with the crystals (Elferink and Riemersma, 1980; Wiessner et. al., 1987; Mandel and Riese, 1991).

It is assumed that urinary flow through the renal tubules is laminar in which case the flow velocity should be very small near the epithelium and may even be zero at the epithelium (Schulz and Schneider, 1981). Thus crystals would be travelling at different speeds and moving in and out of various speed zones which would increase the prospects of crystal aggregation. Crystal movement should also be influenced by their morphology because of the Stokes drag (Robertson and Peacock, 1985). Calcium oxalate crystallizes both as COM and COD, which have different morphologies, and should have different magnitudes of drag. Even the spherulitic form of calcium oxalate monohydrate is not a compact unit. Urine trapped between the crystallites would put a drag on its movement. There is also the possibility that urinary flow through the renal tubules is not laminar. It has been shown that in the papillary collecting ducts urine moves as discreet boluses and is propelled by peristaltic waves that occur at regular time intervals (Reinking and Schmidt-Nielsen, 1981). In that case, there may be a significant turbulence which would increase the probability of crystal contact and aggregation. Thus crystal aggregation may be accomplished by a variety of mechanisms and any or all of them may be involved in crystal retention within the renal tubules.

Acknowledgements:

This work was supported by NIH grants # 5 PO1 DK 20586 and 1RO1 DK41434.

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Discussion with Reviewers

W.G. Robertson: Would the authors agree that Finlayson's original calculations may not have taken all possible factors into account when he concluded that a "free-particle" mechanism was not possible within the renal collecting system?

B. Hess: Would the authors agree that Finlayson's calculations of the expectation of free and fixed particles are valid only for calcium oxalate particles and may not be extrapolated to other forms of renal stones?

Authors: Finlayson proposed that free particles can not grow fast enough to cause stone disease in the upper

urinary tract. His calculations were based on the growth of most common of the stone crystals namely calcium oxalate. The question of mass accretion through crystal aggregation was not addressed. Other stone crystals may grow at rates different from that of COM and thus Finlayson's calculations may not apply. But as Finlayson pointed out, linear growth rates of most stone salts are not known. Our study has clearly demonstrated that crystal aggregation is common in the renal collecting system and that these aggregates can be large enough to occlude the renal tubules. A number of recent studies have demonstrated that urine of human stone formers promotes crystal aggregation. Thus free particles accreting mass by aggregation may be retained within the renal tubules and cause stone disease in the upper urinary tract.

W.G. Robertson: Have the authors any evidence that constrictions in the renal system, such as the "kinked" tubules reported by Grave in 1982 (Br. J. Urol. 54:569-574), might be the cause of some trapping of crystals and/or aggregates?
Authors: It is definitely possible. The changes in luminal diameter as would occur at the kink sites and as happens at the junction of proximal tubules and loops of Henle's may actually facilitate crystal aggregation as we have illustrated here in Fig. 6.

N.S. Mandel: Had the crystals physically dilated the tubules or did the tubules dilate in response to some stimulus in the presence of the crystals?
Authors: Tubular dilatation appears to be the result of luminal occlusion by crystals.

N.S. Mandel: Within the tubules, did the crystals selectively adhere to either the principal or intercalated epithelial cells?
Authors: As illustrated in Fig. 5, in the thick ascending limb of the loop, crystals appear to preferentially adhere to smooth surfaced cells. But we have not examined enough attachment sites to make a categorical statement about the selectivity of crystal adherence.

N.S. Mandel: Can you relate the calcium and oxalate concentrations in stone forming rats to those in stone forming and non stone forming humans.
Authors: On a normal laboratory diet, adult rats do not form calcium oxalate crystals or stones. Rats have to be made hyperoxaluric to become stone formers. On the other hand the major difference between idiopathic stone formers and non stone forming individuals is in the crystallizatioin inhibitory potential of their urine and not in urine's calcium or oxalate content. Thus in rat models of calcium oxalate urolithiasis, crystals are formed primarily because of the increased urinary oxalate, while in humans, because of the decrease in their urine's inhibitory activity. Thus the instigators of crystal formation in rats are different from those in the humans. Therefore urinary calcium or oxalate concentrations in experimentally induced hyperoxaluric rats can not be related to those in human stone formers.

M.I. Resnick: What is the reason for crystal adherence to tubular epithelial cells? Are matrix proteins or other macromolecules important in this process? Can the authors theorize whether these are products of the tubular cells?
Authors: Certain macromolecules of cellular origin may be involved in crystal adherence to the epithelial cells. These macromolecules may be formed as a result of injury to the epithelial cells as suggested by Mandel and associates (1991) or they may be a normal product of tubular cells but be abnormal in nature. Eventually these crystal associated macromolecules may become incorporated in the stones as matrix.

D.J. Kok: The COD crystals appear to be in the same size range as COM-aggregates. However, while the authors did find obstruction of tubules by COM-aggregates after 1 hour, after 6 hours and after 3 days, there is no mention of obstruction by single large COD crystals. Can this be explained by a phase transformation or by shape factors?
Authors: As mentioned in the paper, only plates of COM were seen adhering to the epithelium. Single crystals of COD were observed in the tubular lumina as is illustrated in Fig. 2, but did not appear adherent to the epithelial surfaces. In this very model of CaOx nephrolithiasis (Khan et. al. 1982) we have previously seen COD crystals aggregating with the COM crystals. There may also be a preferential binding between COM and epithelial cells.