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Animal models of kidney stone formation: an analysis

Abstract Calcific kidney stones in both humans and mildly hyperoxaluric rats are located on renal papillary surfaces and consist of an organic matrix and crystals of calcium oxalate and/or calcium phosphate. The matrix is intimately associated with the crystals and contains substances that can promote as well as inhibit calcification. Osteopontin, Tamm-Horsfall protein, bikunin, and prothrombin fragment 1 have been identified in matrices of both human and rat stones. Hyperoxaluria can provoke calcium oxalate nephrolithiasis in both humans and rats. Kidney-stone-forming rats are hypomagnesuric and hypocitraturic during nephrolithiasis. Human stone formers may have the same disorders. Males of both species are prone to develop calcium oxalate nephrolithiasis, whereas females tend to form calcium phosphate stones. Oxalate metabolism is considered to be almost identical between rats and humans. Thus, there are many similarities between experimental nephrolithiasis induced in rats and human kidney-stone formation, and a rat model of calcium oxalate nephrolithiasis can be used to investigate the mechanisms involved in human kidney stone formation.

Kidney stone formation is a complex process and the result of a cascade of events, including crystal nucleation, growth, and aggregation, and crystal retention within the renal tubules [8, 10–12, 36, 43]. Under appropriate conditions, retained crystals evolve into stone nidi, thus establishing a base for stone growth [23]. A number of theoretical, chemical, and animal models have been developed [12] in efforts to understand the mechanisms involved such that therapeutic agents and protocols can be developed and their efficacy, tested. "Why study models [12]?" Models are studied because they allow us to dissect a complex process into individual segments and study these separately as well as together. Good models are uncomplicated, permit critical testing of various hypotheses, and can be used to assess the value of therapy.

Some stone models pertain to only one event and one aspect of the process. For example, studies that use various types of crystallizers [10] are concerned with crystal nucleation and growth. Tissue-culture studies currently being used to investigate the interaction between oxalate and/or calcium oxalate crystals and renal epithelial cells are modeling only these specific events. Theoretical computational models assess only the crystallization potential of urine and similar solutions. Animal models, on the other hand, are employed in efforts to understand all aspects of the pathogenesis, including the anatomical and physiological role of kidneys and renal tubules. Rats are the animals most commonly used for the study of nephrolithiasis [28], although rabbits and dogs have also been used. Most of the data available on renal physiology are also based on experiments with rats, rabbits, and dogs. Almost 80% of stones worldwide are composed of calcium oxalate (CaOx), often in association with calcium phosphate (CaP). As a result, CaOx nephrolithiasis has been studied in greater detail. My comments in this paper are based mainly on the studies of experimental CaOx nephrolithiasis promoted in rats by induction of hyperoxaluria. CaP nephrolithiasis is discussed in less detail.

A model is defined as an imitation, a copy, or a replica of something that exists. For the development of a model or the determination of the relevance of a model a complete knowledge of the object to be modeled is essential. For the purpose of the present discussion, therefore, we should have an understanding of human kidney stones and the process of kidney stone formation. In addition, a knowledge of human and rat kidney anatomy and urinary chemistry is necessary.

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Normal kidneys

There are basic anatomical differences between kidneys of humans and those of animals [47] such as dogs, rabbits, and rats; the latter have been extensively used to study renal ultrastructure and physiology and have been employed in the development of models of many renal diseases. The kidneys of these animals are much smaller, are unipapillate, and have fewer urinary tubules, a simpler pelvis, and a smaller urinary space. However, the medulla-to-cortex volume ratio is very similar, being 1:2 in the rat, the dog, and the human and 1:16 in the rabbit. An appreciation of these differences is necessary when we discuss the size of a nephrolith produced by a kidney. A small kidney with a simple and small pelvis is incapable of making a large staghorn and can generate only a miniature imitation of the human stone. Since the rat is the animal most commonly used as a stone model and most of the present discussion is focused on rat models of stone disease, a comparison of some of the anatomical features of human and rat kidneys is provided in Table 1.

Renal epithelial cells also produce many macromolecular modulators of crystallization [9]. Knowledge about their normal renal distribution and, in limited cases, their occurrence in nephrolithic kidneys has been obtained from studies of rat and mouse kidneys using ultrastructural and in situ hybridization techniques. In normal kidneys, Tamm-Horsfall protein (THP) is localized in the thick ascending limb of the loop of Henle (TALH) [3]; nephrocalcin (NC), in the proximal tubule [9] as well as the TALH; osteopontin (OPN, or uropontin), in the thin and thick ascending limbs of the loop and in papillary surface epithelium in the renal calices [14, 35]; and urinary prothrombin fragment 1 (UPFT-1, or crystal matrix protein), in the distal convoluted tubules and the TALH [49].

Nephrolithiasis in humans

Human kidney stones are polycrystalline aggregates of crystals and organic matrix [5, 8, 10]. CaOx crystals are the main component of up to 80% of stones worldwide. Fractured stone surfaces reveal crystals arranged in concentric laminations and radial striations. Organic matrix is pervasive throughout a stone [30] and contains lipids [37, 34], proteins, and carbohydrates [5, 52]. Ultrastructurally, the stone matrix contains both fibrillar and amorphous elements and is organized in well-pronounced concentric laminations and radial striations [30, 39]. Demineralization of stones produces crystal ghosts, spaces representing the dissolved crystals surrounded by the associated organic matrix. The stone matrix is periodic acid-Schiff (PAS)-, colloidal iron-, and alcian blue-positive, indicating the presence of both neutral and acidic mucosubstances [52]. The matrix is also Sudan black-positive, which is indicative of the presence of phospholipids [31]. Lipids and proteins have also been localized in stone matrix using various ultrastructural techniques. Membranous cellular degradation products are seen between the crystals. Malachite green-positive lipids are identified in close contact with crystals [34]. OPN is seen occluded in the crystals as crystal matrix [39]. Both the concentric laminations and the interlamellar substances (radial striations) label heavily for OPN. THP is found on crystal surfaces and dispersed between the crystals (unpublished personal observations).

Calcific stone formation is associated with various disorders, including renal tubular acidosis, hypercalciuria, hyperoxaluria, hypocitraturia, hypomagnesuria, and hyperuricosuria [8, 43]. These disorders have a variety of causes but ultimately result in abnormal urinary pH and in excretion of calcium, oxalate, citrate, magnesium, and uric acid, respectively. In male CaOx stone formers, urinary oxalate may increase from a normal 0.28 mM/24 h (volume 1.5 l) to 0.59 ± 0.3 mM/24 h [19], calcium may increase from 3.28 mM/l in normals to 3.96 mM/l in stone formers [8], and citrate may decrease from 2.13 mM/l in normals to 1.81 mM/l in stone formers [8]. As a result, there is an increase in urinary supersaturation with respect to CaOx and/or CaP.

Hyperoxaluria is caused by either overproduction or intestinal overabsorption of oxalate [8, 18, 43]. Primary hyperoxaluria, in which oxalate is overproduced because of disturbances in the oxalate biosynthetic pathway, is rare. Idiopathic CaOx stone formers are only mildly hyperoxaluric but the physicochemical constraints are such that in urine, CaOx crystals would form more readily with slight oxalate excess than with calcium excess [11, 45]. Approximately 60% of stone formers are hypercalciuric, 30% are hypocitraturic, and 10% are hypomagnesuric [42].

Nephrolithiasis is defined as the formation of solid phases in urinary passages [1, 10, 11]. The deposition of salts in renal parenchyma is called nephrocalcinosis. This distinction is necessary because nephrocalcinosis is quite common and may or may not evolve into nephrolithiasis. Some studies have found interstitial calcific deposits in almost all autopsied kidneys examined [2]. Incidences of nephrocalcinosis increase with age, whereas nephrolithiasis is mostly a disease of the third to fifth decades of life.

Table 1 Morphological differences between kidneys of rats and humans

	Size (cm) length, width, thickness	Weight (g)	Number of papillae	Number of nephrons
Humans	12 × 6 × 4	160–175	5–6	850,000–1,200,000
Rats	1.6 × 1 × 0.9	0.75–1.2	1	30,000–31,000

Kidney stones originate in renal tubular lumina and are located in the renal calyces and pelvis, anchored to the papillary surfaces [1]. In idiopathic stone formers, crystals are restricted to the renal medulla and papilla. However, in nephrolithiasis associated with primary hyperoxaluria, crystal deposits are seen in all parts of the kidneys, including the cortex, and crystals can be seen in all segments of the nephron, including the proximal tubules.

Whereas normal urine contains many inorganic and organic inhibitors of crystallization of calcific salts, urine from stone formers is considerably less inhibitory [11, 46] and may actually promote the formation of calcific crystals [26]. Magnesium and citrate are inhibitors of crystallization since they can reduce the saturation of calcium oxalate by complexing oxalate and calcium, respectively. It is hypothesized that stone formers excrete either lower amounts of or structurally abnormal inhibitors [20].

An individual's gender and sex hormones appear to play a significant role in nephrolithiasis, which is more prevalent in males than in females [42]. In addition, CaOx stones are more common in males, whereas stones produced by females mostly comprise CaP. However, males and females have an equal tendency toward CaOx nephrolithiasis during childhood. Observations that hepatic glycolate oxidase is directly related to circulating testosterone and increased serum testosterone levels result in increased production of oxalate by the liver, suggest that lower testosterone levels may offer protection to women and children against CaOx nephrolithiasis [10].

Nephrolithiasis in rats

This subject has been reviewed in many recent publications [6, 23, 29]. Documented cases of spontaneous urinary stone formation in rats are rare, and there is no report of spontaneously formed CaOx stones in the rat upper urinary tract [29]. Excess urinary excretion of crystallizable substances of choice with or without manipulation of urinary pH and/or deficient excretion of crystallization inhibitors have been the principal mechanisms used to induce experimentally crystallization in the urine and formation of stones in the kidneys.

CaOx nephrolithiasis

CaOx kidney stones are produced in rats by the induction of acute or chronic hyperoxaluria [22, 29] using a variety of agents such as sodium oxalate, ammonium oxalate, hydroxy-L-proline, ethylene glycol, and glycolic acid. Hyperoxaluric agents have often been used in association with vitamin D or a magnesium-deficient diet and, sometimes, with a pH-reducing protocol of ammonium chloride administration. Lithogenic agents are generally dispensed orally in food or water or by gavage but have also been injected intraperitoneally.

Acute hyperoxaluria caused by intraperitoneal administration of sodium oxalate (3, 7, or 10 mg/100 g rat body weight using sodium oxalate at 28 mg/ml in 0.9% normal saline) resulted in increased urinary excretion of oxalate and an almost instant appearance of CaOx crystals in lumina of the renal proximal tubules [33]. Crystals were later seen in collecting ducts of the cortex and papilla. The amount and duration of urinary excretion of excess oxalate and the size, number, and location of crystals within the kidneys depended on the amount of sodium oxalate given. The largest amount of oxalate was excreted within the first 6 h of the challenge. During this period, nephrolithic rats on 3 mg sodium oxalate excreted more than 200% more oxalate in urine than control rats and those on 10 mg sodium oxalate excreted more than 500% more oxalate than control rats. At the lower dose, crystals were restricted to the tubular lumens and cleared the kidneys within a few days. At higher doses, crystals were initially located in tubular lumina. They were later seen in the interstitium. Apparently, some crystals and crystal aggregates remained small, did not adhere to the renal epithelium, moved with the urine, and were flushed out. Larger crystals moved slowly. The crystals and their aggregates that were attached to the renal epithelium or were too large to move with the urine migrated to the interstitium. Renal papillary tips and the corticomedullary junction were the preferential sites of crystal retention.

Urinary excretion of oxalate increased rapidly and significantly during chronic administration of ethylene glycol (EG) as a 0.75% aqueous solution in drinking water to male Sprague-Dawley rats (Table 2). Excretion of calcium, magnesium, and citrate decreased concomitantly. Urinary CaOx supersaturation increased accordingly.

Table 2 Urinary chemistry of male rats given 0.75% EG in their drinking water[a]

Day	Calcium	Oxalate	Magnesium	Citrate	CaOx relative supersaturation
0	6.94 ± 0.7	1.12 ± 0.1	17.6 ± 0.9	29.5 ± 2.2	7.98 ± 1.1
7	2.70 ± 0.3	3.83 ± 0.3	14.8 ± 1.7	18.0 ± 2.8	18.03 ± 2.5
14	1.95 ± 0.5	4.86 ± 0.5	12.7 ± 1.3	15.4 ± 2.8	14.68 ± 3.1
21	2.16 ± 0.8	4.20 ± 0.6	14.1 ± 1.8	11.12 ± 2.4	16.09 ± 2.0
28	2.04 ± 0.4	4.15 ± 0.6	13.1 ± 1.2	9.9 ± 2.6	18.53 ± 2.8
35	2.06 ± 0.4	4.11 ± 0.6	13.3 ± 1.7	9.7 ± 2.1	19.68 ± 3.01

[a]Data represent mean values ± SD expressed in m*M*/l

Chronic hyperoxaluria induced by the administration of 0.75% EG alone or with 2% ammonium chloride (AC) to male rats produced crystalluria, which was followed by CaOx nephrolithiasis. Combined treatment with EG + AC resulted in persistent crystalluria in all rats by day 3 and in nephrolithiasis by day 7. It took rats approximately 12 days of chronic administration of EG alone to show persistent crystalluria and about 3 weeks to start depositing crystals in their kidneys. Initially, small dipyramidal crystals were seen in the urine. Later, most of the urinary crystals were large aggregates of dumbbell-shaped CaOx monohydrate crystals and twinned CaOx dihydrate crystals. Ministones measuring 75–200 μm in diameter were seen in the bladder aspirate. A magnesium-deficient diet accelerated and exaggerated crystalluria and nephrolithiasis in male rats receiving 1% EG in drinking water. The crystals were located in both the cortex and the medulla [48]. Administration of magnesium oxide to male rats receiving a 1% aqueous solution of EG significantly reduced their urinary oxalate excretion and stopped their CaOx nephrolithiasis [50].

During chronic hyperoxaluria, crystals were initially distributed randomly in the renal medulla. Eventually, collecting ducts at the renal papillary tip and papillary base were the preferred sites of crystal deposition [23–25]. Most crystals were intraluminal aggregates. After 4–6 weeks, crystals were also seen between the tubular epithelial cells as well as inside the epithelial cells and the interstitium. After only 1 week of EG + AC treatment or 8 weeks of EG alone the kidneys of some rats had nephroliths or stones attached to their renal papillary surfaces. These stones contained both CaOx mono- and dihydrate crystals and reached a size of over 1000 μm, occupying and calcifying the entire papillary tip. Examination of the papillary stones by light microscopy and scanning and transmission electron microscopy revealed that they originated in the lumina of collecting ducts near the renal papillary surface [24]. The outer segment of the papillary stone was well organized and appeared striated.

CaOx nephroliths contained a PAS- and colloidal iron-positive organic material. Ultrastructural examination of demineralized stones revealed an organic matrix consisting of amorphous and fibrillar elements [24]. Well-delineated crystal ghosts contained concentrically laminated and radially striated matrix, which at places appeared to be connected to the basal lamina of the tubular epithelium. Cellular degradation products were common between the crystal ghosts and were often seen intimately applied to ghosts' surfaces. The matrix stained positively for OPN and THP [14, 15, 39]. Osteopontin (OPN) was primarily present as occlusions in the crystals and was localized in concentric laminations and radial striations. Tamm-Horsfall protein (THP) covered crystal surfaces and was present between the crystals. Inside the renal tubules, THP appeared to connect one crystal with another [14].

As discussed above, OPN and THP are localized at specific sites in the kidneys and are generally restricted to renal epithelial cells and tubules in the cortex. Staining for THP and OPN, however, was strikingly enhanced and altered in nephrolithiasis [14, 15]. Both were abnormally localized in renal medulla, where they were closely and concurrently associated with crystal deposits.

Both acute and chronic hyperoxaluria resulted in increased urinary excretion of enzymes [22, 32, 33]. Excretion of lysosomal enzyme, *N*-acetyl-β-glucosaminidase, increased in mild chronic hyperoxaluria without crystal deposition in the kidneys. Crystal deposition was associated with increased urinary excretion of the membrane marker enzymes alkaline phosphatase, leucine aminopeptidase, and gamma-glutamyl transpeptidase. The renal tubular epithelium of nephrolithic kidneys was damaged [29]. Severe damage was restricted to the tubules containing the crystals. The proximal tubular brush border was distorted by clubbing of microvilli, their localized loss, and formation of blebs. Degenerative changes in epithelial cells included an increase in the number of lysosomes, swelling of mitochondria, dilatation of endoplasmic reticulum, cytoplasmic edema, and vacuolization. Some cells appeared to burst open and release their contents into the tubular lumen, whereas others sheared away from the basal lamina. Renal papillary surfaces were badly damaged. The intercellular spaces between the intact epithelial cells appeared enlarged. Numerous dividing nuclei were found in the intact epithelial cells.

Intratubular nephroliths were almost always found admixed with cellular membranes. Experimental shedding of the epithelial brush border in the urine metastable with respect to CaOx promoted the nucleation of CaOx crystals [16]. It is suggested that lipids of the membranous cellular degradation can act as heterogeneous nucleators of calcific crystals [34]. In addition, the injury caused by exposure to elevated levels of oxalate and CaOx crystals may reduce the crystallization-inhibitory activity of the urine [17].

All studies described above were carried out in male rats. EG solutions of low concentration that induce CaOx nephrolithiasis in male rats do not produce similar results in females [25]. Administration of 1% EG for 4 weeks produced CaOx nephrolithiasis in 3/13 males as opposed to 0/12 females; urinary acidification increased the incidence to 5/6 males and 1/9 females [38]. In an attempt to understand the role of gender and sex hormones, 0.5% EG was used to induce CaOx nephrolithiasis in male and female normal and gonadectomized rats [37]. Low-level CaOx crystallization was seen in all rats, but of the normal males, 5/7 had kidney stones. Only 1/7 castrated males produced kidney stones. None of the female rats, normal or castrated, produced any kidney stone or massive crystal deposition in the kidneys. In another study, 0.75% EG treatment in association with urine acidification produced crystals in kidneys of both male and female rats, but only male rats contained crystal deposits in and on their renal papillae. CaOx relative supersaturations were similar in both male and female rats. The results of another study

showed that in males a 0.2% solution of EG was enough to produce CaOx nephroliths, whereas in females a solution of 1% or more EG was required to obtain comparable results.

In conclusion, CaOx nephrolithiasis can be induced experimentally by hyperoxaluria and is associated with enzymuria. The renal papillary tip and calyces are the preferential sites for crystal deposition in chronic hyperoxaluria. Chances for crystal deposition elsewhere in the kidneys, including the renal cortex, increase with increasing levels of urinary oxalate and duration of the hyperoxaluric state. Magnesium deficiency exacerbates nephrolithiasis. Testosterone plays a significant role. Experimentally produced crystals and stones contain an organic matrix of carbohydrates, lipids, and proteins.

Calcium phosphate nephrolithiasis

Rats consuming a semipurified diet spontaneously produce calcium phosphate (CaP) nephrolithiasis, which is considerably more common in females than in males [40]. Calcification starts intraluminally in the proximal tubules with microliths blocking the tubules at the corticomedullary junction. There is vesiculation and shedding of the microvillous brush border into the tubular lumen, where the vesicles become incorporated into the growing nephrolith. Estrogen together with dietary levels of calcium, phosphorus, and magnesium plays a significant role. It was shown that ovariectomy resulted in the cessation of calcification. Replacement therapy with estrogen following castration produced CaP nephrolithiasis in both male and female rats [13].

A magnesium-deficient diet also induces CaP nephrolithiasis [41], which follows a developmental pattern similar to that described above for semipurified diets. Minute spherical nephroliths start in the tubular lumen of the loop of Henle, aggregate, and lodge in the hairpin bend of the loop, appearing as a semicircular band at the corticomedullary junction in the cross section of the kidneys. They stain positively with von Kossa and alizarin red, indicating the presence of calcium and phosphorus, and are associated with a PAS-positive organic matrix. A high level of phosphate in the diet also causes CaP deposition in the renal tubules in the outer zone of the renal medulla [6]. Intraluminal CaP deposits developed in weanling female rats given daily injections of 0.5 *M* neutral sodium phosphate for 10 days. Nephroliths developed in the terminal segments of the proximal tubules at the corticomedullary junction. However, a similar treatment of young adult rats caused nephrocalcinosis and produced CaP deposits in basement membranes of the proximal tubules. The injection of calcium glucanate also produced CaP deposits in the basement membrane of the proximal tubular epithelium. CaP deposition after parenteral calcium or vitamin D appeared to involve mitochondria.

CaP stone formation has recently been described in female genetic hypercalciuric rats [7]. These inbred rats excrete excessive amounts of calcium, resulting in high relative supersaturation with respect to brushite and CaOx. Stones consisting of poorly crystalline CaP were found in the kidneys and ureters. The principle mechanism of hypercalciuria in these rats is suggested to be an increase in the intestinal absorption of calcium.

CaP nephroliths contain poorly crystalline biological apatite and an organic matrix. The matrix is composed of both amorphous and fibrillar components. In addition, it contains cellular degradation products, including membranous vesicles.

In conclusion, female rats are more prone to CaP nephrolithiasis than male rats. Increased urinary excretion of calcium and phosphate and decreased excretion of magnesium promote CaP nephrolithiasis. Estrogen appears to play a significant role in the pathogenesis. Nephroliths consist of crystals and an organic matrix.

Foreign-body stones

Vermeulen et al. [51] developed a foreign-body model of production of urinary stones. Foreign bodies of a variety of substances were implanted in urinary bladders. The diet was modified or a lithogen was added to the drinking water to produce a stone of desired composition. Paraffin bodies did not encrust but induced the formation of free-lying stones in the bladder. All other materials tested encrusted and the stones grew. Male Sprague-Dawley rats produced struvite stones, whereas females deposited apatite on the foreign bodies. The addition of EG to drinking water or a pyridoxine-deficient diet resulted in the formation of CaOx stones. Changing of the urinary ambient conditions by administration of EG for 2 weeks at 2-day or 2-week intervals resulted in the formation of urinary stones of mixed composition [29] containing CaOx and struvite and/or CaP.

Historically, most such studies were performed with zinc foreign bodies. We developed a model in which sectionable plastic foreign bodies were implanted. This modification enabled us to study the encrustation process in greater detail using various light microscopy and ultrastructural techniques [27]. Coating of the foreign body with organic material initiated the encrustation. The stone grew by confluent crystal growth and aggregation, and its fractured surface demonstrated characteristic concentric laminations and radial striations. The matrix consisted of amorphous and fibrillar elements mixed with cellular degradation products.

Comparison between rat and human nephrolithiasis

Similarities between human and rat CaOx kidney stones and their pathogenesis are listed in Table 3. Stones formed in kidneys of humans and rats are identical at the ultrastructural level in both the nature and the

Table 3 Salient features of CaOx nephrolithiasis in humans and chronically but mildly hyperoxaluric rats

	Humans	Rats
Stone composition	CaOx mono- and dihydrate crystals and organic matrix	CaOx mono- and dihydrate crystals and organic matrix
Matrix composition	Carbohydrates, lipids, proteins	Carbohydrates, lipids, proteins
Matrix proteins	OPN is occluded in the crystals; THP is present between crystals and on their surfaces	OPN is occluded in the crystals; THP is present between crystals and on their surfaces
Stone location in the kidneys	Renal papillary surface	Renal papillary surface
Main cause of nephrolithiasis	Hyperoxaluria	Hyperoxaluria
Inhibitor status	Stone formers may excrete less citrate and magnesium	Urinary excretion of citrate and magnesium is decreased
Nucleation	Most probably heterogeneous	Most probably heterogeneous
Role of gender	CaOx stone formers are mostly males	Male rats are prone to form CaOx stones
Renal injury	In idiopathic stone formers, kidney functions are normal but enzymuria may occur	Kidney functions appear normal; enzymuria occurs concomitantly with hyperoxaluria and nephrolithiasis

composition of their crystals and matrix. Stone initiation by deposition of crystals in collecting ducts of the renal papilla and the eventual location of stones on the papillary surfaces are also similar in humans and mildly hyperoxaluric rats. However, rat kidney stones are very small. Their diminutive size appears to be a result of small kidneys with single papillae and tiny urinary spaces (Table 1). The main cause of stone formation in both humans and rats appears to be chronic mild hyperoxaluria. Like humans, all rats with similar urinary oxalate and CaOx supersaturation do not produce CaOx nephroliths. This indicates that factors other than supersaturation are involved in kidney stone formation. It is also interesting that normal rats have a much higher urinary oxalate concentration (1.12 m*M*/l) than normal humans (0.28 m*M*/l), yet the rats require an even greater excretion of oxalate to produce CaOx crystals.

Many stone-forming humans are hypocitraturic. Experimental nephrolithiasis in rats is also associated with a reduction in the urinary excretion of citrate. Urinary citrate concentration is very high in rats (29.5 m*M*/l; see Table 2) as compared with humans (2.99 m*M*/l), and it decreases progressively during both CaP and CaOx nephrolithiasis (Table 3).

A large number of idiopathic stone formers are hypercalciuric. CaOx nephrolithiasis experimentally induced in rats by the administration of EG, however, is associated with lower than normal urinary excretion of calcium.

It has been suggested that human stone formers produce structurally abnormal THP, which may not inhibit crystal aggregation. Our studies of THP in the rat model of CaOx nephrolithiasis did not reveal any significant quantitative or qualitative structural difference in THP from normal versus stone-forming rats (unpublished results). Both THPs performed similarly in crystal aggregation assays and had similar amino acid contents.

Experimentally induced renal crystal deposition is always associated with cell injury. Although damaged cells can be identified at the earliest times at which crystals are seen, it is equivocal as to whether damage occurs prior to or subsequent to crystal deposition. However, enzymuria in the absence of nephrolithiasis indicates the possibility of impairment prior to crystallization and suggests that challenge to the renal epithelial cell may be an initiating event. The development of stones on Randall's plaques [44], enzymuria of proximal tubular origin [4], and functional or structural tubular abnormalities and/or tubular damage [21] implicate renal injury in human nephrolithiasis.

The interaction between crystals and organic material appears critical in the attachment and growth of urinary stones. All crystals, whether experimentally induced in rats or spontaneously formed in humans, contain organic material occluded within and on their surfaces. It appears to provide architectural integrity, a complex scaffolding without which the stone would crumble and fall apart.

Conclusions

This paper briefly compares the important features of kidney stone formation in humans and experimental nephrolithiasis in rats. Obviously, the pathogenesis of kidney stone formation can be studied in the rat model as long as limitations of the model are understood and objectives of the study are clearly defined. CaOx stone formation is not a spontaneous phenomenon in most animals, but the near identity in terms of oxalate metabolism in humans and rats allows several strategies for experimental modeling of stones in rats. Rat models of nephrolithiasis have proved helpful in our understanding of the initial events; the site of early response to hyperoxaluric challenge; the nucleation, aggregation, and retention of crystals; and the involvement of macromolecules during these processes in the kidneys. Various experimental manipulations permit the dissection of two key processes of stone formation, namely, stone nucleation and nidus formation in the study of nephrolithiasis and stone growth in the study of foreign-body

encrustation. In addition, a study of these mechanisms over time in the rat model gives a more dynamic view than can be obtained in investigations of stones and their formation in humans.

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