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CALCIUM OXALATE UROLITHIASIS IN THE RAT: IS IT A MODEL FOR HUMAN STONE DISEASE? A REVIEW OF RECENT LITERATURE

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Abstract

Calcium oxalate stone disease is the most common human urinary stone disease in the Western Hemisphere. To understand different aspects of the disease, calcium oxalate urolithiasis in the rat is used as a model. Spontaneous calcium oxalate urolithiasis is very rare in rats. Thus the disease is experimentally induced and the rats are generally made hyperoxaluric either by administration of excess oxalate, exposure to the toxin ethylene glycol, or various nutritional manipulations. All the experimental models show renal injury associated with crystal deposition. Calcium oxalate crystals are in most cases intraluminal in renal tubules and often attached to the basal lamina of the denuded epithelium. Rat renal papillary tips and fornices appear to be the preferential sites for the deposition of large calcium oxalate calculi. Where urinary supersaturation of calcium oxalate has been studied the crystal forming rat urines are shown to have higher urinary supersaturation of calcium oxalate than their controls. Oxalate metabolism in the rat is nearly identical to that in humans. Thus, in a number of respects, experimental calcium oxalate urolithiasis in the rat is similar to calcium oxalate stone disease in man.

KEY WORDS: Urolithiasis in Rat, Urinary Stone Disease, Stone Formation, Crystal Nucleation, Renal Tubular Injury, Epithelial Necrosis, Ethylene Glycol, Foreign Body Stone, Pyridoxine Deficiency, Oxalate Metabolism in Rat.

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Introduction

Urolithiasis, the precipitation of salts in urine, is a process common to many animals including man. The precipitation results in the formation of crystals which move freely and are harmlessly excreted by the animal, or are retained in the urinary tract resulting in stone disease. Thus crystalluria, nephrolithiasis, and lower urinary tract stone disease are special manifestations of urolithiasis. Every individual passes crystals of calcium phosphate and or calcium oxalate in the urine making crystalluria the most common form of urolithiasis in man. Urinary stone disease, on the other hand, afflicts approximately 1 of 1000 individuals in the United States. Importantly, seventy to eighty per cent of these stones contain calcium oxalate. In contrast to man, spontaneous urolithiasis is quite rare in rats, especially the calcium oxalate type. Nonetheless, because the rat is the most easily available and commonly used laboratory animal, a number of experimental models have been developed for the study of calcium oxalate urolithiasis. In this brief review various aspects of calcium oxalate urolithiasis in the rat, with reference to humans and other mammals, will be discussed.

Oxalate Metabolism

In mammals urinary oxalate is derived from exogenous and endogenous sources [51]. In man from 5 to 15 percent of urinary oxalate comes from dietary sources [51,82,84,106] of which three to five percent is derived from dietary glycolate [46,84]. Absorption of oxalate through the intestine is dependent on the availability of soluble oxalate because soluble forms are more readily absorbed. Their absorption is inversely related to the amount of available calcium to convert soluble to insoluble calcium oxalate. Other factors influencing oxalate absorption in humans are magnesium, iron, trace metals [51,106] bile salts, and fatty acids in the intestinal luminal fluids. Oxalate is chiefly absorbed by a passive, non-energy dependent, non-carrier mediated simple diffusion process [4]. Some recent studies have shown carrier mediated as well as active uptake of

oxalate in certain experimental systems. Pinto and Paternain [89] demonstrated the presence of an oxalate binding protein in the cytosol fractions of the brush border cells of human and rabbit intestinal mucosa. They hypothesized that at low concentrations of oxalate, active binding took place, whereas at higher concentrations simple diffusion occurred. Dijkuizen et. al. [21] demonstrated an energy dependent uptake of oxalate by membrane vesicles isolated from the bacterium Pseudomonas oxalaticus.

Approximately 15% of dietary oxalate in rats is absorbed from the intestine, accounting for about one third of the urinary oxalate [52]. Passive diffusion appears to be the main mechanism: intestinal uptake of oxalic acid increases linearly with increasing concentration [4,9,78]. Caspary [9], using everted sacs from various parts of the gut of female Wistar rats, showed the highest uptake rates by colonic intestinal tissues and lowest by duodenal tissue. In contrast, Madorsky and Finlayson [78], using isolated, intact, washed segments of the gut of Harlan male rats, showed the highest ^{14}C oxalate absorption by jejunal segments and lowest by colonic segments. Madorsky and Finlayson [78] also showed that the initial rate of ^{14}C oxalate absorption by intestinal segments was higher than absorption after 5 minutes suggesting that oxalate might inhibit its own absorption or that the rapid intake of oxalate in the first five minutes was an anomalous behaviour and an experimental artifact.

Oxalate absorption in the rat intestine is also influenced by the amount of calcium in luminal fluids. On a low calcium diet, a 30% to 50% rise in urinary excretion of oxalate has been observed [51,52]. An inhibitory effect of calcium on absorption of oxalic acid has also been shown by using everted sacs of various segments of rat intestine [9]. The influence of calcium is explained by the decrease in available absorbable intraluminal oxalate due to formation of insoluble calcium oxalate.

Intestinal absorption of oxalate in rats may also be influenced by the presence of bile and fatty acids [9,104]. Unabsorbed fatty acids bind intraluminal calcium which decreases the amount of calcium available for binding oxalate [82]. Also, bile salts may increase colonic absorption of oxalate by a non-specific alteration of mucosal permeability [9,23].

More than half of the dietary oxalate in man or rat is destroyed by bacteria in the large intestine and less than half of ingested oxalate can be accounted for in the faeces and urine. However, faecal excretion of oxalate is considerably higher in the rat than in man [51,52]. Though most rat fecal oxalate is derived from dietary sources, small amounts may originate in the liver [51]. Oxalate transported from plasma to rat intestinal lumen has been demonstrated. Dobson and Finlayson [24] injected ^{14}C labelled oxalate into the femoral vein of nephrectomized rats and recovered the label in various gut segments. In man about 90% of ^{14}C oxalate injected intravenously is recovered in the urine within 48 hours and little or no activity is detected in the faeces or respired air [25,53]. Following intraperitoneal or subcutaneous injections in the rat, 50% to 70% of ^{14}C labelled oxalate was recovered in the urine, a significant amount was found in the bone [19,52], and up to 1% converted into CO_2 [19,118]. When ^{14}C oxalate was given to rats through a throat probe 25% was recovered in urine and 73% in faeces [3]; within 30 minutes of oral administration, ^{14}C labelled oxalate was cleared by the kidney and entered the bladder, and 2 hours later present in the bone.

Eighty five percent of the oxalate found in mammalian urine is endogenously produced [51,122]. Oxalate is a non-essential end product of metabolism and is excreted unchanged in the urine. Thirty to fifty percent of urinary oxalate is derived from oxidative metabolism of ascorbic acid and about 40% comes from glycine. Hydroxyproline, serine, tryptophan and aromatic amino acids contribute smaller amounts. While ascorbate, glyoxylate, and glycollate are the immediate precursors and can directly convert to oxalate, the other precursors are metabolised to oxalate via glyoxylate or glycollate. Glyoxylate is generally considered to be the major immediate precursor.

In rat too, a conversion of known precursors to oxalate has been demonstrated [17,52, 118] and glyoxylate and ascorbic acid metabolism are shown to be mainly responsible for the endogenous production of urinary oxalate, Citric acid also appears to be a significant source of urinary oxalate in the rat [52]. Various amino acids also contribute to rat urinary oxalate but their importance as precursors most probably depends upon their availability. The importance of amino acids as a source of urinary oxalate in the rat is indicated by the marked effect of protein intake on urinary oxalate. Oxalate excretion fell by 60% on a low protein diet and increased by over 100% with a high protein diet [52]. Similarly when rat diet was supplemented with 3% glycine and 5.2% hydroxyproline, faeces and urine contained unexpectedly high amounts of oxalate.

The liver plays a major role in oxalate metabolism: major enzymes involved in oxalate biosynthesis such as glycolic acid dehydrogenase and glycolic acid oxidase, are restricted to liver both in rat and man [93]. Intestinal mucosa also plays a role in production of oxalate from a variety of precursors. Ribaya and Gershoff [91] incubated homogenates of rat intestinal mucosa with various ^{14}C labelled oxalate precursors and measured the amounts of radioactivity recovered as $^{14}CO_2$ and ^{14}C oxalate. Significant amounts of sodium 1-^{14}C glyoxalate converted to ^{14}C oxalate.

Great variation exists in oxalate concentrations of various body tissues. Human kidneys contain more oxalate (0.4 mg/100 g wet wt) on a wet weight basis than other tissues [54]. Oxalate concentration is higher in rat kidneys than in human kidneys and oxalate in male rat kidneys is greater than in the female averaging 38.8 mg/100 g wet wt in males, and 31.1 mg/100 g wet wt in females [92]. A concentration gradient exists between the renal cortex and papilla in man and rat [48,49,123]. Rat renal papilla contains the maximum amount of oxalate (16.2 mg/100 g wet wt),

Table 1.
Calcium Oxalate Urolithiasis in Rat

S.No.	Strain and Sex	Method of Induction	Response	Ref.
1.	Germ free Male, Female	Spontaneous	Bladder stones in 50% males and 2% females.	43
2.	Sprague-Dawley Male	Spontaneous	Stones in bladder.	88
3.	Charles River Male	Daily intraperitoneal injections of 1% sodium oxalate	After 2 wks crystals in renal cortex, medulla, papilla.	114
4.	Holtzman Male	Single intraperitoneal injections of 2.5% sodium oxalate	Within 15 min of injection crystals in renal cortex; later in all parts of kidney.	57
5.	Sprague-Dawley Male	Sodium Oxalate in diet	Within a week crystals in renal cortex, within 4 to 6 wks stones at renal papillary tips.	42
		Ammonium Oxalate in diet	Within a week stones at renal papillary tips, in fornices, and pelvis. Incidence of stone formation increased with time.	
		Ammonium Oxalate and ammonium chloride in diet	Greater incidence of stone formation than with ammonium oxalate alone.	
6.	Sprague-Dawley Male	Intraperitoneal or subcutaneous implantation of potassium oxalate containing mini-osmotic pumps	Crystalluria within a week, single and twinned as well as aggregated crystals.	63
7.	Sprague-Dawley Male	Single intraperitoneal injection of sodium oxalate in addition to potassium oxalate in mini-osmotic pumps	Crystalluria; after 6 wks crystals in renal tubules; macroscopic crystalline deposits (stones) in bladder.	63
8.	Carworth Farms Nelson CFN Male, Female	4% Diethylene glycol mixed in food	In two year feeding study bladder stones in male rats.	117
9.	Sprague-Dawley Male, Female	Ethylene glycol or ethylene glycol and ammonium chloride in water at various concentrations	Within 3 days crystals on papillary tips, few rats with crystals in renal tubules. Acidification increased severity of crystal deposition. Males had crystals at all concentrations of ethylene glycol while females only at 1% or more.	77
10.	Sprague-Dawley Male	1% ethylene glycol in water + a magnesium deficient diet	Crystalluria; crystal deposition in renal cortex, medulla and papilla.	103
11.	Wistar Male	0.5% ethylene glycol in water + 0.5 µgram Vitamine D once a day by gavage	Crystalluria after 3 wks; after 4 wks crystals in renal tubules, on papillary surface; stones in pelvis, ureters, bladder.	85
12.	Wistar Male Sprague-Dawley Male	Intraperitoneal injection of 2.5 g/kg 4-hydroxy-L-proline	Within 24 hr crystals in renal tubules crystalluria.	108
13.	Wistar Male	3% glycolic acid in diet	Within 3 wks crystals in renal cortex, medulla, papilla; stones in pelvis, ureters, bladder.	11
14.	Wistar Male Weanling	Low phosphate diet (P 0.16%)	Stones in pelvis in 9 wks and bladder in 14 wks	13
15.	Sprague-Dawley Male	Low phosphate diet (P 0.07%)	Microstones in renal pelvis after 10 months.	120
16.	Charles River CD Male	Pyridoxine deficient diet + 3% glycine	Crystalluria; in 6 wks stones on renal papillary tips, in fornices, pelvis, ureters, bladder.	1
17.	Charles River CD, Male Female Castrated	Pyridoxine deficient diet	Renal papillary tip encrustation, stones in pelvis, ureters, bladder. After 3 months males were most affected and females least affected; after 6 months all rats had similar deposits.	36

followed by medulla (6.12 mg/100 g wet wt), and cortex (3.4 mg/100 g wet wt) [123].

Composition of Stones

Of the various aspects of urolithiasis, the presence of stone is the one element which most commonly symbolises stone disease. All human urinary stones consist of two basic components, crystals and matrix [29,79]. In urinary stone disease of man, up to 97% of the stone is crystalline, the rest is proteinaceous matrix. The crystalline component may be cystine, uric acid, mono and dihydrates of calcium oxalate, magnesium ammonium phosphate, various types of calcium phosphates and urates, and other materials that are excreted in urine in concentrations sufficient for precipitation. In rats, stones also consist of organic matrix and crystals [34]. The crystalline component of stones may be citrate, mono and dihydrates of calcium oxalate, calcium phosphate, magnesium phosphate, magnesium ammonium phosphate and other substances experimentally induced to precipitate in the urine. Studies of rat stone matrix are limited [8,34]. Phosphate stones contain 19 amino acids, with glutamic acid being the most abundant and arginine, methionine, histidine, cysteine and cystine occurring in insignificant amounts [10]. In addition to amino acids, rat kidney stones contain glucose, fatty acids, and cholesterol, but lack hexosamine, uronic acid, sulphate, and sialic acid [8].

Spontaneous Oxalate Urolithiasis in Rat

Documented cases of spontaneous urinary stone formation in rats are rare (Table 1). Although renal calcium phosphate and/or ammonium magnesium phosphate stones have been reported, there are no reports of spontaneously formed oxalate stones in the rat upper urinary tract. Rarely, oxalate stones have been found in the bladder. Of 100 male Sprague-Dawley rats in a toxicity study, only 2 rats were found to have oxalate calculi, and these were associated with calcium carbonate and calcium or ammonium phosphate [88]. Mixed calcium oxalate and citrate bladder calculi were found in 50% of male germ free rats on a semi-synthetic diet with a composition within the limits of dietary standards [43]. Genetically related conventional rats on the same diet did not form any calculi. The calculus forming germ free rats had high urinary calcium, high citrate, high pH, and low urinary phosphate. The tendency to form calculi disappeared and the urinary values became similar to those of noncalculi forming rats on contamination of germ free rats by intestinal flora from the conventional rats.

Experimentally Induced Oxalate Urolithiasis in Rats

To understand different aspects of the oxalate stone disease in humans, oxalate urolithiasis has been induced in the rat by various means. The main mechanism is production of hyperoxaluria by addition of oxalate or an oxalate precursor to the diet, or by intraperitoneally injecting them into the rat (Table 1). Hyperoxaluria has also been induced by other dietary manipulations including pyridoxine-deficient diets, low phosphate diets, and high protein diets.

Oxalate Induced Urolithiasis:

Repeated intraperitoneal injections of sodium oxalate into male Charles River rats on regular Purina rat chow and water resulted in a deposition of calcium oxalate crystals throughout the kidney [114]. Rats tolerated daily repeated injections of about 50 mg/kg with very few side effects. In a modification of this method male Holtzman rats were injected with various amounts of 0.22M sodium oxalate in 0.9% saline and the kinetics of calcium oxalate nephrolithiasis were studied [57]. The LD50 was R 2.5×10^{-4} but no rat succumbed to 1.1×10^{-4}. 1.1×10^{-4} moles of sodium oxalate resulted in non-specific cytologic damage which resolved 24 hr post-injection. At this dose, urine volume increased 50% in the first 24 hours, and 80% during the second 24 hours. By the 3rd day post-injection urine volume was normal. Renal tissue calcium increased for the first 4 hours. Subsequently it subsided as a first order process. It was concluded that most of the increased calcium was derived from glomerular filtrate and precipitated as calcium oxalate crystals which were virtually all intratubular.

We have used the sodium oxalate injection method to study the pathogenesis of calcium oxalate urolithiasis in the rat. Male Sprague-Dawley rats received single injections of 3, 5, 7, or 9 mg of sodium oxalate per 100 g body weight utilizing 0.22M sodium oxalate solution in 0.9% saline. The animals were sacrificed from 15 min to 2 wks after injection, and their kidneys were examined by light microscopy, and scanning and transmission electron microscopy [59,60,61,67]. Calcium oxalate crystals appeared in the proximal tubules within 15 minutes of the sodium oxalate injection in as low a dose as 3 mg/kg body weight. Crystals apparently moved with urine flow as they were absent 72 hours post-injection. Within a week the kidney was morphologically normal. The size, number, and location of calcium oxalate crystals depended on the amount of sodium oxalate injected and the time interval after the injection. The papillary tip and cortico-medullary junction were the preferential sites for crystal deposition after longer periods of injections of higher doses of sodium oxalate [59,61]. Tubular epithelium from proximal tubule to the collecting ducts was damaged. The damage depended on the amount of sodium oxalate injected [59,60,61] and was mostly restricted to the tubules containing the crystals. The first noticeable change was seen in the proximal tubules whose brush border was distorted by clubbing of the microvilli and the formation of blebs (Fig. 1a, b). Later, microvilli were lost from localized areas of the brush (Fig. 2a, b). Epithelial cells contained increased numbers of lysosomes, some of which were autophagic vacuoles (Fig. 3). General cellular necrosis was seen in all parts of the nephron. Degenerative changes included swelling of the mitochondria (Fig. 4), dilatation of

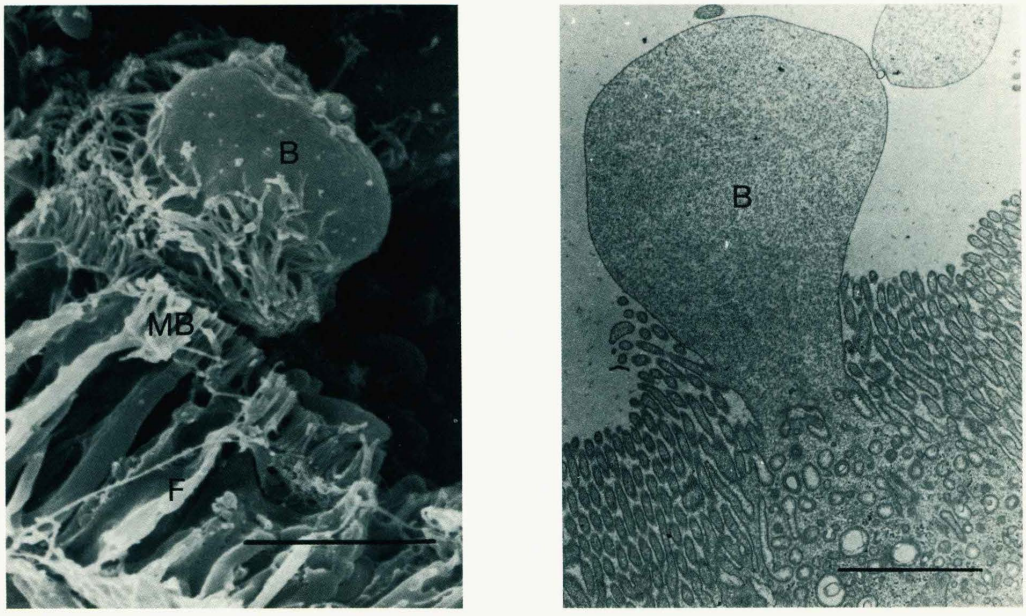


Fig. 1a. SEM of proximal tubular epithelium showing lateral membrane folds (F) of tubular cells and microvillous brush border (MB). The brush border is distorted by the formation of large blebs (B) projecting into the tubular lumen. Bar = 5 μm. 1b. TEM of a section through the brush border and a bleb similar to one seen in 1a. The bleb (B) has a diffuse cytoplasm which is continuous with the cytoplasm of the cell. Bar = 1 μm.

endoplasmic reticulum, cytoplasmic edema, and vacuolation. Luminal cell membranes of degenerating cells appeared to have burst releasing their contents into the tubular lumen (Fig. 4). Focally, cells were sheared from the tubular basement membrane (Fig. 5) contributing to the cellular debris present in all parts of the nephron (Fig. 6). The papillary tip surface was badly damaged. Its covering epithelium was lost exposing the underlying basement membrane [61]. Crystals of both calcium oxalate monohydrate and dihydrate were identified by morphology. Calcium oxalate monohydrate crystals were present as aggregates of monoclinic prismatic plates while calcium oxalate dihydrates appeared as tetragonal bipyramids present individually or in aggregates [60, 67]. Crystals were intraluminal in the nephron and almost always associated with flocculent eosinophilic material which stained positively with periodic acid Schiff reaction, Alcian blue and colloidal iron (Fig. 7). Transmission electron microscopy revealed the material associated with calcium oxalate crystals (Fig. 8) to be cellular debris consisting of membranes, vesicles, and cellular organelles in various stages of degradation (Fig. 6). Crystals were often found attached to epithelial cells, and, in totally denuded tubules, were attached to the basement membrane [59,61].

In a chronic study, 1 ml of 2.5% sodium oxalate solution in 0.9% saline was injected in rats twice a day for a period ranging 49 days to 10 months [20]. Calcium oxalate crystals were found in all renal tubular segments as well as in the interstitium. Regressive changes were evident not only in tubular epithelium but also in the glomerular epithelium. Thickening of glomerular and tubular epithelial basement membranes was described. The first crystals were interpreted as deposited in phagolysosomes.

In a recent study [42] male Sprague-Dawley rats were fed diets supplemented with sodium or ammonium oxalate and calcium or ammonium chloride. Rats were sacrificed at weekly intervals for six weeks. The animals fed sodium oxalate had initial deposition of calcium oxalate monohydrate crystals in cortex and cortico-medullary junction. After six weeks crystals were preferentially deposited at the papillary tips. The urine was more alkaline (pH 7.4) than those of the controls (pH 6.9) and had crystals of triple phosphate. Animals on a diet supplemented with ammonium oxalate had a more acidic urine (pH 5.6 to 6.2) and, depending on the amount of added oxalate, renal or bladder stones. Renal deposits were present either in the papilla or the fornices. Supplementation of diet with either calcium chloride or ammonium chloride resulted in the acidification of the urine, but calcium chloride resulted in lowering of urinary oxalate probably because of oxalate binding in the gut.

In the experiments described above oxalate was administered either through diet or intraperitoneal injection. Because the amount of food consumed by individual rats may vary, such methods can give inconsistent results. Methods utilizing single injections have the serious defect that they produce sudden, acute, overloading surges causing high supersaturations of urinary salts and transient crystalluria. To meet these objections we induced urolithiasis in rats by subcutaneously or intraperitoneally implanting mini-osmotic pumps (Alza Corporation, Palo Alto, CA) loaded with a saturated solution of potassium oxalate [63,64]. Osmotic pumps deliver their contents at a specific rate for a given period of time. The urine of experimental animals had abundant crystals of calcium oxalate, calcium phosphate, triple phosphate mixed with amorphous calcium phosphate, and an amorphous viscid material which was mostly calcium phosphate. No crystals were deposited in the kidney. A direct correlation was evident between supersaturation and crystalluria. However, crystal number did not correlate well with the degree of supersaturation. In this respect the experimental results resembled human crystalluria [119]. Urinary oxalate levels of experimental animals were higher than their controls and this increase was proportional to the increase in urinary supersaturation of calcium oxalate. Urinary calcium levels were not significanlty altered. One group of animals, injected with sodium oxalate in addition to the potassium oxalate containing osmotic pump, had calcium oxalate stones in their bladder and their kidneys had deposits of calcium oxalate crystals. In this respect rats behaved like stone formers who showed an increase in size and aggregation of calcium oxalate crystals on addition of oxalate to their diet. Lower inhibitory activity in stone formers' urine was thought to be responsible for this situation [97].

Urolithiasis induced by Ethylene Glycol Administration

Ethylene glycol is one of the precursors of oxalate and has long been known as the cause of calcium oxalate crystal deposition in kidneys of man and animals when it is accidentally or intentionally ingested. Glycoaldehyde, glycollate and glyoxylate are on its metabolic pathway to oxalate and carbon - dioxide [40,93,118]. Most of the ingested ethylene glycol is eliminated unchanged in the urine and the bulk of the rest is oxidised to carbon-dioxide [40]. The amount of oxalate excreted is dependent upon the animal species as well as the dose. In albino rats only about 0.5% to 1.1% of the dose has been shown to be converted to oxalate. At a lower dose level of 0.1 g/kg rat body weight given by subcutaneous injections, 23% of the ethylene glycol was converted to carbon-dioxide while 35% was excreted in the urine, with none of it converted to oxalate. At 1 g/kg dose level about 1.1% of the ethylene glycol converted to urinary oxalate.

When a 1% solution of ethylene glycol was given to male and female rats in drinking water small oxalate calculi were produced in male rats but were absent in female rats [107]. In a similar study, male Sprague-Dawley rats given ethylene glycol in the diet were shown to be more susceptible to crystal deposition in kidney and renal calculus formation than the female [5]. Male rats on diets containing over 0.2% ethylene glycol showed renal calcium oxalate crystal deposition and at 0.5% or more ethylene glycol, renal stones were produced. On the other hand in female rats renal crystal deposition was limited to those on 1% and 4% ethylene glycol and calculi were found only at 4% ethylene glycol. The earliest lesions showed tubular epithelial necrosis. Crystals were deposited on the basement membrane. The amount of deposited crystal was proportional to the concentration of ethylene glycol in diet.

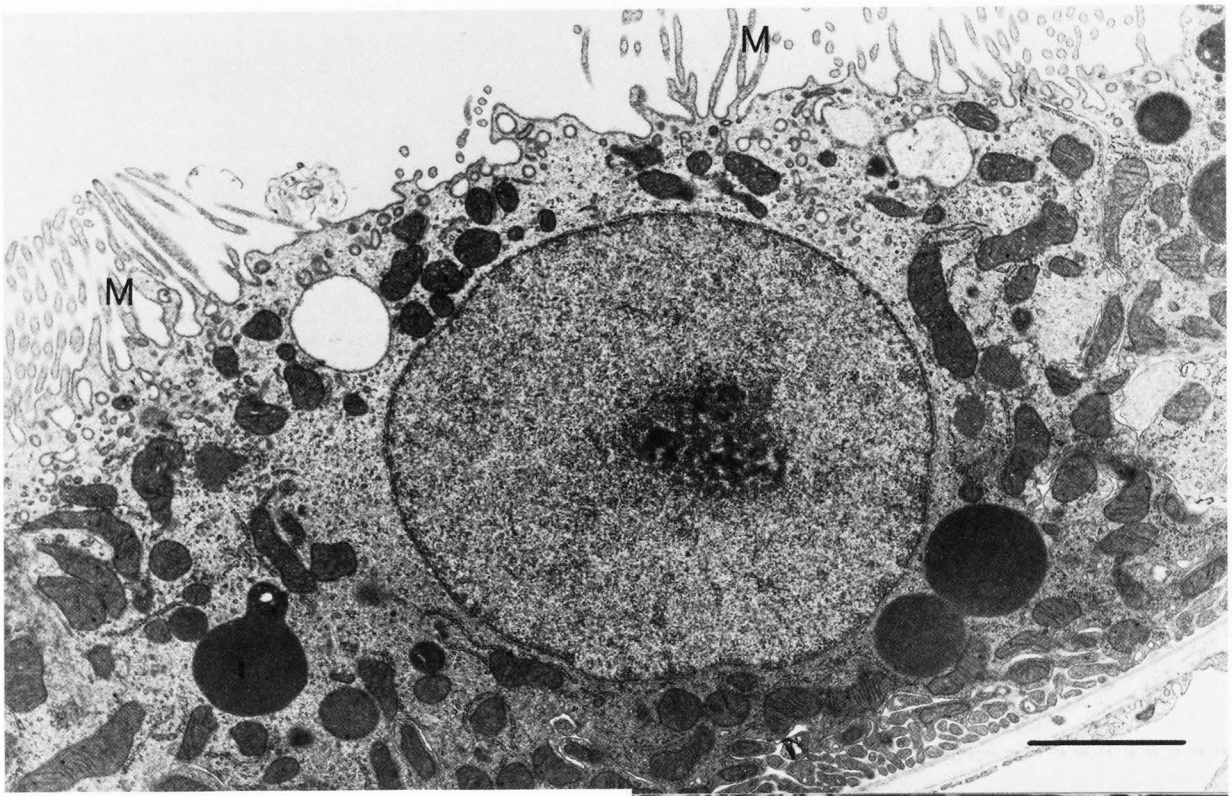
Acidification of urine in conjunction with administration of ethylene glycol increases the severity and incidence of urolithiasis [77,99]. When rats drank water containing both ammonium chloride and ethylene glycol, renal crystal deposition appeared severe [77]. Kidneys were enlarged and stippled with yellowish white flecks on their outer surfaces. Acidification caused an increase in deposits within renal papilla and on papillary tips. In addition it resulted in the deposition of calcium oxalate monohydrate rather than a mixture of mono- and dihydrate [77].

Recently it was shown that when male Wistar rats were given 0.5% ethylene glycol in water in addition to 0.5 microgram of vitamin D administered by gavage, 77.3% of the rats produced stones in the renal pelvis, ureter and bladder [85]. Typical calcium oxalate monohydrate crystals were found in the dilated tubules whose epithelium was partially destroyed. There was a marked increase in the weight of the kidney. Both urinary oxalate and calcium increased and renal insufficiency was moderate. There were no deaths during the four week experiment.

Induction of Oxalate Uroliathiasis by Other Oxalate Precursors

Other oxalate precursors used to induce urolithiasis are hydroxyproline [108,109], glycolic acid [11,12], and glyoxylic acid [81]. Hydroxyproline is directly converted into glyoxylate. Administration to male Sprague-Dawley [108] and male Wistar rats [109] by intraperitoneal injection of 10 ml/kg rat body weight, resulted in the deposition of calcium oxalate crystals in the renal tubules. The volume and weight of the kidneys doubled in 24 hours [108]. Calcium oxalate dihydrate crystals formed in the first 2 hours and later transformed to thermodynamically stable calcium oxalate monohydrate [108].

Glyoxylate and glycollate are immediate precursors of oxalate and their administration results in oxalate urolithiasis. Three percent glycolic acid administered in diet to male Wistar rats resulted in calcium oxalate crystal deposition in kidneys and formation of uroliths up to 4 mm in diameter in renal pelvis, ureters and bladder [11,12]. Crystals were present throughout the renal cortex and medulla.



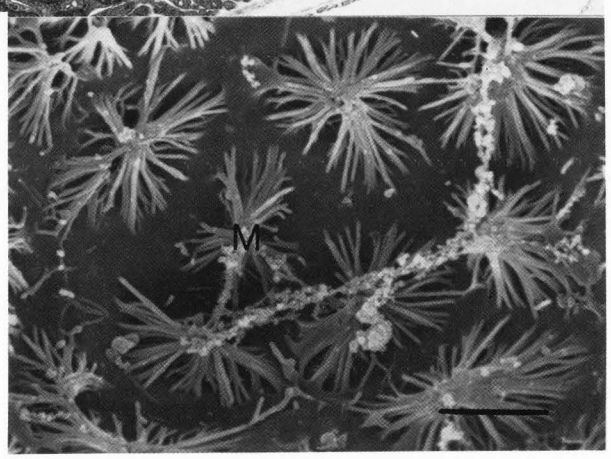


Fig. 2a. TEM of a section through proximal tubular epithelium showing focal loss of microvilli. M, intact microvilli. Bar = 2.5 μm. 2b. SEM of the luminal surface of proximal tubular epithelium showing areas with microvilli (M) surrounded by areas of focal loss of microvilli. Bar = 5 μm.

Pyridoxine Deficiency and Oxalate Urolithiasis

Pyridoxine (Vitamin B-6) deficiency causes hyperoxaluria and oxalate urolithiasis in the cat [38] and rat [1], and may also be a significant etiological factor in the formation of human oxalate renal calculi [27,28,39]. Since pyridoxine in the form of pyridoxal-5'-phosphate acts as a coenzyme for a number of transaminases it was speculated that transamination of glyoxylate to glycine was reduced in pyridoxine deficiency resulting in an accumulation of glyoxylate and its subsequent conversion to oxalate [35,38]. However, no increase was detected in glyoxylate levels of heart muscles of pyridoxine deficient rats [47], and in pyridoxine deficiency, glycolate has been shown to be a better precursor of oxalate as compared to glyoxylate or glycine [100,101]. Thus the reasons for pyridoxine deficiency causing hyperoxaluria remain undetermined. A more recent study has shown that pyridoxine deficiency results in a carrier mediated oxalate transport in experimental rats as opposed to simple diffusion in control rats [28]. Such a transport mechanism results in enhanced intestinal absorption of oxalate leading to hyperoxaluria.

Vitamin B-6 deficiency not only elevates urinary oxalate but also urinary calcium [80], and increases the activity of neuraminidase and other lysosomal enzymes [22]. Neuraminidase removes sialic acid from the glycosaminoglycans (GAGs) and GAGs are important inhibitors of

crystallization [94,95]. Urinary citric acid levels decrease [35] which may be important because citric acid is a known inhibitor of calcium oxalate crystallization [83]. Urinary phosphorous and uric acid levels are not known to be affected by pyridoxine deficiency. Thus hyperoxaluria and hypercalciuria, in association with decreased inhibitor activity, may cause oxalate urolithiasis in pyridoxine deficiency.

On a pyridoxine deficient diet, male Charles River [1] and Wistar [80] rats formed calculi generally restricted to the tubules of renal medulla and papilla, and to renal papillary tips and calyceal fornices although ureteral and bladder stones were sometimes present [1,80]. The calculi were mainly calcium oxalate monohydrate with calcium oxalate dihydrate on the surfaces and traces of calcium phosphate [1,123]. Parts of the papillary tip stones extended into the ducts of Bellini suggesting the stones originated in collecting ducts from a lesion similar to Randall's type II plaque [65,90,123]. In addition, submucosal crystals present on the lateral aspects of renal papillae resembled Randall's type I plaque [90].

Kidney changes, in addition to the deposition of calcium oxalate crystals, included increased weight with papillary swelling and transitional epithelial hyperplasia as well as cortical tubular dilatations, and epithelial degeneration in those rats subjected to long periods of pyridoxine deficiency. The crystals were associated with a PAS positive substance and in male Charles River and Wistar rats were predominantly intraluminal. Crystals deposited in the kidneys of male rats of TAC:SD/NfBR strain were however, predominantly interstitial [73]. It was suggested that the crystals induced by a pyridoxine deficient diet originate in the renal interstitium and later move to a intraluminal position [73].

Alteration of urinary pH affected the extent of calcium oxalate deposition in pyridoxine deficient rats. More urinary stones of larger sizes were formed at low urinary pH [1].

Low Phosphate Diet

It has long been known that a low phosphate diet results in an increase in urinary calcium and citrate leading to the formation of citrate stones in both weanling and mature rats [105,112, 113]. While no significant change occurs in urinary oxalate levels of rats on low phosphate diets [13,52], recent studies have shown that a low phosphate diet may result in the formation of calcium oxalate urinary stones in rats. A moderately low phosphate diet with high calcium/phosphorus ratio (0.16% P, 0.56% Ca, 3.52% Ca/P) resulted in calcium oxalate urolithiasis in male Wistar weanling rats [13]. Renal papillae contained intratubular deposits of calcium oxalate in addition to subepithelial plaques of calcium. Urolithiasis became severe with time. After 14 weeks on the diet bladder stones were present and renal pelvis had visible stones. The calculi were composed mainly of calcium oxalate, with less than 10% calcium citrate. Mature male Sprague-Dawley rats produced calcium oxalate dihydrate crystalluria and pelvic stones on a diet containing less than 0.7% phosphate [120]. No calcium deposits were found in renal medulla or papilla. The urine showed marked hypercalciuria and became highly supersaturated with respect to calcium oxalate within one week of stay on the low phosphate diet.

Development of Calculi upon Foreign Bodies

Regional fixation of a growing stone has been hypothesized as necessary for the development of upper urinary tract stone disease [33,90]. Once a fixed nidus is formed the stone grows by encrustation [69]. With this in mind Vermeulen and his colleagues [115,116] developed a foreign body model for the study of stone disease. They implanted various types of foreign bodies in urinary bladders of male rats and studied the growth of the encrustation [115]. Paraffin foreign bodies did not encrust, though they did result in the formation of free lying stones in the bladder. All other foreign bodies tested encrusted and grew. Though the average pH was 6.8 the stones consisted of magnesium ammonium phosphate. It was later found that male Sprague-Dawley rats deposited struvite on foreign bodies whereas female Sprague-Dawley rats produced apatite [77], and that stones of desired composition could be induced by manipulation of diet or administration of a lithogen [6,76,77]. Addition of ethylene glycol to the drinking water, or a pyridoxine deficient diet resulted in the formation of calcium oxalate stones which morphologically resembled clinical stones and contained both calcium oxalate monohydrate and dihydrate crystals. Acidification of urine however, caused the formation of pure calcium oxalate monohydrate stones and also increased the rate of stone growth [6,76,77]. As discussed earlier ethylene glycol administration generally results in crystal deposition in kidney tissue. Renal crystal deposition can however be avoided by limiting ethylene glycol to approximately 0.75% in the drinking water [87].

Although zinc discs were used as a foreign body for such studies, studying such stones by microscopy is difficult because zinc cannot be sectioned. To overcome this, plastic foreign bodies were introduced [69]. Oxalate stones on plastic foreign bodies induced by ethylene glycol were sectioned following decalcification by EDTA treatment and examined by light microscopy and scanning and transmission electron microscopy. The encrustation was joined to the foreign body by a thin amorphous organic layer upon which crystals of calcium oxalate nucleated. The crystals were surrounded by an amorphous limiting coat, and the stone contained cellular debris. Apparently, the deposition of an organic layer on the foreign body was the initiating step in the formation of the crust and the stones grew by confluent crystal growth and aggregation. The matrix was acquired by adsorption of macromolecules on crystal surfaces and incorporation of cellular debris. Crystals of calcium oxalate have been shown to adsorb proteinaceous coat from solutions in in vitro experiments [62,72] and calcium oxalate crystals of urinary stones on

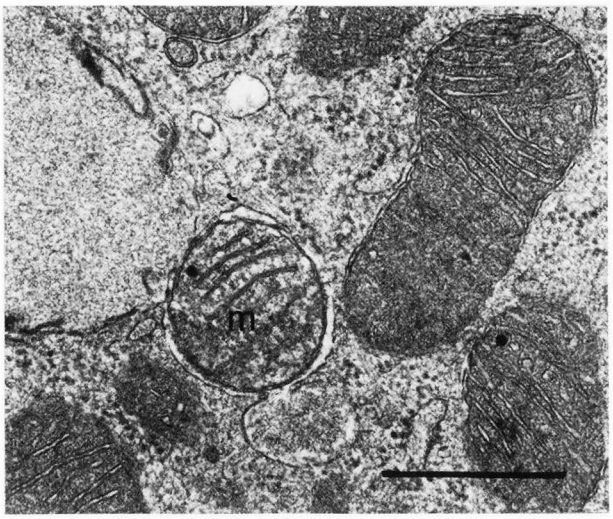


Fig. 3. TEM of a section through proximal tubular epithelial cell showing autophagic vacuole with a mitochondria (m). Bar = 0.5 μm.

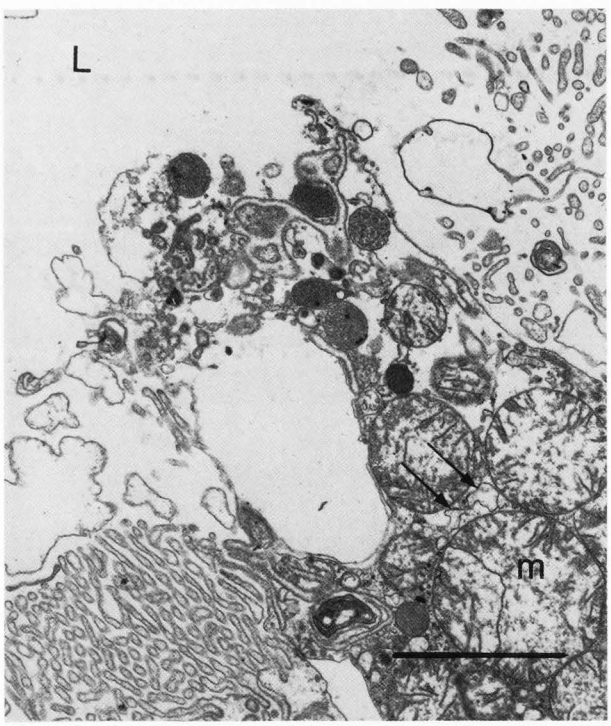


Fig. 4. TEM of a section through luminal end of a degenerating proximal tubular epithelial cell showing swollen mitochondria (m), cellular edema, vacuolation, and dilation of endoplasmic reticulum (unlabelled arrows). The luminal plasma membrane is broken at the apex and is releasing cellular contents into the tubular lumen (L). Bar = 2.5 μm.

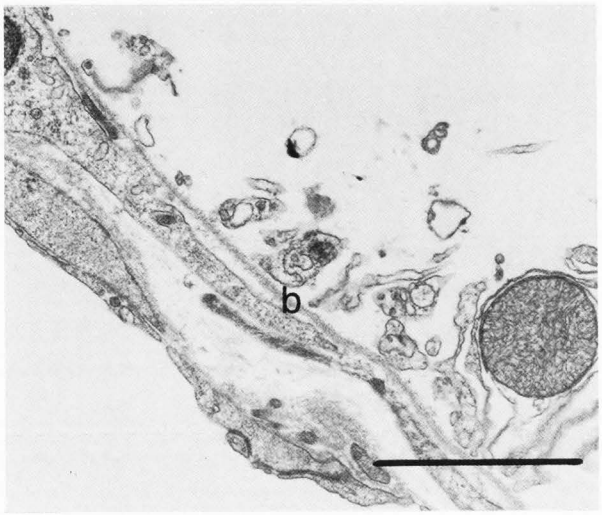


Fig. 5. TEM of a section through the thin loop of Henle showing shearing of the epithelial cells from their basal lamina (b). Bar = 2 μm.

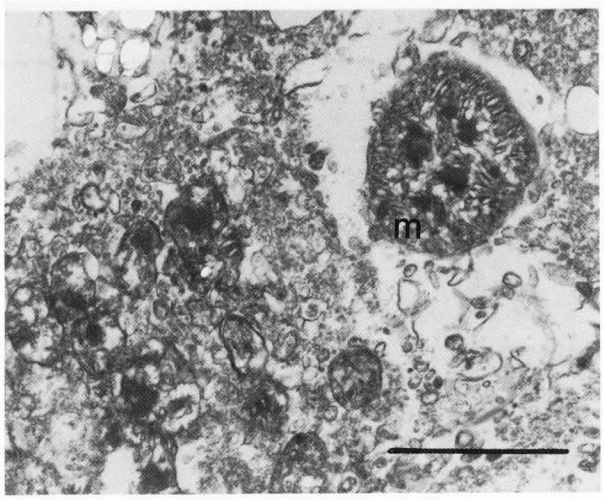


Fig. 6. TEM of a section through a tubular lumen with cellular debris. The debris contains various degenerating cellular organelles of which mitochondria (m) are still recognizable and contain flocculent densities. Bar = 1 μm.

decalcification form crystal ghosts and contain organic material within crystal boundaries [66, 68].

Magnesium and Oxalate Urolithiasis

That dietary magnesium depletion in rats produces hypomagnesemia and hypomagnesuria [41, 121], and causes deposition of calcium phosphate in renal tubules has long been known [18,86,121]. Recent studies have shown that magnesium deficiency also enhances calcium oxalate renal tubular deposition in rats on experimental

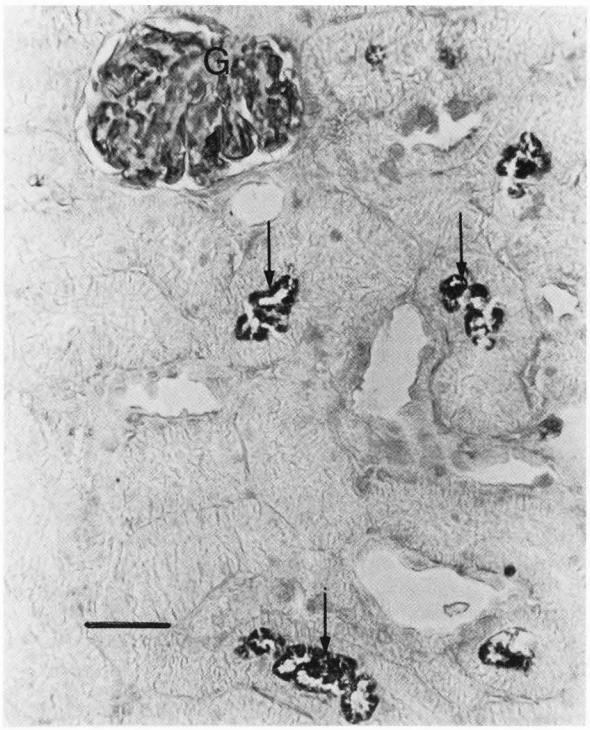


Fig. 7. A section through the renal cortex stained with colloidal iron at pH 2.5. The glomerulus (G) and the crystal associated debris (unlabelled arrows) in tubular lumen are stained. Bar = 30 μm

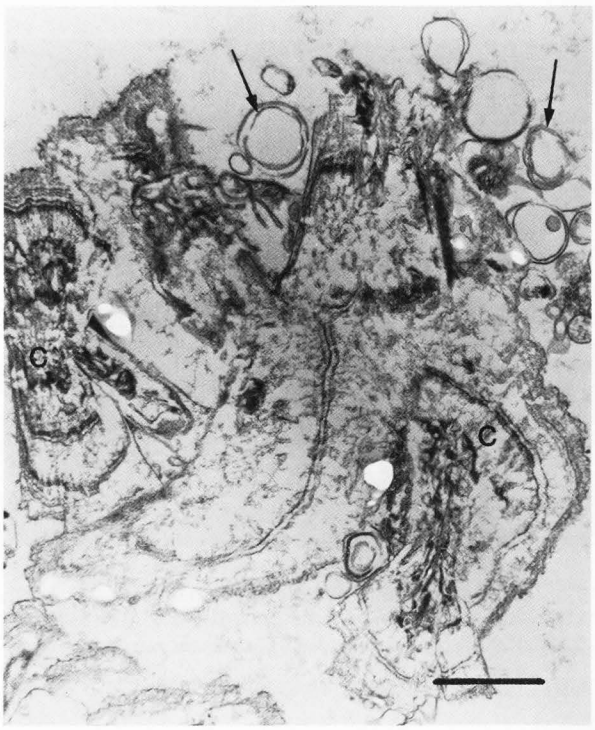


Fig. 8. TEM of a section through tubular lumen containing decalcified calcium oxalate crystals (C) or cellular debris (unlabelled arrows) which in this micrograph consists of membranous vesicles. Bar = 2 um.

hyperoxaluric protocol induced either by pyrodoxine deficiency [37] or by administration of ethylene glycol [102,103]. Clinically low urinary magnesium/calcium ratios are found in calcium oxalate stone formers [55,74,111] and reduction of stone recurrence in renal calcium oxalate stone formers occurs on prophylactic treatment with magnesium hydroxide [55,56]. The mechanism by which magnesium exerts its beneficial effect on urolithiasis has been studied in vivo and in vitro. Hyperoxaluric male Sprague-Hawley rats given magnesium oxide, magnesium chloride, sodium bicarbonate, or ammonium chloride, were studied [6]. At comparable urinary pH, magnesium oxide was more efficacious than sodium bicarbonate in preventing stone formation, and fewer stones were formed on magnesium chloride than on ammonium chloride, thus proving the beneficial effect of magnesium independent of urinary pH. Magnesium may provide protection by increasing the solubility of calcium oxalate [26,45], or magnesium/calcium ratio [55], or by complexing with oxalate [83] and making it unavailable for binding with calcium.

Hyperuricosuria and Oxalate Urolithiasis

Clinical studies have shown a positive correlation between hyperuricosuria and hyperuricemia, and calcium oxalate stone formation [14,15,16,44]. Experimentally, the relation between hyperuricosuria and stone formation has been studied in male Wistar rats by inducing urinary excretion of uric acid in the presence of hyperoxaluria [50]. Hyperoxaluria was induced by adding ethylene glycol to drinking water of rats in a daily dose of 0.8%, and hyperuricosuria was induced by administering 2% oxonic acid by a duodenal tube. Oxonic acid is a specific blocker of uricase, the enzyme responsible for metabolism of uric acid to allantoin in rats. After thirty days, more calcium oxalate calculi were seen in renal parenchyma, pelvis, and fornices on a combined regimen of oxonic acid and ethylene glycol than with ethylene glycol alone and no oxalate crystals were seen on oxonic acid treatment alone. Calcium oxalate deposition in renal parenchyma was associated with tubular epithelial damage and more crystals were seen in medulla than in cortex.

It has been suggested that sodium acid urate, uric acid and calcium oxalate share sufficient structural similarities to allow epitaxis [16,75] and that urate crystals act as nucleation agents for heterogeneous crystallization of calcium oxalate. This mechanism of facilitation of calcium oxalate crystallization by urate particles, and the relevance of epitaxis hypothesis has been questioned [31,32]. An alternate hypothesis is that uric acid may

promote calcium oxalate stone disease by interfering with naturally occurring urinary inhibitors of calcium oxalate crystal growth. It has been shown that human urines containing more urates have less inhibitors and the addition of urate in vitro reduces and removal of urates restores inhibitory activity [94,95].

Experimental Calcium Oxalate Urolithiasis in Rat As A Model for Human Calcium Oxalate Stone Disease

Although calcium oxalate stone formation is not a spontaneous phenomenon in most experimental animals, near identity in oxalate metabolism in man and rat permit several strategies for experimental modelling of stones in the rat. The value of such manipulation is that dissection of the two key processes involved in stone formation, that of nucleation as in the study of calcium oxalate nephrolithiasis, and growth as in the study of encrustation of foreign bodies, is possible. Furthermore, study of these mechanisms of stone formation over time gives a more dynamic view than is possible in studying the human stone. Virtually all experimental models employed utilize hyperoxaluria as the basic perturbation, whether by administration of excess oxalate [57], exposure to the toxin ethylene glycol [77], or manipulation of nutrition as exemplified by pyridoxine deficiency [1]. Although the point is debated as to whether hyperoxaluria or hypercalciuria is more important in human stone formation [98], physical chemical constraints are such that calcium oxalate crystals in urine would more readily form with small oxalate excess than with calcium [30,96,98], making the hyperoxaluric rat a valuable model for calcium oxalate stone disease.

The study of the interaction between the formed calcium oxalate crystals and renal or urothelial tissues points in a direction important for the understanding of attachment and retention of crystals for, without such retention, stone disease would not be possible [33]. With few exceptions, renal crystal deposition in experimental systems are associated with cell injury or necrosis [1,59,80,102,103]. Although damaged cells can be identified at the earliest times that crystals are formed, it is unclear whether damage occurs prior or subsequent to the crystal formation. In either case, crystals are often found associated with cell debris and such debris is a common component of human stones. Loss of epithelial cells exposes the basement membrane which may provide for crystal attachment (1,61, 123]. Such a crystal fixation to basement membrane or other structures in the absence of urinary obstruction would be essential for retention and stone growth. The renal tubular damage has also been implicated in human urinary stone disease. Matrix substance A and Tamm Horsfall mucoprotein both are associated with renal injury and both are found in urinary stones and are present in greater concentrations in urines of stone patients than normal individuals [7,58,70,71,110]. Urinary enzyme pattern of urinary stone patients is suggestive of renal tubular damage [2]. Morphologically, damage to the renal tubular epithelium has been found in papillectomy specimens from a stone patient [65]. The presence of Randall's plaques [90] in stone patients is also suggestive of the involvement of renal injury in the stone disease. What role does renal injury play in the evolution of stone disease? Whether the injury is primary and thus an initiating factor, or secondary but capable of promoting increased crystallization or secondary with no role whatsoever has not been resolved. These are some of the questions still to be answered and have been discussed in greater details in a number of reviews [7,29,31,70,71,110].

The reaction of calcium oxalate crystals with organic materials appears to be a generally critical mechanism in attachment and growth. With foreign body models as an example, no crystal-foreign body interplay exists until the foreign body is covered with an organic film [69]. Central too, is the fact that all experimentally induced or spontaneously formed calcium oxalate crystals, when closely examined, will have an organic coating [62]. In the construction of a stone, the organic coating, in conjunction with other organic materials forms a complex scaffolding. This material, the matrix, may play a significant cementing role in the genesis of stone. Indeed, one is hard put to visualize a stone without matrix.

Experimental stone formation in the rat is not as yet completely understood. Rat calcium oxalate stone matrix has to be examined both morphologically and biochemically. The important topic of inhibitors has to be explored both in the normal and stone forming rats. It may be that an analysis of the reasons why rats are resistant to calcium oxalate stone is a more important point in understanding human stone formation than is the experimental induction. Further exploration of these questions will lead to a more comprehensive understanding of human stone disease and will permit a study of the elements needed for effective treatment.

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Discussion with Reviewers

J.G. Gregory: Your rats, infused with potassium oxalate through the osmotic pump form crystals, but no renal deposits. Is this a dose related phenomenon?

Authors: We did not investigate this point. All of our experiments were done using a saturated solution of potassium oxalate. In other experimental systems crystal deposition in renal tissue is dependent on the amount of lithogen used. For example changing the dose of ethylene glycol results in various crystal locations and by altering dosage one can induce crystal deposition either in the upper or in lower urinary tract (Text Reference 87).

J.G. Gregory: Did electron microscopy of the animals so treated show any evidence by electron microscopy of renal tubular injury?

Authors: The kidneys of these animals were studied only by light microscopy which did not reveal any overt cellular injury.

J.G. Gregory: Are you proposing that calcium oxalate crystals, formed renally, injure the renal tubules, producing a proteinaceous material that leads to crystal aggregation and then stone formation? If so, this is an hypothesis that should be addressable by use of the osmotic pump model in which increasing concentrations of oxalate are added intravenously. Do you have data to supplement this area? Against this possibility, is the observation of many hyperoxaluric patients who for months have passed massive quantities of crystals and crystal aggregates without ever forming a renal calculus an observation that makes one certain that crystal production itself is an insufficient stimulus for stone formation?

Authors: There are a number of possibilities. As you suggested, calcium oxalate crystals formed within the renal tubules may injure the tubular epithelium and initiate stone formation. Alternatively, cellular debris from injured renal epithelium, irrespective of the cause of the injury, may provide nidi for heterogeneous nucleation of crystals. Thus the crystals may induce renal epithelial injury and may also be initiated by it. Whether one or both mechanisms operate has not been clearly established, nor is it known whether they act independently or in conjuction.

The observation that hyperoxaluric patients pass massive quantities of crystals and crystal aggregates without ever forming a renal calculus, in itself, neither proves nor disproves the hypotheses mentioned above. Crystalluria is only an indication of supersaturated bladder urine. Experimental work mentioned in our paper has shown that crystals may be present in the urine

without ever being seen in the kidneys. In our opinion, renal injury increases the probability of renal stone formation.

R. Tawashi: It is fascinating that controlling delivery rate of oxalate ion using the mini-osmotic pump system has an effect on the site of crystallization and the nature of crystals formed. Do you have evidence or any idea how the control delivery rate of Ca^{++} might have on crystalluria?

Authors: The point you raised is an interesting one. We have not yet studied the effect of controlled delivery rate of Ca^{++} on crystalluria. But we are planning some experiments with this particular aspect of urolithiasis in mind.