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Insights on the pathology of kidney stone formation

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Abstract The purpose of these studies was to test the hypothesis that Randall's plaque develops in unique anatomical sites of the kidney and that its formation is conditioned by specific stone-forming pathophysiologies. To test this hypothesis, we performed intraoperative mapping studies with biopsies of papilla from the kidneys of 15 idiopathic calcium oxalate (CaOx) stone formers, four intestinal bypass for obesity patients and ten brushite stone formers, and obtained papillary specimens from four non-stone formers after nephrectomy. Both light and electron microscopic examination of tissue changes along with infrared and electron diffraction analyses of mineral composition were performed. Distinct patterns of mineral deposition and papillary pathology were discovered in each of the three different stone forming groups. CaOx stone formers had predictable sites of interstitial apatite crystals beginning at the thin loops of Henle and spreading to the urothelium. These plaque areas are termed Randall's plaque and are thought to serve as sites for stone attachment. The papilla and medullary tubules appeared normal. The intestinal bypass patients only had intraluminal sites of crystalline material in the medullary collecting ducts. The brushite stone formers had the most severe form of cortical and medullary changes with sites of Randall's plaque, and yellowish intraluminal deposits in medullary collecting ducts. All deposits were determined to be apatite. The metabolic and surgical pathologic finding in three distinct groups of stone formers clearly shows that "the histology of the renal papilla from a stone former is particular to the clinical setting". It is observations like these that we believe will provide the insights to allow the stone community to generate better clinical treatments for kidney stone disease, as we understand the pathogenesis of stone formation for each type of stone former.

Keywords Kidney calculi · Pathology · Calcium oxalate · Hydroxyapatite · Human

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Introduction

Urolithiasis is a common disease, with a prevalence of 3–5% in the United States (US). It causes considerable morbidity, occasional mortality, and costs $1 billion per year in the US [1] for hospital treatment of stone alone and up to $2 billion when including costs associated with lost employment. Appropriate evaluation and treatment of metabolically active stone disease could save nearly $2,200 per year per patient in related retreatment costs [2]. There have been significant advances in treatment modalities for urolithiasis in the past 20 years: percutaneous renal access for removal of large stone burdens, shock wave lithotripsy and advances in endoscopes and energy sources for intracorporeal stone fragmentation [3]. Metabolic evaluation for kidney stone disease has also advanced and can now identify factors involved in recurrence and guide medical therapy for kidney stone disease [2, 4]. However, advances in our understanding of the pathogenesis of kidney stone formation and clinically significant urolithiasis have not paralleled advances in diagnosis and treatment.

The exact etiologic cascade of events which lead to urolithiasis is unknown. Hypotheses ranging from oxalate-induced renal injury to insufficient urinary inhibitors of calculogenesis, to nidus formation with epitaxy,

have been proposed [5, 6]. Crystal formation and growth are common events in the urinary tract of humans. Urine in most humans is supersaturated with varying breakdown products and substances (calcium, oxalate, urate, cystine, phosphate) which can form stones if the upper limit of metastability for the substance is surpassed [7]. Kidney stones form in only a small percentage of people with supersaturated urine, however, due to a balance between the upper limit of metastability, crystal formation and inhibitory substances like citrate, magnesium, and urinary proteins.

Since he first described them, Randall's plaques have interested researchers who study the pathogenesis of common calcium oxalate (CaOx) stones [6]. Plaque, defined as sites of interstitial crystal deposition at or near the papillary tip**,** are common in kidneys of calcium oxalate stone formers (100%), and not rare, but less, in people who do not form them (43%) [8]. Anchored to renal tissue in the renal papillae, and of crystalline composition, they seem an ideal site on which overgrowths of calcium oxalate or phosphate could grow into stones. In fact, Randall showed examples of calcium oxalate stones shaped as though they had grown over renal papillae. They had, at the apex of their interior, where the papillary tip would have been, patches of calcium phosphate, the same material he found in the plaques themselves.

The goals of our resent studies were to test two separate hypotheses. First, that plaque is a specialized disease that begins as apatite in a unique region of the kidney because of local driving forces and anatomy. Second, that stones which arise from causes radically different from the common CaOx stone do not necessarily arise on plaque. To test these hypotheses, we performed intraoperative papillary and cortical biopsies during percutaneous nephrolithotomy of plaque in kidneys of three types of stone formers (idiopathic-calcium stone formers, patients with stones due to obesity-related bypass procedures, brushite stone formers) and obtained papillary and cortical specimens form non-stone formers after nephrectomy [9].

Idiopathic calcium oxalate stone formers

Our surgical and morphological analyses were performed on 15 idiopathic CaOx stone formers who were well characterized metabolically [9]. During the endoscopic examine of the papillary tip, the suburothelial sites of Randall's plaque appeared as irregular, whitish lesions just as described by Randall (type 1 plaque) (Fig. 1a). These sites of plaque are thought to be where a CaOx stone would be fixed to the kidney tissue, thereby allowing the stone to slowly grow. Mapping studies performed on 14 of the 15 patients revealed the fractional plaque coverage for the CaOx stone formers to be significantly higher than the controls (7.6% vs 0.5% respectively) [10]. When the values for fractional plaque coverage were correlated with urine data, urine volume and calcium excretion, only higher calcium and lower urine volume were determined to be involved in the formation of interstitial plaque. Furthermore, percent plaque coverage directly correlated with the number of stones formed even when corrected for the duration of stone disease [11]. No correlation was found between plaque coverage and duration of stone disease.

Both light and electron microscopic examination of the papillary biopsies found plaque to originate in the basement membrane of the thin loops of Henle (Fig. 1b, c) and then appear to spread through the interstitial space of the inner medulla to lodge beneath the urothelium lining the urinary space (Fig. 1b). The deposits within the basement member appear as single, laminated spheres as small as 50 nm with as many as seven alternating light and dark rings or layers (Fig. 1c). The light areas represent crystal that we found to be composed of hydroxyapatite as determined by infrared analysis and electron diffraction. The electron dense areas represent what we assume to be an organic layer based on prior studies of kidney stones [12, 13]. We recently determined that the electron dense material indeed contains osteopontin and that this protein is positioned between the interface of the light and dark rings (Fig. 1c) [14]. The single crystalline deposits appear to migrate into the interstitium where they are surrounded by a layer of electron dense material thought to be matrix forming islands of plaque that incompletely or complete encompass nearby tubules (Fig. 1c, d). The plaque eventually migrates to the urothelium and where it is thought to serve as an anchoring site for stone formation and growth within the urinary space. No oxalate crystals were identified within the renal tubules or the interstitium. Furthermore, we found no evidence of generalized cell injury or associated interstitial inflammation. In fact, none of the morphological methods used have found evidence of cell injury to those tubules that have apatite deposits in their basement membrane or encompassing them in the near interstitial space.

Intestinal bypass stone formers

It is well known that some patients who have undergone obesity-related intestinal bypass surgery develop kidney stones as a result of that procedure. In our study, we examined the kidneys of four intestinal bypass patients [9]. In contrast to the idiopathic CaOx stone formers, papillary biopsies from these stone forming patients lacked type 1 Randall's plaque. No evidence of Randall's plaque was noted by endoscopic examination of the renal papilla, but instead small nodular deposits appeared to project off of the urothelium near the openings of the ducts of Bellini (Fig. 2a). By histologic examination, we found crystals only in the lumens of a few inner medullary collecting ducts as far down as the ducts of Bellini (Fig. 2b, c). Those intraluminal deposits that filled a tubule appeared to obstruct a number of nephrons, as evidenced by the dilation of these tubules

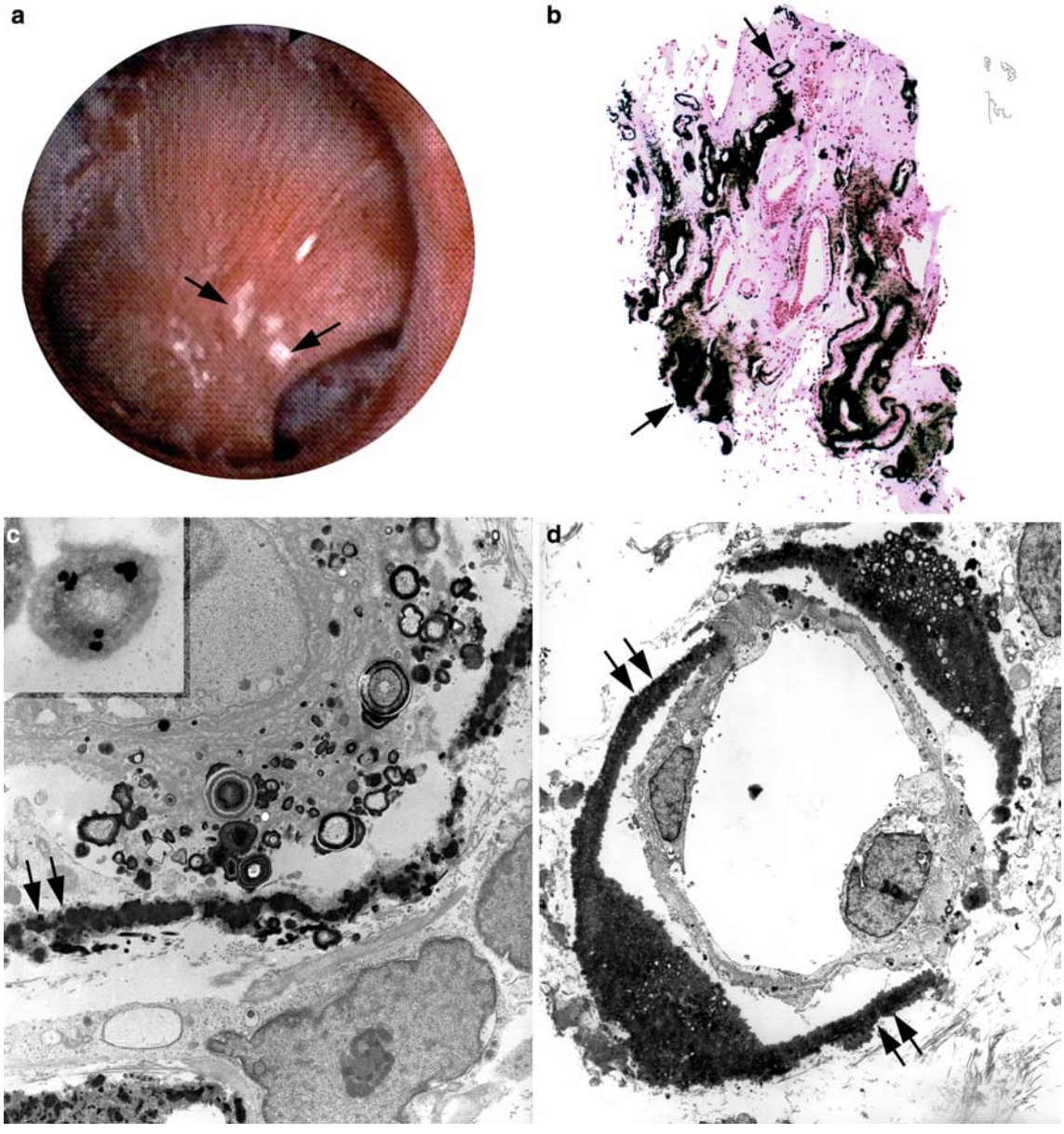

Fig. 1 Endoscopic and histologic micrographs of papillary and biopsies from idiopathic calcium oxalate stone formers. **a** A video recorded papilla at the time of the percutaneous nephrolithotomy for stone removal. Sites of suburothelial Randall's plaque are seen (*arrows*) as the whitest areas near the tip of the papilla. **b** A low magnification light micrograph of a portion of a papillary biopsy taken at a site of Randall's plaque during stone removal. Sites of calcium deposits (*arrows*) were detected using the Yasue metal substitution protocol for calcium histochemistry. Initially the deposits are located within the basement membranes of thin loops of Henle (**c**) and then appear to migrate into the interstitial space forming incomplete (**c**) to complete (**d**) rings (*double arrows*) of mineral that surround these tubular segments. Note that the crystalline deposits located in the tubular basement membranes appear as spheres with alternating light and dark layers (**c**) and that osteopontin (*small dark circles*) is located at the interface between the light and dark rings (insert **c**). Magnification, ×100 (**b**); ×15,000 (**c**); ×13,000 (**d**)

and others proximal to the obstruction. These upstream dilated tubules contained cast material and evidence of cell injury. No crystalline deposits were found in the basement membranes of the thin loops of Henle or the interstitial space. Those tubules filled with crystals showed evidence of cell injury with a cuff of interstitial fibrosis surrounding the tubules (Fig. 2c, d). Both infrared and electron diffraction analyses determined the deposits to be biological apatite.

Brushite stone formers

The third group of stone formers comprised brushite stone forming patients, a rapidly rising stone forming group. About 15% of stone formers produce predominantly calcium phosphate stones, and about one fourth of these patients form stones containing brushite ($CaHPO_4$). Brushite stone patients make many more

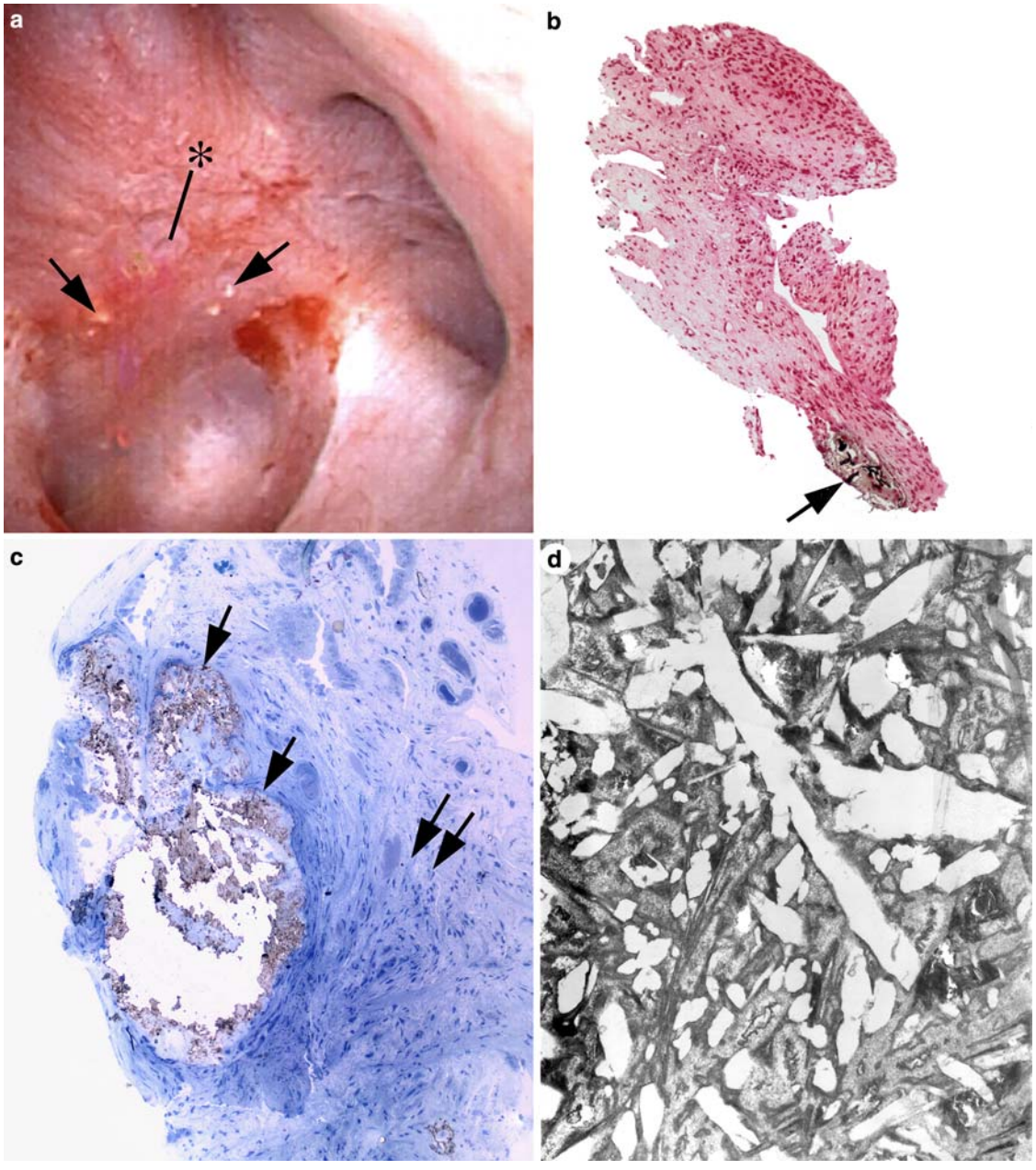

Fig. 2 Endoscopic and histologic micrographs of papillary and biopsies from intestinal bypass patients for obesity with kidney stones. **a** A video recorded papilla. In contrast to the common CaOx stone former, intestinal bypass stone formers lack sites of suburothelial Randall's plaque but show nodular-appearing structures (*arrows*) near the tip of the papilla and occasionally dilated openings to the ducts of Bellini (*asterisk*). **b** A low magnification light micrograph of a portion of a papillary biopsy taken at the site of a nodular-appearing structure. Sites of calcium deposits (*arrow*) were detected only in the lumens of a few inner medullary collecting ducts and ducts of Bellini. **c** A higher magnification light micrograph that shows a complete loss of tubular lining cells (*arrows*) in those inner medullary collecting ducts filled with crystalline deposits determined to be hydroxyapatite. In addition, extensive interstitial fibrosis (*double arrows*) is noted in the interstitial space surrounding such ducts. **d** The crystalline patterns of the intraluminal mineral deposits. Magnification, ×100 (**b**); ×400 (**c**); ×19,000 (**d**)

stones and they make them more often than CaOx stone formers. In addition, the brushite stones are harder than the CaOx stone, making them resistant to shock wave lithotripsy (SWL) treatment. We studied ten brushite stone formers who ranged in age from 23–59 years. Some of these patients had received up to 25 stone removal procedures that included SWL and percutaneous nephrolithotomy [15]. In general, these stone formers had high urine volume and Ca excretion, and low citrate excretion compared to our CaOx patients. Three patients were unique in that one had a UPJ obstruction fixed at the age of 7, another was a chronic uric acid

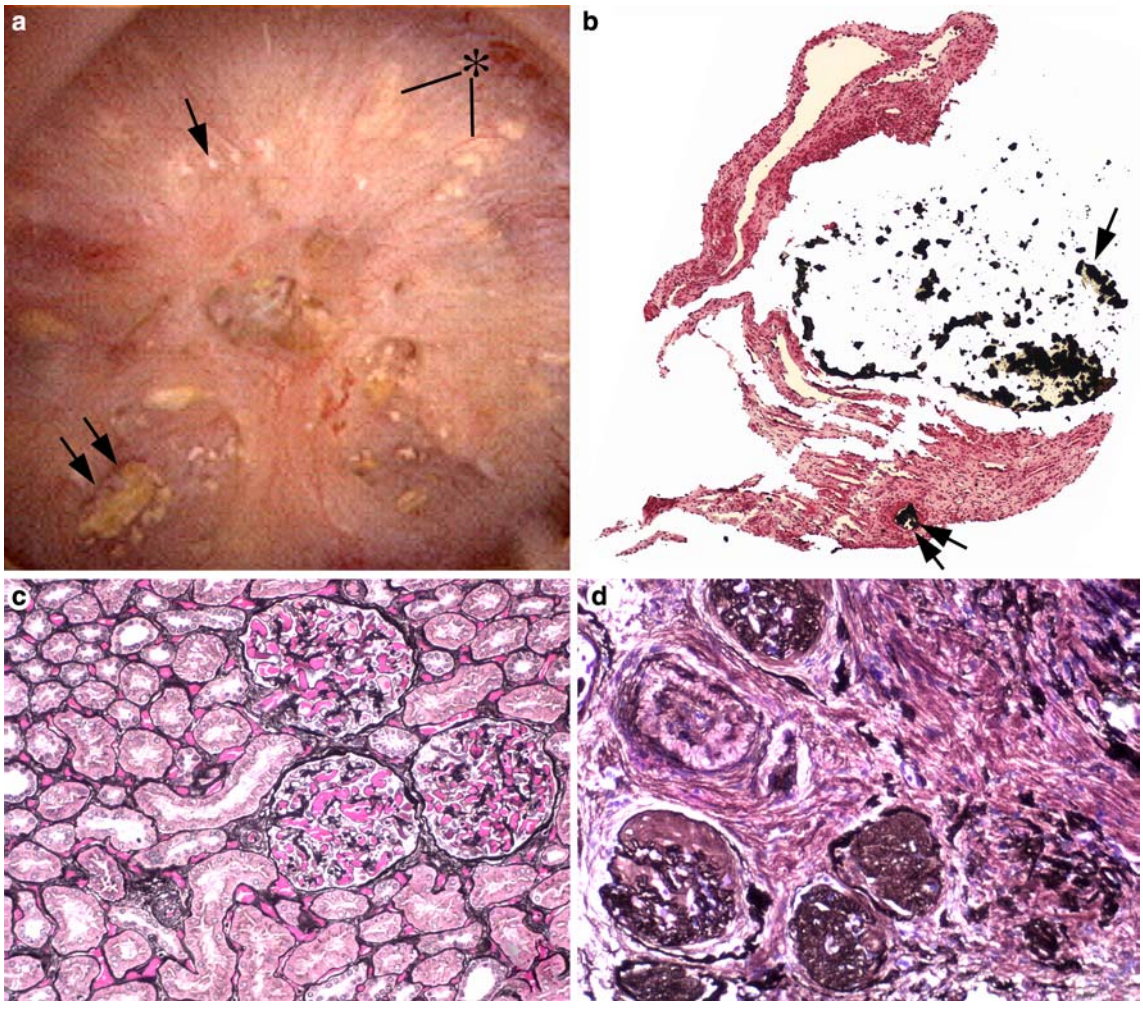

Fig. 3 Endoscopic and histologic images showing papillary pattern of crystal deposits in brushite patients. **a** An example of a papilla from a brushite patient that was video recorded at the time of stone removal. The papilla from brushite stone formers shows three types of crystalline patterns. Type 1 shows the irregular areas of whitest material (*arrow*) termed Randall's plaque located beneath the urothelium and commonly found in idiopathic CaOx stone formers. In addition, these papilla possess sites of yellowish crystalline deposits (type 2) at the opening of ducts of Bellini (*double arrow*). Usually, type 2 deposits are associated with dilated openings to ducts of Bellini. These enlarged openings appear as pits or depressions at the tip of the papilla. The third type of crystalline pattern is seen as yellowish mineral deposits located in lumens of inner medullary collecting ducts (*asterisk*) at single sites or in multiple tubules organized in a spoke and wheel-like pattern around the circumference of the papilla. **b** A low magnification light micrograph of a portion of a papillary biopsy taken at a site of a type 2 deposit. The sites of calcium deposits are again stained black by the Yasue method. Note the enormous amount of Yasue-positive material filling an enlarged inner medullary collecting duct (*arrow*) and a more normal sized duct (*double arrow*). The brushite stone patients were found to have pathologic changes in the cortex. Cortical tissue from a normal individual is seen in **c**, while **d** shows the cortex of a brushite patient. The pathologic changes seen in the brushite biopsy include glomerular opalescence, tubular atrophy and interstitial fibrosis. Magnification, ×100 (**b**); ×1,000 (**c**); ×1,000 (d)

stone former over treated with potassium citrate, and the last was a quadriplegic at the age of 22, while the others were originally common CaOx stone formers.

Endoscopic examination of the renal papilla of these patients revealed three kinds of deposits. The first pattern of deposition was sites of interstitial Randall's plaque identical to that described for the idiopathic CaOx stone formers (Fig. 3a). Like bypass stone formers, brushite stone formers have apatite crystallization within terminal collecting ducts (type 2 deposit), associated with collecting duct cell death and papillary interstitial inflammation and fibrosis. This deposition pattern was also seen as yellow crystals projecting from the opening of ducts of Bellini into the urinary space. The third pattern (type 3 deposit) was suburothelial yellow deposits on the sides of the papillary tips radiating in a spoke and wheel pattern around the circumference of a papilla, extending from the papillary tip to the fornix. Type 2 and 3 deposits were never encountered in CaOx, or controls while type 2 deposits are common for bypass stone formers. In other words, brushite pathology is an amalgam of CaOx and bypass stone disease plus type 3 deposits; but collecting duct plugging and cell injury are far more severe in the

brushite stone disease, as is interstitial inflammation and fibrosis. Both infrared and electron diffraction analyses determined that all three types of deposits were biological apatite.

A new morphological feature of the papilla of the brushite stone formers was the presence of pits or depressions primarily at the papillary tip (Fig. 3a). These pits were usually associated with dilated openings of ducts of Bellini but occasionally were found on the sides of the papilla. The dilated ducts of Bellini were enlarged as much as 20 times above normal by enormous intratubular crystal deposits (Fig. 3b).

The severity of the collecting duct and papillary interstitial disease led us to evaluate cortical injury in all three groups [15]. The cortical biopsies were evaluated for glomeruloscelorsis, interstitial fibrosis and tubular atrophy (Fig. 3c, d). The papillary and cortical abnormalities in brushite stone formers are, in fact, so marked as to constitute a significant and hitherto unrecognized renal disease not present in CaOx or bypass stone formers. In order to perform statistical analyses on the qualitative description of papillary and cortical changes, we combined scores for tubular atrophy and interstitial fibrosis into a single score and left glomerular changes as a separate category. Clearly brushite stone formers had a grossly higher score for atrophy/fibrosis than the other stone formers. Also, the brushite stone formers had significantly more SWL procedures. To evaluate the hypothesis that the higher atrophy/fibrosis score in brushite patients was due to their larger number of SWL treatments, we performed ANOVA. SWL was marginally significant ($P = 0.059$) and the adjusted score for brushite stone formers exceeded that of CaOx stone formers. Brushite stone formers also had a higher glomerular pathology score but SWL was not related to this pathological change. These findings, taken together, delineate a hitherto undescribed kidney lesion in stone forming patients.

Discussion and conclusions

Idiopathic calcium oxalate stone formers

The data from these studies clearly supports the statement that "the histology of the renal papilla from a stone former is particular to the clinical setting of that stone former". Each of the three different stone forming groups (common CaOx, intestinal bypass for obesity and brushite) had particular morphological changes that correlated with their metabolic profiles. The common CaOx stone formers without systemic or bowel diseases formed apatite interstitial plaque that appear to act as an anchored nucleation site for CaOx stone formation, as originally described by Randall [6]. The pathophysiology of the initial site of crystallization may well be linked to hypercalciuria in that high urine calcium levels and low urine volumes are risk factors for stone growth. Crystal deposition appears to begin within the basement membrane of the thin loops of Henle and then migrate into the interstitial space of the papillary tip. High calcium movement through tubules, as occurs in hypercalciuria, if coupled with increased water extraction, could increase calcium concentrations along the nephrons, and create conditions favorable for the accumulation of calcium salts in the interstitial spaces that could in turn lead to calcium solid phase deposits. Our morphological observations support such a hypothesis in that crystalline deposits of biological apatite are consistently found in the basement membrane of the thin loops of Henle in the deep medulla, the site where one would predict a rise in ion (calcium and phosphorous) movement into the interstitium opposed by removal by the vasa recta. Cell injury does not appear to be a perquisite for the initiation of stone formation and growth in patients with common CaOx stone disease. While there may be cellular changes that were not detectable with the morphological methods we employed, it is hard to imagine that such events are critical to stone formation.

Our papillary mapping observations that show a correlation between papillary plaque coverage with number of stone events when corrected for the duration of stone disease supports the hypothesis that the pathogenesis of CaOx stones begins with Randall's plaque. These are the first numerical observations of plaque coverage and stone number with sufficient detail to test the plaque-stone hypothesis. Furthermore, our 24-h urine data indicated that two primary factors, urine volume and calcium, are involved in the formation of interstitial plaque which supports the assumption of Randall that papillary plaque is the progenitor of urinary stone formation. We suggest that future studies need to focus on designs that examine the ability of plaque coverage to predict future stone events. Hopefully, more sensitive, less invasive radiographic protocols can be found to correlate the direct endoscopic observations we have made so that plaque determinations can be quickly and easily performed in a large group of stone patients using prospective study designs.

Intestinal bypass patients for obesity

The intestinal bypass and brushite stone formers each present a different pattern of papillary changes that appear to be specific to their peculiar pathophysiology and very different from that seen in common CaOx stone formers. The intestinal bypass patients only have intratubular deposits, but to our surprise the mineral was again biological apatite. The tubules plugged with apatite show extensive cell damage to complete cell loss with a cuff of interstitial fibrosis around these tubules. We believe that stones form through a completely different process in the bypass patients than what we have described in idiopathic CaOx stone formers. Furthermore, the metabolic status of the bypass patients does not favor stone formation by Randall's plaque.

The unique morphological finding observed in the bypass patients was the presence of intraluminal deposits in the distal end of the ducts of Bellini and inner medullary collecting ducts. How this process is initiated is unclear, but once these ducts are obstructed, a cascade of injurious events could be set in place. Further damage of the cells of the medullary collecting ducts could alter pH regulation and lead to a more alkaline urine, thereby supporting apatite crystal retention. Thus, a vicious circle could be initiated that would only lead to additional cell injury and a further loss of pH regulation.

Brushite stone formers

The brushite stone formers presented by far the most severe morphological changes to the renal papilla and cortex of the three groups of stone formers studied here. Clearly, they share a common pathogenesis with idiopathic CaOx stone formers in that sites of Randall's plaque are easily and consistently found in the papilla of both stone formers, and they generally show high urine calcium. In addition, some of our brushite stone formers began as CaOx stone formers. However, the brushite stone formers deviated from the CaOx stone formers in that they possessed intratubular plugs of apatite. This finding is like that for the bypass patients.

The factors driving the intraluminal deposition of apatite appear to be multiple. One of our patients was a uric acid stone former who was treated with large amounts of potassium alkali and thereafter formed brushite stones. Another patient had an ureteropelvic obstruction in his youth that might have triggered a mild collecting injury as the result of the obstruction. A surprising finding was the fact that some our brushite patients had ten to 25 shock wave lithotripsy (SWL) treatments. It is well known that a clinical dose of shock waves consistently damages the renal papilla, which may appear as papillary necrosis. The histological finding of regions of tubular atrophy and interstitial fibrosis in the medullary collecting duct of our brushite patients is suggestive of a link between the number of SWL treatments and the onset of brushite stone disease. Such damage could reduce the ability of the collecting ducts to regulate tubule fluid pH so that luminal pH rises and fosters calcium phosphate crystallization. Injury to the medullary collecting ducts may also result in tubular ectasia in the cortex and glomeruloscelorsis.

In conclusion, our metabolic and surgical pathologic findings in three distinct groups of stone formers clearly show that "the histology of the renal papilla from a stone former is particular to the clinical setting". It is observations like these that we believe will provide the insights to allow the stone community to generate better clinical treatments for kidney stone disease.

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