Randall's plaque: Pathogenesis and role in calcium oxalate nephrolithiasis

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The purpose of these studies was to test the hypothesis that Randall's plaque develops in unique anatomical sites of the kidney and their formation is conditioned by specific stone-forming pathophysiologies. We performed intraoperative papillary biopsies from kidneys of idiopathic-calcium oxalate (CaOx), intestinal bypass for obesity, brushite (BR) and cystine stone formers (SF) during percutaneous nephrolithotomy. Tissues were examined by infrared analysis and light and electron microscopy. Our analysis revealed a distinct pattern of mineral deposition and papillary pathology for each type of SF. CaOx SF had interstitial apatite crystals beginning at thin loops of Henle. These deposits termed Randall's plaque are thought to serve as sites for stone attachment. No tubular injury was noted. Intestinal bypass patients possessed intraluminal apatite deposits in inner medullary collecting ducts (IMCD) with associated cell injury. BR SF showed the most severe form of cortical and medullary changes with sites of Randall's plaque, and yellowish intraluminal deposits of apatite in IMCD. Cystine SF had plugging of ducts of Bellini with cystine crystals and apatite deposits in IMCD and loops of Henle. Intratubular sites of crystalline deposits were always associated to adjacent regions of interstitial fibrosis. The metabolic, anatomic, and surgical pathologic findings in four distinct groups of SF clearly show that 'the histology of the renal papilla from a stone former, is particular to the clinical setting'. We believe our approach to studying stone disease will provide insights into the pathogenesis of stone formation for each type of SF that will lead to improved clinical treatment.

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PLAQUE IN IDIOPATHIC CALCIUM OXALATE STONE FORMERS

Patients who form calcium oxalate (CaOx) renal stones without any systemic disorder apart from familial (so-called idiopathic) hypercalciuria (IH) represent the most common kind of kidney stone patient and are often referred to as 'idiopathic calcium oxalate SF' (ICSF). It is among these patients that research on Randall's plaque has led to new discoveries and the reasonable beginnings of a pathogenetic scheme that connects physiological abnormalities and a characteristic renal histopathology to the clinical manifestation of stone formation. Among the systemic disorders that must be excluded, we include primary hyperparathyroidism, sarcoidosis, hyperthyroidism, glucocorticoid excess, renal tubular acidosis (RTA), hyperoxaluric states of all varieties, patients whose stones contain above 50% calcium phosphate, and of course patients with non-calcium stones of any kind. These criteria define the phenotype for which this portion of the review is valid. Whether or not the pathogenetic elements we describe here are germane to other kinds of stone formers (SF) remain untested.

CaOx stones grow over interstitial plaque

In ICSF, many stones are found attached (Figure 1a and b) to the renal papillae.[1] The attachment site is over a whitish deposit called Randall's plaque (Figure 1). When stones are removed, the plaque undersurface is easily seen (Figure 1c and d). In our recent survey of 24 kidneys in 23 ICSF, we found plaque in 100% of cases and in 48% (11 patients) stones were found attached to areas of plaque.[1] Within these 11 people, 49 papillae had attached stones. In the 24 kidneys, we inspected 172 papillae and found plaque on 156 (91%).

Our attachment rates are likely to be underestimates. We made our observations during percutaneous nephrolithotomy (PNL). Access to the renal interior is via a papillum and attached stones on that papillum may easily be dislodged by the procedure itself and the papillum of access cannot be reliably examined. Patients who require PNL are those with the largest target stones (generally above 2 cm) and these large stones frequently are unattached. For those stones in ICSF that are attached, attachment to plaque has thus far been the invariable rule without an exception. For those stones found unattached, we cannot as yet say where they

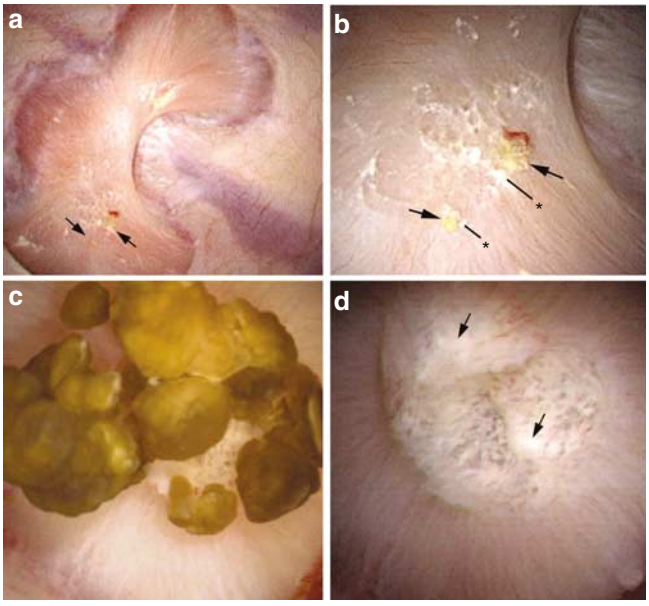

Figure 1 | Digital photographs obtained with an endoscope at the time of PNL showing calcium oxalate monohydrate stones attached to the renal papilla of two different idiopathic CaOx SF. The compound papillae in (**a**) and (**b**) show sites of irregular whitish suburothelial material termed Randall's plaque (single arrows) that are associated with two small stones (asterisks). Numerous stones attached to a papilla tip (**c**) and after these stones were removed (**d**), and they appear to have been attached to sites of Randall's plaque (single arrows).

originated. Evidence from stone morphology has suggested a papillary origin for unattached stones.[2,3] This remains an open investigative area.

If indeed CaOx stones require plaque to anchor them and permit development into clinically relevant stones, we should expect that patients with more stones would have a higher fraction of their papillae covered by plaque. This is indeed the case (Figure 2a).[4] The relationship holds even after adjustment for the number of years of stone formation (Figure 2b). This kind of correlation does not tell us whether plaque fosters stones, or the converse, but given our histopathology and gross findings, the former is overwhelmingly the more reasonable alternative.

Characteristics of interstitial plaque

The earliest and most minimal deposits of plaque are in the basement membrane of the thin loops of Henle (Figure 3a).[5] This location is invariably involved with plaque and is the only location where plaque can be found in isolation. In many regions, plaque becomes very dense around thin loops and appears to spread from the loops into the surrounding interstitial space (Figure 3b). Often, as in this illustration, one finds plaque migrating down the papillary tip towards the urothelium. Plaque is never found in the lumens of tubules or vessels, or within epithelial cells; it is uniquely located in the basement membranes and the interstitium. Therefore, as expected, the renal epithelial cells invariably appear normal, as does the cellular interstitium.

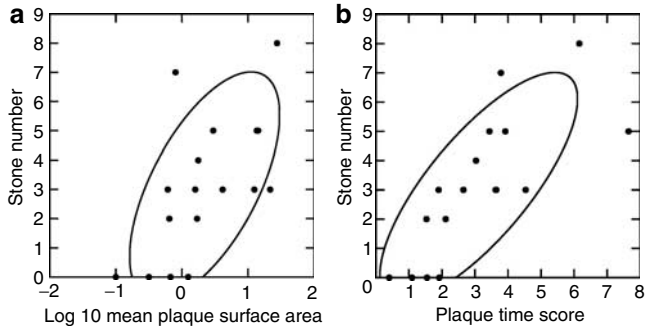

Figure 2 | (a) Number of stones vs log transformed mean plaque surface area. Nonparametric ellipse of containment includes two standard deviations. (**b**) Number of stones vs multivariate regression equation from general linear model, including stone disease duration and plaque surface. Plaque time score = 1.788 + 1.386 × log 10 mean plaque surface area + 0.082 × time. Reprinted with permission from the *Journal of Urology*.[4]

Within the basement membranes, plaque consists of individual laminated particles in which zones of crystal and organic matrix overlay each other in a tree-ring pattern (Figure 3c and d). In the interstitium, the particles fuse to form a syncytium of crystal islands in an organic sea (Figure 3e and f). At no time have we found crystals not coated by an organic layer. This means it is most unlikely that CaOx stones grow over plaque crystals themselves. Rather, CaOx must grow over the organic material that invariably coats plaque crystals.

Relationship of plaque to the urothelium

Not only does the organic layer intervene between CaOx and plaque crystals, the urothelium is present as well. Plaque without stones appears at surgery almost always covered by a shiny, intact urothelial layer. Moreover, the urothelial layer is coated by a complex glycoprotein mixture that includes a variety of glycosaminoglycans.[6] This means CaOx must somehow localize itself to plaque areas despite at least two intervening barriers, urothelium and its organic coating. This implies some complex biology, perhaps induced by plaque and affecting urothelium, about which nothing is presently known. In essence, the events involved in the transition from plaque to plaque with stone remain, at this time, unclear.

Nature of the plaque crystal

Using high-resolution Fourier transform infrared microspectroscopy (FTIR) and electron diffraction, the crystal component of plaque particles is calcium phosphate (CaP) in the form of apatite.[5] The apatite is seemingly identical to that found in bone. Given that the apatite microparticles are forming in the type IV collagen of basement membranes and type I in the interstitial space, the process is most closely analogous to that of bone formation. Whether this analogy will be fruitful in terms of new research remains to be tested. An example of what may be a similar process is coronary artery calcification, in which cell transformation to osteoblast character has been documented.[7] Although CaOx crystals can

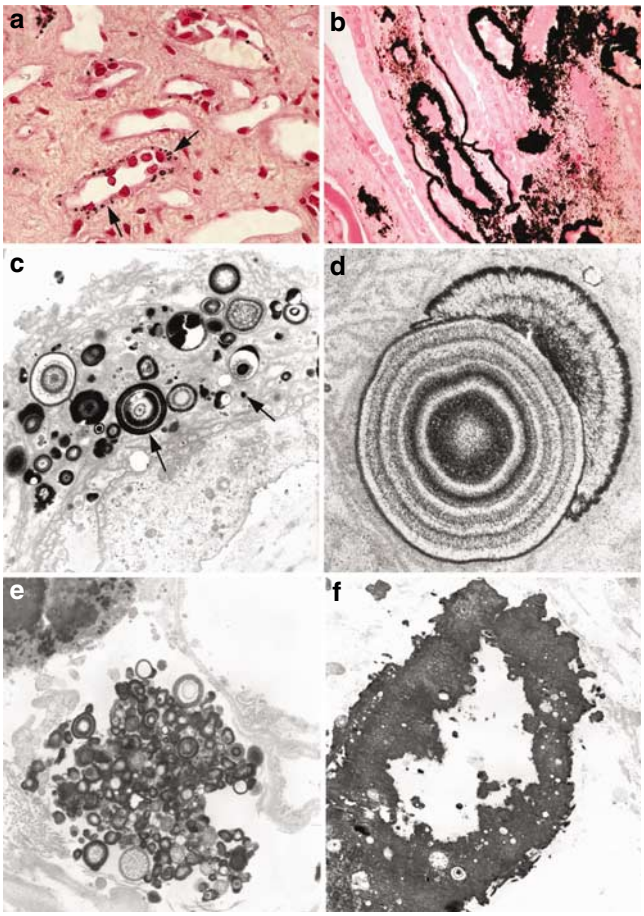

Figure 3 | Histologic images showing sites of Randall's plaque and its progression. (**a**) The initial site and size of calcium deposits in the papillary tissue of a CaOx patient as seen by light microscopy, while (**c**) and (**d**) show these same structures by transmission electron microscopy. Sites of crystalline material (arrows) are first noted in the basement membranes of the thin loops of Henle. The individual deposits are as small as (**c**) 50 nm and (**d**) grow into multi-laminated spheres of alternating light and electron dense rings. Extensive accumulation of crystalline deposits occur around the loops of Henle and spread into the nearby interstitial space (**b**) extending to the urothelial lining of the urinary space. Individual deposits accumulate in the interstitium forming an island of mineral encased in a matrix material (**e**). These islands can completely surround individual tubules (**f**). Original magnification, (**a**) $\times$ 900; (**b**) $\times$ 500; (**c**) $\times$ 25 000; (**d**) $\times$ 70 000; (**e**) $\times$ 13 000; (**f**) $\times$ 10 000. Panels (**c** and **e**) reprinted with permission from the *Kidney International*.[10]

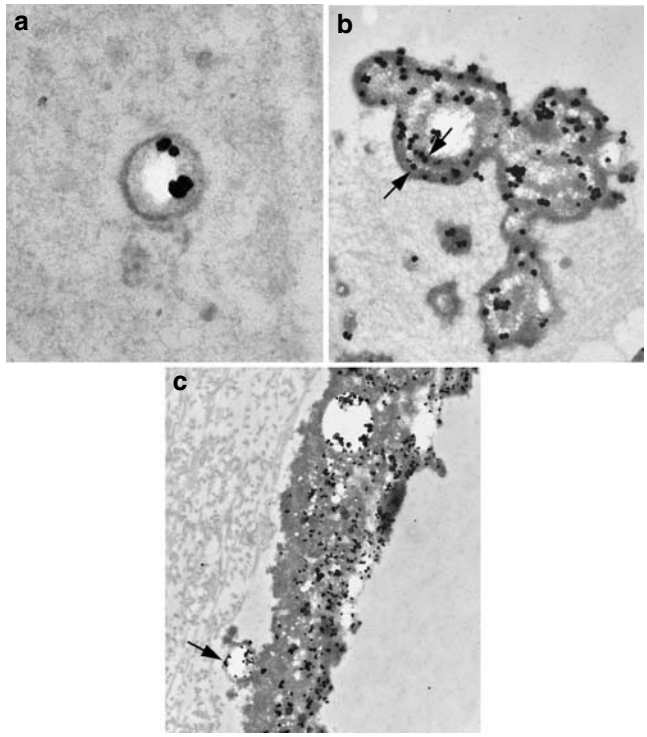

Figure 4 | Immunoelectron microscopic localization of osteopontin to the basement membrane of loops of Henle and the medullary interstitium. (**a**) A high magnification TEM showing immunogold label indicating osteopontin localization within single crystals that is found at the interface of the crystalline material and the organic layer. This particle was located in the basement membrane of a loop of Henle. (**b** and **c**) Immunogold label (arrows) localization of osteopontin within crystalline deposits that are coalescing to form regions of interstitial plaque. Original magnification, (**a**) $\times$ 50 000; (**b**) $\times$ 35 000; (**c**) $\times$ 14 000. Reprinted with permission from the *Kidney International*.[10]

be found in tissues of animals induced to form stones via oxalate loading, that crystal is never present in interstitial plaque. In fact, thus far, in our studies of a range of human SF, we have never found CaOx in the renal interstitium.[5,8,9]

Nature of plaque matrix

To date, we have identified only osteopontin among the constituents of matrix (Figure 4a–c).[10] The osteopontin appears to localize preferentially at the matrix–apatite crystal interface. This is not surprising given the well-known affinity of osteopontin for calcium crystal surfaces.[11] The role of osteopontin in plaque formation is unknown. Osteopontin can inhibit nucleation, growth, and aggregation of calcium phosphate crystals, and if anchored to collagen or crystal surfaces could also serve as a nucleating site for new crystals. Obviously, there are many other molecules in the organic matrix beside osteopontin that remain to be discovered. This is an important area of potential new research.

Mechanism of plaque formation

Using 24-h urine collections from ICSF and normal subjects, the fraction of papillary surface covered by plaque varies with 24-h urine calcium excretion and inversely with urine volume and pH (Figure 5).[12] The wide range of urine calcium excretions reflects the fact that a majority of ICSF have IH. The correlation of plaque with hypercalciuria suggests some common and perhaps causal connections. IH is generally thought to arise from increased tissue vitamin D response.[13] Serum 1,25-dihydroxy vitamin D levels are above normal as is intestinal calcium absorption. Linkages between IH and plaque could therefore be via the increase of calcium movement from blood to urine or via vitamin D response of renal cells in the medulla and papillum. Because there is no animal model of human plaque thus far, research in this area is very incomplete.

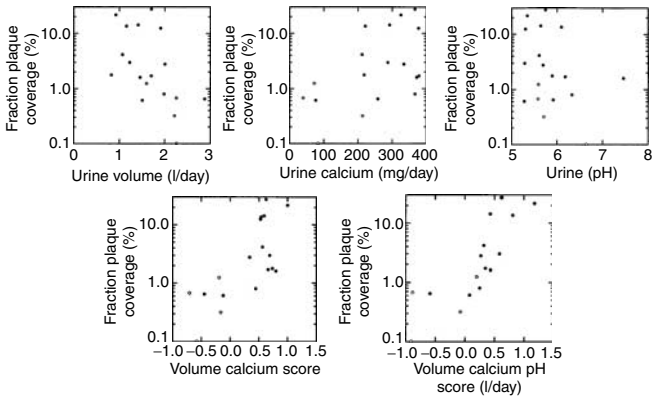

Figure 5 | Fractional plaque coverage per papillium varies inversely with urine volume (upper left panel) among SF (closed circle) and non-stone-forming control subjects (open circle). Plaque coverage varies with urine calcium excretion (upper middle panel) and is inverse to urine pH (upper right panel). A composite multivariate score using urine volume and calcium excretion (lower left panel) and one that includes urine pH as well (lower right panel) strongly correlate with plaque coverage. Reprinted with permission from the *Kidney International*.[12]

Given that plaque forms in the thin loops of Henle, one is not surprised that its abundance increases with water conservation. Obvious candidates for a link between water conservation and plaque would be interstitial calcium concentration or even osmolality itself. This is an unexplored area of research. In producing acid urine, inner medullary epithelial cells could concomitantly increase interstitial fluid pH. Since apatite crystals form preferentially in alkaline media, this might be an explanation for the link between urine pH and plaque abundance. Until an animal model of plaque can be obtained, this matter cannot be fully explored.

CaOx SF WITH OBESITY BYPASS SURGERY LACK PLAQUE

One clue to pathogenesis is the absence of plaque in this type of SF. Their urine is acidic[14] but their urine volumes are high, and urine calcium excretion rates are in the normal range. Elevated urine oxalate is the putative cause of their stones, and no attached stones were ever observed on any papillum. This pattern supports the notion that high urine calcium or IH itself, or perhaps urine osmotic concentration, are more central to plaque formation than an acid urine pH. Of interest, these patients form intratubular crystal masses in their inner medullary collecting ducts (IMCD) (Figure 6a and b). IMCD cells are injured in regions of deposits and the surrounding interstitium is inflamed and fibrotic. Such cell injury and interstitial reaction is never found in ICSF. Although stones are CaOx with little or no CaP, and although urine pH is low, these IMCD deposits are apatite. Apatite is unstable at the average urine pH of 5.57 found in these patients, meaning that local tubule pH must depart from that of the bulk phase urine. These patients are an instructive natural experiment and illustrate the range of pathology one can encounter in CaOx SF.

PLAQUE IN BRUSHITE SF

Like ICSF, Brushite (BR) SF have IH and also have plaque.[8] Stones are attached to papillae, but as extensions into the lumen of long IMCD and Bellini duct (BD) apatite plugs, and not as overgrowths on interstitial plaque. These IMCD and BD plugs were described by Randall[15] as his Type 2 form of plaque and present themselves as yellow elongate, distinct suburothelial deposits easily distinguished from white interstitial plaque with its vague serpiginous borders (Figure 6c and d). Like bypass SF, BR SF have cell injury and interstitial reaction in regions of IMCD deposits (Figure 6c and d), but the process is far more severe. Gross papillary morphology is abnormal with retraction and pitting deformities (Figure 6c). Involved IMCD are massively dilated with extensive cell necrosis and denuding of basement membranes. Although the stones are BR (calcium mono-hydrogen phosphate), the deposits are apatite, an entirely different crystal.

We did not observe stones growing attached to interstitial plaque regions. In part, this could reflect the massive volume of BR stones that would interfere with detection of tiny attached stones. Likewise, the deformity of the papillae could affect our ability to visualize such stones. It is our impression, however, that they are not present.

Our current formulation is that BR SF begins often as CaOx SF because many patients initially form such stones and BR SF has IH about as often as do CaOx SF. What distinguishes patients with CaP in stones from those with only CaOx is mainly higher urine pH.[16] Because of IH and perhaps efficient water conservation, BR SF may form plaque when producing CaOx stones. As their urine pH rises, for reasons we do not as yet know, CaP becomes the predominant stone mineral and stones attached to interstitial plaque become rare enough that we have not as yet been able to document them. An alternative formulation is that IH leads to plaque in BR SF but BR stones simply do not form over plaque the way CaOx stones apparently do. It is clear that BR SF, like bypass SF, offer unique research opportunities.

PLAQUE IN CYSTINE SF AND NON-STONE-FORMING SUBJECTS

Patients with cystinuria form cystine stones. Biopsy of their papillae reveals plugging of BD with masses of cystine crystals (Figure 6e).[9] This is expected as BD contains the same final urine that clearly produces cystine crystals and stones. Unexpectedly, they form apatite deposits in their IMCD and even their thin loops of Henle (Figure 6f). Mechanisms responsible for apatite deposits could include alkali loading in treatment of cystine stones, or obstruction of IMCD by BD plugs of cystine with resulting acidification defects that permit apatite crystallization. Interstitial plaque is present at the abundances found in non-stone-forming people. Cystine stones grow attached to BD cystine deposits and are also found frequently free in the renal pelvis. They are not attached to the small amounts of plaque.

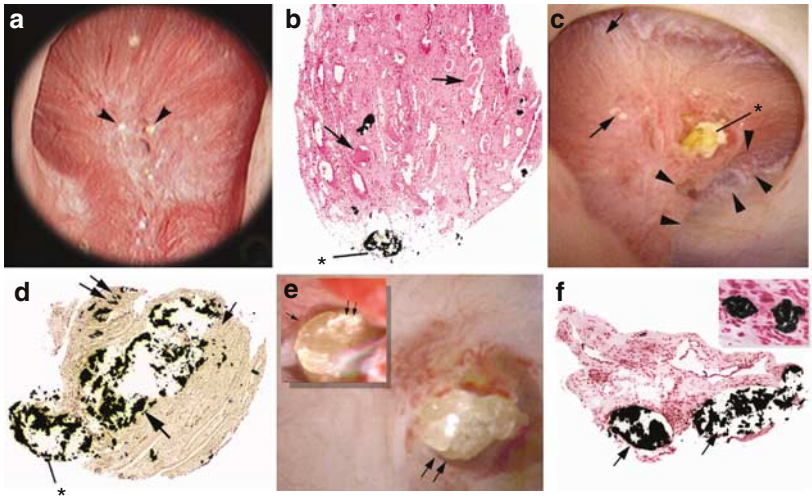

Figure 6 | Endoscopic and histologic images of crystal deposition in human papillary tissue from three different types of SF. (**a**) An example of a papilla from an intestinal bypass stone former that was video recorded at the time of PNL. Distinct sites of Randall's plaque material are not found on this papilla; instead, several nodular appearing structures (arrowheads) were noted near the openings of ducts of Bellini. (**b**) A low magnification light microscopic image of a papillary biopsy specimen also from an intestinal bypass stone former. Crystal deposition was only found in the lumens of a few collecting ducts as far down as the ducts of Bellini (asterisk). Note dilated collecting ducts (arrows) with cast material and regions of fibrosis around crystal deposit-filled collecting ducts. (**c**) An example of a papilla from a BR patient that was video recorded at the time of PNL. This papilla shows the irregular white areas of crystalline deposits (arrows) beneath the urothelium that was described for CaOx patients. In addition, the papilla from BR patients possesses sites of a yellowish crystalline deposit at the openings of ducts of Bellini (asterisk). Note the enlargement of the opening of a duct of Bellini that is filled with crystalline material (asterisk) seen as a depression or 'pit' on the papilla. A large pit (arrowheads) is seen along the side of this papilla and does not appear to be associated with a duct of Bellini. (**d**) A low-magnification light-microscopic image of a papillary biopsy specimen from a BR patient showing an enormous amount of Yasue positive material within an IMCD (arrows). This crystalline material is seen protruding from the opening of the duct of Bellini (asterisk). In addition, Yasue positive material is seen in the interstitium of the renal papilla surrounding thin loops of Henle (double arrows) as we have previous described for CaOx SF. (**e**) The papillary morphology by endoscopic examination of a cystine stone former at the time of PNL. Papilla from cystine SF varied from normal to flattened with greatly enlarged openings of the ducts of Bellini with protruding plugs of crystalline material (double arrow). Small sites of suburothelial white plaque (not shown), termed Randall's plaque, were noted. The insert in (**e**) shows a plug of cystine at the end of a duct of Bellini. The papillary histopathology of the cystine patients varied from normal to regions of plugging, dilation, and injury of IMCD (arrows, **f**). Intraluminal plugging with crystals was noted in thin loops of Henle (insert, **f**). Original magnification, (**b**) $\times$ 90; (**d**) $\times$ 100; (**f**) $\times$ 150. Panels (**a** and **b**) reprinted with permission from JCI.[5] while panels (**c** and **d**) were reprinted with permission from *Kidney International*.[8]

AN ATTEMPT AT SYNTHESIS AT THE PRESENT TIME

Somehow, perhaps because of IH, plaque forms and migrates beneath the urothelium. Urine crystallization potential is certainly increased by IH and promotes CaOx overgrowth to produce routine ICSF patients. In some of these patients, IMCD lumen pH increases and permits high calcium concentrations from IH and water extraction to express themselves in the formation of apatite crystals that damage cells. Mechanisms for such an increase could include: repeated obstruction from stones, even mild chronic urinary obstruction, protracted use of alkali as potassium or calcium salts, renal damage from extracorporeal shock wave lithotripsy, and initial formation of even the most microscopic CaOx crystals that cannot produce any clinical manifestations but could attack apical cell membranes and disorder pH regulation. Such a mechanism could possibly engender apatite deposits in IMCD of bypass patients. As well, apical membrane attack by cystine crystals or the necessary but extensive use of potassium alkali to control cystine solubility, could lead to the IMCD apatite deposits we have found in such patients.

The fact that plaque forms in cystinuria and normal subjects suggests that even normal amounts of calcium movement through the nephron are sufficient for plaque production. Perhaps when urine volumes are habitually low, it is fostered to a greater extent. Randall found plaque within kidneys from unselected autopsy cases, most of who were not SF. The examples of cystinuria and normal people point away from specialized effects of IH and toward plaque as a part of normal renal mineral handling that is exaggerated in SF because of high rates of calcium movement through the nephron. In fact, a few of our ICSF had relatively normal urine calcium (Figure 5) and correspondingly low plaque abundance. Lacking an animal model, we must be content with what we can learn from patients.

Within the admittedly narrow confines of what we know, at least one treatment stands out as having a great potential for reducing the formation of plaque. Plaque surface area falls as urine volume increases, and such treatment offers no apparent disadvantages or risks. Whether thiazide, which lowers urine calcium, would lower plaque abundance is far less easy to predict. So, as clinicians, we are endorsing from

the vantage point of our newest investigations, what our forebears endorsed long before us as a stable underpinning for the treatment of virtually any stone-forming patient.

LIMITATIONS OF CONTEMPORARY ANIMAL MODELS

Throughout we have alluded to our lack of an animal model for the study of plaque. This is despite at least eight models of hypercalciuria in rodents. By inbreeding, a rat strain has been established with a 10-fold increase of urine calcium above normal. Although the rats are a close simulacrum of human IH, driven by high vitamin D receptor expression, and produce both CaP and CaOx stones, depending on diet, and in this way strongly resemble human SF, they produce no plaque and never form attached renal stones.[17] The ob/ob mouse model of obesity and diabetes is known to have 1,25-vitamin D excess[18] that has been ascribed to leptin deficiency. Despite hypercalciuria, neither plaque nor stones have been described. There are three mouse models of Dent's disease,[19] of which one is persistently hypercalciuric, with hypercalciuria ascribed to high 1,25-vitamin D levels. Plaque has not been described in these mice. Our inspection of the published figures shows intratubular deposits in medullary collecting ducts with peritubular interstitial fibrosis. These changes were reduced by treatment with citrate. In proximal tubule cells, tiny calcium deposits appear to follow basolateral infoldings. Hypercalciuria due to high 1,25-vitamin D levels has been described in a klotho gene knockout mouse.[20] Plaque was not found despite obvious hypercalciuria and hypercalcemia. The authors report deposition of calcium in the kidneys. Published pictures do not permit independent evaluation by us as to localization. Hypercalciuria occurs in mice with TRPV5 gene knockout, but no mention is made of renal calcification. Mice with knockout of NaPi IIa, a sodium-phosphate co-transporter, develop renal phosphate wasting, hypercalciuria, hypercalcemia, elevated 1,25 vitamin D levels, and renal calcification. The calcifications are intratubular across the entire nephron, but no plaque has been described. A mouse model of Cav1 knockout develops hypercalciuria and urinary tract calculi, but no renal calcifications.[19] Knockout of aldose reductase causes hypercalciuria, but there is no description of renal calcification.[19] Most recently, a mouse NHERF-1 knockout model has been described, with increased urinary calcium excretion and elevated 1,25-vitamin D levels.[19] Calcifications are present in the renal papillae of these mice, but further study will be needed to determine if these calcifications resemble plaque seen in human SF.

This large panel of hypercalciuric animals studied by well-established laboratories suggests that plaque formation may be a very specialized event not necessarily unique to humans, but dependent upon more than simply hypercalciuria. It could be that water conservation is imperfect in these animals; the hypercalciuric rats are described as polyuric, as are the aldose reductase knockout mice, the CLC5 knockout mice, and the TRPV5 knockout mice.[19] Alternatively, plaque formation may require more time than is available in the rodent experiments described thus far.

ACKNOWLEDGMENTS

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