Histopathology Predicts the Mechanism of Stone Formation

Andrew P. Evan

Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN 46202-5120, USA

Abstract. About 5% of American women and 12% of men will develop a kidney stone at some time in their life and these numbers appear to be on the rise. Despite years of scientific research into the mechanisms of stone formation and growth, limited advances have been made until recently. Randall’s original observations and thoughts on the mechanisms for kidney stone formation have been validated for idiopathic calcium oxalate stone formers (ICSF) but not for most other stone forming groups. Our current studies on selected groups of human stone formers using intraoperative papillary biopsies has shown overwhelming evidence for the presence of Randall’s plaque in ICSF and that stone formation and growth are exclusively linked to its availability to urinary ions and proteins. Intense investigation of the plaque-stone junction is needed if we are to understand the factors leading to the overgrowth process on exposed regions of plaque. Such information should allow the development of treatment strategies to block stone formation in ICSF patients. Patients who form brushite stones, or who form apatite stones because of distal renal tubular acidosis (dRTA), or patients with calcium oxalate stones due to obesity bypass procedures, or patients with cystinuria, get plugged inner medullary collecting ducts (IMCD) which leads to total destruction of the lining cells and focal sites of interstitial fibrosis. These stone formers have plaque but at levels equal to or below non-stone formers, which would suggest that they form stones by a different mechanism than do ICSF patients.

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INTRODUCTION

There may be many types of stone disease, each requiring a specific plan of medical management. The idea that different types of stones are formed in different ways, and that the complications that develop in one group of patients may be different from those of others, is a new concept. Little attention has been paid to how stones are formed in each of the different groups of stone formers, whether stone formation involves pathological changes in the kidney, and if there are specific systemic effects associated with the development of stones of a given type. If “stone disease” is really many separate, distinct stone diseases, then the best medical management for a given patient needs to be tailored to his specific disease, and to treat not just the kidney, but solve the systemic complications as well. Our goal is to characterize the various types of stone disease in order to understand their specific renal abnormalities and systemic complications, so that treatment can be targeted specifically and effectively.
THEORIES OF STONE FORMATION AND GROWTH

Fixed and Free Particle Theories

Three theories of stone formation and growth are currently being investigated. The “Free Particle” concept suggests that crystal nuclei form by homogenous nucleation in the lumen of the nephron under conditions of a phase change (due to increasing supersaturation) in the dissolved salts present in the ultrafiltrate. The theory then states that these nuclei would have to grow to sufficient size to eventually lodge in the lumen of the distal nephron resulting in tubular obstruction. The second theory is termed the “Fixed Particle” model. Like the “Free Particle” model, crystal nuclei also form in the lumen of the nephron, but these crystals then adhere to the apical surface of the tubular epithelium. While a number of mechanisms have been proposed to model this crystal-cell attachment step, the most commonly cited model requires renal cell injury, probably as a result of high tubular oxalate levels. Once the crystal-cell attachment step has occurred, the crystal nuclei would be fixed in position and exposed to the potentially supersaturated ultrafiltrate that would facilitate further growth of these crystals. Clearly both of these theories could result in plugging of the nephron and lead to intratubular calcification, termed tubular nephrocalcinosis. Randall in his historic paper in 1940 described such findings of intraluminal calcification (papillary lesion type II) or nephrocalcinosis in only 23 cases and compared it to the more common finding (204 cases) of interstitial plaque (papillary lesion type I) and placed the type II lesion in a different category than type I [1]. We have shown that patients who form brushite stones [2], or who form apatite stones because of distal renal tubular acidosis (dRTA) [3] or patients with calcium oxalate stones due to obesity bypass procedures [4] or patients with cystinuria [5], plug (tubular nephrocalcinosis) their inner medullary collecting ducts (IMCD) which leads to total destruction of the lining cells and focal sites of interstitial fibrosis. However, it is unclear how the “Free or Fixed Particle” theories could lead to clinical stone formation (i.e. a stone in the renal pelvis large enough to obstruct the ureter) in such stone forming patients, except that either the “Free or Fixed Particle” process or both are occurring in the renal pelvis independent of events in the tubular lumen.

Randall’s Plaque Theory

The third theory suggests that crystals in the urine can become attached to a site of exposed crystalline deposits of interstitial calcium phosphate (termed Randall’s plaque) following loss of the normal epithelial covering of the renal papilla. An anchored nidus of urinary crystals could form as an overgrowth on the interstitial plaque, permitting a fixed stone to form and potentially grow over many years. This theory is explicit in stating that sites of interstitial plaque are the initiating lesion. Alexander Randall presented his data and theory over six decades ago after examining 1,154 pairs of cadaveric kidneys [1]. After carefully opening the renal pelvis of a kidney, he examined
each papilla visually and with a hand lens. His first twenty-seven kidneys were normal in appearance. However, the next kidney revealed an “innocent” appearing lesion that was a cream-colored area near the papillary tip and appeared to be subsurface or subepithelial in location. This lesion was seen in 20.5% of all the kidneys he studied. By light microscopy, the lesion was found to be a plaque of calcium salts deposited in the interstitial tissue and definitely not intraluminal in location. Evidence of inflammation was rarely found. The deposits were localized initially to interstitial collagen material and tubular basement membranes. Subsequently the deposits appeared to fill much of the intertubular space. Chemical analysis of the regions of plaque showed calcium, nitrogen, carbon dioxide and phosphorus. His next major observation was finding a small stone (2 mm) that projected into the lumen of the renal pelvis. It was firmly attached to an area of visible calcium plaque and found to be composed of calcium phosphate. Such stones were found attached to papillae in 65 kidneys. Randall was able to study several stones still attached to the papillary wall and concluded from these studies that 1) attached stones are growing from and are supported by interstitial calcium plaque, 2) the sites of interstitial plaque can lose their epithelial cell covering allowing the plaque to be exposed to the calyceal urine, and 3) that some detached stones have a concave surface with patches of phosphate material (possibly representing the attachment site to the interstitial plaque). Fig. 1 shows an attached stone we have observed in our human studies. This attached stone is probably very similar to what Randall observed. Note that the stone has a concave surface that faced the papilla with a projection of mineral (probably calcium phosphate) and a convex smooth surface that faced the urinary space just like Randall’s description. More recent studies of Cifuentes-Delatte [6] and Daudon and colleagues (7, a paper in this proceedings) have also observed a “footprint” of the papilla as a depressed zone (“umbilication”) on the concave surface of the stone which corresponds to the shape of the tip of the papilla and this concave surface contains mineral detached from interstitial plaque. These findings give support to Randall’s observations and ideas. The observations Randall made from 1,154 pairs of post-mortem kidneys were earth-shattering in their importance and originality. However, what Randall was not able to determine, because of the techniques available to him in the 1930’s, was the composition of the mineral in the plaque, the type of crystalline material that formed right at the site of overgrowth, and the transition of mineral type (i.e. calcium oxalate, uric acid, calcium phosphate) from the site of attachment into the body of the stone.

**RANDALL’S PLAQUE IS UNIQUE TO IDIOPATHIC CALCIUM OXALATE STONE FORMERS (ICSF)**

Our group set out to test the hypothesis that regions of Randall’s plaque develop in unique anatomical sites of the kidney, and that their formation is conditioned by specific stone-forming pathophysiology. To test this hypothesis, we employed state-of-the-art digital endoscopic equipment during percutaneous nephrolithotomy (PNL) for stone removal. We chose to study ICSF patients as our primary group of stone formers in that they represent about 75% of all stone formers [4]. This protocol allowed us to
obtain digital images of all papillae for plaque surface determinations and permitted us to obtain papillary biopsies of regions with and without plaque. During intraoperative endoscopy, we identified irregularly shaped regions of whitish material generally located on the papillary tip and usually surrounding the openings of the ducts of Bellini (Figs. 2A & B), the same plaque material described by Randall. The plaque is located deep to the epithelium (subepithelial) with a smooth outer surface, except where it is pitted due to the removal of an attached stone (Fig. 2B). Light microscopic examination of Yasue stained papillary biopsies revealed minute deposits of mineral first laid down in the basement membrane beneath cells of a specific portion of the urinary tubule, the thin limbs of Henle as they pass near the tip of renal papilla—close to where urine drips into the renal pelvis (Figs. 2C & D). Initially, these mineral deposits are seen as electron dense, spherically shaped objects of about 50 nm, but larger deposits are seen as multilayered spheres with alternating light (mineral layer) and dark (matrix layer) rings or bands (Fig. 2C, insert). The individual deposits appear to then coalesce on the collagen bundles in the interstitial space and become embedded in an electron dense matrix material generating islands of plaque. These islands extend down to the basal
FIGURE 2. Endoscopic and histologic images of Randall’s plaque in a ICSF patient. Panel A is a light microscopy photomicrograph showing a papilla from an ICSF patient that possesses a large cream-colored area (arrow) at the tip of papilla. This is the interstitial plaque material Randall discovered. Panel B shows a papilla from another ICSF patient imaged by endoscopic digital equipment at the time of a PNL procedure for stone removal. Note numerous irregular whitish areas (arrows) of Randall’s plaque scattered around the papilla. Histologic examination of a light microscopic Yasue stained section of a papillary biopsy with minimal plaque shows spherically shaped, brown-black deposits only in the basement membranes of the thin loops of Henle (panel C, arrow). The insert at the lower right side of panel C shows such a deposit by transmission electron microscopy. Note the multilayered appearance with alternating light and dark rings. Panel D shows a denser region of plaque where the individual deposits are embedded in a continuous layer of calcified matrix material.
side of the epithelium and encase the ducts of Bellini. In order to determine the mineral composition of the interstitial plaque, we developed a protocol to analyze these small sites of crystalline material using μ-Fourier transform infrared microspectroscopy (μ-FTIR) (Fig. 3). The mineral in the plaque was determined to always be biological apatite (calcium phosphate). In addition, we have identified osteopontin as one of the proteins forming the matrix layers [8]. Other potential matrix proteins are bikunin, prothrombin fragment 1, and fetuin-A. Ryall and colleagues have reviewed the macromolecules relevant to stone formation in a paper in this proceedings [9]. No evidence of cell injury, inflammation, interstitial fibrosis or intratubular crystalline material was detected in any of the ICSF papillary biopsies.

Our biopsy and mapping studies have now included not only ICSF patients but patients with brushite stones [2], those who form apatite stones because of distal renal tubular acidosis (dRTA) [3], patients with calcium oxalate stone due to obesity bypass procedures [4], and patients with cystinuria [5]. A table in Coe’s paper in this proceedings summarizes the different histopathologic changes for each these stone formers [10]. Randall’s plaque is unique to ICSF while the other stone forming groups show varying degrees of plugging of their inner medullary collecting ducts (IMCD), which leads to total destruction of the lining cells and focal sites of interstitial fibrosis. The non-ICSF stone forming groups that we have studied so far have regions of Randall’s plaque equal to or below the levels of non-stone formers.

FIGURE 3. New μ-FTIR method to determine mineral type in a histologic section. Our group developed a new histologic approach to analyze small mineral deposits in kidney tissue [1]. Single 4-micron sections of paraffin embedded tissue were cut and placed on low-E glass for μ-FTIR analysis. Such a section is seen in panel A through the microscope of a Perkin-Elmer AutoImage infrared microscope interfaced to a Perkin-Elmer Spectrum 200 Fourier transform spectrometer at a low magnification. This section was stained by the Yasue method so the small brown-black regions represent calcium deposits (denoted by the asterisks). This same section is seen at a much higher magnification in panel B.
STONES IN ICSF ARE ATTACHED TO THE PAPILLUM

Counting Attached Stones

Randall was convinced that interstitial plaque was a prerequisite for stone formation and growth. Unfortunately, he was not able to collect kidneys from carefully selected groups of stone formers, so his theory was stated in very general terms. That is to say, he was not able to determine if all stone formers made plaque or if only a selected group presented such a histopathologic characteristic. Our research program has allowed us that selectivity. So we began collecting data at the time of PNL to determine if all stones in ICSF patients were attached to a papillum, and if so, were they attached at regions of

FIGURE 4. Endoscopic and $\mu$-CT profile images of a small stone attached to a papilla tip. Panel A shows the endoscopic view of a 0.5 mm attached stone surrounded by white plaque. This stone is highlighted in panel B while the plaque is enhanced in panel C. The entire stone-tissue sample is seen in panel D immediately after biopsy retrieval of this sample. The arrow indicates the stone while the arrowheads point out a large region of white plaque that was associated with this stone. The plaque-stone interface is clearly seen in the $\mu$-CT profile image of this sample (E). Again the stone is indicated by the arrow and the regions of plaque by several arrowheads.
Randall’s plaque. Figs. 4A-C show such a stone fixed to the papillum at a site of plaque. Matlaga and colleagues show similar images of attached stones in their paper in this proceeding [11]. The hypothesis of this study stated that if Randall’s plaque were a prerequisite to future stone formation in ICSF patients, all or most observed stones would be seen attached to the papilla at sites of plaque. Matlaga presented evidence at this symposium in support of this hypothesis in that 11 of 23 patients studied retrospectively had attached stones, with 91% of these stones at sites of plaque [11,12]. These numbers are much higher than those reported by Randall. We are conducting a new prospective study to determine if all stones are indeed attached and, if so, are they attached by means of plaque. Such a study requires the surgeon to inspect and video record all papillae prior to stone removal. This same study would need to be repeated in other types of stone formers to determine the validity of the hypothesis. Those stone forming groups that do not make interstitial plaque should form stones by a process very different than ICSF patients. Those with intraluminal plugging of the ducts of Bellini (like cystine and brushite stone formers) may have pelvic stones attached to the distal ends of such plugs and thereby mimic the Randall’s type II papillary lesion [1]. Other types of stone formers may simply form free-floating stones in the renal pelvis.

**Stone-Plaque Junction**

As stated earlier in this paper, those attached stones that have been plucked off from the papilla, generally have been found to have an irregular concave surface that fit against the papilla and a smooth convex surface that faced the urinary space (Fig. 1). Often the concave surface was found to have a stock or umbilicus (Fig. 1A, arrow) thought to be made up of plaque and that this plaque was in continuity with the interstitial plaque before the stone was detached from the papilla. Daudon and colleagues have reported at this symposium an analysis of 5,401 stones with an umbilication [7]. They found the main mineral component of this plaque material to be carbapatite (91%) with lesser amounts of amorphous carbonated calcium phosphate, sodium hydrogen urate or uric acid. Matlaga and colleagues using μ-CT have also examined the mineral content of three stones that were observed during PNL to be attached to sites of Randall’s plaque [11]. Their proceedings report shows each of these stones to have a small region of apatite in an otherwise large volume of calcium oxalate. These observations again support the ideas laid out by Randall [1]. Thus, calcium oxalate stones in ICSF patients appear to grow like stalactites adherent to the tips of kidney papillae and, typically, are made of layers of calcium oxalate surrounding a core of apatite.

All of these findings lead us to the next critical question: What is the mineral that forms the overgrowth on the interstitial plaque? By answering this question, we will have insight into the initiating event for stone growth on interstitial plaque, which can then be possibly used to determine ways to block this process from occurring. Randall desperately attempted to address this question but failed. We presently have the techniques necessary to carry out such an analysis. The first step requires the selection of a documented ICSF patient and one that requires PNL to remove a stone burden.
At the time of surgery, the smallest (< 0.5 mm), visible attached stone should be selected and biopsied with its underlying papillary tissue so that the entire stone-plaque interface can be sectioned (Fig. 4). If the stone is small, one should be able to cut the sample without demineralizing the stone to allow for μ-FTIR analysis and still keep the plaque/stone junction intact and minimize tissue/stone shredding. A set of serial sections should be prepared for sequential analysis by light microscopic histochemistry and μ-FTIR. Similar samples will need to be demineralized and studied by transmission electron microscopy. Such experiments are currently on their way in our laboratory.

**DISRUPTION OF THE UROTHELIAL BARRIER**

Randall’s theory of stone formation also requires erosion of the epithelium over sites of plaque as a means of exposing the interstitial plaque to the urine in the renal pelvis. Such an event would allow minerals and proteins from the urine to attach to the regions of exposed plaque, and initiate stone formation. To date no one has actively worked on this problem. Bergsland has a paper in this proceedings detailing the known properties of the papillary epithelium, which again is sparse [13,14]. She presents several pathophysiological factors that might cause the epithelial barrier to become leakier and thereby induce a loss of epithelial integrity. Additional mechanisms of epithelial cell injury and loss are presented in Fig. 5. In panel A, the interstitial plaque positioned at the basolateral cell surface of the urothelial cells induces alterations in the integrity of the apical surface of these cells allowing attachment of crystals to the cell surface. In this model, the epithelial layer is not lost but becomes incorporated into the stone-plaque

**FIGURE 5.** Six different models illustrating potential pathways for the loss of epithelial integrity at the renal papilla. The star represents crystal, while the irregular gray structures show sites of Randall’s plaque at the basolateral cell surface. See text for details.
complex. In panel B, urinary crystals attach to and damage the epithelial cells allowing these crystals to merge with the interstitial plaque. The third model (panel C) suggests that calcium and oxalate ions enter the epithelial cells, form intracellular crystals which then injure these cells and allow the crystals to merge with the interstitial plaque. In panel D, the interstitial plaque grows through or between the epithelial cells and into the urinary space allowing overgrowth to occur. The fifth model (panel E) suggests that the interstitial plaque triggers apoptosis or necrosis of the overlying epithelial cells allowing the plaque to be exposed to the urine and subsequent overgrowth. In panel F, a random apoptotic event occurs in epithelial cells overlying a site of interstitial plaque, resulting in a loss of those epithelial cells and exposing the plaque to the urine. It may be that none of these models are useable; however, the issue of how a compromise in epithelial cell integrity might occur during the process of kidney stone formation needs serious investigation.

**SUMMARY**

In summary, Randall’s original thoughts on the mechanisms for kidney stone formation appear valid for idiopathic calcium oxalate stone formers (ICSF) but not for most other stone forming groups. Our current studies on selected groups of human stone formers using intraoperative papillary biopsies have shown overwhelming evidence for the presence of Randall’s plaque in ICSF patients and that stone formation and growth are exclusively linked to its availability to urinary ions and proteins. Intense investigation of the plaque-stone junction is needed if we are to understand the factors leading to the overgrowth process on exposed regions of plaque. Such information should allow the development of treatment strategies to block stone formation in ICSF patients. Stone formation in brushite, dRTA, cystine and intestinal bypass stone formers appears to occur by other mechanisms, which are not yet well understood.

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