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STUDIES ON STRUCTURE OF CALCIUM OXALATE MONOHYDRATE RENAL PAPILLARY CALCULI. MECHANISM OF FORMATION

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Abstract

A scanning electron microscopy study of the ultrastructure of 18 calcium oxalate monohydrate papillary calculi was performed with the purpose of establishing the main steps in calculus formation. It is concluded that these calculi originate in a "core" located near the central part of the calculus. Significant quantities of organic matter as well as calcium phosphates can be found in the "core" and at the surface of adhesion to the papilla and, in some cases, fibers and calcified tubules can also be found in the contact zone. In no case did this material affect the crystalline structure of the calculi, indicating that its formation follows the calculus genesis. The study of the compact columnar zone revealed that its formation starts in a practically continuous surface formed by organic matter and crystals that surround the core. This layer favors the growth of oriented calcium oxalate monohydrate crystals upon it. Based on these observations, a feasible mechanism of papillary calcium oxalate monohydrate calculus formation is proposed.

Key Words: Calcium oxalate monohydrate, renal papillary calculi, structure, genesis.

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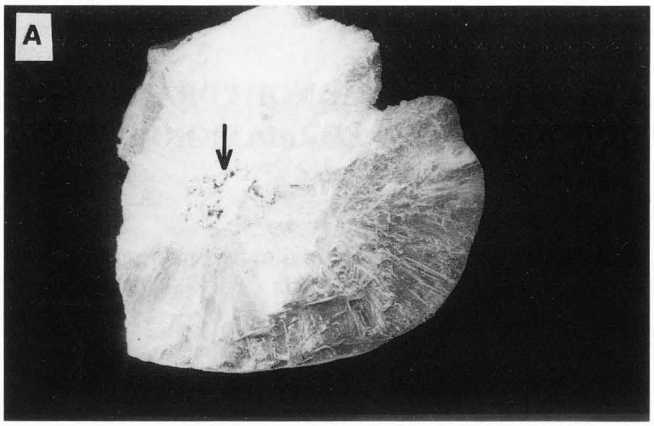
Introduction

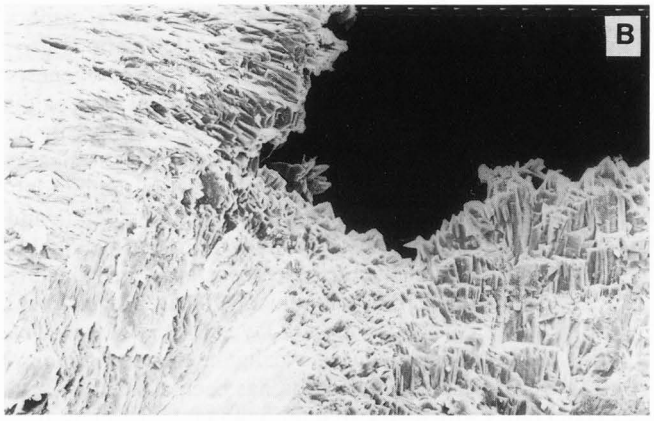
In the 1930's, Randall published several papers describing the presence of papillary calcifications proposing them as inducers of renal calculogenesis (Randall, 1937, 1940). The most common of such calcifications, named Randall's plaque, is claimed to be formed by subepithelial deposits of calcium phosphates measuring 1 to 2 mm in diameter located in the interstitial tissue of the papillae. If these deposits ulcerate and are exposed to calyceal urine, they initiate the calculus formation. Obviously, if these deposits are responsible for the calculus origin, they must be located at the focal point of the concentric growth layers of the stone. The role of Randall's plaques in calcium oxalate monohydrate (COM) calculi formation has been studied by several authors with different opinions about the importance of this lesion as a major cause of renal stone formation. Thus, a number of researchers believe that only a few stones might originate by this mechanism (Anderson, 1969, 1979; Resnick and Boyce, 1979). Others (Prien and Frondel, 1947; Cifuentes *et al.*, 1987) assign an important role to the subepithelial plaques located in the interstitial regions of the papillae. Therefore, the aetiology of stone disease has not yet been totally elucidated. In this paper, we study the fine structure of COM papillary calculi in an attempt to clarify the mechanism of calculi formation. Such knowledge is fundamental to the development of further efficient medical treatments of the disease.

The aim of this paper is to perform a detailed analysis of the crystalline organization and structure of the papillary COM calculus. This analysis must allow us to determine the mechanism, and consequently the main steps, in COM calculus formation. The study will be organized considering three main parts of the calculus: **a**) the "core"; **b**) the surface of calculus adhesion to the papilla that coincides with the calculus concave zone, in which some authors (Randall, 1937, 1940; Cifuentes *et al.*, 1987) localize the Randall's plaque; and **c**) the compact columnar structure.

Materials and Methods

Renal stones from 18 patients were used in this





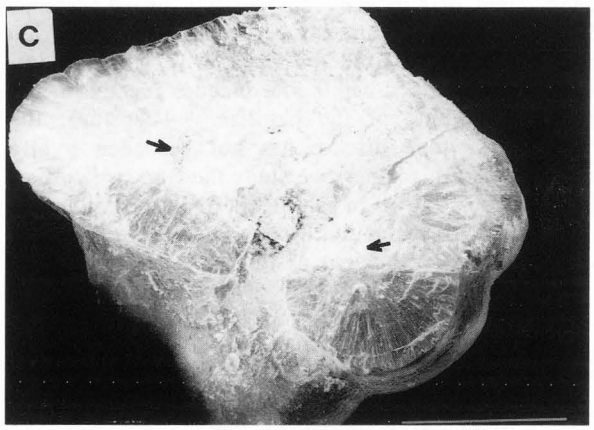


Figure 1. Cross-section of two typical COM papillary calculi. (**A**) The position of a unique central "core" is evidenced. Bar = 2 mm. (**B**) Magnified view of a cross-section of the above stone corresponding to the zone of attachment to the papilla in which tightly arranged columnar crystals forming the striated layer can be seen. Bar = 100 μm. (**C**) The position of two "cores" in this stone is marked by arrows. Bar = 1 mm.

study. Table 1 presents the main characteristics of the stone collected.

The composition of the calculi was determined by infrared (IR) spectroscopy with potassium bromide discs using a Perkin-Elmer 683 spectrometer. Stones and stone fragments were studied with a Hitachi S-530 scanning electron microscope (SEM) equipped with an EDAX X-ray microanalysis attachment.

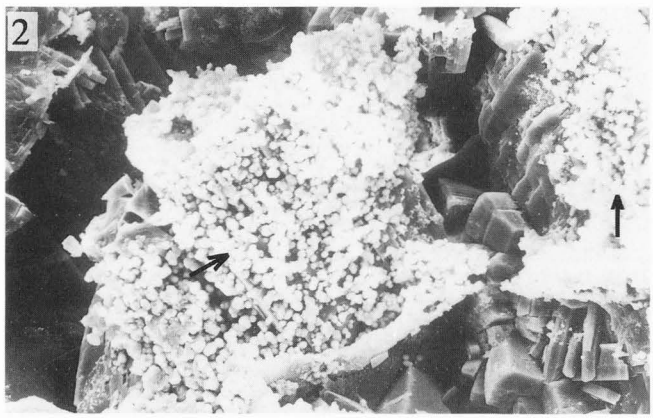
Fresh and carefully collected papillary COM calculi were selected exclusively for the results presented in this paper. Only spontaneously passed renal stones, composed mainly of whewellite, and with a rough-surface concave face (see Fig. 1), were considered. In our case, these calculi constituted about 20% of all passed renal stones. Once collected, these calculi were rinsed with water, dried, and stored at room atmosphere. For SEM, the whole calculus or fractured fragments were fixed on a metallic plate using silver paint and were gold coated.

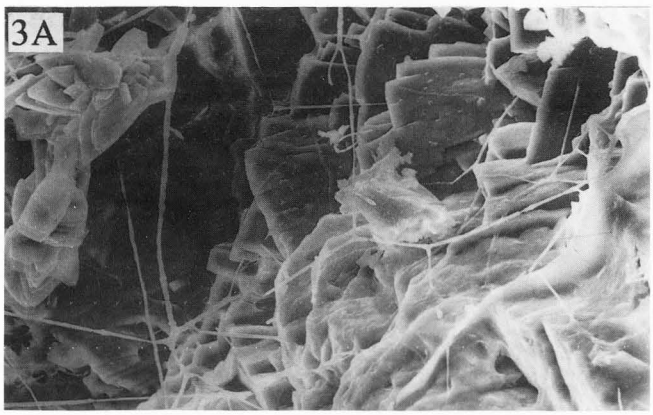
The zone of contact between the calculus and the papilla was first studied by stereoscopic microscopy and then by SEM. Next, the calculi were fractured into two nearly equal fragments along a plane perpendicular to the union zone with the papilla to obtain a fracture surface that gives us the maximum information about the structure of the zone of union with the papilla and of the localization of the cores. These stone fragments were also studied by stereoscopic microscopy and by SEM.

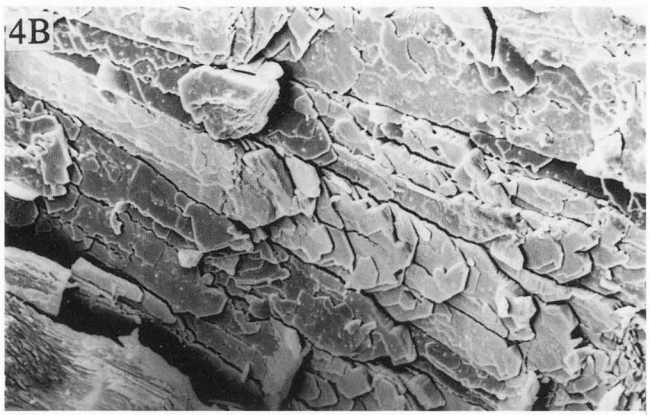
The main urinary biochemical parameters were determined for all patients. Subjects were on free diet at the time of urine collection and none of them were undergoing pharmacologic treatment of any kind. Metabolic evaluation included calcium, magnesium, phosphate, oxalate, uric acid, creatinine, and citrate. Urinary calcium and magnesium were determined by atomic absorption spectroscopy (using Perkin-Elmer 703 spectrometer) in the presence of 0.5% lanthanum; phosphate and creatinine were determined by applying the well known photometric methods based on the formation of the yellow phosphomolybdovanadic acid (Marczenko, 1976) and on the kinetic reaction with picrate without deproteinization (Pesce and Kaplan, 1987); uric acid, citrate, and oxalate were determined by the Boehringer Mannheim kits number: 704156, 139076 and 755699, respectively.

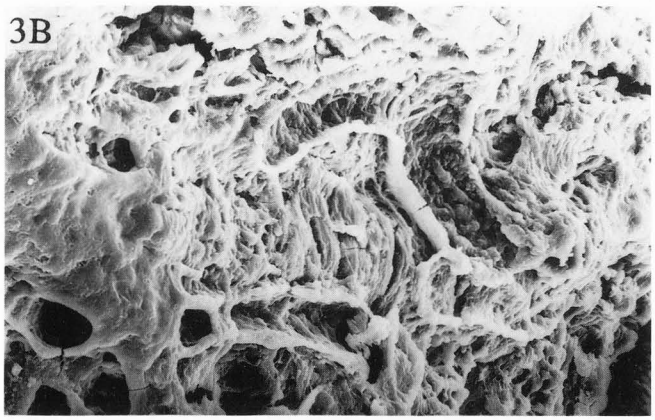
Results

The results of the study of the papillary COM calculi are shown in Table 1 and Figures 1-4. As can be seen, in a number of calculi, several "cores" were detected and in some cases the presence of phosphates was identified (Fig. 2). It is important to keep in mind that the detection of only one "core" did not imply the impossibility of the existence of others. It is possible that due to the particular feature of the calculus fracture, a number of "cores" remained hidden. The same can occur when phosphates were not detected in the "core". The study of the calculus zone corresponding to the implantation on a papilla, in almost all cases, revealed the









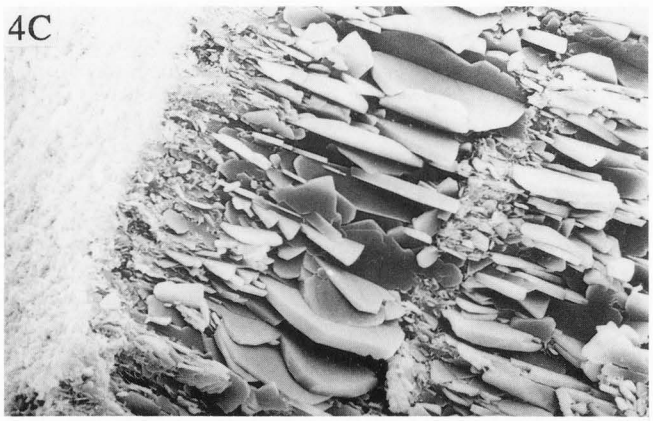


Figure 2. Spherulitic crystals of calcium phosphate (determined by EDAX) found in a stone "core" (marked by an arrow). COM crystals can be seen behind such spherulitic crystals. Bar = 100 μm.

Figure 3. Images of the surface of calculus adhesion to the papilla. **(A)** Organic fibers. Bar = 50 μm. **(B)** Organic matrix and calcified tubules. Bar = 100 μm.

Figure 4. Images of the compact columnar structure of COM papillary calculi. **(A)** Limit of a "core" where a continuous surface formed by organic matter and crystals can be appreciated. "Rosette" structures can be seen in the core (marked by arrow). Bar = 200 μm. **(B)** and **(C)** Magnified view of the columnar structure of two different stones. Bars = 20 μm (B) and 50 μm (C).

TABLE 1: Main characteristics of the studied COM papillary calculi.

Patient No.	sex	age	Calculus size[a]	No. of cores observed	Phosphates in the core	Characteristics of the union zone with the papilla
1	M	41	3 x 3 x 4	1	-	No other substances except COM
2	F	40	4 x 4 x 3	2	detected	No other substances except COM
3	M	44	5 x 2 x 3	1	-	A lot of organic matter
4	F	40	3 x 2 x 3	1	detected	A lot of organic matter and fibers
5	F	16	4 x 2 x 2	1	-	A lot of organic matter and phosphates
6	M	54	2 x 3 x 3	1	-	A lot of organic matter
7	F	59	5 x 5 x 5	2	detected	A lot of organic matter, phosphates and calcified tubules
8	M	50	4 x 3 x 2	1	-	A lot of organic matter
9	M	61	5 x 3 x 3	2	-	A lot of organic matter
10	M	68	7 x 3 x 4	3	-	A lot of organic matter
11	M	35	5 x 3 x 4	3	detected	No other substances except COM
12	F	61	2 x 2 x 2	3	-	A lot of organic matter
13	M	42	4 x 2 x 5	2	-	A lot of organic matter, phosphates and calcified tubules
14	M	54	4 x 4 x 3	1	detected	A lot of organic matter, phosphates and fibers
15	M	41	3 x 2 x 2	1	-	Phosphates, a lot of organic matter and calcified tubules
16	M	48	3 x 2 x 4	3	detected	A lot of organic matter
17	F	39	4 x 3 x 3	2	detected	Bacteria and organic fibers
18	M	53	3 x 3 x 3	2	detected	Phosphates and organic matter

[a]base x base x height in mm x mm x mm M = Male; F = Female

presence of important quantities of organic matter and in some cases the presence of organic fibers, phosphates, or even calcified tubules (Table 1 and Fig. 3). Micrographs of the compact columnar structure (Figs. 4A-4C) of COM calculi show that these crystals have a main growth direction perpendicular to a base surface.

Table 2 summarizes the main urinary biochemical parameters of 18 patients with papillary COM calculi, and shows that the most frequent biochemical alteration corresponded to hypocitraturia (in approximately 30% of individuals), whereas the following were detected only in a few cases: hypercalciuria, hyperoxaluria, hyperphosphaturia, hyperuricosuria.

Discussion

In view of the observed general structure of the COM papillary calculus (see particularly Fig. 1), the origin of these calculi obviously takes place in a "core", as has been previously described (Grases *et al.*, 1993b; Söhnel and Grases, 1993). Nevertheless, as observed (Table 1), generally several "cores" can be identified in a normal COM papillary calculus. Considering the calculus geometry, it can be postulated that the first "core" formed must be located near the central part of the calculus and if there were several in such a position, the first one must be the closest to the papillary zone. The presence of several cores formed at different times near the papillary zone determines the morphology of the calculus and implies the presence of a cavity in the zone of union between the calculus and the papilla. Thus, the appearance of new cores in the vicinity of the papillary walls (Figs. 5D and 5E) can be considered to produce a displacement of the first formed core, generating the cavity.

The presence of organic matter in the calculus cavity (zone of implantation on a papilla) can be attributed to the necrosis of a small area on the tip of the papilla in which the calculus is formed, which can be acute as the presence of calcified renal tubules confirmed. Accordingly, it is interesting to consider that important quantities of organic matter can also be found in the "core", thus supporting the idea of an area between the "core" and the papilla as the first sites of the calculus formation. Cellular membranes have been demonstrated to have an active role for nucleation of calcium phosphate (Boskey, 1981). Table 1 shows that when calcium phosphate appears on the surface of adhesion to the papilla, it is also generally detected in the "core". In fact, the presence of calcium phosphate in a significant proportion of COM monohydrate calculi has already been reported (Grases *et al.*, 1993b; Khan and Hackett, 1993). If the Randall's plaque is to be considered as responsible for the calculus origin, the material containing phosphorous, calcium and a certain amount of organic matter without a well defined structure, located in the

TABLE 2. Main urinary and serum parameters corresponding to "pure" COM stone formers (serum values between parenthesis).

pat. no.	pH[a]	Diuresis[b] (ml)	Ca[C] mg/l	Mg[C] mg/l	Phosphate[C] mg/l	Oxalate[C] mg/l	Uric acid[b] mg/l	Creatinine[C] mg/l	Citrate[C] mg/l
1	5.42	1500	97 (100.6)	71.1 (20.3)	447 (33.4)	20.8	495 (53.8)	887 (11.0)	516
2	6.50	1820	102 (91.3)	37.5 (19.5)	579 (35.1)	12.7	466 (60.6)	733 (11.1)	175
3	5.49	1130	73 (93.4)	84.8 (18.3)	781 (35.3)	22.8	360 (46.8)	815 (7.7)	249
4	5.60	2500	58 (97.3)	23.4 (17.1)	604 (27.6)	16.0	340 (45.6)	370 (9.0)	220
5	6.68	965	208 (99.3)	71.6 (18.1)	1034 (40.1)	20.6	507 (35.3)	1327 (7.4)	654
6	6.89	1965	171 (93.7)	56.0 (19.9)	263 (26.5)	13.6	386 (53.0)	566 (9.6)	443
7	6.06	1700	88 (94.5)	40.4 (19.6)	718 (27.7)	14.7	308 (43.6)	765 (9.3)	445
8	5.94	1400	101 (94.2)	74.5 (18.9)	716 (33.4)	27.3	398 (50.6)	795 (9.3)	361
9	5.74	1380	162 (99.3)	58.6 (21.9)	489 (21.6)	25.3	348 (75.8)	1360 (12.1)	566
10	5.29	2200	44 (97.7)	25.7 (18.7)	371 (31.4)	16.9	310 (81.4)	652 (12.0)	249
11	5.84	2975	122 (96.5)	34.0 (19.5)	534 (34.2)	11.7	345 (57.8)	534 (10.7)	117
12	6.08	1230	97 (97.5)	55.2 (19.4)	430 (24.0)	35.4	506 (51.9)	1150 (9.4)	376
13	6.56	2000	93 (92.1)	55.2 (20.1)	402 (32.1)	17.2	426 (56.8)	704 (11.7)	322
14	5.28	1600	79 (97.2)	59.7 (20.2)	407 (26.0)	17.2	302 (43.9)	962 (11.0)	205
15	5.40	1650	102 (93.0)	52.0 (17.9)	498 (35.9)	13.3	430 (62.8)	933 (10.9)	349
16	5.55	1275	145 (92.0)	71.9 (21.1)	570 (33.5)	18.9	452 (48.0)	1170 (11.5)	400
17	5.93	1170	193 (100.4)	59.0 (19.7)	740 (28.9)	38.0	412 (43.8)	950 (8.6)	292
18	5.13	1815	105 (94.3)	53.8 (18.6)	657 (34.1)	17.2	448 (58.1)	735 (11.1)	418

Normal excretion values in our laboratory: Ca 149 mg/l; oxalate 23 mg/l; uric acid 460 mg/l; phosphate 770 mg/l; Mg 62 mg/l; Citrate 450 mg/l. Diuresis 1.31.

[a]Determination carried out in urine accumulated over a 2-hour period following overnight fast.

[b]Determination carried out in 24-hour urine. [C]Determination carried out in 24-hour urine collected on HCl.

concave zone of the studied COM papillary calculi, cannot be considered as an authentic Randall's plaque, although it can be easily confused as such. Those results would then be in agreement with authors postulating that the existence of authentic Randall's plaque (as subepithelial calcification that initiates calculus growth) is not a frequent phenomenon (Anderson, 1969, 1979; Prien, 1975; Resnick and Boyce, 1979). If the cause and origin of the calculus formation were assigned to the authentic Randall's plaque, it should play a relevant role in the calculus crystalline organization, this has seldom been seen. Although, in our study, several apparent Randall's plaques can be detected, in no case did they exercise any influence on the crystalline structure of the calculi, thus demonstrating its formation after the calculus genesis, for in these cases, it is the core or cores that determine the calculus crystalline structure.

Another interesting question regarding the structure of COM papillary calculi is that of the compact columnar structure that surrounds the "cores", both constituting the major part of the calculus and being responsible for its hardness. If the fine structure of a core is considered in detail, the central part is found to contain primary COM aggregates or single crystals (generally with the {110} crystallographic face with maximum surface, minimum growth) with a great void space between them, whereas the edge of the core is composed of a practically continuous surface formed with organic matter, crystals, and crystal detritus (Fig. 4A). This layer enhances the growth of COM crystals on its surface. The simultaneous growth of crystals with different nuclei orientation, can form an oriented aggregate of crystals. The initial crystals are formed on a given surface and they will grow according to their orientation with respect to the common base and to the different growth rates of each surface. The greater the inclination of the crystals to their base, the sooner their growth stops, because they collide with other crystals formed around them. Only those crystals having an orientation in the direction of the main growth perpendicular to the base surface, will continue growing, and will finally form a packed columnar crystals aggregate whose base is the initial surface (Figs. 4B and 4C).

Based on the above considerations, we conclude that the formation of a significant number of COM papillary calculi is initiated by a first and decisive step where

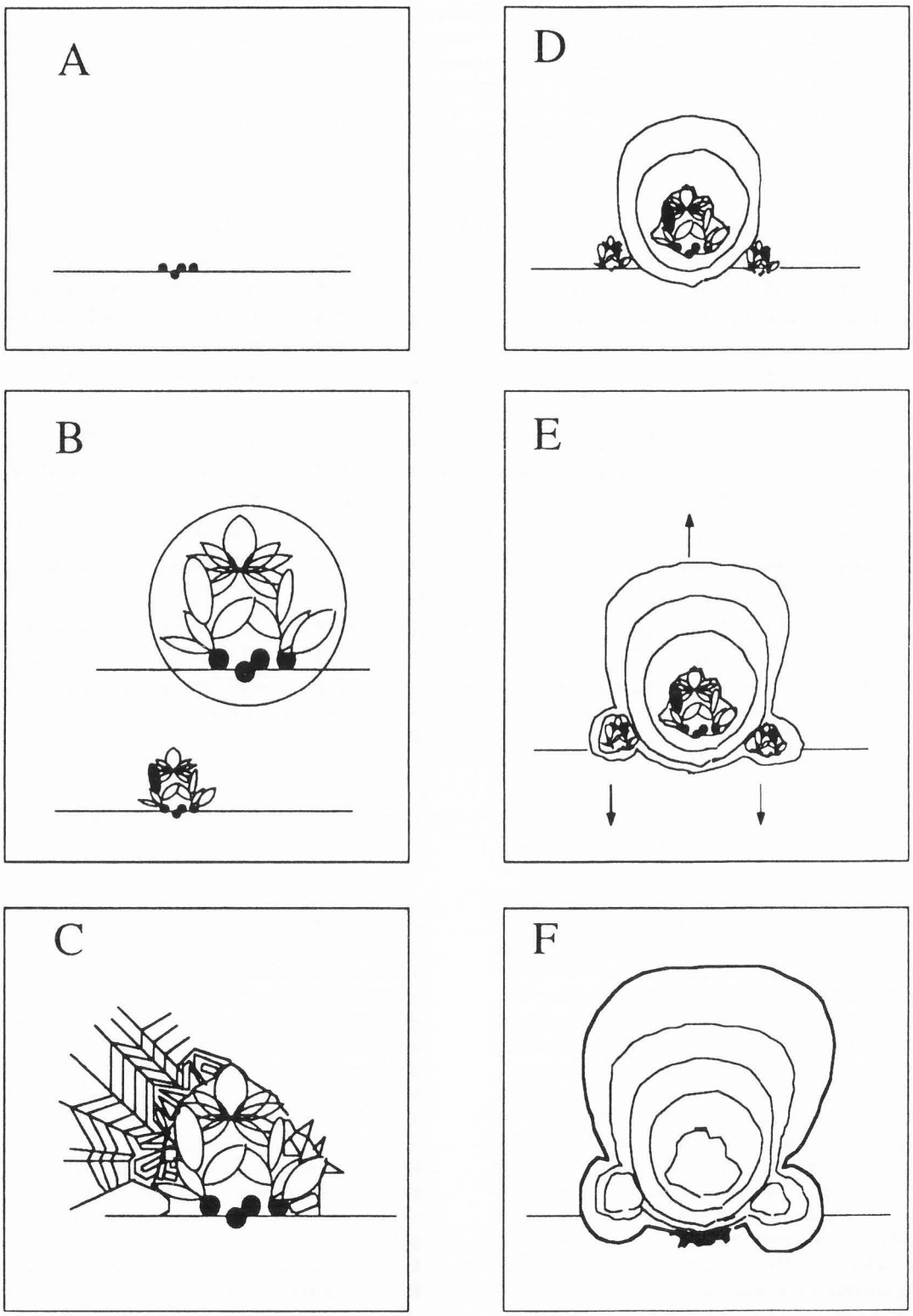


Figure 5. Proposed mechanism of COM papillary calculi formation. (**A**) Heterogeneous nuclei formation. (**B**) First "core" development. (**C**) Progress of the compact columnar zone. (**D**) Appearance of new "cores". (**E**) Formation of successive layers. (**F**) Definitive calculus morphology.

some foreign particles (calcium phosphates, uric acid, etc.) appear and attach themselves to the walls of the renal papilla (Fig. 5A). These solid particles, according to the theories and evidence (Grases *et al.*, 1993a; See and Williams, 1992) would be formed on sites in which the protective continuously renewed glycosaminoglycan layer, that lines the inner renal walls, was damaged, destroyed, or perhaps just reduced. Such particles would act as heterogeneous nucleants of COM and would induce the formation of loosely arranged twinned and intergrown plate-like crystals and/or particles of "rosette" structure with considerable void space among crystals, thus constituting the "core" of a COM calculus (Fig. 5B). The increase of the material (crystals and organic matter) at the "core" boundary stops growth, generating a continuous layer on which columnar COM crystals grow (Fig. 5C). Successive layers of such compact structure increase the calculus size. Formation of new "cores" close to the wall of the renal papilla, would induce the formation of the typical concave cavity characteristic of papillary COM calculi, where apparent Randall's plaques frequently appear (Figs. 5D, 5E, and 5F).

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Discussion with Reviewers

S.R. Khan: Could organic fibers shown in Figure 3A be bacterial or fungal in origin since stones were washed and stored at room temperature, an ideal environment for such contamination?

O. Le May: In **Materials and Methods**, nothing is mentioned about fixation of the samples, the stones being only rinsed and air-dried before their observation by SEM. Under, these conditions, do you consider that organic matter and fibers could be well preserved, and therefore, identified?

Authors: Only fresh spontaneously passed renal calculi were included in the presented study. In spite of this, organic fibers could appear as a consequence of bacterial contamination. However, in such cases, when the fibers were observed (e.g., Fig. 3A), the presence of crystalline structures grown on the fibers (this is only possible during calculus formation) and/or the presence of fibers in zones preserved from environmental contamination, such as, the inner cores, was also detected. Thus, the presence of fibers is not an artifact.

W.G. Robertson: Why should uric acid or calcium phosphate crystals form in the urines of the patients described in this study and why should such crystals adhere to the papillary tip?

Authors: The occurrence of urinary pH values less than 5.5 insolubilizes uric acid and induces uric acid crystal formation. This formation is favored by higher concentrations of uric acid and by the absence of crystallization inhibitors, such as glycosaminoglycans. Urinary pH values above 6 favor insoluble calcium phosphate formation, enhanced at higher calcium and phosphate excretion levels and diminished in the presence of higher quantities of crystallization inhibitors such as citrates. In the

calculi of the patients described in our study, calcium phosphate crystals appear as the most frequent heterogeneous nucleator, as is demonstrated by their presence in the core. Nevertheless, in some cases, in spite of the clear detection of calcium phosphates in the core, the measured urinary pH values were less than 6 (patients no. 4, 11, 14, 16, 18). This apparent contradiction can be explained by considering that the tabulated pH value is a representative value for each individual and during the day the existence of higher pH values is possible. Also, pH values can vary seasonally (i.e., due to dietary changes) or due to circadian rhythm. Moreover, these pH values were measured some days after stone expulsion and consequently do not necessarily correspond to the same values that were present during the stone formation.

Finally, taking into account the kidney hydrodynamic conditions, after the appearance of a calculus, the development of encrustations, covering almost the entire inner surface exposed to urine, could be expected. However, it was found that when crystalline formations appear, they develop only on a limited number of isolated sites. Therefore, a protective layer that efficiently prevents nucleation and fixation of crystals, has to be assumed to cover the urinary tract walls. Crystals then can develop only on sites where the protective layer is destroyed, damaged or perhaps just reduced. Experimental observations support both the existence of a protective layer and the confinement of crystal formation to areas of damaged layer (Grases *et al.*, 1993; See and Williams, 1992). We can, therefore, safely conclude that the majority of COM stone nuclei are formed by crystals originating directly on sites of the upper urinary tract wall with a damaged, or just reduced, protective layer.

W.G. Robertson: What percentage of all stones do the authors consider to start in the way postulated in this paper?
O. Le May: During your sample selection, did you determine the proportion of papillary calculi with regard to other renal stones?
Authors: In our case, the percentage of COM papillary calculi compared to other spontaneously passed renal stones was approximately 20%. This implies that in these cases, the stones start their formation through a mechanism similar to that postulated in this paper.

O. Le May: In one case (no. 17) you observed bacteria. Do you think that these bacteria could also participate in the formation of the calculus?
Authors: Bacteria was detected only in the papilla union zone and not in the core. Consequently, it is probable that bacteria appeared as a consequence of some local infection provoked by the injury caused by the presence of the calculus and not vice-versa. Nevertheless, it is clear that bacterial attack constitutes an important portion of organic matter and detritus that contributes to the calculus growth.

O. Le May: In Figure 5D, new cores appear after an initial central core, but do you think that a calculus with several cores could be also the result of the "fusion or aggregation" of different calculi formed simultaneously?
Authors: It is possible that in some cases, several cores growing simultaneously in near proximity, generate by "fusion", a unique calculus. This probably implies the existence of large areas with a damaged or reduced glycosaminoglycans layer, which would generate calculi with a flat morphology.

O. Le May: Recently, calcium oxalate dihydrate (COD) crystals have been identified with SEM on the rough surface of COM stones (Iwata *et al.*, Scanning Microsc, **6**: 231-238, 1992). Have you observed such surface COD deposits in your calculi?
Authors: No calcium oxalate dihydrate (COD) crystals have been detected in the rough surface of the 18 spontaneously passed COM calculi studied in this paper. Nevertheless, we have observed the COM and COD association on several other occasions. This means that, in agreement with the opinion of Iwata *et al.* (1992), such association appear infrequently, and some abrupt change must occur to start COD growth after formation of the COM layer.

W.G. Robertson: Some kit methods, particularly for oxalate, are not ideal for good quantitative work. Many workers have found that, unless extra precautions are taken, the oxalate-kit method underestimates oxalate excretion. Indeed, I suspect that the oxalate values in this study are underestimated since I would expect higher urinary oxalate excretions to accompany the relatively low urinary calcium excretions reported. Otherwise, there is no explanation for the formation of calcium oxalate crystals.
Authors: Certainly, the exact determination of urine oxalate content still remains as an important analytical problem and the kit method used in the present paper is no exception. Nevertheless, before selecting this methodology, we assayed different kit procedures and indeed found the enzyme oxalate oxidase underestimation you mention. The recovering studies, using kits based on the oxalate descarboxilase as initiator enzyme (the kit used in the paper), were quite acceptable. On the other hand, it is true that in the studied patients presented in this paper, only some very occasional hypercalciuria and/or hyperoxaluria and/or hyperuricosuria could be detected. Thus, these stone-formers could be classified as "idiopathic" if only the urinary biochemical parameters were considered. Nevertheless, recent papers have clearly demonstrated, as stated in the answer to the second question, that a non-adequate renewed solid surface, independently from its nature, in contact with normal urine, sooner or later, develops stone forming compounds. The presence of permanent solid deposits of calcium phosphates or uric acid then can induce COM calculi formation in such cases in spite of the presence of absolutely normal urine.