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A Study of the Ultrastructure of Urinary Calculi by Scanning Electron Microscopy

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Summary. A study of urinary stones obtained from patients after surgery in the Medical College Hospital, Trivandrum, under the scanning electron microscope showed the presence of calcium oxalate and calcium biphosphate crystals as the main constituents. However, the pattern of the different phases of crystal growth was not uniform. Within the crystal lattice, fibrous structures, possibly of protein matrix, were invariably observed. Electron microscopy may be usefully adapted as a particularly suitable method for ultramicroscopic investigation of the fine structure of urinary stones including single crystal surface structure, section of urinary calculi and for possible presence of hitherto unknown components within the calculus.

Key words: Urinary calculi, Stone ultrastructure, Electron microscopy.

Introduction

Several reports are available in the literature of both qualitative and quantitative analysis of urinary stones, both in India and outside [5, 6, 9]. The advent of the scanning and optical electron microscopes in recent times offers a new avenue of investigation for identification and determination of the phases of calculogenesis, thus supplementing the information obtainable by means of wet chemical processes alone. In a recent report on electron microscopic photographs of urinary crystals and sections of urinary calculi Hesse et al. [4] have demonstrated characteristic whewellite and calcium phosphate crystals in the stone patient. These authors have also given evidence about mixed phase crystal formation and inclusions in a phase in sections of urinary calculi.

The classical chemical analysis of urinary stones necessarily involves initial disintegration of the stone into powder form or dissolving the stone powder in suitable solvents for subsequent qualitative and quantitative analysis. Such an approach rules out any attempt at investigating the in situ and de facto crystalline topography of the stone in its native state. We are presenting in this report typical photographs of the urinary stone in its native state obtained from electron microscopic scanning. Such a study has given information regarding the determination of different mineral phases, inclusions and crystalline composition. Further, we are also reporting the characterization of polished sections of urinary calculi using the scanning electron microscope.

Materials and Methods

The stones were obtained from patients after surgery at the Trivandrum Medical College Hospital. The retrieved stones were immediately washed free of blood and mucous in a stream of tap water and allowed to dry at room temperature. Stones of a size ranging from 0.5–1.5 cm in diameter were taken for the study of the surface topography of the calculi by scanning electron microscopy. Sections were prepared from stones that are 2 cm or more in diameter by using a stone splitting machine, devised indigenously. Surface and cut sections were studied in 30 stones. The sections prepared were less than 1.5 cm in diameter. For electron microscopic studies, the stones and sections were further manipulated. A JEOL JSM 35 C scanning electron microscope was used for the study. The stone samples were glued to brass studs with silver paste and were made conductive by sputtering with gold to approximately 100 Å thickness. The treated specimens were introduced into the vacuum chamber and viewed through the microscope. The signals emitted from the specimen surface, when the electron beam hit it, were taken up by the detector, amplified and picked up by the cathode ray tube of the display screen on which the image was obtained. The interesting pictures were photographed immediately.

The scanning electron microscopic photographs were read based on the results of the qualitative and quantitative analysis and infra-red spectroscopic study of the stones, as also comparing with reference photographs [2, 3, 7].

Results and Discussion

Figure 1 is the scanning electron micrograph of a weddellite (calcium oxalate dihydrate) calculus surface structure. It

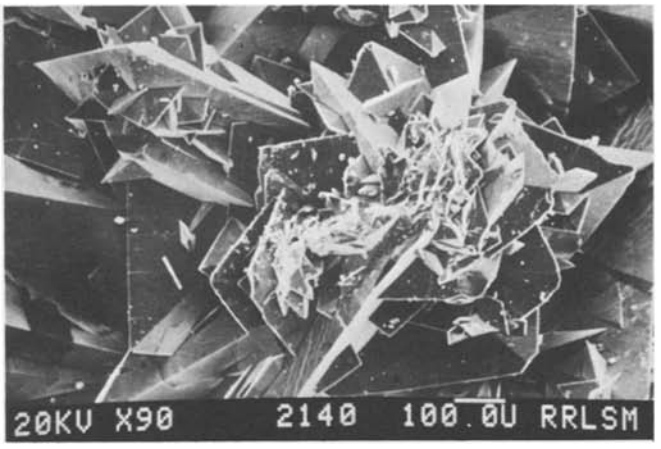

Fig. 1. Surface of a weddellite calculus. x43, scanning electron microphotograph (2140)

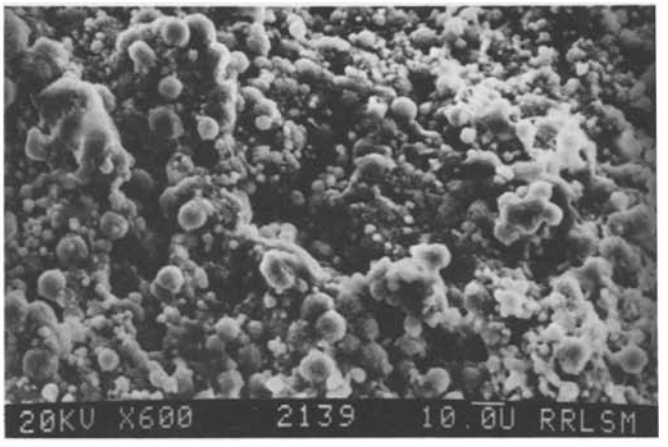

Fig. 3. Surface of a brushite calculus. x300 (2139)

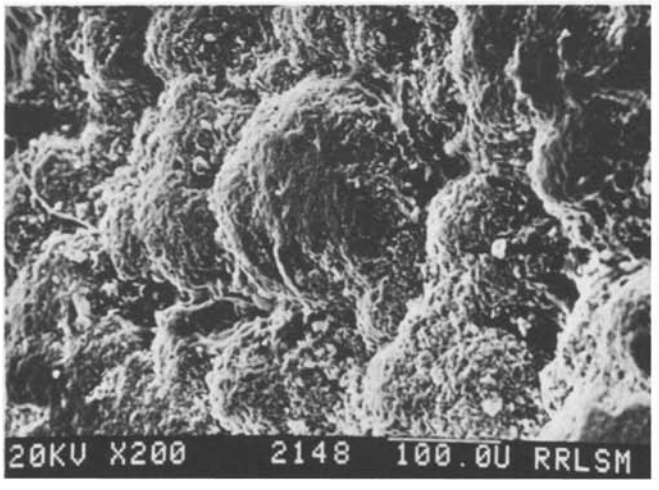

Fig. 2. Surface of a whewellite calculus. x100 (2148)

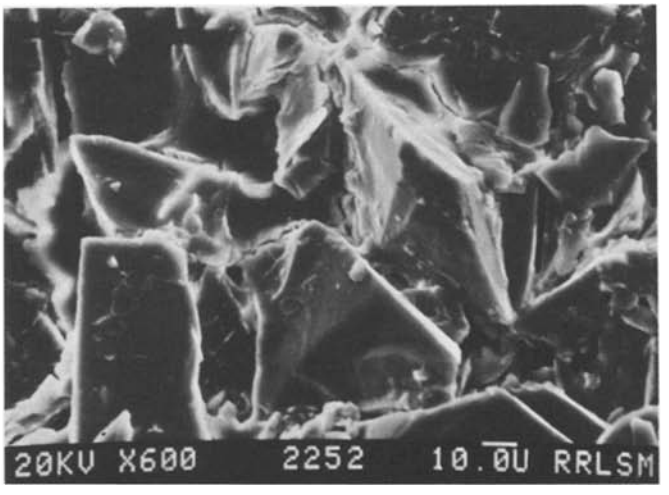

Fig. 4. Struvite – weddellite calculus. x100 (2146)

may be seen that the deposition of crystals that form the stone is very irregular and unoriented. Figure 2 is a photograph showing a whewellite (calcium oxalate monohydrate) calculus surface; note the significant difference between a whewellite and a weddellite calculus. Figure 3 shows a brushite (calcium biphosphate) calculus surface. Figure 4 depicts the surface of a mixed calculus – struvite (magnesium ammonium phosphate) and weddellite. Figure 5 shows the surface topography of a uric acid – whewellite mixed calculus. These photographs represent surface topography of the stones. It is interesting that Figs 2 and 3 show a similar pattern of crystal deposition although they are whewellite and brushite calculi respectively, having different chemical composition as determined by routine qualitative and quantitative chemical analysis. Figure 6 is a struvite calculus showing a fibrous network in between, which could be the protein matrix. Figure 7 shows a whewellite calculus with a distinct protein matrix. Figure 8 shows the cut section texture of a struvite – brushite mixed calculus. These high magnification photographs of the cut sections of the different types of stones clearly indicate interstitial protein matrices within the crystal layers of the stone. The same stones when analysed by routine chemical methods do not give any evidence of the presence of protein material in the composition of the stone. However, there are reports in the literature suggesting that some urinary stones contain a thick putty like polysaccharide matrix in the stone [10] and that in certain matrix stones 70% by weight of the stone is formed of a mucoproteinous gelatinous substance [8]. In the present study we have been able to demonstrate the occurrence of protein matrix in urinary calculi through high magnification electron microscopic scanning. The exact function of protein matrix within the stone has not yet been elucidated. It is possible that the protein matrix, which may be of a mucoprotein nature, may either promote heterogenous nucleation or the mucoprotein matrix may

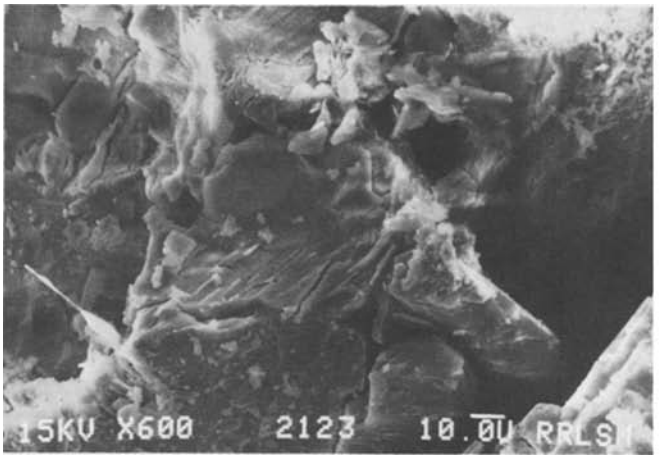

Fig. 5. Surface of uric acid – whewellite mixed calculus. x300 (2123)

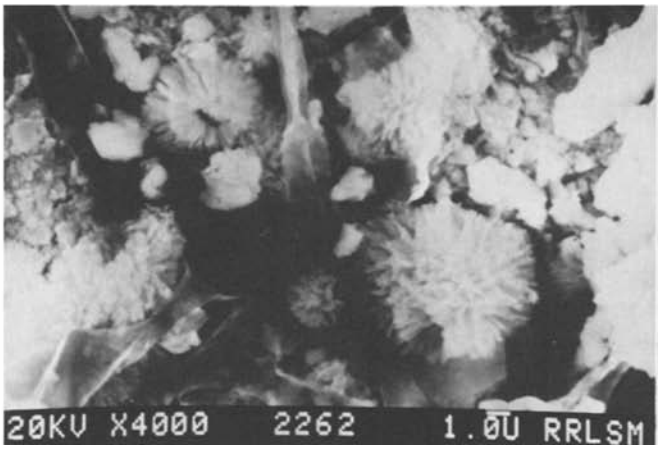

Fig. 7. Cut section of a whewellite calculus with protein matrix in between. x1,920 (2262)

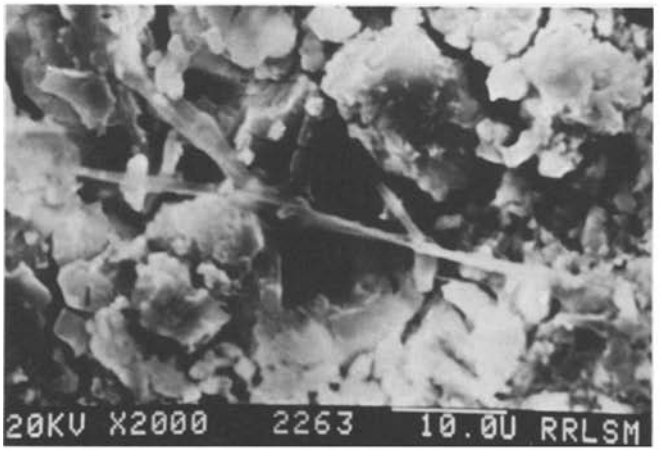

Fig. 6. Cut section texture of a struvite calculus. Note the fibrous strands in between. x1,000 (2263)

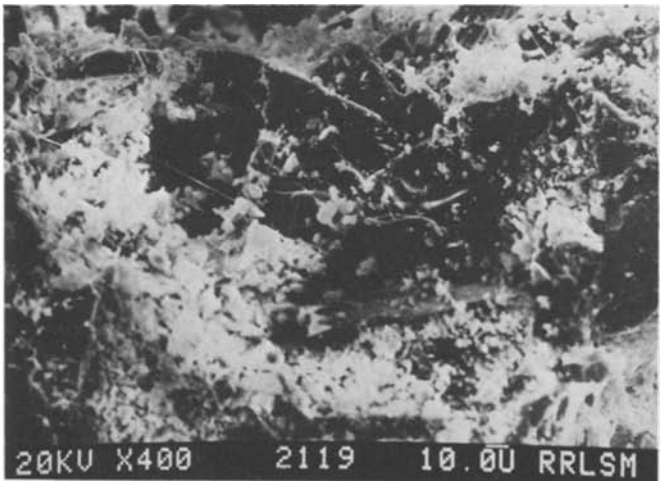

Fig. 8. Cut section of struvite – whewellite brushite mixed calculus. x200 (2119)

act as an inhibitor of crystallization. It may be pointed out in this connection that among the various inhibitors of crystallization believed to be present in urine of both normal and stone patients, mucoproteins or glycosaminoglycans account for the major inhibiting activity [1].

The adoption of an electron microscope for studying urinary stones definitely helps in a more detailed investigation of urinary stones as shown in the present study, together with X-Ray diffraction, IR-spectroscopy, spectrophotography and chemical analysis. The results of the present study have clearly demonstrated the usefulness of scanning electron microscopy for the investigation of thin sections of the stone and may help in accurate analysis of urinary calculi which is a precondition for investigation into the different phases of urinary calculus formation and growth.

References

1. Felix R, Monod A, Booge L, Hausea NM, Fleisch H (1977) Aggregation of calcium oxalate crystals. Effect of urine and various inhibitors. Urol Res 5:21–28
2. Hesse A, Bach D (1982) In: Harnsteine – Pathobiochemie und klinisch-chemische Diagnostik. Georg Thieme, Stuttgart New York, pp 154–179
3. Hesse A, Lange P, Berg W, Bothor C, Hienzsch E (1975) Scanning electron microscope and microprobe investigation of phosphate phases in uroliths. Urol Int 34:81–94
4. Hesse A, Hicking W, Bach D, Vahlensieck W (1981) Characterisation of urinary crystals and thin polished sections of urinary calculi by means of an optical microscopic and scanning electron microscopic arrangement. Urol Int 36:281–291
5. Kirby JK, Pelphrey CF, Rainey JR (1957) The analysis of urinary calculi. Am J Clin Pathol 27:360–362

6. Marickar YMF, David J, Abraham PA (1976) Study of urinary stones in Kerala. Ind J Surg 38:480–484
7. Meyer JL (1977) In: Van Reen R (ed) Idiopathic urinary bladder stone disease. DHEW Publication No. (NIH) 77–1063, pp 83–108
8. Spector AR, Gray A, Prier EL Jr (1976) Kidney stone matrix. Differences in acidic protein composition. Invest Urol 13:387–389
9. Thind SK, Nath R (1970) Biochemical characteristics of urinary stones in Chandigarh area. Bull PGI 4:21–23
10. Wickham JEA (1976) The matrix of renal calculi. In: Williams, DI, Chisholm GR (eds) Scientific foundations of urology, chap 47. Heinemann, London, pp 323–327

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