known to block dopamine inhibitory effects[7,10], caused no alteration in the snail cyclic AMP. The snail dopamine receptors also seem to be similar to those of the vertebrates[19], since fluphenazine strongly counteracts the increase in cyclic AMP caused by dopamine, and apomorphine also mimics the effect of dopamine. Moreover, these receptors are unlike the β-adrenergic receptors of the vertebrates[19] in that they are not influenced by noradrenaline or propranolol. Although 5-hydroxytryptamine is more potent than dopamine in stimulating adenylate cyclase in the snail nervous system, which supports the results of Cedar and Schwartz[11], it appears as if the receptors for the 2 amines are different, since tubocurarine, fluphenazine and haloperidol had no influence upon the 5-hydroxytryptamine effect at concentrations which drastically inhibited dopamine stimulation of adenylate cyclase. To conclude, the present results provide support for the idea that a dopamine-sensitive adenylate cyclase may be the excitatory receptor for dopamine in the snail nervous system. Further studies on the biochemistry and pharmacology of this dopamine-sensitive adenylate cyclase and its relationship with physiological processes in neurons are, however, necessary not only to clarify the type of receptor, but also to locate their sites.

Investigation of the microstructure of kidney stones (oxalate type) by high voltage electron microscopy and electron diffraction

K. El-Sayed[1,2] and V. E. Cosslett

Department of Physics, Electron Microscopy Section, Old Cavendish Laboratory, Free School Lane, GB–Cambridge CB2 3RQ (England), 26 November 1976

Summary. The organic matrix of this type of urinary calculus contains 3 components, which differ in form and in amorphous/crystalline content. Deposition of crystalline material in the early stages of mineralisation seems to be epitaxially related to the orientation of the organic matrix.

Optical microscopy and X-radiography show that urinary calculi are composed of a series of layers of crystalline components and a lesser amount of organic matrix[3–5]. The crystalline layers contain oxalates and phosphates of calcium, varying in calculi of differing provenance. Boyce and Garvey[6] found that the matrix is made up of fibrils and amorphous interfibrillar material.

X-ray diffraction and microprobe analysis have been successful in investigating the nature and orientation of the crystalline regions[4,5,7] but electron microscopy has been little used, because of the difficulty of cutting sufficiently thin sections. Watson[8] applied a replica technique and obtained micrographs of hexagonal crystals from some specimens, provisionally identified as cystine. Catalina and Cifuentes Delatte[9] examined the calcium oxalate sediments deposited from urine, and obtained some electron micrographs and diffraction patterns. These showed a type of spherulitic crystal growth, but the diffraction patterns were diffused and probably dominated by organic matrix material deposited with the crystalline components[5].

Mohammed et al.[10,11] succeeded in obtaining thin sections of calculi and studied them by electron microscopy at 80 kV and by electron diffraction. They showed that the organic matrix is often arranged in layers alternating with crystalline components, but sometimes seems to be present in screw dislocated crystals. They were unable to distinguish structure in the organic matrix, or to find evidence for the nature of its role in crystal growth.

The availability of electron microscopes operating at very high voltage now makes it possible to study relatively thick sections, and advances in the techniques of microtomy facilitate the cutting of suitable sections from friable materials such as calculi. The present study utilized these developments to obtain detailed information on the structure of the organic matrix and its morphological relations with the crystalline regions.

Materials and methods. Sections were prepared from 3 stones collected by surgical operation from 3 different patients, 1 woman and 2 men. Some 200 sections, cut from all parts of a stone from the centre to the periphery, were examined in an AEI EM6B electron microscope at 80 kV and in the Cavendish high voltage microscope at 600 kV.

Embedding: Araldite was used as embeddiug material, several ratios of resin, hardener and accelerator being tried in order to obtain good coherent sections. Best results were obtained with a composition of 5 c^3 resin, 8 c^3 hardener and 0.4 c^3 accelerator, instead of that usually used for biological tissues: 10, 10 and 0.5 c^3 respectively.

Sectioning: Great difficulty was encountered in cutting and collecting thin sections from calculi. Glass knives prepared with different wedge angles were investigated. A 40° angle was found satisfactory for cutting, and allowed sections to be collected safely. Trouble was experienced from the water in the collecting trough wetting the surface of the stone so that the latter dragged sections with it during the motion of the microtome. To avoid this the level of the water in the trough must be lowered, and

1 Address: Dept. of Physics, Ein-Shams University, Cairo (Egypt).

2 Acknowledgments. The authors are indebted to Ass. Prof. N. Allouba of Ein-Shams University (Cairo) for supplying the kidney stones and medical information, and to the Physiology and Zoology Departments of Cambridge University for assistance in cutting the sections. The work was performed during the tenure by K. E. S. of a research bursary from the British Council.

3 J. A. Carr, Br. J. Urol. *25*, 26 (1953).
4 K. Lonsdale, Nature Lond. *217*, 56 (1968).
5 K. Lonsdale, Science *159*, 1199 (1968).
6 W. H. Boyce and F. K. Garvey, J. Urol. *76*, 213 (1965).
7 M. H. Nguyen, S. Offret and C. Laurence, Micron *7*, 37 (1976).
8 J. H. L. Watson, J. Urol. *75*, 940 (1956).
9 F. Catalina and L. Cifuentes Delatte, Archos esp. Urol. *2*, 147 (1958).
10 A. A. Mohammed, K. El-Sayed, A. M. Ibrahim and N. Allouba, Proc. Egypt. Acad. Sci. *23*, 2 (1970).
11 A. A. Mohammed, K. El-Sayed and A. M. Ibrahim, Ein-Shams Bulletin (Cairo), No. 17, 10 (1972).

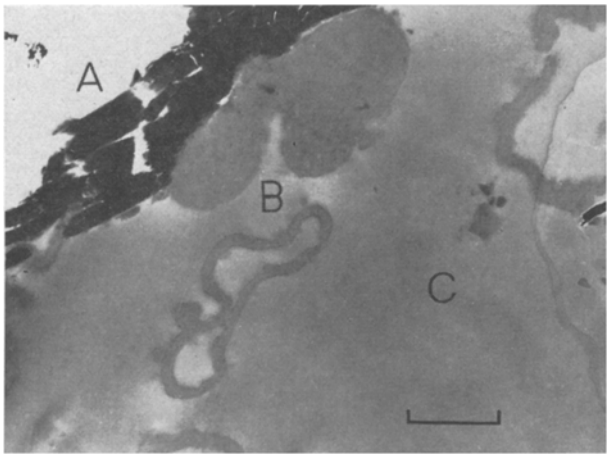

Fig. 1. Section through the organic matrix of a calculus showing the 3 types of structural component, A, B, C. (80 kV). ×6000. Scale mark, 2 μm.

the surface tension reduced to ensure that the upper surface of the knife remains wet; 10% of acetone in water was found to be satisfactory.

Results. Electron micrographs and the corresponding diffraction patterns were obtained from samples of the organic matrix and the crystalline material, and from the early stages of deposition of the latter on the matrix.

Both the matrix and the crystalline material were found to consist of more than one component, characterised by morphology and structure. The crystals are small in size near the centre and larger in the outer regions. From X-ray diffraction patterns the presence of both wedellite (calcium oxalate dihydrate) and whewellite (calcium oxalate monohydrate) was confirmed.

3 components can be identified in the organic matrix. Sections through this region of a stone (as in figure 1) show a dense fairly regular material (A), larger irregular inclusions (B), and an amorphous continuous matrix (C). The presence of crystalline material in forms A and B was established by electron diffraction. At higher magnification (figure 2) form A is seen in thinner areas to contain small crystallites dispersed among fine fibrillar material. Form B has a complex and irregular structure. Thinner sections (figure 3) often show a variety of internal components within a boundary membrane.

The continuous matrix (form C) in which forms A and B are embedded is amorphous in nature, giving no diffraction pattern. Attempts to characterize it by electron microprobe analysis (in publication) showed that it contains also a certain proportion of calcium salts.

Electron diffraction patterns from region B of the organic matrix display a number of broad rings (figure 4), showing that the crystalline material present is small in average particle size, whereas form A gives a single crystal pattern as well. The mean values for the interplanar spacings are shown in the table: A_1 and A_2 refer to the two components in form A.

A_1	B	A_2	H
5.46 Å		5.35 Å	5.26 Å
3.51	4.00 Å		
		3.07	3.08
2.51	2.50	2.68	2.72
			2.63
1.79	1.74	1.78	1.78
		1.56	
1.47	1.48		1.54
	1.176		

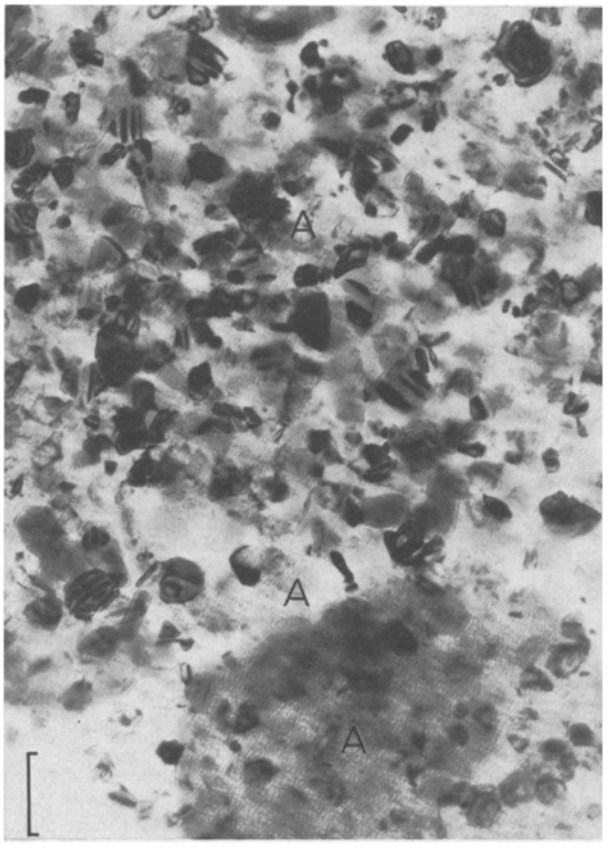

Fig. 2. Section through composite material, showing small crystallites and the microfibriller nature of the matrix (A). (600 kV). × 50,000. Scale mark, 0.2 μm.

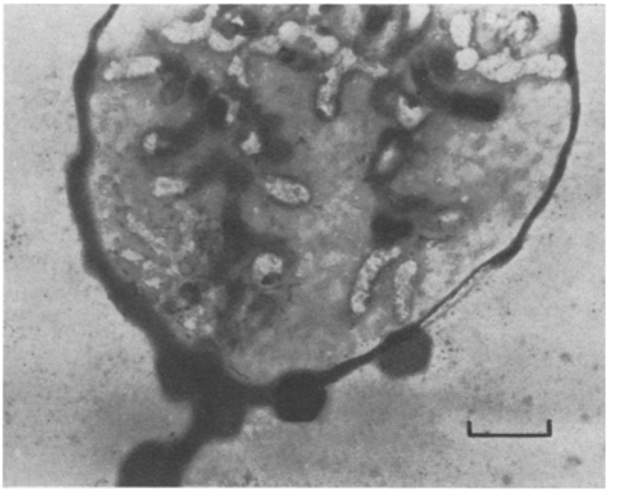

Fig. 3. In thin section, form B of the matrix shows a well-defined envelope and internal particles of virus-like form. (80 kV). ×100,000. Scale mark, 0.1 μm.

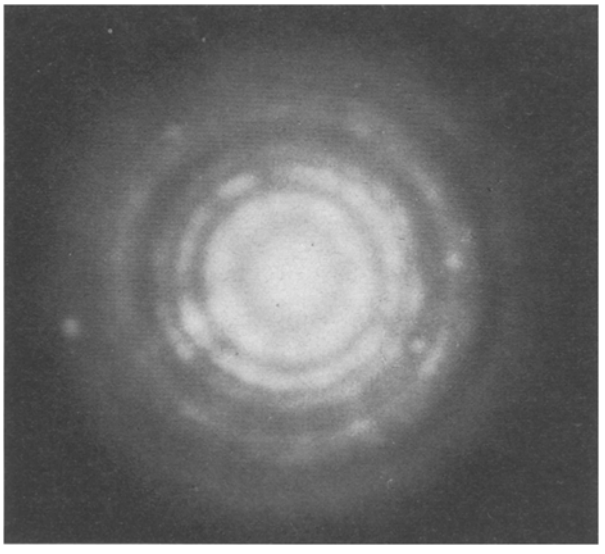

Fig. 4. Electron diffraction pattern from a region of the matrix containing form B. (600 kV).

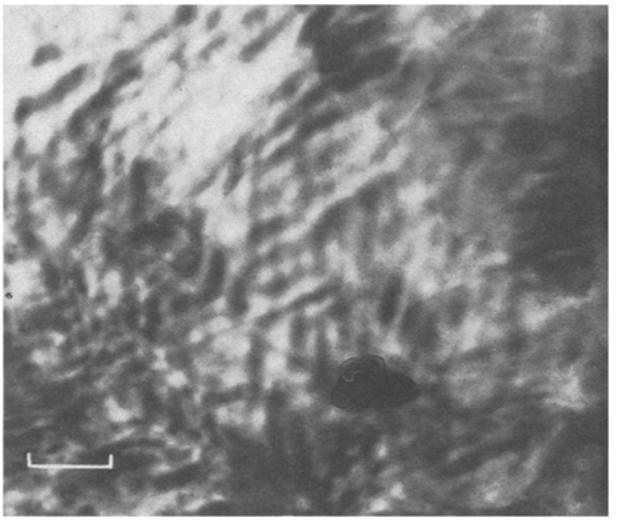

Fig. 5. Section through a region between the organic matrix layer and a fully crystalline layer, showing particle growth in preferred orientations. (600 kV). ×20,000. Scale mark, 0.5 μm.

Additional rings sometimes occurred at 4.20, 3.65 and 3.00 Å.

There appears to be a common broad-ring component of forms A and B, characterized by the spacings 2.51, 1.79 (1.74) and 1.47 (1.48) Å, mixed with a component specific to form A (with 5.46 and 3.51 Å spacings) and a component specific to form B (with 4.00 Å spacing). We could not identify these components with any of the crystalline forms of calcium oxalate normally present in kidney stones. They may be mixed crystals formed by oxalates having varying degrees of hydration in the early stages of mineralisation and thus of very small particle size. Alternatively these components may consist solely or primarily of proteins, some of which give good electron diffraction patterns even when unstained[12]. The well-defined hexagonal pattern A_2 corresponds most nearly to that of hydroxyapatite (H in the table), within our experimental error.

At later stages of mineralisation the crystallites are larger and better defined. Figure 2 is typical of a region in which the crystals are about 0.1 μm across and sufficiently thick to show extinction fringes. The fibrillar nature of the matrix is visible at A and elsewhere. The crystallites tend to orientate themselves in the fibrillar direction and in 2 directions at 60° to it. The diffraction pattern from these particles corresponds to the 3-dimensional lattice of calcium oxalate monohydrate (whewellite). A section cut in the region between the organic matrix and the crystalline material showed dense deposits orientated in several crystallographically related directions (figure 5), typical of the epitaxial growth of one crystalline substance on another. Here it is probably due to crystallisation of calcium oxalate on the organic matrix, the preferred directions being those of a hexagonal planar lattice.

Discussion. The structure of the matrix and the formation of calculi. These results show that the organic matrix in calculi has a more complex structure than hitherto assumed, and its relation to the crystalline layers is equally complex. Component A of the matrix, composed of very small crystallites, may be an early stage of mineralisation in which discrete centres of crystallisation form in the truly amorphous component (C) quite randomly, as the diffraction patterns show.

The other component (B) of the matrix appears to be a combination of organic and crystalline material. It is cellular in form yet shows a fairly well-defined crystal structure by diffraction. In some sections small rod-shaped or virus-like particles are visible (figure 3), in size about 1500 Å long and 500 Å across. It has been suggested that bacteria play a role in the nucleation of kidney stones[13–15]. We hesitate to identify component B as a streptococcus, but the evidence present here tends to support the hypothesis that bacteria are involved in the formation of calculi.

Epitaxial growth of the crystalline layers. Evidence of the orientated growth of crystals in tissue was first obtained by Clark[16], who showed that hydroxyapatite in bone was laid down with the c-axis parallel to the collagen fibres. Lonsdale[4,5] reported that the crystalline components in kidney stones showed strong preferred orientation, but attributed this to the epitaxial growth of one crystalline component on another, e.g. calcium oxalate on calcium phosphate (apatite).

The micrographs presented here demonstrate the alignment of crystallites with the fibrillar structure of the matrix and diffraction patterns indicate that true epitaxy occurs (figures 2 and 5). This is circumstantial evidence for the epitaxial growth of crystalline material on the organic matrix itself, as well as on other crystals. But as a calculus is of 'onion-skin' construction, a layer of the organic matrix is itself deposited on a crystal lattice. The fibrillar structure of the matrix may arise as much from that circumstance as from the form of its component macromolecules. The orientation we have observed in crystalline layers on the matrix could therefore be described as a second-order epitaxy, the ordering effect of a previously deposited crystalline layer being transmitted through the matrix to the next layer.

12 R. P. Ferrier, Adv. Opt. El. Microsc. *3*, 200 (1969).
13 A. Randall, Ann. Surg. *105*, 1009 (1937).
14 E. C. Rosenow and J. C. Meissner, Archs intern. Med. *31*, 807 (1923).
15 I. M. Nielsen, J. Urol. *75*, 4 (1956).
16 J. H. Clark, Am. J. Physiol. *98*, 328 (1931).