

Analysis of vaterite microspherolith deposits on a pure cholesterol gallstone by X-ray diffraction, X-ray microanalysis and infrared absorption techniques

H. Yamamoto¹, T. Sakae², and H. Schäfer¹

¹ Institute of Pathology, University of Hamburg, Martinistrasse 52,
D-2000 Hamburg 20, Federal Republic of Germany

² Institute of Anatomy, Nihon University, School of Dentistry at Matsudo,
Chiba 271, Japan

Summary. Minute globular concretions of light-green and light-brown colour were found as deposits in the pits at the rough surface of a pure cholesterol gallstone. They were analyzed by scanning electron microscopic, X-ray diffraction, X-ray microanalytical and infrared absorption spectrometric methods. In scanning electron microscopy, the concretions appeared as ovoid microspheroliths varying from 5 to 30 μm in size. The microspheroliths presented smooth and rough surfaces; the reason for these different types of surface remained unclear. X-ray diffraction and infrared absorption analysis revealed that the microspheroliths were mainly composed of vaterite and the host plate-like crystals were cholesterol. By energy-dispersive X-ray microanalysis, large quantities of calcium without significant quantities of phosphorus were detected in the microspheroliths. Calcium was absent in the plate-like crystals and could not be detected in the central part of the stone. In the pigmented periphery of the stone, some silicon and iron were found. The presence of calcite, aragonite and iron-containing pigment materials in the concretion is suggested. From the textural point of view, the analytical data strongly suggest that the stone-forming conditions suddenly changed from a cholesterol-favourable stasis condition to a condition favoring the deposition of calcium carbonate possible as the result of haemorrhage.

Key words: Gallstone – Microspherolith – Vaterite – X-ray diffraction – X-ray microanalysis

The components of gallstones have been investigated by a variety of analytical methods. Cholesterol and calcium carbonate are the most frequent of the organic and of the inorganic deposits in gallbladders (Sutor and Lewis 1971).

Offprint requests to: H. Schäfer at the above address

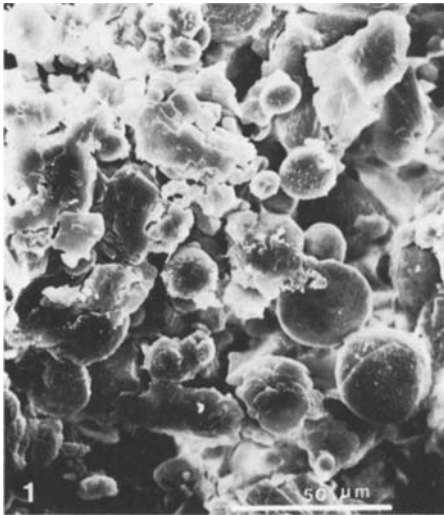


Fig. 1. Aggregation of microspherulites and fragments of plate-like cholesterol crystals at the surface of the gallstone: bar = 50 μm)

Cholesterol and calcium carbonate gallstones are formed under entirely different conditions. Rains (1964) traced back the classical controversies about the pathogenesis of cholesterol gallstones to Naunyn vs Aschoff and Bachmeister. The latter declared that a single radial cholesterol stone could occur without any inflammation in the gallbladder; that is, stone could form without infection, although the former authors stated “without infection – no stones”. One point with which both opinions agreed was that stasis in gallbladder was necessary in order to give the stone time to grow. Calcium carbonate gallstones, in contrast with cholesterol stones, are formed in sterile as well as infected bile and the presence of stasis is not necessary (Rous et al. 1924).

Among a number of reports about gallstone texture, there are only a few reports about the deposition of calcium carbonate microspherulites on the surface of pure cholesterol gallstones. In this paper, the mode of occurrence of vaterite microspherulites on a pure crystalline cholesterol gallstone is described as evidence for stone forming conditions changing from cholesterol-favourable to calcium carbonate-favourable.

Materials and methods

The gallstone used in this study was one of the stored collection in the hospital. The appearance of the stone, 10 mm in diameter, was characterized by a whitish, translucent, roughly pyramidal crystalline surface with spotty dispersed light-green or light-brown minute globular concretions in the pits at the surface. The cross-section showed a radial distribution of large translucent crystals, which easily fractured along cleavages; no coloured material was present inside.

Scanning electron microscopical observation was carried out with a sectioned block of the stone including the coloured concretions at the surface. X-ray diffraction patterns of the translucent crystals were obtained using a Rigaku X-ray powder diffractometer, and those

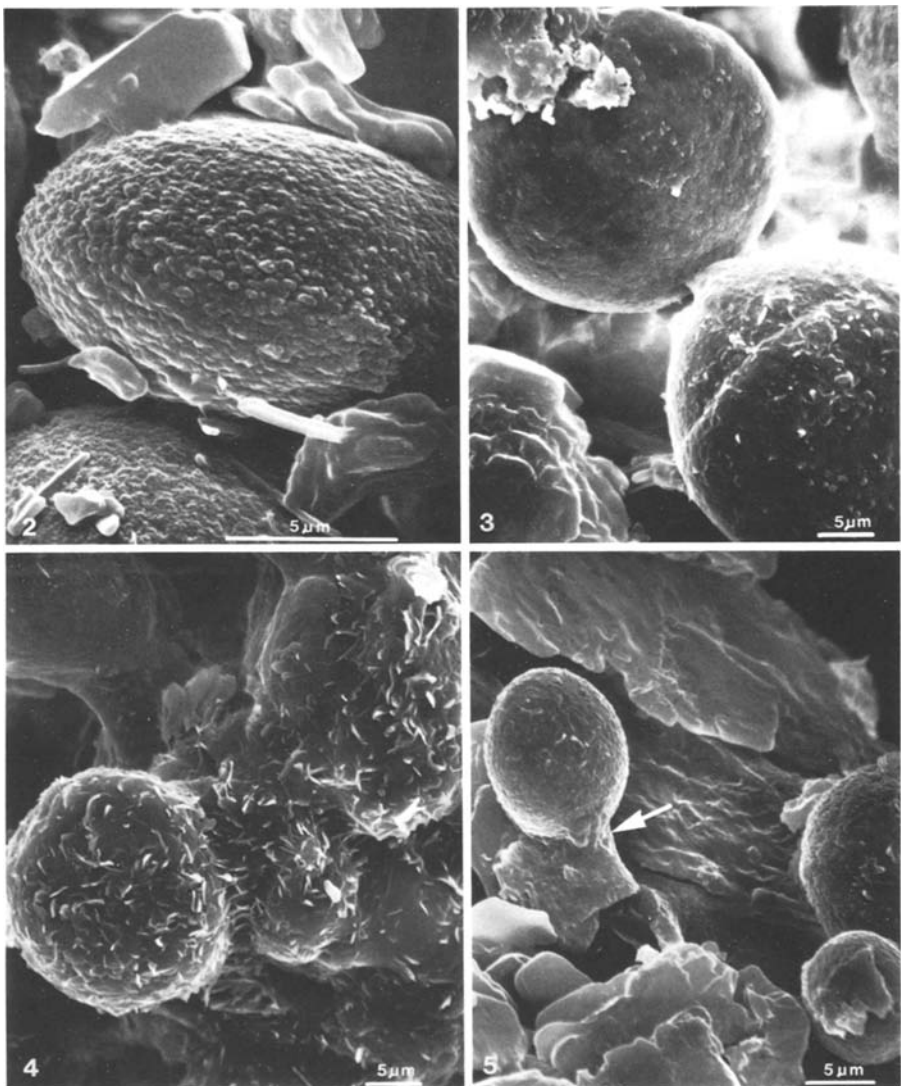


Fig. 2. Rugged surface of a microspherulith. (Figs. 3–6: bars = 5 µm)

Fig. 3. Smooth surface of microspheruliths

Fig. 4. Scaly foliated layer covering the microspheruliths

Fig. 5. Tight connection (arrow) between microspherulith and plate-like crystal

of the small portions of the coloured concretions were obtained using a Rigaku X-ray microdiffractometer. Infrared absorption analysis was carried out employing a JASCO IRA-2 by KBr disk method using a small sample of the surface portion containing the concretions.

X-ray microanalysis was done using an energy-dispersive X-ray microanalytical system 8032 (ORTEC Ltd., Springfield, USA), combined with a EM 400 transmission electron micro-

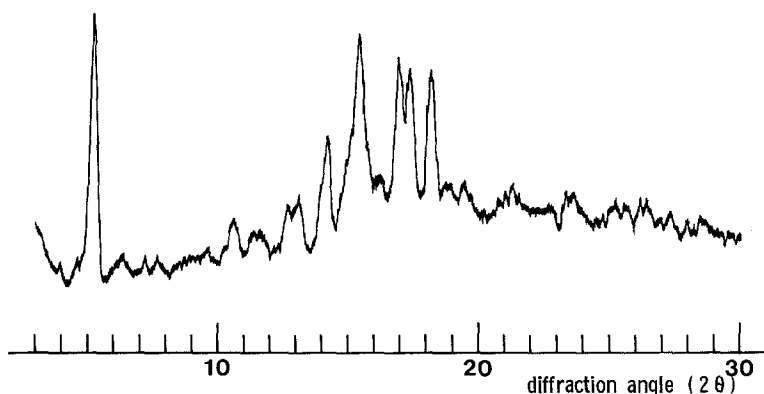


Fig. 6. X-ray powder diffraction pattern of the cholesterol crystals. (Figs. 7–8: Numericals are the diffraction angles, 2θ – degree, with CuK_α radiation)

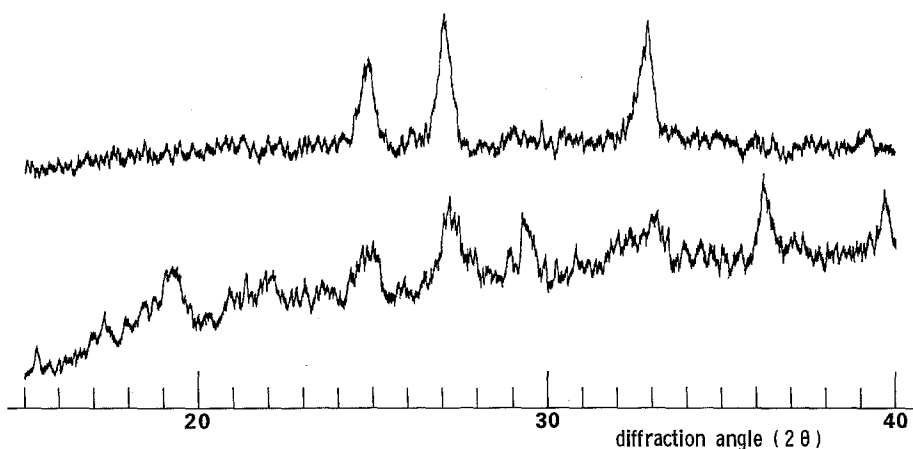


Fig. 7. X-ray microdiffraction patterns of the microspherulith concretions. The upper pattern shows the peaks of vaterite, and the lower more complicated pattern shows the presence of calcite, aragonite and undefined materials

scope (Philips Ltd., Eindhoven, Netherlands) equipped with a PHILIPS scanning device. For this purpose, cross-sections of the peripheral parts of the stone were analyzed under scanning electron microscopical control using a secondary electron image of the uncoated specimen surface. The beam (diameter 20 nm) was directed to the center of the microspheruliths, the surrounding areas or the central part of the stone. The accelerating voltage was 80 keV, the counting time was 200 seconds. The obtained X-ray spectra represented the elemental composition of the illuminated specimen area.

Results

In the scanning electron microscopic picture, the concretions were composed of microspheruliths varying from 5 to 30 μm in size uniformly showing an ovoid shape (Fig. 1). The microspheruliths were not only aggregated in the pits at the surface but also presented as solitary deposits on the

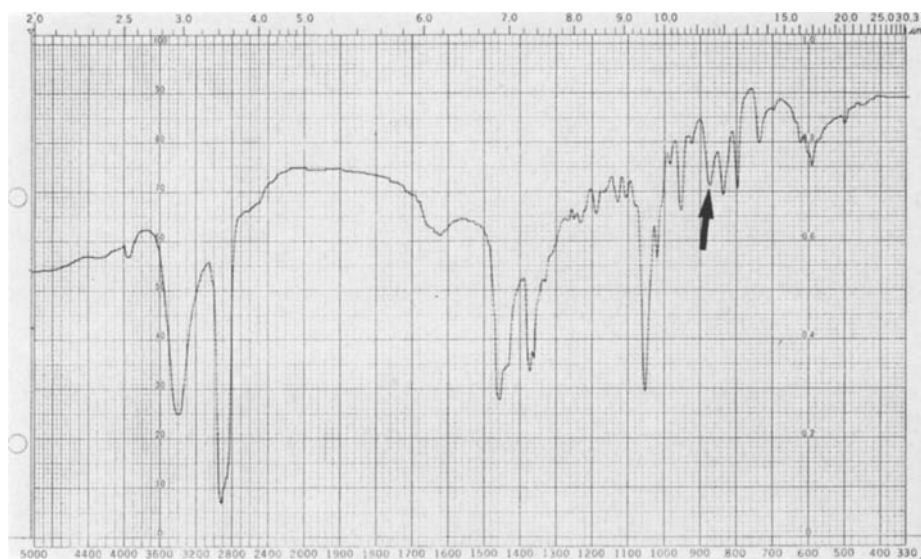


Fig. 8. Infrared absorption spectra showing cholesterol absorptions and calcium carbonate absorption at 870 cm^{-1} (arrow)

surface. The surfaces of the microspheruliths were both, roughly rugged (Fig. 2) or smooth (Fig. 3). The cause of these different appearances could not be determined. On part, the aggregation of microspheruliths and neighbouring plate-like crystals were covered by an undefined scaly or leaf-like layer (Fig. 4). Some of the microspheruliths showed a close connection with the underlying crystals (Fig. 5).

Among the variety of crystal forms of cholesterol such as cholesterol monohydrate, anhydrous cholesterol (Borgren and Larsson 1963a), second form of anhydrous cholesterol (Sutor and Wooley 1969), and cholesterol derivatives (Aprahamian et al. 1978), the X-ray powder diffraction pattern of the translucent fragile plate-like crystals (Fig. 6) was identified as cholesterol (JCPDS (1981): organic card no. 7-742), which is also identical with the second anhydrous form.

Figure 7 shows two X-ray micro-diffraction patterns of the concretions at the periphery of the stone. The upper pattern shows the peaks at 3.59, 3.30 and 2.73 Å indicating vaterite (JCPDS inorganic card no. 24-30c); the lower curve shows a more complicated pattern. The additional peaks in the lower pattern are the contributions of calcite (3.03, 2.28 Å; JCPDS inorganic card no. 24-27c), aragonite (3.27, 2.70, 2.48 Å; JCPDS inorganic card no. 24-25) and some undefined materials (4.6 Å and other weak peaks).

An infrared absorption spectrum of a small portion of the stone containing some of the concretions is shown in Fig. 8. The absorption pattern is in good agreement with that of cholesterol reported by Wei (1982) except one absorption peak at 870 cm^{-1} , which is not due to cholesterol and may be assigned to either vaterite (870 cm^{-1} ; White 1974) or calcite (872 cm^{-1}). Aragonite (851 cm^{-1}) was not detected in this chart.

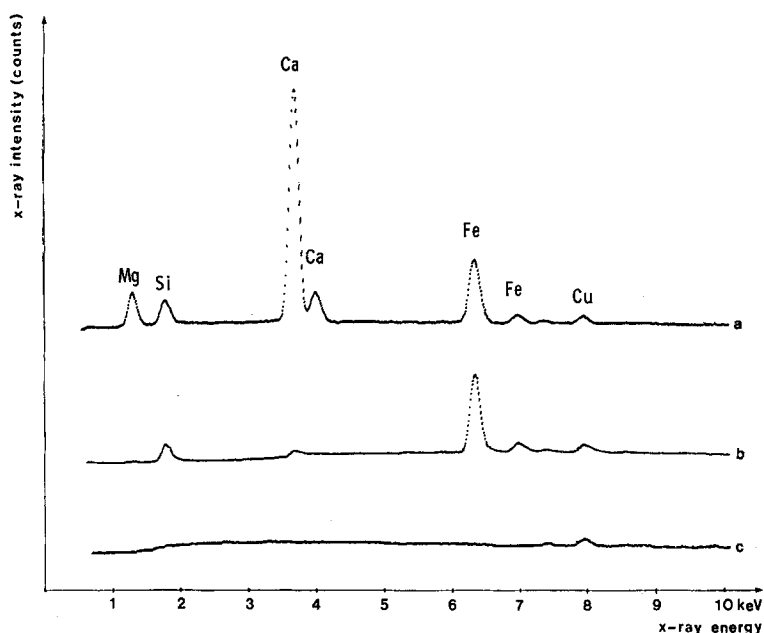


Fig. 9. Energy-dispersive X-ray microanalysis of the gallstone. The X-ray energy (keV; *abscissa*) of the peaks identifies the chemical element; the peak height (X-ray counts; *ordinate*) represents the quantity of an element. **a** Microspherulith at the stone periphery. **b** Area neighbouring a microspherulith in the stone periphery. **c** Plate-like crystal in the central part of the stone

The applied energy-dispersive X-ray microanalytical technique detects chemical elements with an atomic number equal to or higher than sodium. Figure 9 shows some of the X-ray spectra obtained. In the periphery of the stone microspheruliths mainly induced prominent calcium peaks without significant accompanying phosphorus peaks consistent with the presence of calcium carbonate. In addition, in some concretions minute amounts of iron, silicon and traces of magnesium were found. In the pigmented areas neighbouring the microspheruliths significant iron peaks, small silicon peaks, but only traces of calcium or magnesium were detected. The colourless central parts of the stone revealed only "white" X-ray radiation without significant element peaks proposing the presence of material (i.e. cholesterol) composed of elements with an atomic number less than sodium.

Discussion

Three main types of calcium carbonate (CaCO_3) crystals are existing in nature: calcite, aragonite and vaterite. Calcite, a hexagonal (rhombohedral) crystal of low solubility, is one of the most common and widespread minerals in the earth's crust. Aragonite, a crystal with orthorhombic symmetry, is much less common than calcite, to which it is because of its metastability frequently partly inverted. Aragonite with some calcite builds up the substance of pearls. Vaterite is the most unstable form of calcium carbonate

and occurs rarely in nature. In gallbladder, however, there is a particularly favourable, but not clearly defined condition for its formation, and many investigators have reported the occurrence of vaterite as well as aragonite and calcite in gallstones (see Toor et al. 1979). Sutor (1979) stated that in 331 patients the frequency of vaterite was 6.4%, of aragonite 4.6% and of calcite 4.1%.

Epitaxy is the phenomenon of orientated crystal deposition on the surface of a host crystal of different composition. Lonsdale (1968) pointed out the epitaxial relationship between cholesterol and calcium carbonate crystals. Epitaxy promotes crystal deposition, when it possibly otherwise would not occur; he suggested that this situation may exist in case of the mixed type of gallstone. In the present case, however, the cholesterol crystals apparently have not promoted vaterite crystal growth since the vaterites have grown into a spherulithic shape which generally indicates growth in free spaces. Moreover, the observation of complete microspheroliths attached onto a flattened cholesterol crystal indicates that the contact occurred after the growth of the microspheroliths had been completed.

Pepinsky (1941) stated that vaterite in gallstones is apparently stabilized by the presence of bile pigment. The vaterite microspheroliths in this study are of light-green or light-brown colour. The light-green colour is obviously due to the presence of calcium biliverdinate, a small amount of which induces a green colour (Borgren 1963 b) while the light-brown colour may be attributed to calcium bilirubinate and, in addition to iron, which could be detected by X-ray microanalysis. The vaterite in this study is also supposed to be stabilized by calcium biliverdinate. In addition to calcium carbonate, calcium bilirubinate and calcium biliverdinate may contribute to the calcium peaks, which could be observed in the X-ray microanalytical spectra not only of the microspheroliths but also of the adjacent areas around the microspheroliths in the stone periphery.

As mentioned above, the genesis of cholesterol deposits is controversial. It is, however, agreed that deposition of calcium carbonate in bile is mainly caused by alterations of bile acids which exert a protective effect by keeping calcium in solution by diminishing ionization. Rous et al. (1924) suggested that it was infection of gallbladder which caused a failure of that organ to acidify bile, favouring the formation of calcium carbonate microspheroliths. The occurrence of calcium carbonate microspheroliths in this study is explained by following this theory. It is noteworthy that only concretions in the pits were coloured, while the cholesterol crystals were not coloured in this case. This fact and the X-ray microanalytical detection of iron only in the pigmented stone periphery strongly suggest that conditions with bleeding promoting formation of calcium carbonate occurred, but that these conditions were not present during formation of the cholesterol crystals.

In addition to the above mentioned components our X-ray microanalytical data indicated the presence of silicon and magnesium in the peripheral parts of the stone. There are few reports on the presence of silicon in gallstones. It was proposed that it might exist as silicate (Aprahamian et al. 1978), but corresponding peaks in the X-ray powder or microdiffraction

patterns or in the infrared spectra could not be observed and the chemical nature and role of silicon in the stone remained unclear.

The texture of a gall stone gives an important indication of the growth rate. If cholesterol and calcium carbonate (or their precursors) are precipitating simultaneously, the resulting stone is a mixed-type stone. Rapid growth occurs in the gallstones composed of microliths, examples of which are shown by Juniper and Woolf (1956). Rains (1964) suspected that the described microspheroliths had begun to form and had been aggregating over a period of 3–6 weeks. A large single cholesterol stone with well-developed crystal facets, on the other hand, took 8 years to grow. For fast growing multiple stones, containing aggregated hemispheres (250 μm or more in diameter) with radiating plates at their centers, a growth time of about 2 years has been reported (Wolpers and Blaschke 1971). Toor et al. (1979) concluded from their experiments of cholesterol monohydrate crystal nucleation that a single stone with a moderate amount of twinning required about 10 years to reach a diameter of 1 cm and that small multiple stones of intensely twinned type required about 1 year to reach a radius of 250 μm .

On the base of these data, the present cholesterol gallstone is assumed to have taken some years to grow and ultimately to obstruct the cystic duct resulting in hemorrhage, which promoted calcium carbonate deposition on its surface.

Acknowledgements. We thank Prof. emer. T. Sudo for helpful discussions and Mr. A. Okuda for preparation of the SEM photographs. This study was supported by the Grant for the Scientific Research from Nihon University.

References

- Aprahamian M, Arnaud JP, Eloy R, Adolf M (1978) Contribution à l'étude des calculs biliaires. Analyse morphologique, radiologique et cristallographique. *J Chir (Paris)* 115:297–304
- Borgren H, Larsson K (1963a) Crystalline components of biliary calculi. *Scand J Clin Lab Invest* 15:457–462
- Borgren H, Larsson K (1963b) On the pigment in biliary calculi. *Scand J Clin Lab Invest* 15:569–572
- Borgren H (1964) The composition and structure of human gall stones. *Acta Radiol Suppl* 226:18
- JCPDS (1981) Joint committee on powder diffraction standards. Powder diffraction files. JCPDS international center for diffraction data. Pennsylvania
- Juniper K jr, Woolf WE (1956) Biliary tract studies. I. X-ray diffraction analysis of gallstones; correlation with occurrence of microspheroliths in bile. *Am J Med* 20:383–391
- Lonsdale K (1968) Epitaxy as a growth factor in urinary calculi and gallstones. *Nature* 217:56–58
- Pepinsky R (1941) X-ray diffraction analysis of calcium salts in gallstones. *Phys Rev* 60:168
- Rains H (1964) Gallstones, causes and treatment. Wiliam Heinemann Med Books, London
- Rous P, Drury DR, McMaster PD (1924) Obervations on some causes of gallstone formation. II. On certain special nuclei of deposition in experimental cholelithiasis. *J Exp Med* 39:77–97
- Sutor DJ (1979) The composition of gallstones. *Hepatology (N.Y.)* 4:19–29
- Sutor DJ, Lewis D (1971) A statistical survey of the composition of gallstones in eight countries. *Gut* 12:55–64

- Sutor DJ, Wooley SE (1969) X-ray diffraction studies of the composition of gallstones from English and Australian patients. *Gut* 10:681–683
- Toor E, Evans DF, Cussler EL (1979) The nucleation of cholesterol monohydrate crystals in model bile solutions. In: Fisher MM, Goresky CA, Shafier EA, Strasberg SM (eds) *Gallstones*. Plenum Press, New York, p 169–181
- Wei Ta-C (1982) Quantitative determination of chemical composition of gallstones by infrared absorption spectrophotometry. *J Formosan Med Ass* 81:145–157
- White WB (1974) The carbonate minerals. In: Farmer VC (ed) *The infrared spectra of minerals*. Mineralogical Society, London
- Wolpers C, Blaschke R (1971) Electron microscopy of human gallstones. In: Arceneaux CJ (eds) *29th Ann Proc Electron Microscopy Soc Amer Boston*, pp 296–297

Accepted November 26, 1984