Transmission and Scanning Electron Microscopic Evidence for Cytoplasmic Deposition of Strontium Sulphate Crystals in Colonial Radiolaria

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SUMMARY

Colonial radiolaria are multicellular marine protozoa (Sarcodina) that reproduce by flagellated swarmers, each containing a vacuolar-enclosed crystal of celestite ($SrSO_4$). The crystal morphology (an elongated square prism with pairs of triangular end faces) is unusual compared with crystals produced directly from solution. The crystals of radiolarian swarmers are deposited within a cytoplasmic envelope, separate from the surrounding vacuolar wall, and are subsequently enclosed by an organic coat (ca. 500–1000 Å† thick) apparently deposited by the cytoplasmic envelope. The enclosing biological structures may account for the unusual morphology of the crystals, and offer further evidence that the process of crystallization, and ultimate crystal morphology, can be influenced markedly by surface chemistry at organo-mineral interfaces.
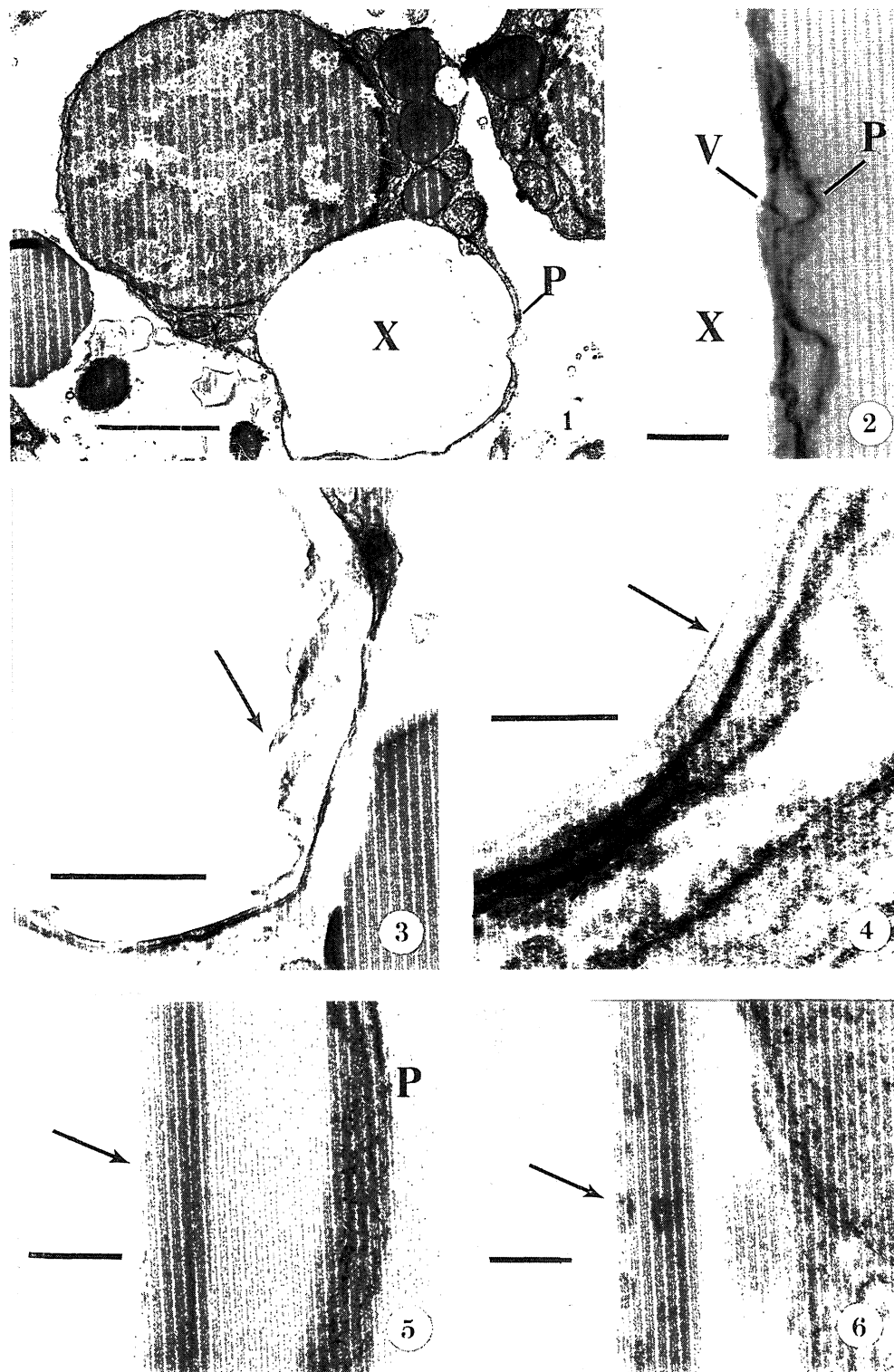
1. INTRODUCTION

Alkaline earth sulphate deposits, e.g. barium and strontium sulphates, have been identified in a wide variety of aquatic organisms including multicellular algae (see Kreger & Boeré 1969; Raven et al. 1986), unicellular algae such as desmids (Brook et al. 1980; Brook 1981), in some ciliated protozoa, possibly as geosensory statoliths (Fenchel & Finlay 1986), and in radiolarian reproductive swarmers where strontium sulphate crystals are readily visible with the light microscope (Hollande & Martoja 1974; Anderson 1976, 1981, 1983). Radiolaria are silica-secreting marine protists (Sarcodina) found in open ocean locations (Anderson 1983). During reproduction, the central mass of cytoplasm becomes segregated into numerous uninucleate, flagellated swarmers, each containing a single $SrSO_4$ crystal. The crystals are always located within a large vacuole separated from the vacuolar membrane by a substantial space (figure 1) that is electron lucent when viewed in ultrathin sections (Anderson 1981; Hughes et al. 1989). It was not known previously whether the crystals are directly enclosed by a living membrane or other organic matter that may influence the deposition of the crystals, but recent crystallographic evidence (Hughes et al. 1989) suggested that there is some biological constraint on crystal formation as the morphology (an elongated square prism with pairs of triangular end faces) was different from geological and synthetic $SrSO_4$ crystals (simple tabular forms). The long axis of the crystal is orientated along the crystallographic a axis and the

† Å $= 10^{-10}$ m $= 10^{-1}$ nm.

trapezoidal faces of the crystal lie perpendicular to the [0$\bar{1}$1] and [023] zone axes, as found in non-biologically produced crystals (Hughes et al. 1989). Evidence is presented here that a very thin cytoplasmic envelope, and in later stages of $SrSO_4$ deposition a thin organic coat, is closely associated with the crystal surface.
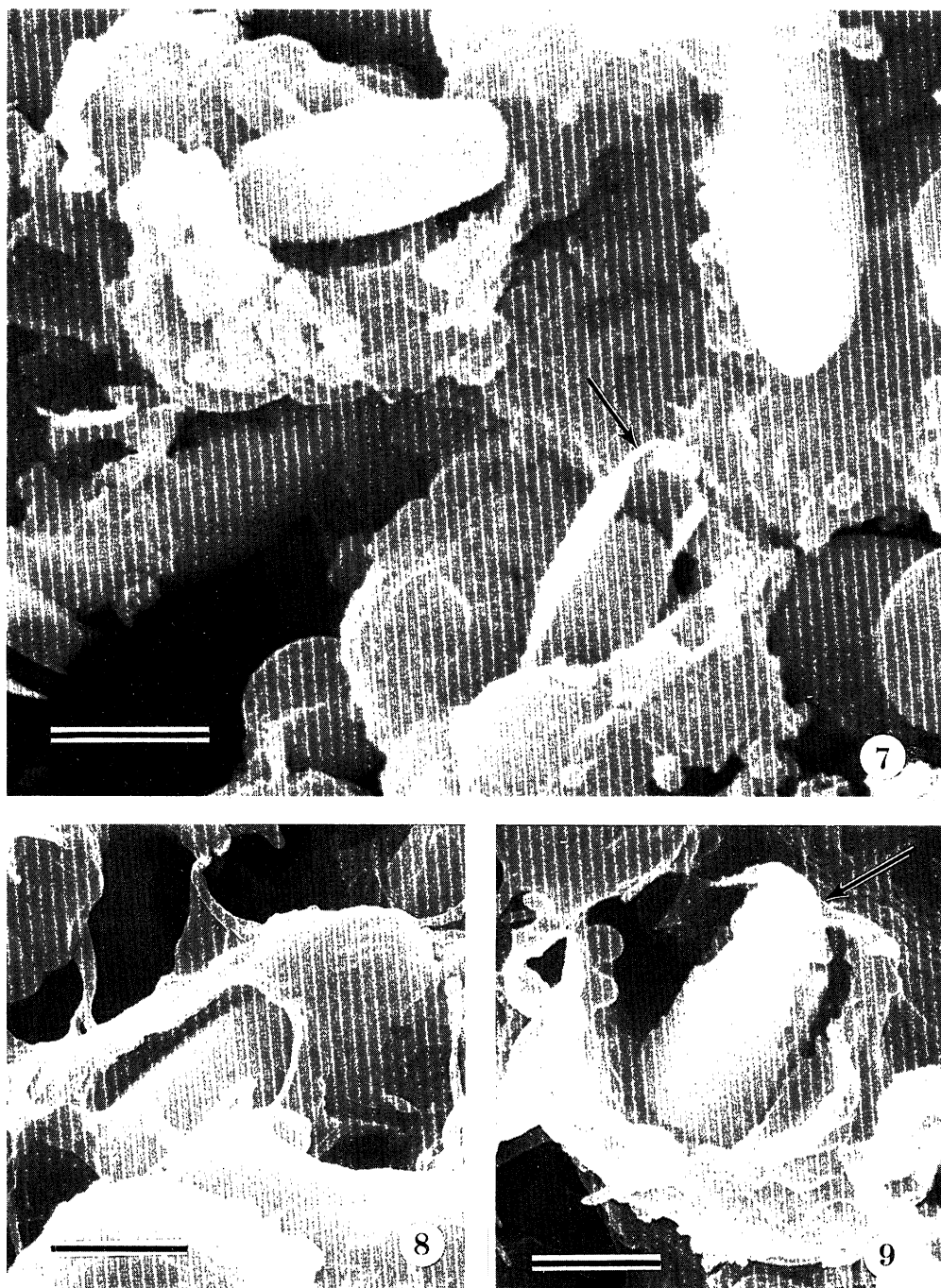
2. MATERIALS AND METHODS

Samples of colonial radiolaria (Collozoum and Collosphaera spp.) were collected from surface water in open ocean locations near Bermuda, or at a site from Piscadera Bay, Curaçao, Dutch West Indies. Fixation and preparation for transmission electron microscopy (TEM) was according to Anderson (1976); however, post-staining with uranyl acetate was used to increase visualization of organic structures. Ultrathin sections were obtained with a Porter-Blum MT-2 ultramicrotome, stained with a solution of 20 g l^{-1} uranyl acetate in 70% (by volume) aqueous ethanol, and observed with a Philips 201 electron microscope. For high resolution scanning electron microscopy (SEM), samples were fixed in electron-microscopic grade glutaraldehyde, stored in a saturated solution of strontium sulphate to prevent dissolution of the crystals (Hughes et al. 1989), returned to the laboratory, briefly rinsed with distilled water, immediately freeze-dried after deposition on SEM stubs, and individual radiolarian central capsules containing the swarmers were cleaved with a high-quality, single-edge, chrome-plated razor blade while viewed with a dissecting microscope. The cleaved specimens were plated with gold–palladium and observed with a Cambridge Stereoscan 250 Mk2 scanning electron microscope.

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Vol. 329. B

Figures 1–6. Transmission electronmicroscopic views of ultrathin sections through radiolarian swarmers containing strontium sulphate crystals. The crystal is fractured out during sectioning and appears in profile (X, figure 1) surrounded by the vacuolar space and the enclosing vacuolar wall limited by a plasma membrane (P). A high magnification view near the edge of a crystal-containing vacuole shows a region where the outer vacuolar wall (P) is in close proximity to the cytoplasmic membranous envelope (V) enclosing the crystal surface (X). The crystal-enclosing membranous envelope is approximately the same thickness (500 Å) as the outer cytoplasmic wall surrounding the vacuolar space. Incomplete crystal surfaces (arrow, figure 3) are deeply penetrated by the cytoplasmic envelope (compare with figure 9). A very thin (50 Å) organic deposit (arrow, figure 4) is observed inside the cytoplasmic envelope and lying immediately adjacent to the crystal surface. Thicker organic coats (figures 5 and 6) are observed at later stages of development and sometimes contain membranous vesicles and tubules (arrow, figure 6). Bars = 2 μm (figure 1), 2000 Å (figure 2), 1.0 μm (figure 3), 1000 Å (figures 4–6.)

Figures 7–9. Scanning transmission electron microscopic views of cleaved, crystal-containing vacuoles within reproductive swarmers prepared by freeze-drying. A cleaved surface through several swarmers (figure 7) shows a ruptured organic sheath (arrow) that has been fractured during cleavage, clearly exhibiting the very thin quality of the organic coat. A crystal, enclosed by the organic coat (figure 8) is displayed within the vacuole, which has been cleaved open showing the peri-crystalline space and a position of the membrane-enclosed crystal relative to the cell body of the swarmer. An apparently incomplete crystal (arrow, figure 9) is exposed and projects from within the surrounding vacuolar space whose wall has been ruptured during preparation yielding a loose peripheral 'collar' of cytoplasm. The cytoplasmic envelope is closely molded to the incomplete surfaces of the crystal (compare to figure 3) and extends as a thin cytoplasmic strand from the tip of the crystal toward the vacuolar surface at the back (Bars = 2.0 μm.)

6-2

3. RESULTS

A section through a reproductive swarmer, viewed by transmission electron microscopy (figure 1), shows the prominent crystal inclusion (clear profile remaining when the crystal is dissolved during sectioning) enclosed in a large eccentrically located vacuole. A higher magnification view near the edge of the crystal-enclosing vacuole (figure 2) exhibits the outer vacuolar cytoplasmic wall (P) enclosing the thin cytoplasmic envelope (V) immediately surrounding the crystal. A section through the edge of an incomplete crystal (figure 3) shows a membranous envelope (arrow) closely associated with the surface indentations of the crystal. In later stages of crystal development (possibly on those surfaces that have reached maximum deposition of the SrSO$_4$) a thin, non-living, organic coat is deposited, detectable as a 50 Å thick, dense layer (arrow, figure 4) situated inside the membrane surrounding the crystal. At other locations on the large surfaces of the crystal, during later stages of maturation, there are thicker (500–1000 Å) deposits of organic matter (figures 5 and 6). In these stages, the cytoplasmic envelope is not present. A system of interconnected cytoplasmic strands and membranous vesicles (arrow, figure 6) is present within the organic coat separating the crystal surface from the surrounding vacuolar milieu. In this stage the organic coat is *ca.* 1000 Å in thickness.

Cleaved vacuoles of freeze-dried specimens, observed by scanning electron microscopy (figures 7–9), exhibit crystals in varying stages of deposition including the enclosing organic coat (500–1000 Å thick, arrow, figure 7). Crystals exposed within the cleaved vacuole of a swarmer (figure 8) are contained within a smooth organic coat. Incomplete crystals with membranous material closely adherent to the irregular surfaces (figure 9) is consistent with TEM evidence (figure 3) that the cytoplasmic envelope is closely molded to the contour of the crystal surface. A thin cytoplasmic strand extends from the tip of the incomplete crystal toward the surrounding vacuolar cytoplasmic surface.

4. DISCUSSION

The biological control of mineral deposition is a significant issue in a wide range of scientific disciplines spanning microbial to biomedical research fields. Various mineral deposits (including alkaline earth salts, calcium carbonate, calcium phosphate, silicates and metal ionic depositions) ranging from amorphous masses to highly sculptured skeletons (i.e. bone and the ornate shells of some protists) occur in unicellular to advanced metazoan organisms (see, for example, Lowenstam 1986; Mann 1986). Biologically deposited crystals offer an attractive tool for understanding the dynamics of biomineralization as they provide a highly ordered system whose crystallographic organization, chemical composition, and general morphology can be rather precisely analysed to detect possible biological effects during deposition.

The SrSO$_4$ crystals in radiolarian swarmers deviate from expected morphology for crystals deposited in thermodynamic equilibrium with the surrounding solute (Hughes *et al.* 1989). Whereas the crystallographic properties are typical of non-biologically deposited crystals, the unusual morphology suggests biological constraint. Based on these insights, we have made high resolution TEM and SEM examinations of colonial radiolarian swarmers to determine possible sources of biological control of crystal morphology during deposition. The fine structure evidence shows that a cytoplasmic envelope immediately surrounds the growing crystal and probably deposits the mineral. The close apposition of this membranous envelope with the rugose surface of incomplete crystals lends further credence to its dynamic role in regulating SrSO$_4$ deposition. The irregular, creviced surfaces of the tips of the growing crystal deviate markedly from the usually smooth and geometrically regular form expected when crystals are deposited directly from solution. The irregular surface probably does not represent dissolution, because fully formed crystals are found in the same preparation as those with irregular surfaces. Moreover, the enclosing membrane is consistently attached directly to the irregular surfaces and molded to the contours. We have not observed such irregular membranes surrounding complete crystals. Furthermore, it is not likely that shrinkage of the membrane, surrounding partially dissolved crystals, can account for the close apposition of the membrane with the crystal surface, as no evidence of shrinkage, rupture, or distortion of the membrane at other locations in the preparation has been observed. Furthermore, the preparation was frozen in situ and vacuum dried, hence it is not possible for the membranes to have been distorted as a result of surface tension changes associated with air drying. The surrounding vacuolar wall, moreover, is intact (figure 8), with the exception of the fractured surface, and exhibits the characteristic vacuolar space surrounding the crystal as observed in TEM sections (figure 1). Thus there appears to be little shrinkage of membranous structures in the freeze-dried preparations. We conclude that the most likely explanation is that the cytoplasmic envelope is the biomineralizing surface directly responsible for the SrSO$_4$ deposition at the crystal surface, similar in function to the cytoplasmic envelope (cytokalymma) that produces the siliceous skeleton of the radiolaria (Anderson 1983). The siliceous skeleton however, is not enclosed in a non-living organic coat, whereas the SrSO$_4$ crystal contains a 500–1000 Å organic lining at maturity.

The function of the organic coat is not known, but the following hypotheses are offered. The cytoplasmic envelope, while functional as a mineral-depositing surface, may not be an efficient barrier against dissolution once the crystal is deposited. The high dissolution characteristics of SrSO$_4$ in aqueous media, including seawater, could require an additional barrier to maintain the crystal. It would not be energetically favourable to maintain crystal stability by constantly 'pumping' the mineral into a cytoplasmic enclosed space as a means of compensating for dissolution. Hence, the deposition of an organic coat could provide protection against dissolution. Theoretically, the en-
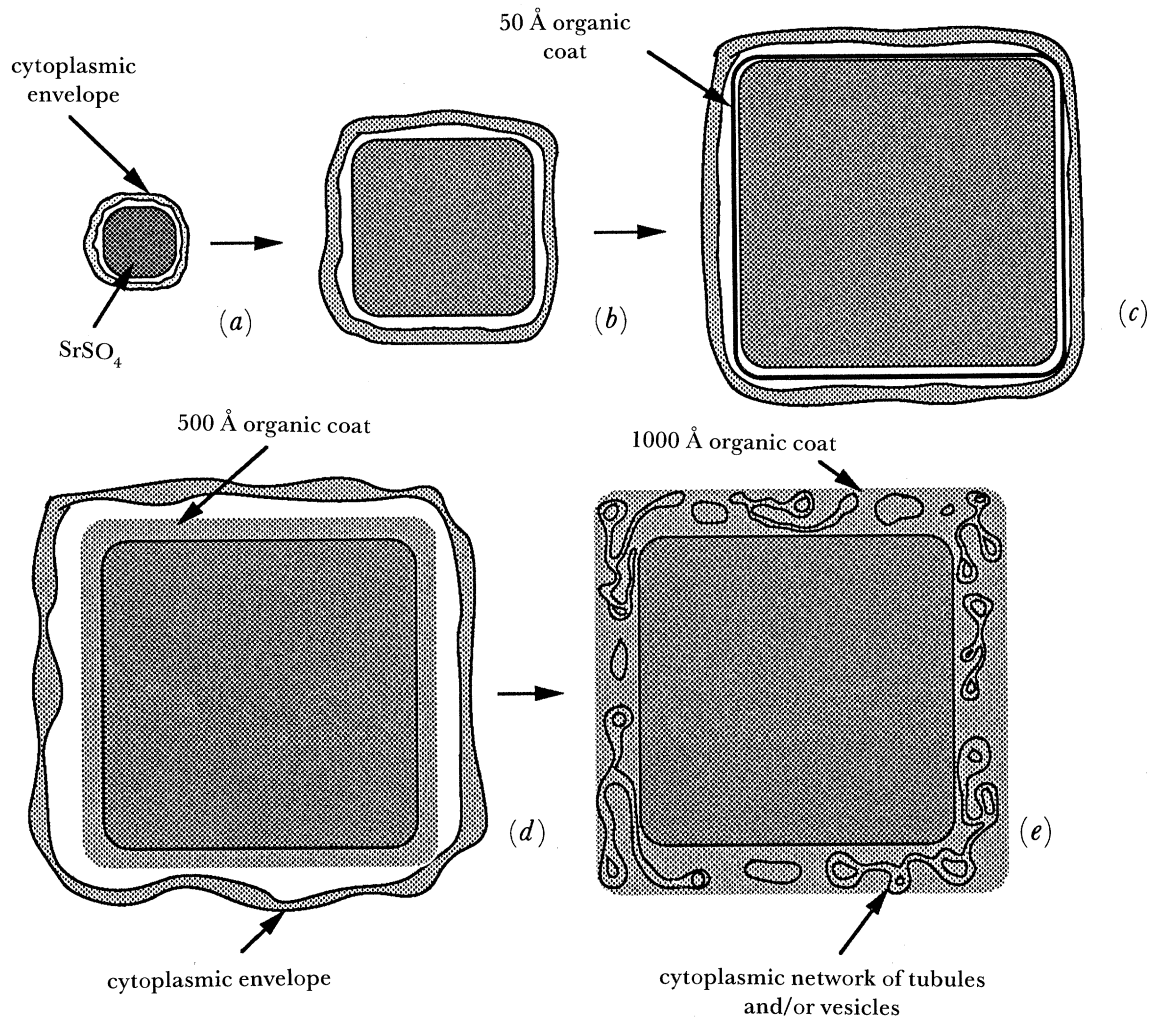
Figure 10. A diagrammatic interpretation (viewed in cross-section) of events during $SrSO_4$ deposition within vacuoles of reproductive swarmers. In the earliest stages (a, b), a thin cytoplasmic envelope immediately surrounds the crystal and deposits the $SrSO_4$ upon the surface of the developing crystal. The vacuolar wall is not figured. At a later stage (c), a very thin organic coat (50 Å) is deposited, perhaps by action of the cytoplasmic envelope, which increases in thickness with maturation of the crystal (d, e), and eventually becomes complete (1000 Å stage), containing internal remnants of the cytoplasmic envelope which at this stage has ceased to function in mineral deposition. The enclosing organic coat may protect the crystal against dissolution.

ergy required to secrete the coat should be less than that required to maintain the crystal by mineral replacement at the cost of metabolic pumping energy. Additional experimental research is needed to document more fully the energy budget of these processes. Moreover, our data suggest that the organic coat is deposited on the mature surfaces of the crystal and is the major residual organic surface in contact with the crystal as the living cytoplasmic envelope disintegrates into a vesicular or tubular network within the secreted coat. If the organic coat is deposited progressively as the crystal matures, this lining in conjunction with the dynamic role of the cytoplasmic envelope may account for the biological constraint influencing crystal morphology. As the Sr^{2+} and SO_4^{2-} ions are concentrated within the cytoplasmic envelope, they would be expected to crystallize in lattices typical of free-formed crystals in solution (as shown by crystallographic evidence), however, the final shape of the crystal would be altered substantially by the geometry of the enclosing organic sheath. Further work is required to

clarify the dynamics of the interaction of the living cytoplasm with the mineral phase, the role of the organic coat, and the physical chemistry of $SrSO_4$ crystallization in the unique environment created by the surrounding biological barriers.

It is unlikely that the morphology of the crystal is completely dictated by the shaping activity of the living envelope, but probably involves the dynamic interaction of biological constraints and physical chemical ordering processes during crystallization. We conclude that the cytoplasmic envelope deposits the $SrSO_4$, and subsequently the organic sheath. The TEM evidence for remnants of cytoplasmic tubules or vesicles within the thick deposits of organic matter, suggests that these are the final stages of cytoplasmic organization remaining after the work of organic deposition is completed.

Given the above assumptions and the empirical data from TEM and SEM analyses, we offer the following diagrammatic interpretation of events during $SrSO_4$ deposition in radiolarian swarmers (figure 10). The

membranous envelope is produced by extension from the surrounding cytoplasm of the swarmer and becomes active as a $SrSO_4$ secretory surface initiating crystal formation. We suspect that the crystal usually forms first at the base of the vacuole, nearest the point of attachment to the cell body, and grows outward toward the periphery. This is consistent with many SEM micrographs showing partially completed crystals with fully formed bases nearest to the cell body and more irregular and incomplete surfaces distally (e.g. figure 9). As the crystal surface approaches the final size, further deposition is halted and the surface may be stabilized by deposition of the organic coat. This progressive process of $SrSO_4$ deposition and stabilization continues until the total crystal has been deposited and enclosed within a protective organic coat, thus possibly accounting for the crystal morphology. We do not know the fate of the crystals when the swarmers produce the next generation. They may be ejected or possibly resorbed. If the latter is the case, the organic coat could be removed, perhaps by enzymic action, at the time of resorption to aid dissolution. There is no evidence of how the $SrSO_4$ mineral would be used during subsequent development of the radiolarian, and our best insight to date is that the crystal is a form of ballast deposited by the swarmer to allow proper positioning in the water column where completion of reproduction is most favourable (Anderson 1983). It is clear, however, that the deposition of such a relatively large crystal requires considerable expenditure of energy. The presence of an organic coat protecting the integrity of the crystal would have marked survival value in conserving the cost of metabolic energy. We do not know if the unique form of the crystal is biologically significant, or if it is an indirect effect of the constraints of the biological surfaces at the mineral interface during crystallization. In either case, it is clear that the $SrSO_4$ crystal morphology can be dramatically altered by geometric constraints and perhaps organo-mineral interfacial chemistry.
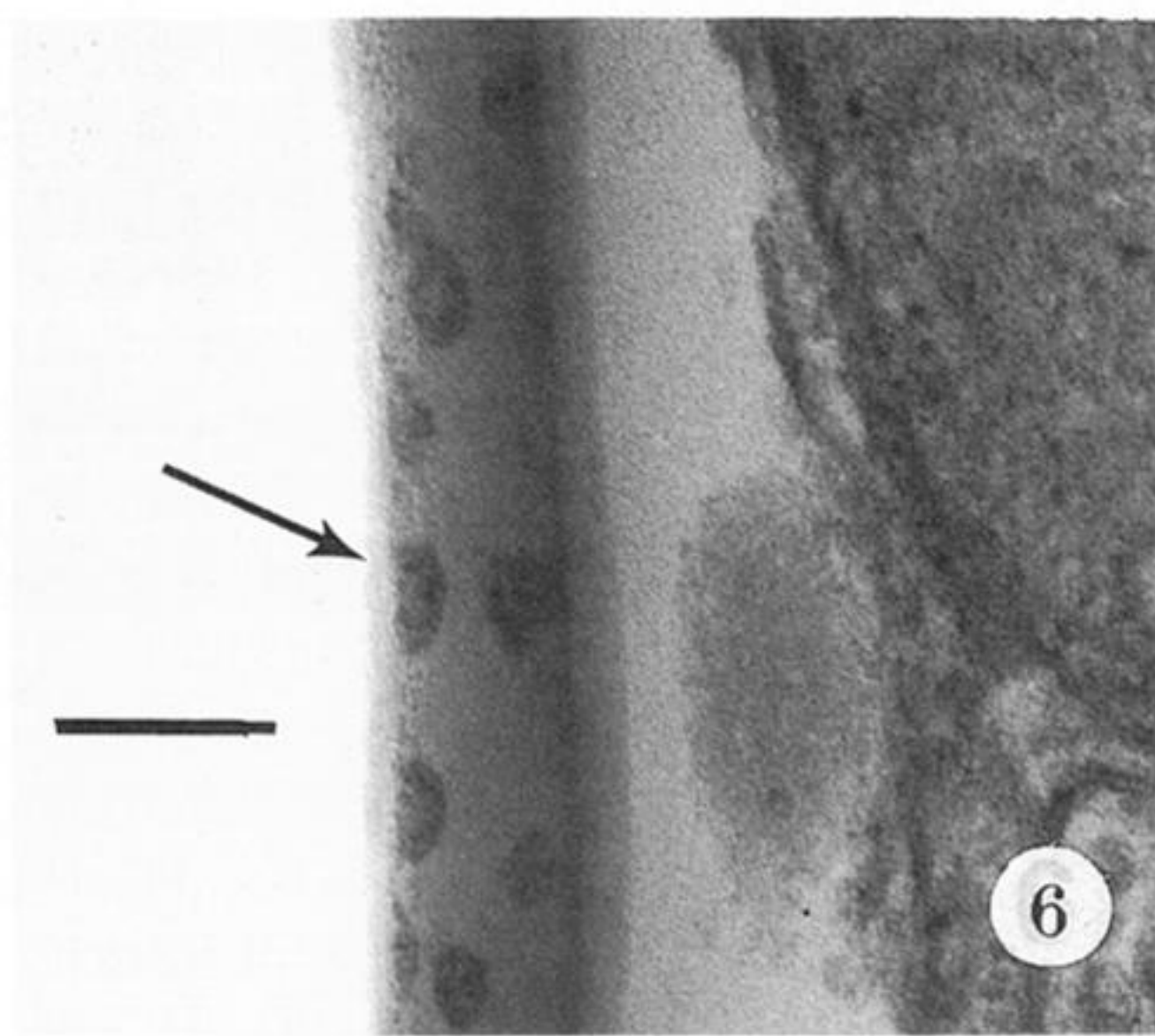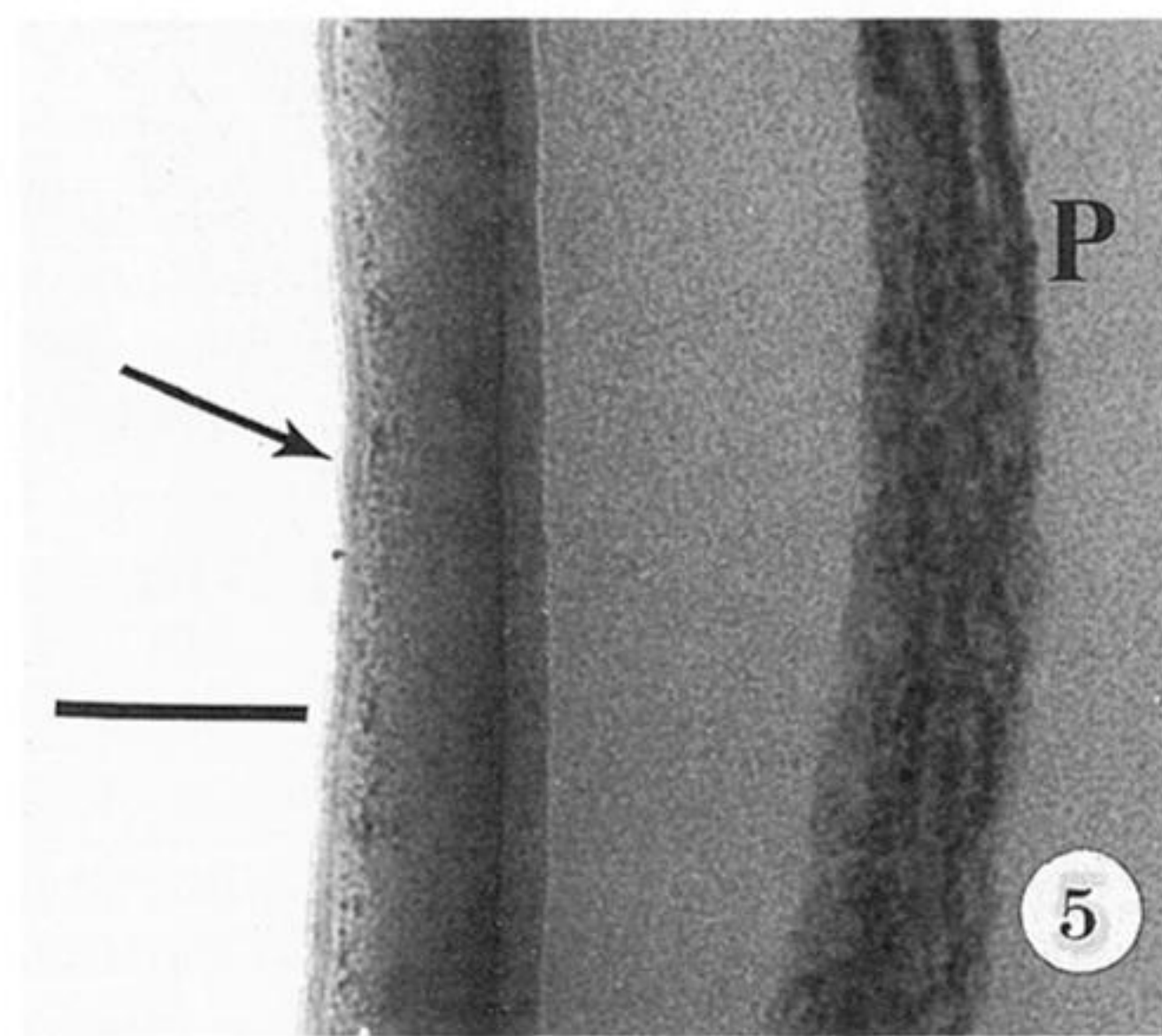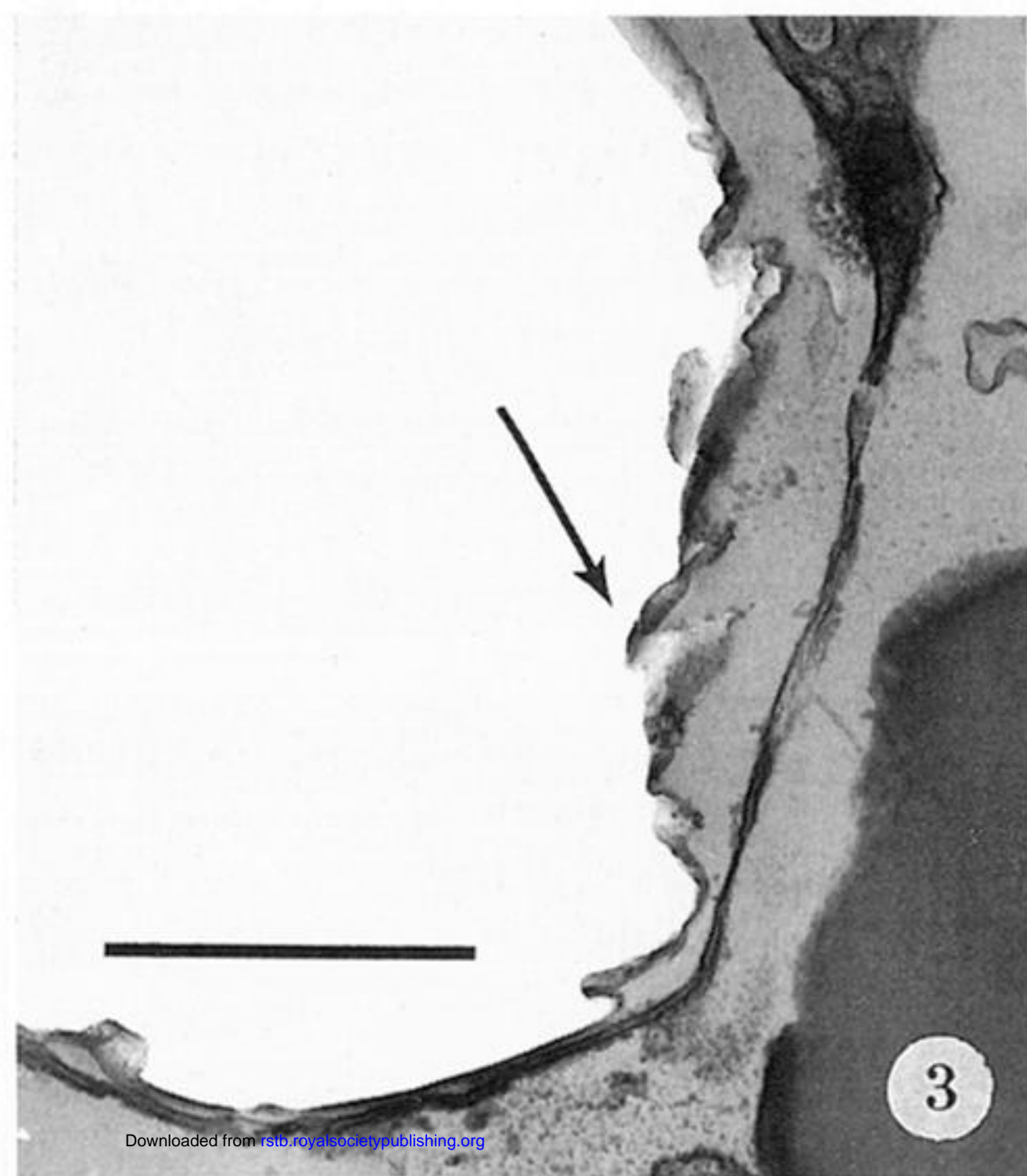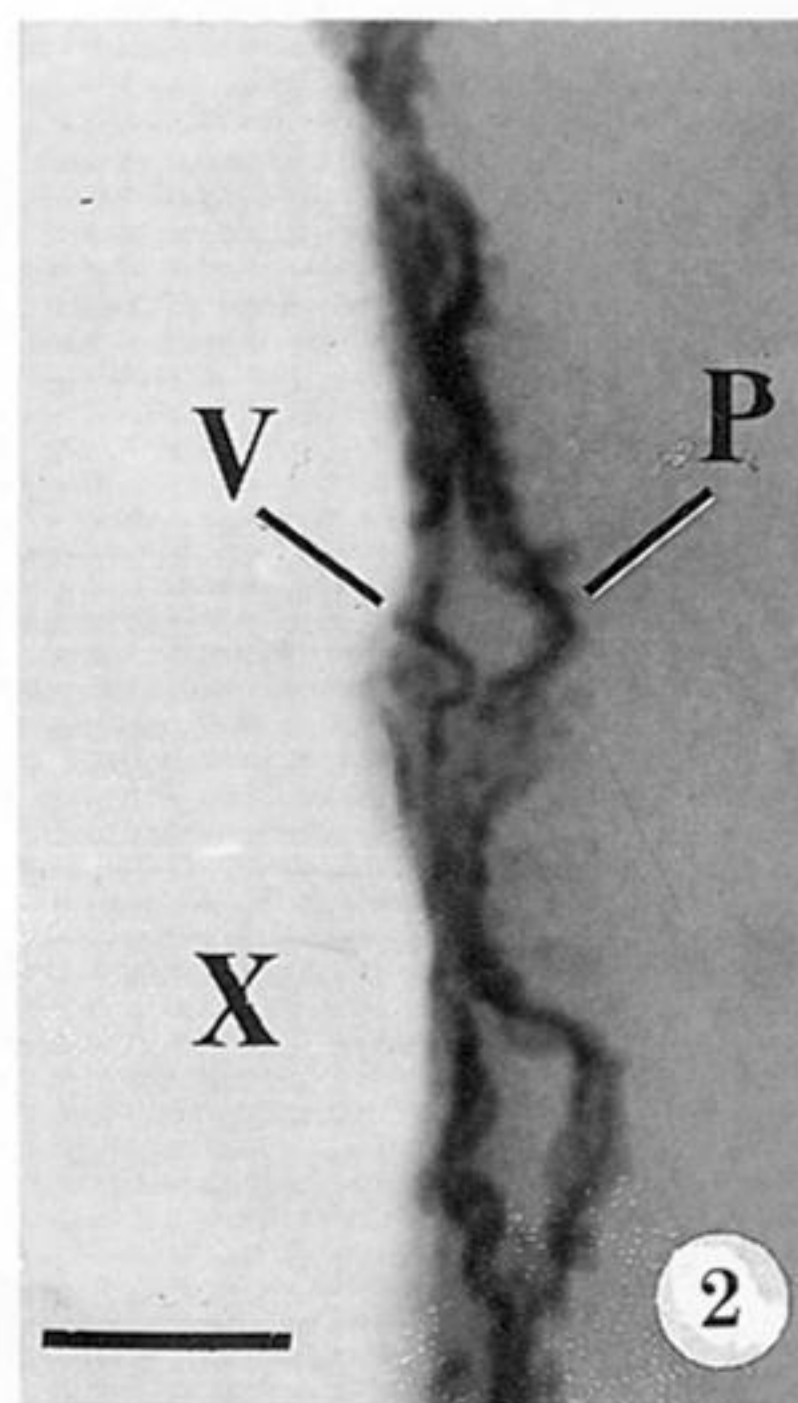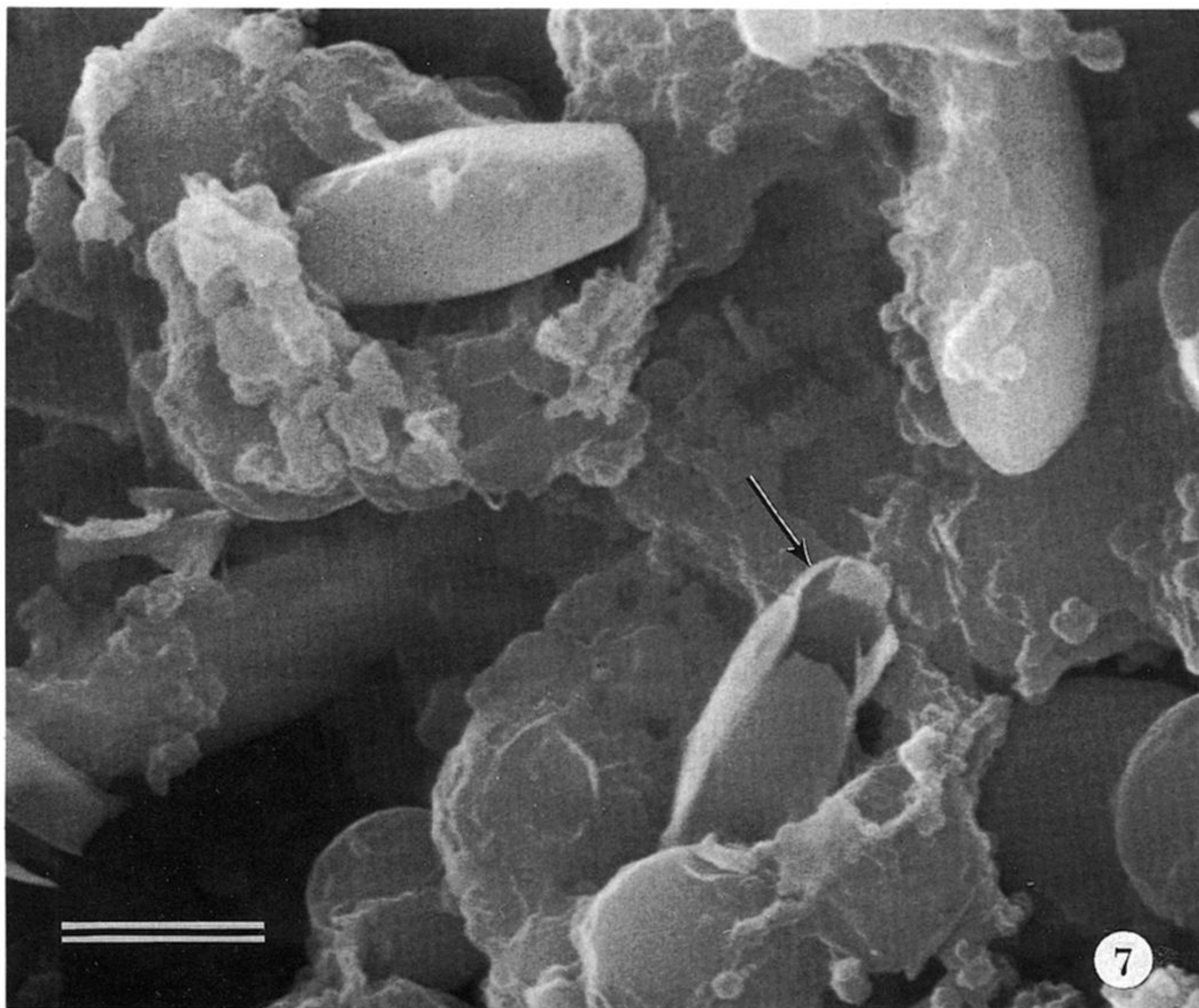
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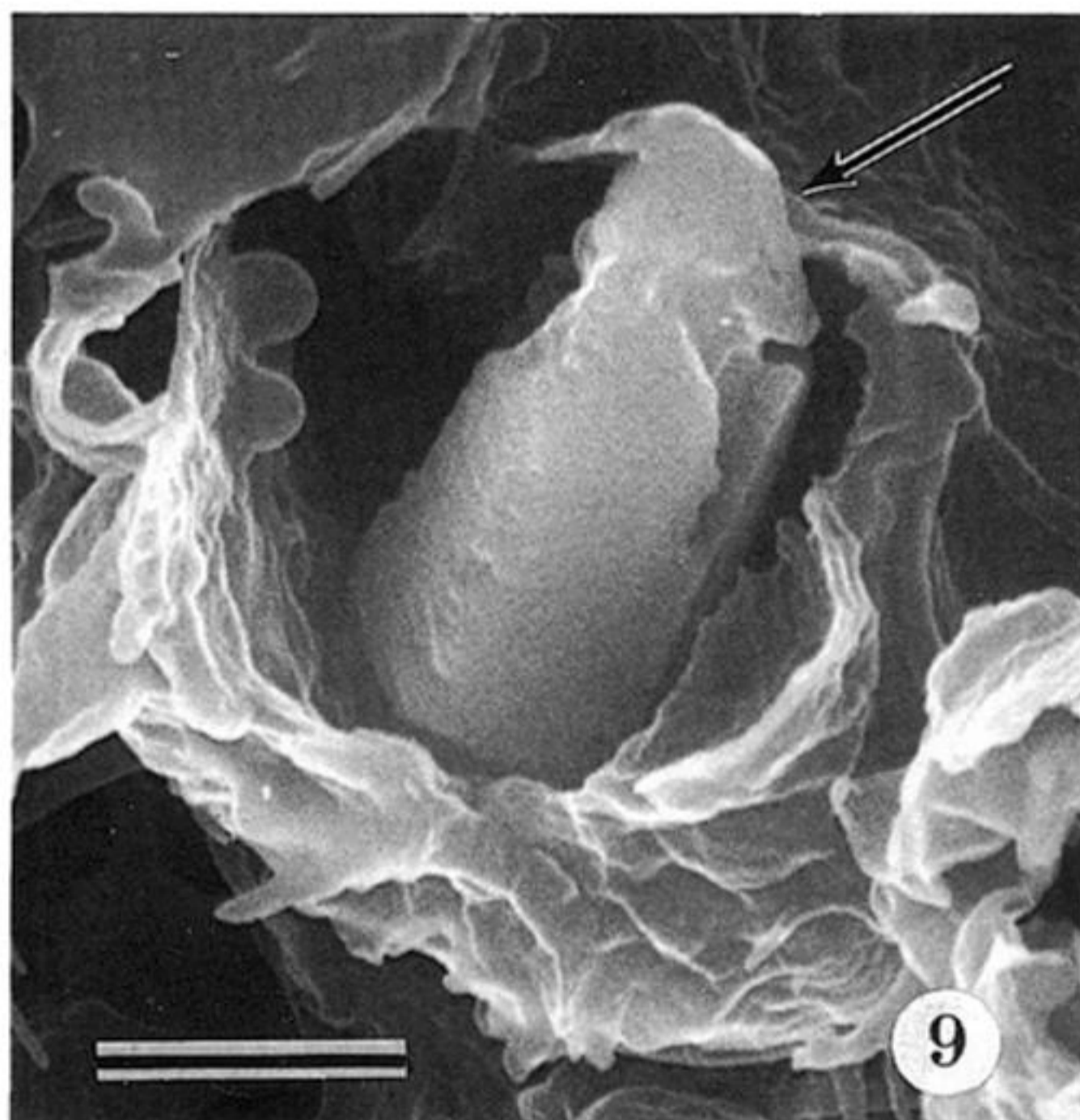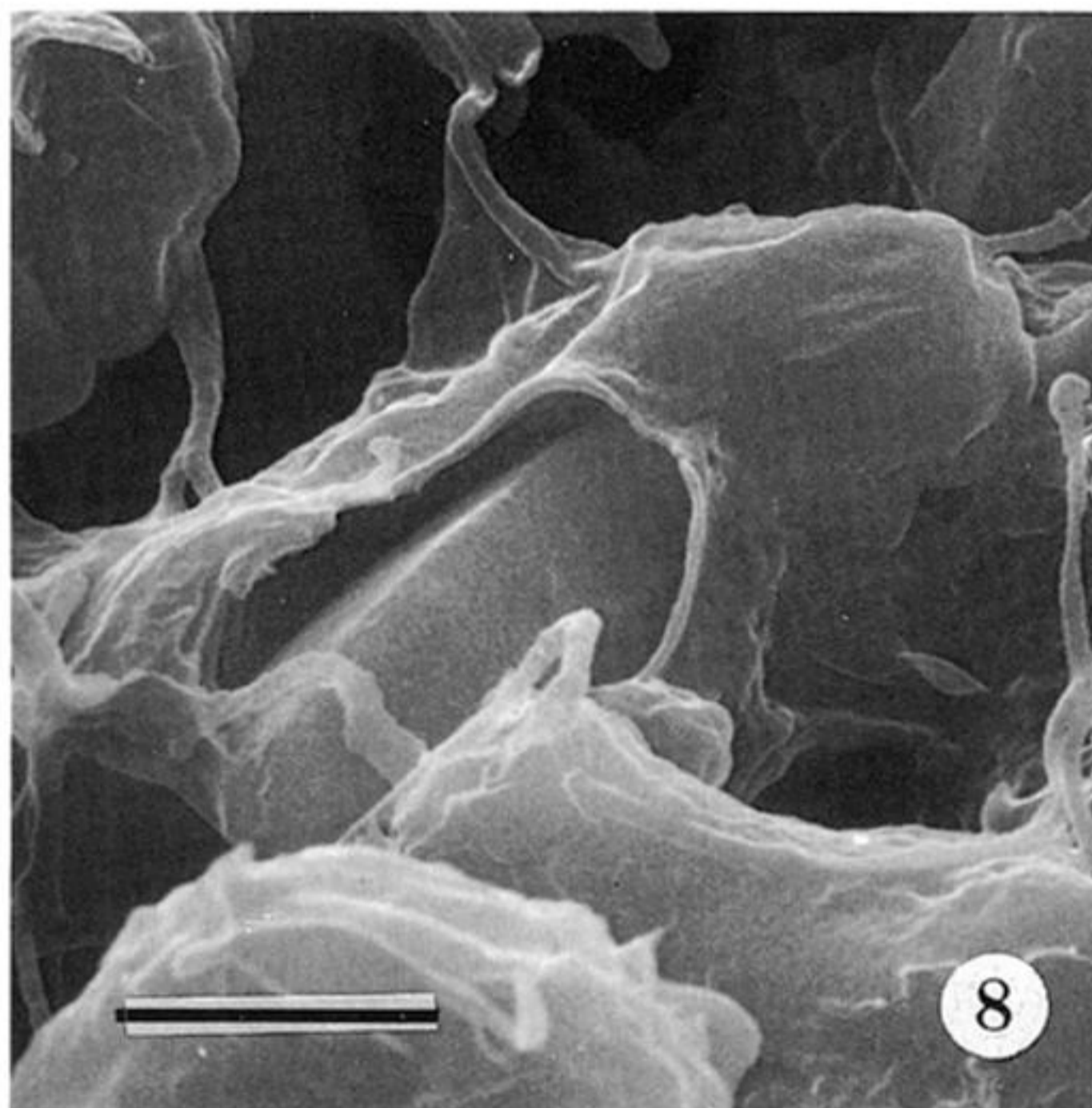
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