Zoomorphology (1986) 106:232–243

Zoomorphology



# Ultrastructural investigation of matrix-mediated biomineralization in echinoids (Echinodermata, Echinoida)

**Konrad Märkel, Ursula Röser, Ute Mackenstedt, and Melanie Klostermann**

Ruhr-Universität Bochum, Lehrstuhl für Spezielle Zoologie und Parasitologie (Arbeitsgruppe Funktionelle Morphologie), Postfach 102148, D-4630 Bochum 1, Federal Republic of Germany

**Summary.** The formation of echinoderm endoskeletons is studied using echinoid teeth as an example. Echinoid teeth grow rapidly. They consist of many calcareous elements each produced by syncytial odontoblasts. The calcification process in echinoderms needs (1) syncytial sclerocytes or odontoblasts, (2) a spacious vacuolar cavity within this syncytium, (3) an organic matrix coat in the cavity. As long as calcite is deposited, the matrix does not touch the interior face of the syncytium. The cooperation between syncytium, interior cavity and matrix coat during the mineralization process is discussed. The proposed hypothesis applies to the formation of larval skeletons, echinoderm ossicles and echinoid teeth.

When calcite deposition ceases the syncytium largely splits up into filiform processes, and the skeleton is partly exposed to the extracellular space. However, the syncytium is able to reform a continuous sheath and an equivalent of the cavity and may renew calcite deposition.

The tooth odontoblasts come from an apical cluster of proliferative cells, each possessing a cilium. The cilium is lost when the cell becomes a true odontoblast. This suggests that cilia are primitive features of echinoderm cells. The second step in calcification involves the odontoblasts giving rise to calcareous discs which unite the hitherto single tooth elements. During this process the odontoblasts immure themselves. The structures necessary for calcification are maintained until the end of the process.

The mineralizing matrix is EDTA-soluble. The applied method preserves the matrix coating the calcite but more is probably incorporated into the mineral phase and dissolved with the calcite.

## A. Introduction

The mineral phase of calcareous echinoderm endoskeletons is well known, but investigations of the underlying tissues and their role in the formation and mineralization of the skeleton are rare. The importance of an organic matrix for the biomineralization of calcareous skeletons has been proved for a variety of organisms (Kemp 1984; Kingsley 1984; Weiner 1984). In echinoderms, the presence of a true mineralizing matrix is still disputed. Weiner (1985) found by biochemical methods "organic matrixlike macromolecules associated with the mineral phase" of echinoid skeletons, but the location of the supposed matrix is unknown.

The main purpose of this investigation is a detailed study of the cells producing the skeleton and to search for the location of the mineralizing matrix. This requires sites of rapid growth and echinoid teeth meet this condition. During feeding, they are abraded anteriorly whilst being restored from the basal end, the plumula. Plumula tissues have been studied by Kniprath (1974) and Chen (1985). Both authors showed that mineral deposition takes place within a preformed cytoplasmic sheath. However, they did not observe the true mineralizing matrix. The calcite skeleton makes ultrastructural studies difficult. In this investigation, a special method (Märkel and Röser 1983a; 1985) is applied which largely avoids the difficulties caused by the mineral phase. Teeth of *Eucidaris tribuloides* (Lamarck, 1816) are used because cidaroids have a shorter plumula than other echinoids. This facilitates the handling of the plumula end to obtain well-defined sections.

*Offprint requests to:* K. Märkel

## B. Materials and methods

*Materials.* Specimens of *Eucidaris tribuloides* were obtained from aquarium supply companies or collected near Santa Marta, Columbia. They were kept for months in artificial sea water and fed with algae and aquarium fish food.

*Methods.* The plumula parts were carefully dissected and fixed in either 5% glutaraldehyde in 0.1 *M* Soerensen phosphate buffer (pH 7.6) or 0.05 *M* cacodylate buffer + 5 m*M* $CaCl_2$ (pH 7.8), both followed by 1% osmium tetroxide and embedded in araldite. The skeleton was exposed, decalcified and the block re-embedded in araldite (Märkel and Röser 1983a; 1985).

Due to the brittle calcite skeleton, relatively large pieces had to be fixed. Thus, the quality of fixation and the staining properties often changed within the same block. The organic matrix coat was most successfully stained by addition of 0.05% ruthenium red to the fixatives. For studies on the cell structures, cacodylate buffer was used and $K_3Fe(CN)_6$ was added to the osmium solution (Karnovsky 1971).

Calcitic tooth elements are small. Normally, the youngest were not exposed before decalcification and so were not decalcified. Ultra-thin sections of these were not possible but semi-thin ones were obtained. During the decalcification process empty spaces replace the calcite plates, leaving araldite lamellae in between. These lamellae often adhere together and either fill or expand the spaces originally occupied by the calcite. For this reason spaces filled with the second application of araldite do not necessarily correspond to the thickness of the calcite plates.

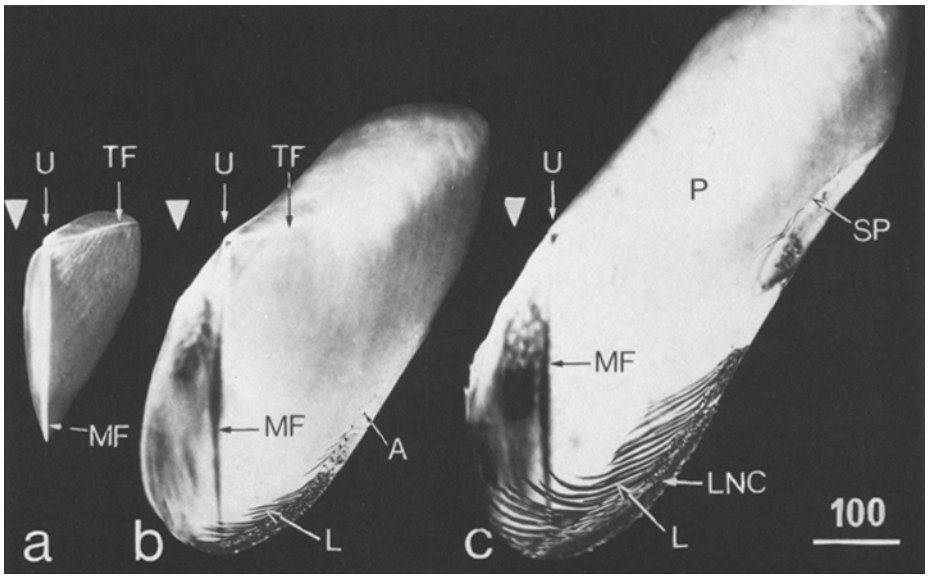


**Fig. 1a–c.** The first stages of tooth element development, crossed polars (Pol.+). Elements of the right hand row are shown (seen from the lantern's main axis). Arrow heads indicate the position of the tooth's median plane. See text and page 242. Scale: μm

## C. Results

### *I. Gross anatomy of the cidaroid tooth*

The structure of the cidaroid tooth skeleton has been described by Märkel and Titschack (1969). For present purposes a short survey of its development is required.

Echinoid teeth consist of (1) a growth region, the plumula; (2) the shaft, which is attached to the lantern; (3) the sharp business end. Each tooth skeleton contains two rows of calcitic tooth elements (Fig. 1) which are symmetrical, but set far enough apart to overlap in the tooth's median plane. New tooth elements are constantly produced in the plumula. They are shifted adorally and assume their definite shape only at the beginning of the shaft.

Each tooth element passes through several stages. The very young elements (Fig. 1a) are triangular primary plates (*P*). They show a growth center, the umbo (*U*), and growth lines. The upper edges of the plates remain for the present at the same level (Fig. 2a). The plates expand only in an oral-adaxial direction, by elongation of the main fold (*MF*) which comes from the umbo. When this stops the plate's oral edge becomes semicircular (Fig. 1b). At this stage the plates expand beyond the mid-line, protruding between succeeding plates of the counter row. From then on the plates move adorally and their umbones are no longer at the same level (Fig. 2a).

Warty adhesive points (*A*, Fig. 1b) arise along the lower edge of the primary plate. They branch off to form thin lamellae (*L*) which cling to the upper face of the plate. Fine calcareous needles and prisms (big needles) arise from the adaxial edge of the lamellae. Furthermore, the primary plate extends in a lateral-apical direction, and the secondary plate (*SP*, Fig. 1c) arises from its outer edge. The secondary plate and the prisms form at right angles to the primary plate, so that the fully grown tooth element (which is not shown) acquires a half-conical shape.

The plumula is covered with a ciliated epithelium (*E*, Fig. 2) and there is a bag (*B*) at its adaxial face. The extreme end of the plumula contains irregularly arranged proliferative cells (*PR*) which give rise to the odontoblasts (Holland 1965). Proliferative cells extend along the abaxial and lateral faces of the plumula; moreover, a cuneiform cord lies in the medial plane dividing the two rows of triangular primary plates (Fig. 2b). The spaces betwen the tooth elements are occupied by odontoblasts which seem to be "interplate cells" (Holland 1965) but belong to two different syncytia, each of which encases one of the adjacent plates (see below). The primary plates are arranged at an angle to the tooth's long axis (Fig. 2a); thus, numerous subsequent plates are cut in cross-section (Fig. 2b).

The calcite elements in the plumula are completely enveloped by the cells which produce them, the odontoblasts. At the beginning of the shaft the odontoblasts give rise to "calcareous discs" which bridge the interplate spaces and unite the hitherto single tooth elements. A rigid tooth skeleton is only formed in the second stage of calcification (see Results II, 2 e).

### *II. Ultrastructure*

Two kinds of cells may be discerned within the tooth; (1) the numerous odontoblasts (Fig. 5, O Fig. 7) and (2) a few phagocytes (*Ph*, Fig. 7b). Granulocytes or spherulocytes, which are abundant in the spine tissues (Märkel and Röser 1983a, b), are not present. The odontoblasts come exclusively from the proliferative cells cap. Scattered phagocytes lie among these cells and in the interplate spaces, i.e., among fully grown odontoblasts. In contrast to the odontoblasts, phagocytes in the interplate space undergo mitoses, i.e., they are not derivatives of the proliferative cells.

#### *1. Ciliated proliferative cells (preodontoblasts)*

The proliferative zone contains irregularly arranged and densely packed cells as well as unstriated and striated fibrils (*f*, Fig. 3b). Mitoses (*MI*, Fig. 3a) occur in this zone. They were not observed by other authors. When labeling with 3H-thymidine, numerous labeled cells are first seen in the proliferative zone (Holland 1965); only later do they migrate into the interplate spaces.

The proliferative cell nucleus is eccentrically positioned (Fig. 3b). The cytoplasm is electron dense and delicate cell structures are largely concealed. Membrane-bound reserve bodies (*rb*, Fig. 3a) are often present. They consist largely

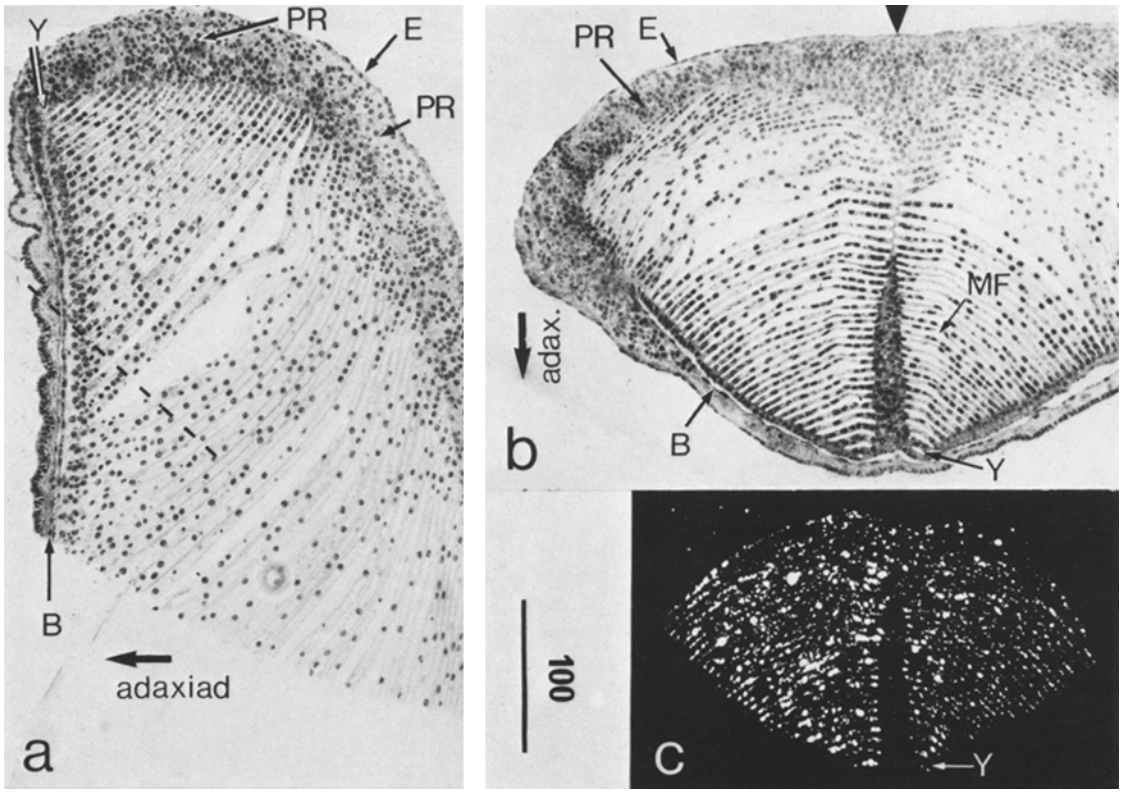


**Fig. 2a–c.** Semi-thin sections through the plumula. **a** Sagittal section. (Broken line indicates the plane of the section of Fig. 7a, b.) **b** Cross section. **c** Part of **b**, Pol. +, showing the preserved calcite. Scale: µm

of lipoids and the centre of those in specimens treated with cacodylate buffer is often dissolved.

A straight, short cilium (*ci*, Fig. 3b–d) 1.0–1.4 µm in length, with a rootlet (*rl*) is the most striking feature of a proliferative cell. Cilia are often cut in sections and we assume all proliferative cells bear one. The bases of most cilia lie in a pouch (Fig. 3c, d). The basal bodies (*bb*) are surrounded by pericentriolar satellites (*s*) with numerous radiating microtubules (*mt*). Golgi complexes (*G*) accompanied by numerous electron dense vesicles (*v*) are arranged around the basal bodies. Their maturing cisternae mostly face the nucleus (Fig. 3e). They give rise to electron-light condensing vacuoles (*cv*) which enclose dark granules and migrate towards the cell surface (*cv 1–3*, Fig. 3c).

Proliferative cells are highly branched into thin, cable-like processes (*cp*, Fig. 5d) filled with microtubules arranged lengthwise. These cable processes are characteristic of proliferative cells and odontoblasts. Their origins from the cell bodies (Fig. 5d, *arrow head*) are rarely cut in sections for they are extremely thin. Here and there the cable processes puff up to form cytoplasmic bladders (*cb*) which contain some electron-dense granules but appear to be otherwise empty. Their content is probably identical with that of the condensed vacuoles (*cv*) mentioned above. They are present throughout the tooth, at least in the plumula and obviously have an important transport function (see below). The connections between the cytoplasmic bladders and the interconnecting cables are rarely cut in sections; thus the bladders often look like extracellular vesicles (*cb*, Fig. 7d).

The extracellular space is continuous throughout the tooth. It contains unstriated and striated fibrils which are often arranged in bundles. They are densely packed in the proliferative zone, but their density decreases distally. The fibrils are often in close contact with the proliferative cells. However, their release from these cells was not seen as distinctly as in the sclerocytes of *Ophioderma* (Märkel and Röser 1985). These fibrils have nothing to do with the deposition of calcite. Chen (1985) observed cells in the proliferative zone with less dense nuclear chromatin than that of the odontoblasts. He calls these cells fibroblasts since they are located near collagen fibrils, but they were not unequivocally proved to release fibrils.

The tooth elements of each row show strictly the same crystallographic orientation (Märkel and Titschack 1969). Perhaps the extracellular fibrils play a role in the adjustment of the odontoblasts and (indirectly) in the adjustment of the calcite's c-axis.

### 2. *Odontoblasts*

*a) Cell structure of odontoblasts.* When developing into true odontoblasts, proliferative cells lose the cilium and the rootlet. The basal bodies become centrioles (*ce*, Figs. 4c, 5b) and are relocated near the nucleus with the Golgi complex. A few odontoblasts lying near the adaxial bag (*B*, Fig. 4a) barely adhere to skeletal structures. They are highly branched so that their cell bodies lie in a three-dimensional network of filamentous cell processes. There are thick ones which often contain mitochondria and thin, cable-like ones (*cp*) with well-developed cytoplasmic bladders (*cb*, Fig. 4d). These protrusions run in all directions and only small sections can be cut lengthwise (Fig. 4e). Thus, it is not known whether the processes fuse and the cells form a syncytium.

Syncytia formed from odontoblasts produce the tooth skeleton (Kniprath 1974). Each primary plate (*P*, Fig. 5a, b) lies in a synplasmic sheath (*sh*) and the mononucleate

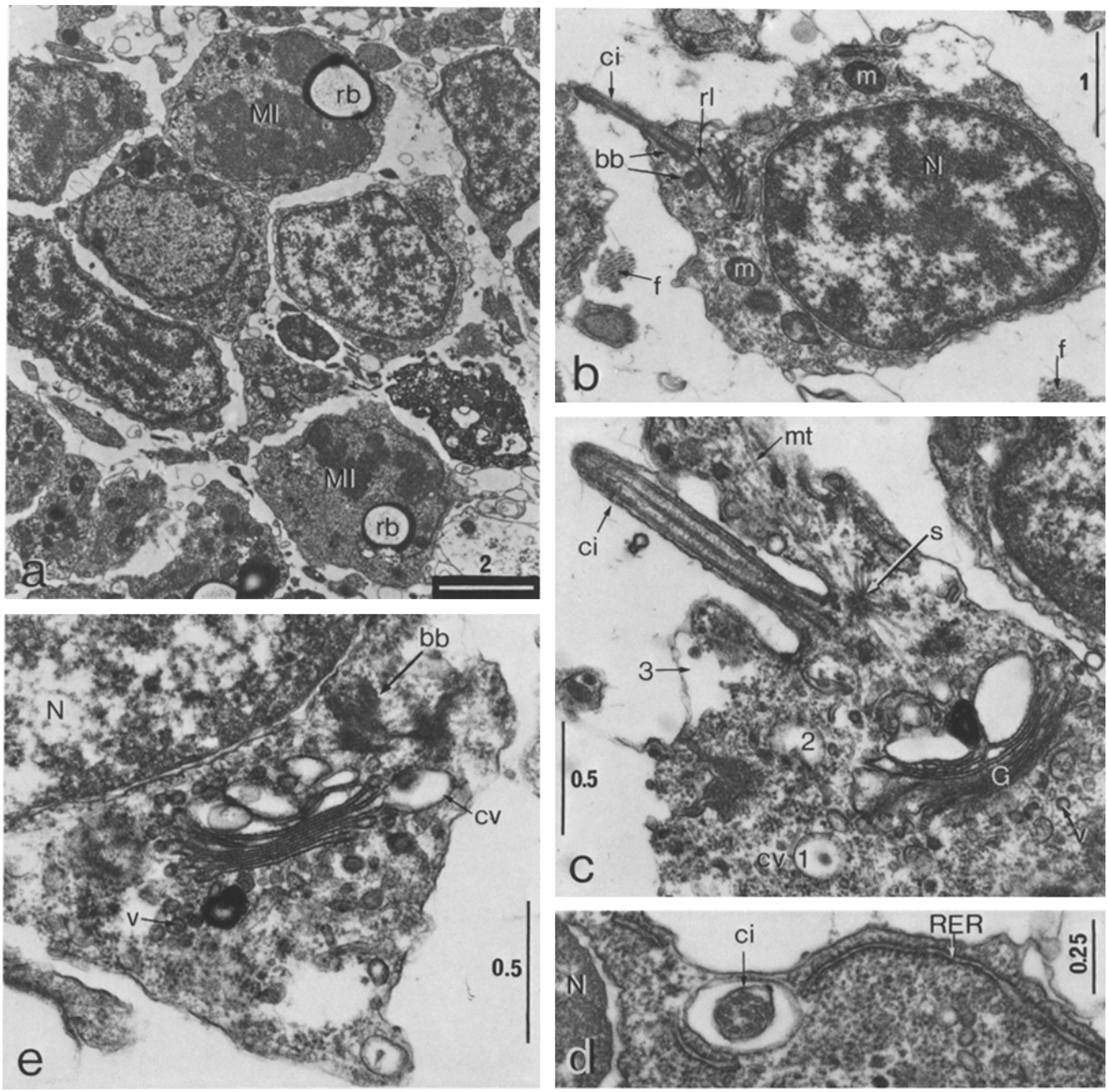


**Fig. 3a–d.** Proliferative cells. **a** Low magnification photograph showing two mitoses (*MI*). **b** A single cell. **c** Cilium and related structures, cut lengthwise. **d** Cilium, cross cut. **e** Golgi complex. Scales: μm

cell bodies arise from the sheath on stalks (*st*) filled with microtubules. At first the cell bodies are crowded but they steadily drift apart at a rate proportional to the expansion of the calcareous plate (Fig. 2). Each tooth element is produced in a sheath of its own. The cell bodies in the same interplate space belong to two different syncytia. The structure of the odontoblasts is largely in line with that of normal sclerocytes. However, they do not have the boundary layer characteristic of sclerocytes (Märkel and Röser 1983a; 1985).

Full-grown tooth elements, especially their imporous primary plates, are large. The interplate cells have to obtain nutrients for metabolism, calcium etc., through narrow interplate spaces connected to the environment only by slit-like apertures between the succeeding plates. After producing the tooth elements, the odontoblasts need a lot of nutrients as when they get to the shaft they have to deposit the voluminous calcareous discs which tie together the single tooth elements. This is probably why they contain a considerable amount of glycogen. This is stored as α-particles in the cell bodies and the cytoplasmic sheath (Fig. 6). The sclerocytes in the spines lack aggregates of glycogen α-particles.

*b) Arrangement of odontoblasts and tooth elements.* Fig. 7a shows a section through half a plumula cut at right angles to the triangular primary plates (see Fig. 2a, broken line). The uppermost and youngest plate (*RY*) shown in the figure is cut through its projecting adoral tip (see Fig. 1a). Note that this is not the youngest tooth plate. The calcite plates

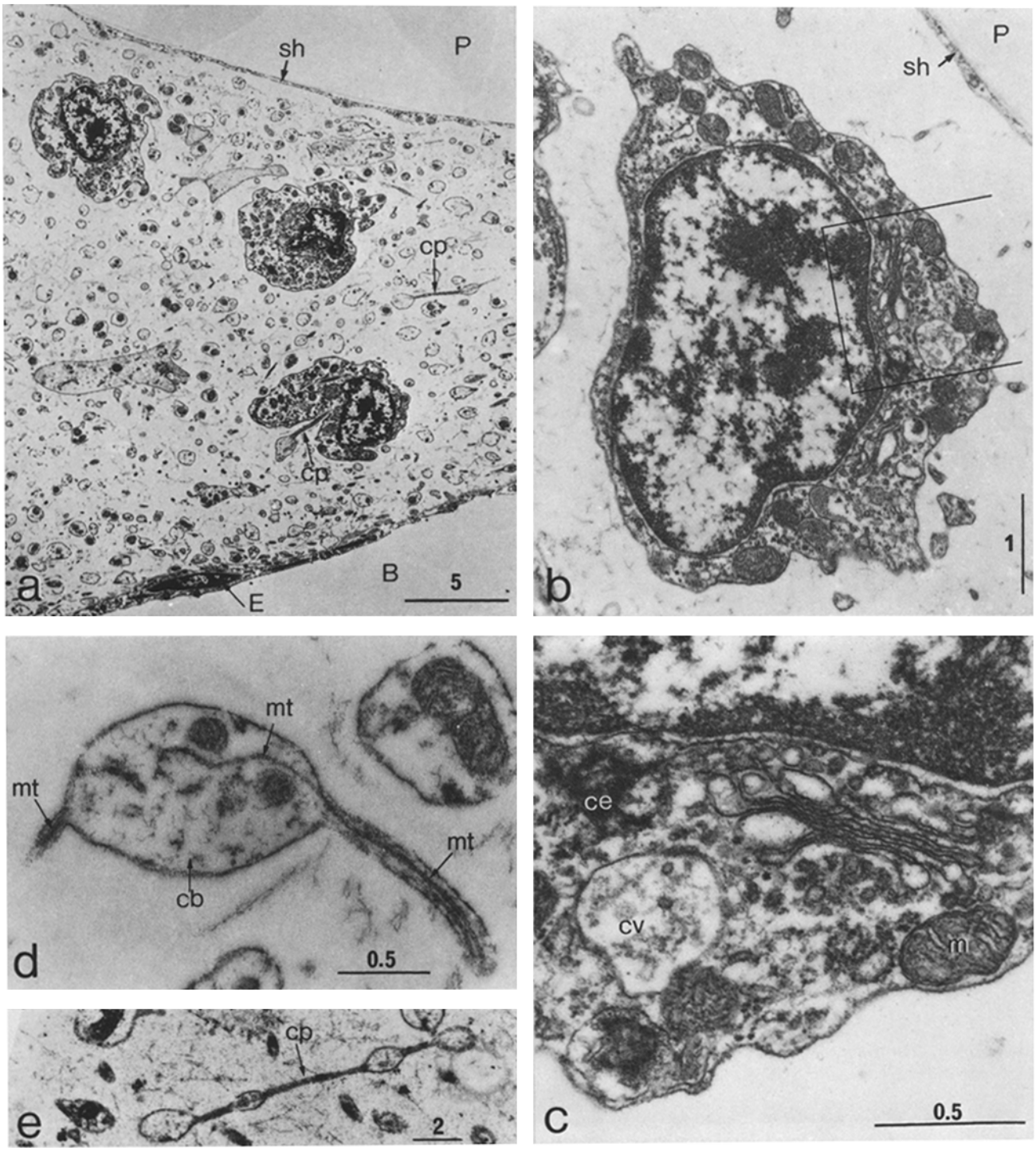


**Fig. 4.** **a** Free odontoblasts near the adaxial bag (*B*). Note the numerous cytoplasmic branches. **b** Cell body, enlarged. **c** Partial view of **b.** **d** Part of a cable process with a cytoplasmic bladder. **e** Cable process with a string of cytoplasmic bladders. Scales: μm

were dissolved and their places occupied by the second application of araldite which (in that case) is more electron-translucent than the first. The main folds (*MF*) of the plates are marked by enlargements. Most of the sheaths do not adhere to the calcite but there is a space in between which appears optically empty (and is filled with the first application of araldite). This syncytium-enclosed vacuolar space is called the interior cavity (*ic*). It is not a simple vacuole but has an intricate shape.

Fig. 7b shows a section through the outer edges of older plates at the semicircular stage. Double rows of odontoblasts are parallel to and extend beyond the interplate spaces. They are obviously ready for incorporation into the syncytia of the interplate cells. Numerous cable processes with their characteristic bladders (*cb*) lie in the region beyond the interplate spaces. The supposed origin of these processes and their bladders is described above (see Figs. 3c, 5d). They probably have an important transport function as they occur deep within the spaces. Fig. 5c suggests that the bladders (*cb* 1–4) finally fuse into the synplasmic sheath.

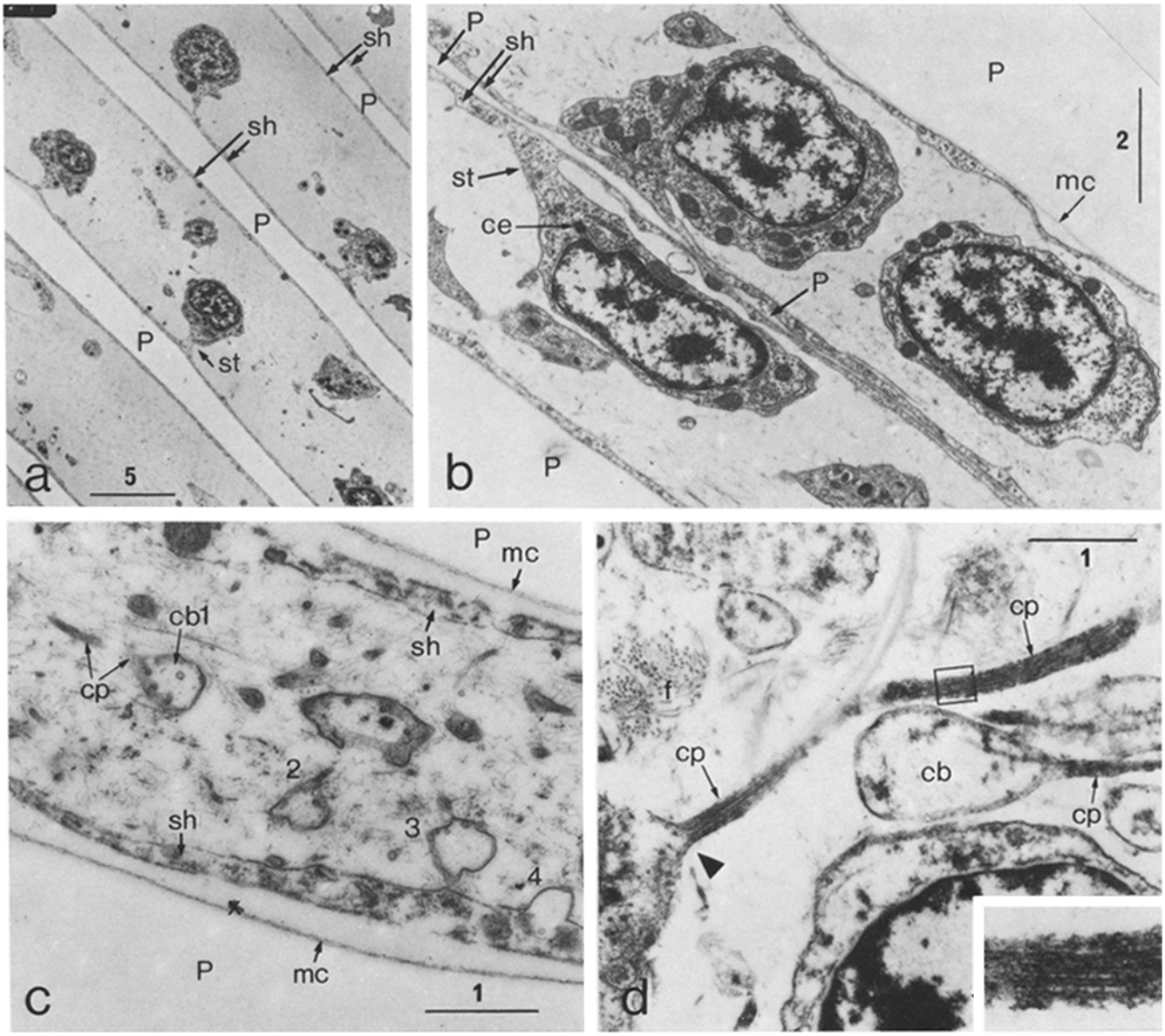


**Fig. 5.** **a** Several stalked odontoblast cell bodies and the respective sheaths (*sh*) at low magnification. **b** Two cell bodies on stalks, from the same syncytium. The space originally occupied by the plate (*P*) collapsed during decalcification. **c** Cytoplasmic bladders (*cb1–4*) in an interplate space. They fuse with the sheath in succession; cable processes (*cp*) partly cut. **d** Cable processes in the proliferative zone. *Left* the origin of a process. *Inset* microtubules within the cable. Scales: μm

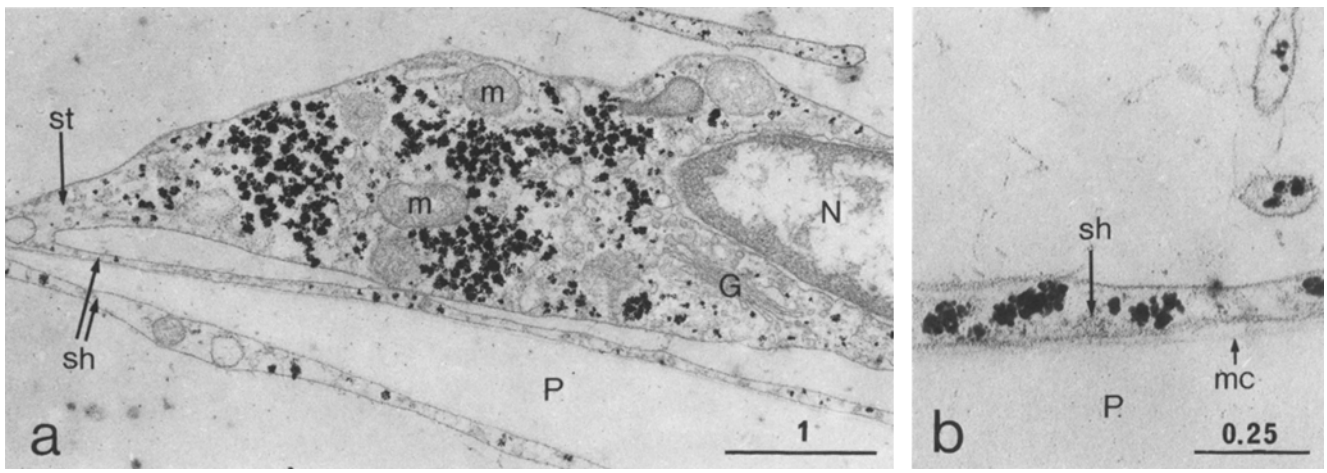


**Fig. 6 a, b.** Glycogen particles (α-particles) as shown by Thiéry's method. Scales: μm

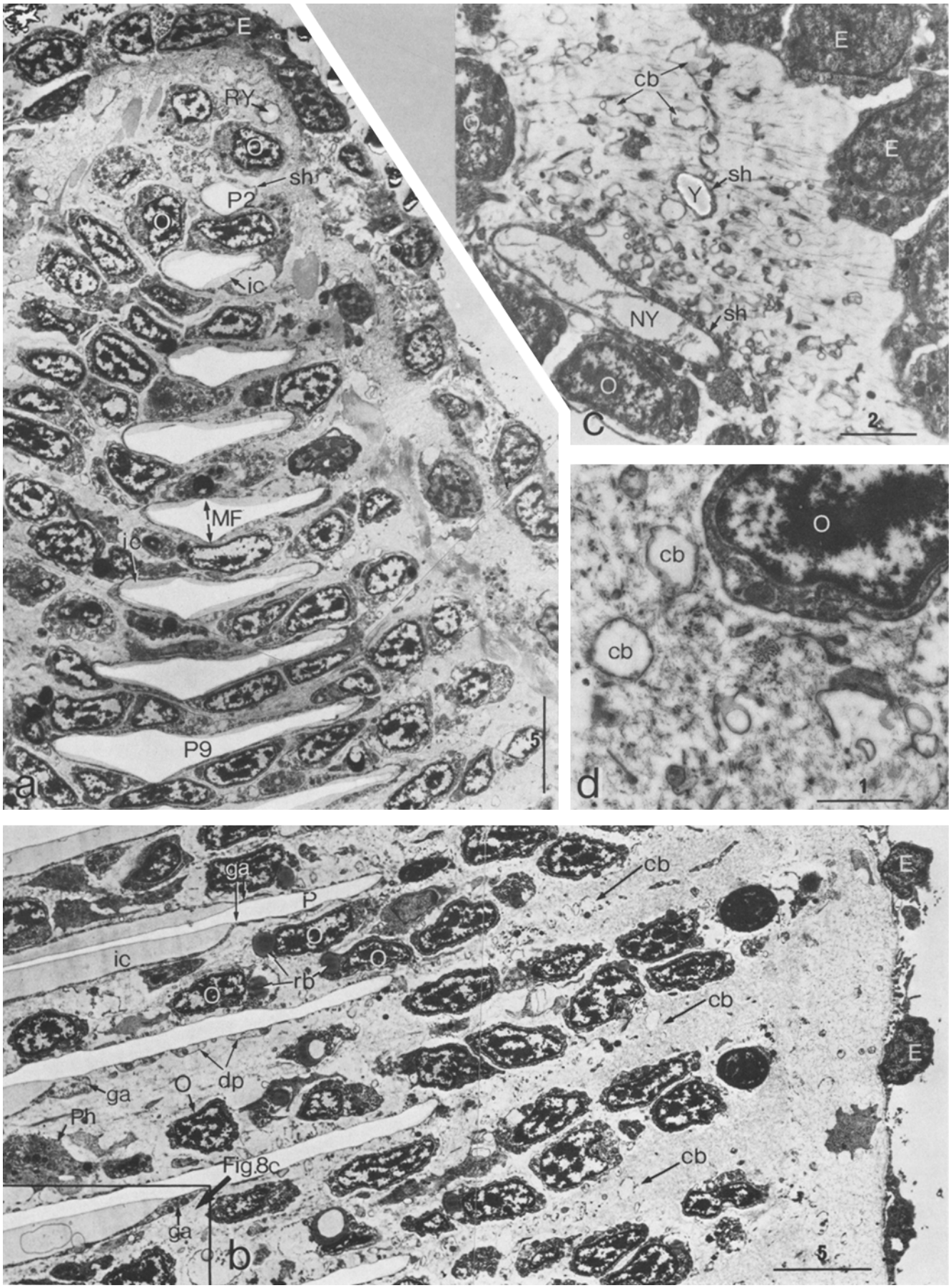


**Fig. 7a–d.** **a, b** Half a tooth cut at right angles to the primary plates (cf. Fig. 2a, broken line). **a** Triangular plates near the adaxial end. RY extreme oral tip of the youngest plate within the section. **b** Lateral edges of older plates. **c** Extreme plumula end, cut lengthwise. The enclosed space *NY* does not yet contain calcite but only supposed matrix material. **d** Cytoplasmic bladders, enlarged. Scales: μm

*c) Matrix coat.* The calcite plate itself is ensheathed in a smooth, continuous matrix coat (*mc*, Fig. 5c) up to 45 nm thick. Towards the outer edge of the plate the coat is often loose and irregularly shaped (Fig. 8a, b). Unlike under normal conditions, the first application of araldite partly penetrated the loose part of the coat indicating that the calcite deposition in this area was incomplete. As shown (Fig. 1a), the trigonal stages of the tooth elements are mainly enlarged sideways and the loose part of the coat lies towards the lateral edge. This suggests that the coat is the organic matrix which mediates the process of calcification. It is obvious that the matrix has to be formed prior to calcite deposition. The matrix hypothesis is strengthened by observations of the formation of the lamellae-needle complex (*LNC*, Fig. 1c). Sections (Fig. 8f, g) show that adhesive points (*A*) and lamellae (*L*) are preformed by outgrowth of the synplasmic sheath (*sh*) and loose matrices are seen within them.

The interior cavity appears empty aside from smooth, thin-walled vesicles (*sv*, Fig. 8c,–f) which become detached from the interior face of the cytoplasmic sheath. They penetrate the sheath (*arrow*, Fig. 8d) but have no visible contents. Several vesicles show invaginations and appear double-walled. The walls are smooth and resemble the matrix coat; moreover, in various places they touch it. Perhaps they contribute some material to the expansion of the matrix.

The matrix is a delicate structure which is hardly preserved during normal decalcification prior to embedding, but is still present if the double embedding method is used. It consists, obviously, of EDTA-soluble material (in the sense of Weiner 1985). Matrix material is probably not restricted to the matrix coat but is also present in the deposited calcite (see discussion).

*d) Structure of resting odontoblasts.* The central parts of the tooth pass through a stage where there is no calcite deposition although the elements at the periphery are still growing. The interior cavity shrinks more and more in fully grown regions until, finally, the sheath touches the calcite plate (Fig. 5a). The cavity is never filled up with calcite.

The calcite trabeculae in fully grown echinoderm ossicles are ensheathed by a trabecle coat (which corresponds with the initial matrix coat). Filiform cytoplasmic "distal processes" take the place of the continuous cytoplasmic sheath (Pilkington 1969; Märkel and Röser 1983a; 1985).

An unexpected finding was that distal processes also occur locally within the plumula (*dp*, Fig. 7b) although the tooth skeleton on the whole is incomplete. Odontoblasts have a second phase of activity during which they produce the calcareous discs. In areas where calcite deposition stops, the synplasmic sheath may split up into distal processes. This is combined with the disappearance of the interior cavity. However, the cavity is preserved in neighbouring areas where the sheath forms gaskets (*ga*) which attach themselves closely to the calcite, tightening the preserved part of the cavity against the extracellular space.

*e) Formation of rigid tooth skeleton.* The functional part of the tooth is a composite material of tooth elements and the calcareous discs which unite them. These two components differ in structure. Scanning electron micrographs of broken teeth show smooth surfaces in the broken faces of the tooth elements but the calcareous discs have a polycrystalline structure (Märkel et al. 1977).

For technical reasons, the investigation of the deposition of the calcareous discs used sections through the prismzone of the tooth. The plate zone is greatly deformed during decalcification (see Fig. 2).

The calcareous discs are produced by the odontoblasts in the second stage of calcification. Before resumption of calcite deposition the odontoblasts have to restore the continuity of the synplasmic sheath and the interior cavity. On the whole, the process of disc deposition resembles the deposition of tooth elements.

While the tooth elements are being formed the volume of the interelement space is much greater than that of the mineral phase. There are few cell bodies (*O*, Fig. 9a) in the extracellular space (*ex*). Locally, protuberances (arrows) arise from the prisms (*pr*) which bridge over the interprism space to combine neighbouring prisms. As mentioned above, the tooth elements and the calcareous discs differ in structure, but in ultrathin sections the borders between them are not seen. The calcareous deposits (*CA*) immure the odontoblasts more and more, and the ratio between calcite and pore space becomes proportionately smaller. Nevertheless, the odontoblasts obstinately preserve their structure (Fig. 9c, d). The odontoblast cell body remains surrounded by a remnant of the extracellular space which still contains fibrils (*f*). The cell body is attached to the synplasmic sheath only by short stalks (*st*). The matrix coat (*mc*) develops a serrated appearance, clearly due to the polycrystalline structure of the calcareous discs. Locally, remnants of the intrasyncytial cavity (*ic*) are seen between the sheath (*sh*) and the coat. In fully grown areas, however, the pore space contains distal processes (*dp*, Fig. 9b) which adhere to the calcite wall.

## D. Discussion

### *I. Matrix material coating the mineral*

The development of echinoderm larval spicules has been thoroughly studied (Okazaki 1975a, b; Blankenship and Benson 1984; Lennarz 1985). Unfortunately, in echinoderm embryology the term matrix is a long-established designation for primary mesenchyme cells and the collagen fibers required to attach the micromeres. Matrix in this sense is not the true mineralizing matrix. The primary mesenchyme comes from micromeres which migrate into the blastocoel. In doing so, they lose their cilia and their mitotic activity ceases (Gibbins et al. 1969). Processes of these cells unite into a syncytial sheath with its cell bodies on stalks. The spicules are formed in a vacuolar space in the syncytium, and do not touch the wall of the sheath. Millonig (1970) described a "dense amorphous membrane of about 200 Å" which surrounds the mineral. This membrane is not found in decalcified specimens. Millonig did not speculate on the function of this membrane, which is obviously the matrix coat. On the whole, there is a striking coincidence between the formation of the mineral skeleton and its production cells in echinoplutei and adults.

Echinoderm biocrystallization (Fig. 10) obviously needs (1) syncytial sclerocytes or odontoblasts which enclose (2) a spacious vacuole, the intrasyncytial cavity, and (3) an organic matrix located within this cavity. The matrix is not part of the cytoplasm. Calcite deposition itself takes place solely within the matrix-enclosed space. As long as

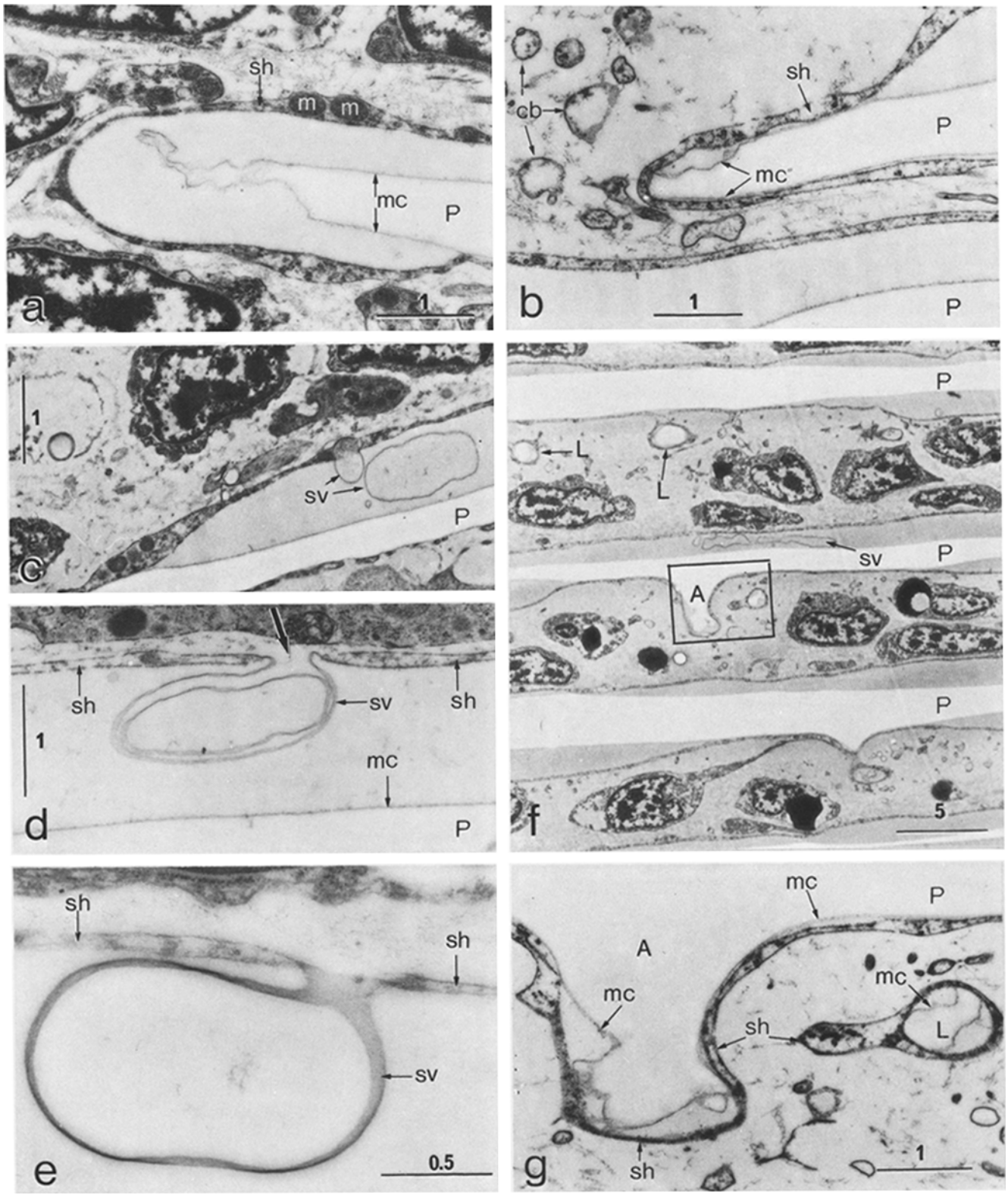


**Fig. 8a–g.** Formation of the matrix coat (*mc*) which preceeds the enlargement of the respective calcite structures. **a** Lateral edge and **b** median edge of the plate. **c–e** Smooth walled vesicles (*sv*) which penetrate the sheath to enter the enclosed cavity. **f, g** Origin of calcite lamellae (*L*) from the lower edge of a tooth element of the semicircular stage. Scales: μm

calcite is deposited, the matrix coating does not touch the cytoplasmic sheath.

The cooperation of the synplasmic sheath, the intrasyncytial cavity and the mineralizing matrix in the formation of the calcite skeleton is clearly demonstrated in adult ossicles. Adult ossicles have a sophisticated structure, especially in the tooth elements which, when fully grown, have a three-dimensional and half-conical shape. Nonetheless, they are

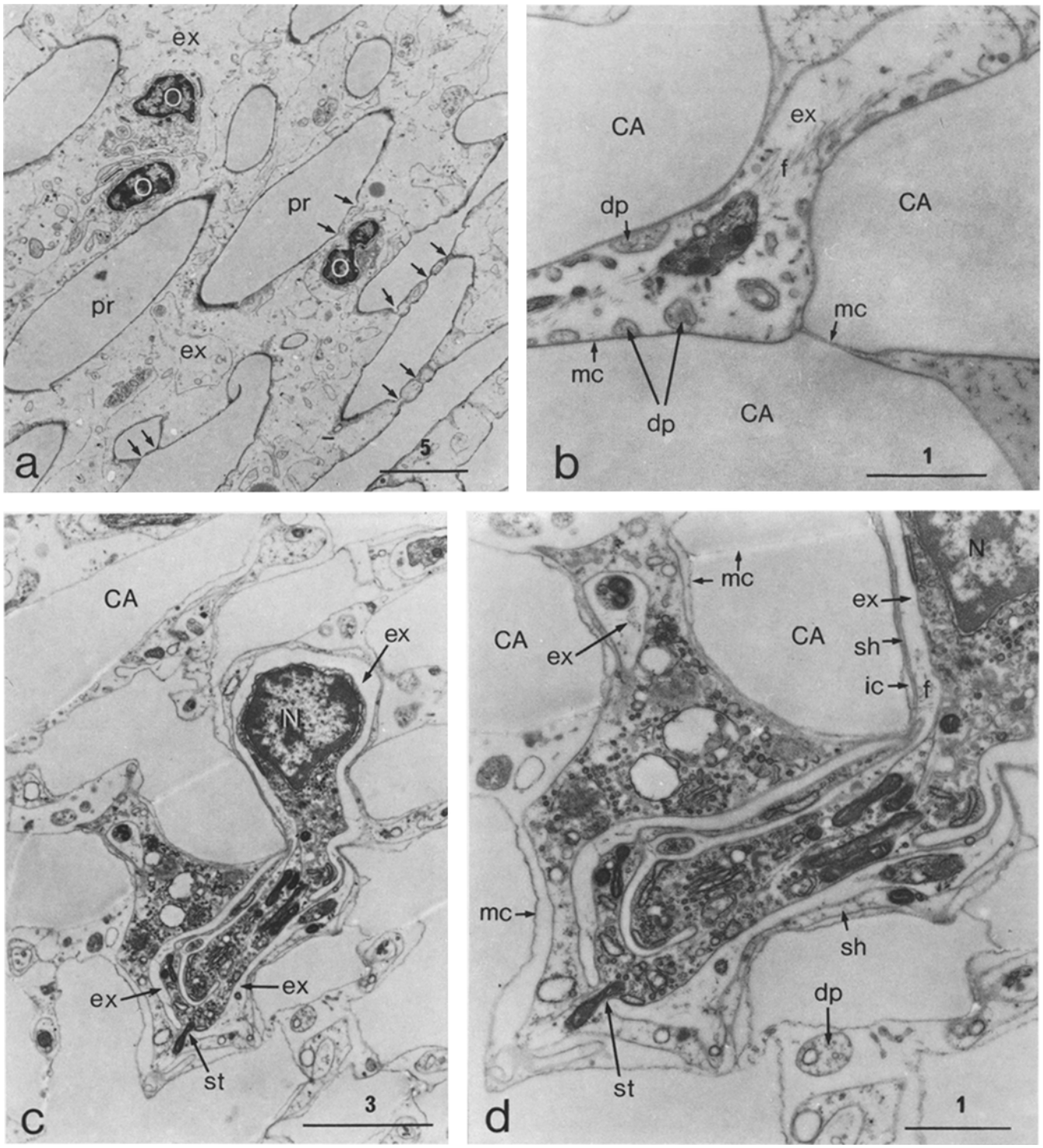


**Fig. 9 a–d.** Development of the calcareous discs. **a** Low magnification photograph of the prism zone at the very beginning of the development of calcareous discs (*arrows*). **b** Pore channel between calcareous deposits. **c, d** Odontoblast immured in calcareous deposits. Scale: μm

optically like monocrystals of calcite. The ossicle's shape is obviously controlled by the syncytium whose cytoplasmic sheath forms a mold slightly before formation of the matrix. The matrix is formed somewhat before calcite deposition and probably controls the *c-axis* orientation of the calcite. The suggested cooperation between syncytium and matrix suggests that shape and crystal orientation are largely independent in echinoderm ossicles. The synplasmic sheath probably controls the activity of the matrix since calcite deposition stops when the sheath touches the coat and the intrasyncytial cavity disappears. The sheath draws up to the matrix-coated calcite: the calcite never fills the initial volume of the interior cavity.

The deposition of calcium carbonate skeletons and the

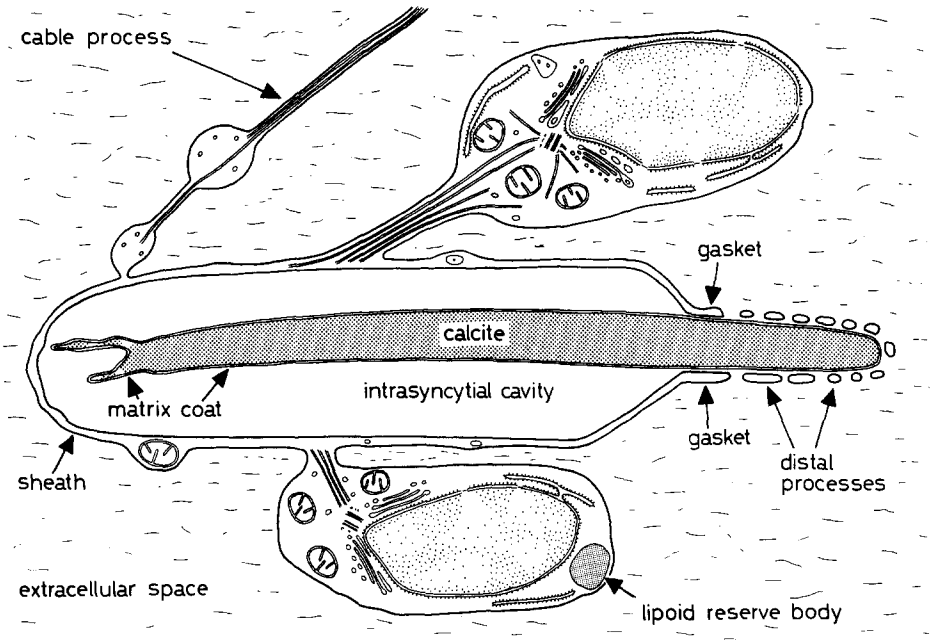


**Fig. 10.** Schematic diagram summarizing the proposed hypothesis on calcite deposition in echinoderms. The calcite plate is enlarged on the *left* of the diagram and full-grown to the *right*. Not to scale

role of the organic matrix in this process has been studied mainly in molluscs (for review see Wilbur and Saleuddin 1983). The mollusc shell is an exoskeleton. Nevertheless, to a limited extent, its formation may be compared with the calcification process in echinoderms. The echinoderm synplasmic sheath has the same function as the mollusc mantle lobe, and the intrasyncytial cavity fulfills the role of the extrapallial space. In both phyla the matrix is non-cytoplasmic. According to Weiner (1984) the biochemical composition of the "macromolecules associated with the mineral phases" share common properties in molluscs and echinoids. The location of the respective molecules is unknown. There is still a serious gap between structural and biochemical findings.

Mesenchymal calcite spicules occur in several non-echinoderm invertebrates (for review see Kingsley 1984). Kingsley and Watabe (1982, 1984) studied the spicule formation in *Leptogorgia virgulata* (Lamarck) (Anthozoa, Gorgonaria). These spicules likewise grow in a sclerocyte vacuolar cavity. Finally, however, the sclerocytes atrophy and the mature spicule is exposed in the extracellular space. In echinoderms, the skeleton remains covered with distal processes throughout life.

### *II. Matrix material incorporated into the mineral phase*

Echinoderm calcite contains a small amount (0.05–0.1%) of organic substances (Pilkington 1969; Inoué and Okazaki 1977) which obviously influence the material properties of echinoderm calcite (Emlet 1982; Burkhardt et al. 1983). Little is known about the distribution of these substances within the mineral phase, and even the crystallographic structure of echinoderm calcite is still under discussion. Normal skeletal elements behave optically like monocrystals of calcite, but recent investigations provide evidence of a microcrystalline substructure (Pearse and Pearse 1975; O'Neill 1981; Emlet 1982). SEM photographs of freshly broken ossicles reveal smooth fracture faces (Märkel et al. 1977), which when rinsed in water for a few minutes show a concentric arrangement of microcrystallites. Perhaps the matrix material is very intimately bound to the mineral, but it is highly soluble and its removal reveals the microcrystalline substructure.

The double-embedding method applied in this investigation preserves the matrix coat which surrounds the mineral phase but the material within the mineral is dissolved with the calcite. This also happens to the organic material in the calcareous discs which have a coarse microcrystalline structure compared to normal ossicles. At the border between mineral components which unite (i.e., tooth elements and calcareous discs) the matrix is preserved at first, but more and more of it vanishes (see Fig. 9). This demonstrates the limitations of this method. Scanning electron micrographs (Märkel et al. 1977) reveal poor adhesion between tooth elements and calcareous discs, indicating that they do not grow together seamlessly.

Remnants of matrix material incorporated into the mineral are perhaps seen in the very first tooth elements (*NY*, Fig. 7c). These elements are barely calcified, and are not representative of the overall composition.

*Acknowledgements:* We thank Professor Dr. A. Ruthmann, Bochum, for helpful discussions and Professor Dr. J. Lawrence, Tampa, Florida, USA for providing us with a copy of Chen's thesis. We also thank Dr. E. Wedler and Mr. K. Müller, Santa Marta, Columbia, for collection of specimens. The research was supported by Deutsche Forschungsgemeinschaft, Bonn.

**Abbreviations**

| | | | |
|---|---|---|---|
| *A* | adhesive point (*LNC*) | *f* | extracellular fibrils |
| *B* | adaxial bag | *ga* | gasket (*sh*) |
| *bb* | basal body (*ci*) | *ic* | interior cavity |
| *CA* | calcareous deposits | *L* | lamellae (*LNC*) |
| *cb* | cytoplasmic bladder (*cp*) | *LNC* | lamellae needle complex |
| *ce* | centriole | *m* | mitochondrium |
| *ci* | cilium | *mc* | matrix coat |
| *cp* | cable-like cell process | *MF* | main fold (*P*) |
| *cv* | condensing vacuole | *MI* | mitosis |
| *dp* | distal processes (*sh*) | *mt* | microtubules |
| *E* | epithelium of the tooth | *N* | nucleus |
| *ex* | extracellular space | *O* | odontoblast |

| | | | |
|---|---|---|---|
| *P* | primary plate | *s* | satellite (*bb, ce*) |
| *Ph* | phagocyte | *sh* | synplasmic sheath (*O*) |
| *PR* | proliferative cell | *SP* | secondary plate |
| *pr* | prism | *sv* | smooth-walled vesicle |
| *rb* | reserve body | *TF* | transversal fold (*P*) |
| *RER* | rough endopl. reticulum | *U* | umbo (*P*) |
| *rl* | rootlet (*ci*) | *v* | Golgi vesicle |
| *RY* | relatively youngest plate | *Y* | youngest tooth element |

## References


Blankenship J, Benson S (1984) Collagen metabolism and spicule formation in sea urchin micromeres. Exp Cell Res 152:98–104

Burkhardt A, Hansmann W, Märkel K, Niemann HJ (1983) Mechanical design in spines of diadematoid echinoids. Zoomorphology 102:189–203

Chen CP (1985) The role of carbonic anhydrase in the calcification of the tooth of the sea urchin *Lytechinus variegatus*. Ph.D-Dissertation, University of South Florida

Emlet RB (1982) Echinoderm calcite: A mechanical analysis from larval spicules. Biol Bull 163:264–275

Gibbins JR, Tilney LG, Porter KR (1969) Microtubules in the formation and development of the primary mesenchyme in *Arbacia punctulata*. I. The distribution of microtubules. J Cell Biol 41:201–226

Holland ND (1965) An autoradiographic investigation on tooth renewal in the purple sea urchin (*Strongylocentrotus purpuratus*). J Exp Zool 158:275–282

Inoué S, Okazaki K (1977) Biocrystals. Sci Amer 236:83–92

Karnovsky MJ (1971) Use of ferrocyanide-reduced osmium tetroxide in electron microscopy. Abstracts of the Am Soc of Cell Biol. Rockefeller University Press, New York, New Orleans, p 146

Kemp NE (1984) Organic matrices and mineral crystallites in vertebrate scales, teeth and skeletons. Am Zool 24:965–976

Kingsley RJ (1984) Spicule formation in the invertebrates with special reference to the gorgonian *Leptogorgia virgulata*. Am Zool 24:883–891

Kingsley RJ, Watabe N (1982) Ultrastructural investigation on spicule formation in the gorgonian *Leptogorgia virgulata*. Cell Tissue Res 223:325–334

Kingsley RJ, Watabe N (1984) Synthesis and transport of the organic matrix of the spicules in the gorgonian *Leptogorgia virgulata*. Cell Tissue Res (1984) 235:533–538

Kniprath E (1974) Ultrastructure and growth of the sea urchin tooth. Calcif Tissue Res 14:211–228

Lennarz WJ (1985) Regulation of glycoprotein synthesis in the developing sea-urchin embryo. Trends in Biochem Sci 10:248–251

Märkel K, Titschack H (1969) Morphologie der Seeigelzähne. I. Der Zahn von *Stylocidaris affinis*. Z Morphol Tiere 64:179–200

Märkel K, Gorny P, Abraham K (1977) Microarchitecture of sea urchin teeth. Fortschr Zool 24:103–114

Märkel K, Röser U (1983a) The spine tissues in the echinoid *Eucidaris tribuloides*. Zoomorphology 103:25–41

Märkel K, Röser U (1983b) Calcite resorption in the spine of the echinoid *Eucidaris tribuloides*. Zoomorphology 103:43–58

Märkel K, Röser U (1985) Comparative morphology of echinoderm calcified tissues: Histology and ultrastructure of ophiuroid scales. Zoomorphology 105:197–207

Millonig G (1970) A study on the formation and structure of the sea urchin spicule. J Submicrosc Cytol 2:157–165

Okazaki K (1975a) Normal development to metamorphosis. In: Czihak G (ed) The sea urchin embryo. Springer, Berlin Heidelberg New York

Okazaki K (1975b) Spicule formation by isolated micromeres of the sea urchin embryo. Am Zool 15:567–581

O'Neill PL (1981) Polycrystalline echinoderm calcite and its fracture mechanics. Science 213:646–648

Pearse JS, Pearse VB (1975) Growth zones in the echinoid skeleton. Am Zool 15:731–753

Pilkington JB (1969) The organization of skeletal tissues in the spines of *Echinus esculentus*. J Mar Biol Assoc UK 49:857–877

Weiner S (1984) Organization of organic matrix components in mineralized tissues. Am Zool 24:945–951

Weiner S (1985) Organic matrixlike macromolecules associated with the mineral phase of sea urchin skeletal plates and teeth. J Exp Zool 234:7–15

Wilbur KM, Saleuddin ASM (1983) Shell formation. In: Wilbur K (ed) The Mollusca, Vol. 4. Academic Press, New York London, pp 236–287


Received March 27, 1986