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The Spine Tissues in the Echinoid *Eucidaris tribuloides*

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Summary. The tissues of a fully grown echinoid skeleton are described using the primary spines of *E. tribuloides* as an example. Cidaroid spines are covered with an external, polycrystalline cortex, but as long as they are still growing they are covered with an epithelium. The mineral skeleton is embedded in the mesodermal stroma tissue which largely consists of fluid. Different types of mesodermal cells float within this fluid, but the sole characteristic stroma cells are the sclerocytes which are anchored to the calcite trabeculae by means of a cytoplasmic stalk. The latter spreads over the surface of the young trabeculae as a thin, continuous sheath, but on fully grown trabeculae it ramifies into numerous filiform processes (*dp*). The sclerocyte cell body is surrounded by a boundary layer which, however, is absent in the distal sheaths or filiform processes. The cytoplasm of the sclerocytes is electron-translucent and contains numerous free ribosomes. Sclerocytes which lie below the epithelium produce the cortex layer, and in the end the extracortical stroma as well as the epithelium vanish, and the cortex becomes external.

Phagocytes within the stroma are at least as numerous as sclerocytes. They have a dense cytoplasm with long, straight pseudopodia protruding from it and running through the midst of the pore space. In normal conditions the pseudopodia do not touch the trabeculae. In a single instance, however, a phagocyte was demonstrated to etch a trabecula. Its etching face was crowded with clear vesicles which are not found elsewhere.

A. Introduction

Calcareous endoskeletons consisting of many ossicles, the stereoms, are characteristic of echinoderms. Each stereom is a three-dimensional lattice of trabeculae which behaves optically like a monocrystal of calcite. The

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stereom is embedded in mesodermal tissue, called stroma, which also fills the interconnecting pore space within the stereom. Histological investigations on the stroma are extremely rare. A first ultrastructural study on the fully grown stroma of an echinoid was carried out by Pilkington (1969), who studied the spine tissues of *Echinus esculentus*, but was mainly concerned with histochemistry. Heatfield and Travis (1975a and b) investigated the ultrastructure of stroma cells of regenerating spines of *Strongylocentrotus purpuratus*, but largely outside the skeleton.

Echinoid spines are especially suitable for investigations on the stroma because each spine skeleton corresponds with a single stereom and its stroma lacks all the special structures necessary in stereoms that are connected with one another. The present investigation deals with the tissues of the primary spines of the cidaroid *Eucidaris tribuloides* (Lamarck). The spine consists of the base (*BA*), the shaft, and the annulus (*A*) in between (Fig. 1a). In most echinoids the whole shaft is covered with an epithelium; in cidaroids, however, the shaft of the fully grown primary spine is covered with an external cortex layer (*SH*), only a short collar (*COL*) is without any cortex (Carpenter 1847; Prouho 1887).

B. Materials and Methods

Materials: Live specimens of *E. tribuloides* were obtained from an aquarium supply company. They were kept in artificial sea water at 22°–24° C and were fed snails and algae.

Methods: Spines were dissected from the specimens. One half of each spine was quickly abraded by means of emery paper moistened with sea water.

The process of fixation took at least 18 h (up to several days) at 4° C in 5% glutaraldehyde in 0.1 M Sorensen phosphate buffer (pH 7.6). By addition of sucrose the osmolarity of the fixation fluid was adjusted to the osmolarity of the artificial sea water. Post-fixation was carried out for 2 h at 4° C with 1% osmium tetroxide in 0.1 M Sorensen buffer (pH 7.6) containing the respective amount of sucrose.

A modified double embedding procedure of Pilkington (1969) was used: The fixed specimens were embedded in araldite. Two faces of the hardened block were ground away to expose the calcite. Decalcification was done with EDTA. Thereafter the specimens were rinsed, dried, and re-embedded in araldite. Silver-thin sections were mounted on coated or uncoated grids and stained with uranyl acetate followed by lead citrate. The sections were examined with a Zeiss 9S-2 electron microscope, operated at 60 kV, or with a Siemens Elmiscop 101, operated at 80 kV.

Semi-thin sections were stained with crystal violet but examined with a phase contrast microscope to distinguish between the first phase of araldite (which fills the pore space) and the second phase (which fills the dissolved stereom).

C. Results

I. The Spine Skeleton

The cidaroid spines are solid spines, i.e., the genuine, monocrystalline skeleton consists of the central medulla (M) surrounded by highly perforated wedges (*W*) which are radial in position (Fig. 1). The monocrystalline stereom is covered with the polycrystalline cortex layer (*CX*) (Borig 1933; Märkel et al. 1971). The cortex is a continuous layer which in places shows

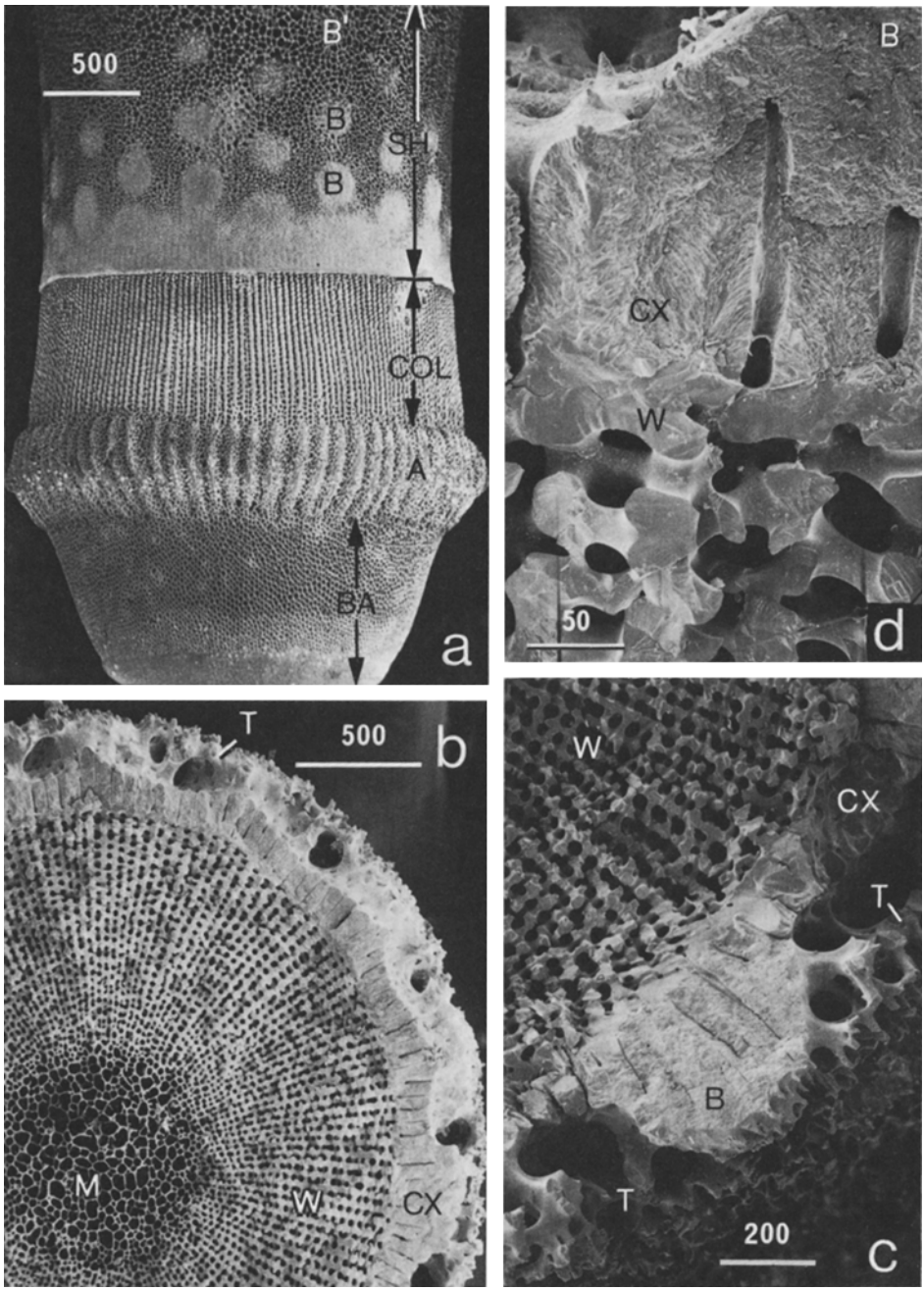

Fig. 1 a–d. SEM-photographs of the spine skeleton. **a** Basal part of the spine. **b** Transversally broken shaft. **c, d** Partial views of **b.** Detailed explanation in the text. Note the glassy appearance of the broken wedges (*W*) in contrast to the polycrystalline cortex (*CX*). Measurements in μm

bosses (*B*); it is provided with straight channels which are continuous with the radial pores running between the wedges. In *E. tribuloides* the surface of the cortex is provided with thorns (*T*) which conceal the bosses (*B'*) and bridge over the spaces between the latter. Eventually the spine's surface shows a scaffolding which resembles the lattice of the stereom trabeculae but is polycrystalline in structure.

II. The Spine Tissues

The young regenerate of the shaft forms a tall cone which arises from the centre of the spine's broad stump (Prouho 1887). The regenerate is completely covered with an epithelium which is clearly separated from the stroma by its basal lamina (Figs. 2, 3a *arrows*). The mesodermal stroma largely consists of intercellular fluid (Fig. 2). Sclerocytes (*s*) are anchored to the stereom trabeculae (*t*) by means of cytoplasmic processes, whereas phagocytes (*p*) show long, straight pseudopodia that extend through the pore space. Cells which contain spherules (morula cells *MC*, granulocytes *GR*) float within the fluid. The sclerocytes are the characteristic cells of the stroma, whereas the other cell types mentioned are generally distributed within mesodermal tissues of echinoderms.

1. The Epithelium

The epithelium is single-layered. Its cell bodies, with the nuclei arranged at different levels, occupy the longitudinal grooves between the outermost trabeculae (*t*) of the regenerating spine skeleton (Fig. 2). Cell processes containing tonofilament bundles (*tf*) run from the nuclear region to the surface (Fig. 3a). These processes are often branched, and the branches may contain pigment granules (*pi*). At the surface they expand horizontally and cover the wedges of the skeleton as a thin layer. By means of desmosomes (*d*) the cells adjoin distally to form a straight surface. The surface is provided with branched microvilli (*mv*) which terminate in electron-dense pommels and bear a distinct cuticular layer (*cu*). Single cilia (*ci*) project through the cuticle; they show a second basal body at right angles to the basal body of the shaft as well as a striated rootlet (Fig. 3b). The smooth endoplasmic reticulum (*SER*) forming small cisternae is distinctly developed especially near the origin of the cilia (Fig. 3b *arrows*); occasionally pinocytotic vesicles also occur. Cell processes run from the nuclear region also in proximal direction, probably towards the basiepithelial nerve plexus. The epithelium is clearly separated from the stroma by its basal lamina (Figs. 2, 3a *small arrows*). The basal lamina is sharply demarcated from the epithelium, whereas fibrils running into the stroma branch off its proximal face. Morula cells (*MC*) are found within the epithelium, and cells obviously filled with excretory products pass to the outside.

The epithelium covers the shaft until the polycrystalline cortex layer has been deposited by the extracortical stroma beneath the epithelium (Fig. 8). By means of the straight channels which run through the cortex the extracortical stroma is connected with the stroma of the spine's interior.

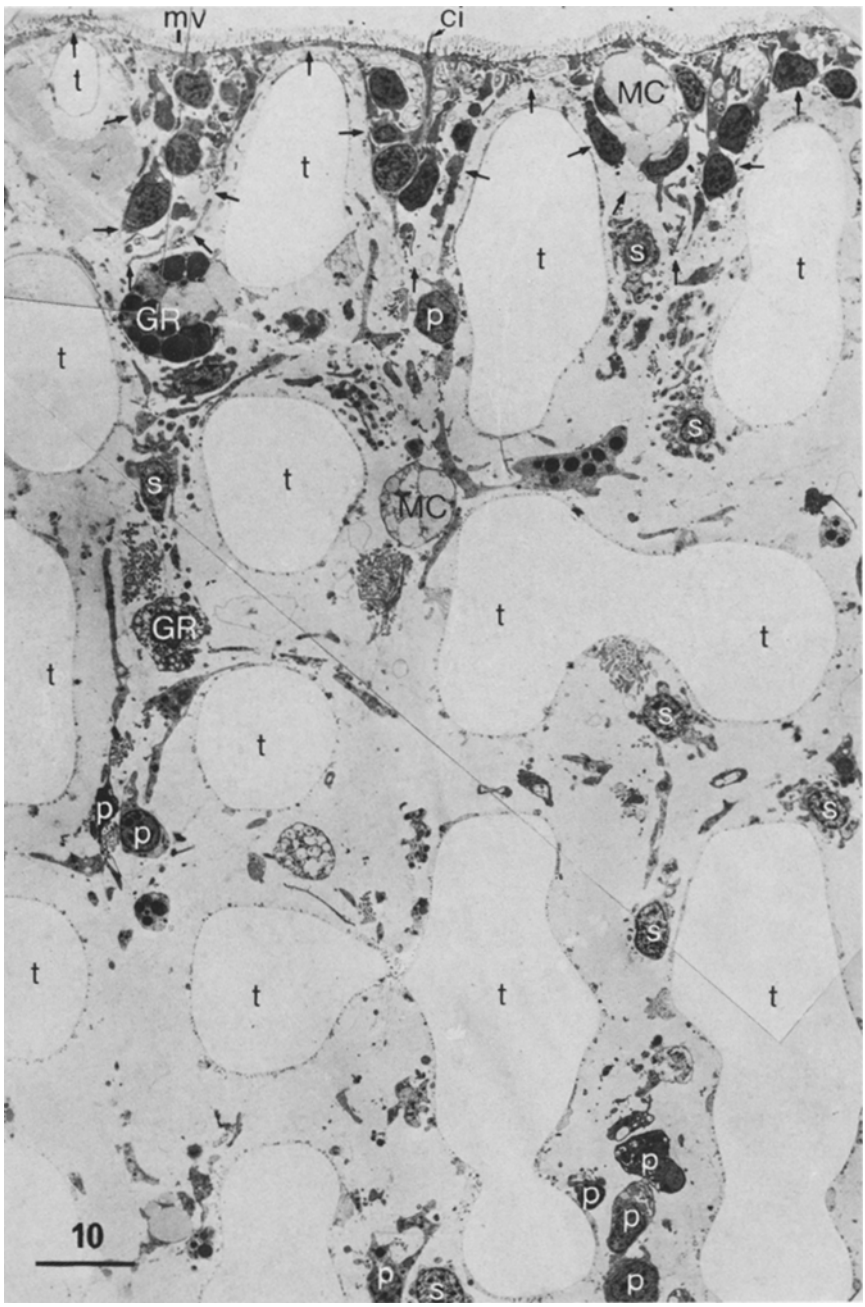

Fig. 2. Low magnification photograph of a transverse section through a young spine (cortex still lacking). *ci* cilium, *GR* granulocyte, *MC* morula cell, *mv* microvilli, *p* phagocyte, *s* sclerocyte, *t* calcite trabecula (dissolved). Note the radial arrangement of the trabeculae. *Small arrows* indicate the basal lamina. Measurements in μm

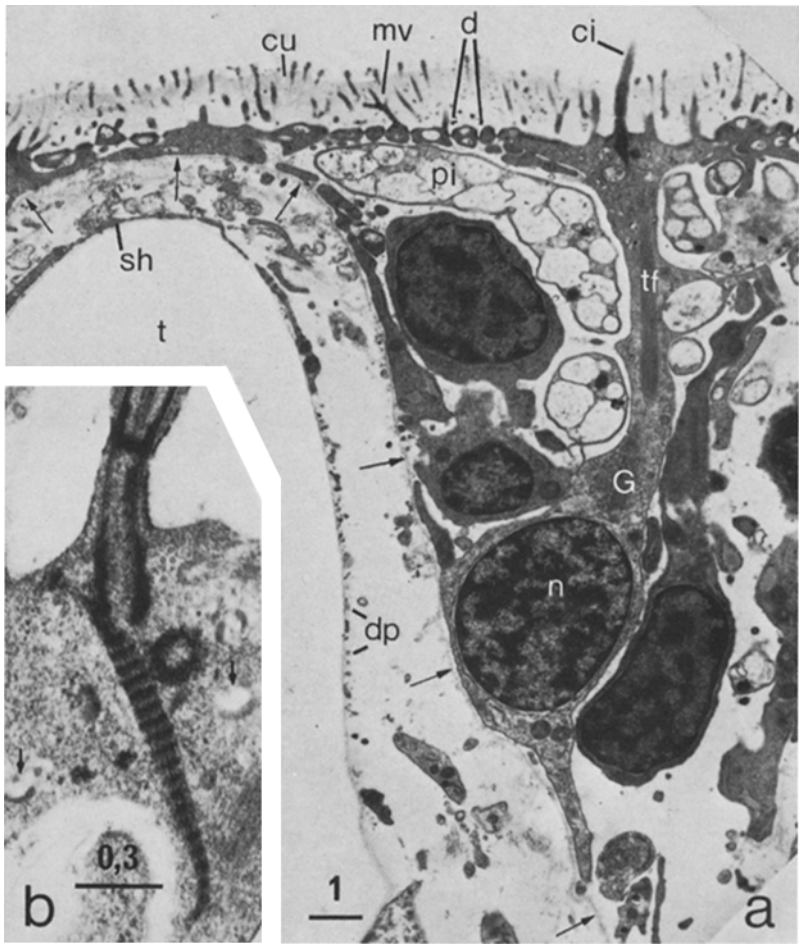

Fig. 3a, b. Epithelium. **a** An epithelial cell, cut lengthwise. *G* Golgi complex, *n* nucleus, *tf* tonofilament bundle, *pi* pigment, *arrows* indicate the basal lamina. **b** Basal part of a cilium, the *arrows* indicate SER-cisternae. Measurements in μm

Finally the epithelium as well as the extracortical stroma vanish, and the cortex becomes external. Thereafter sessile organisms may settle on the shaft. The collar part of the shaft is covered with an epithelium throughout life.

2. *The Stroma*

a) The Sclerocytes. The sclerocytes (*s*) are the characteristic cell type of the stroma. Their cell bodies lie in the pore space, but they are closely attached to the calcite trabeculae by means of distal processes (*dp*). Their cytoplasm is conspicuously electron translucent. The most characteristic feature of the sclerocyte is a continuous, electron-dense boundary layer

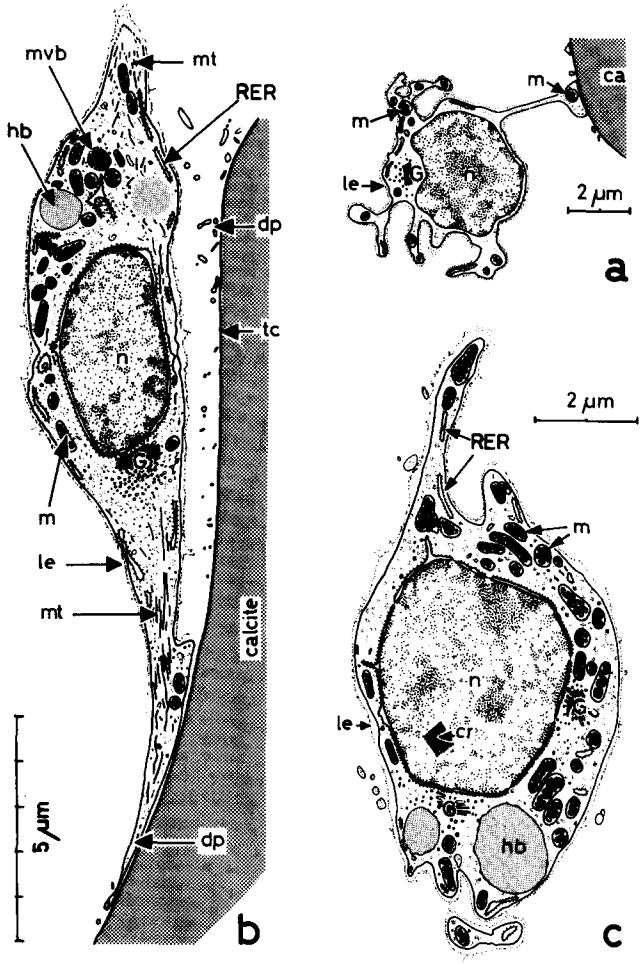

Fig. 4a–c. Sclerocytes drawn from photographs. **a** Young sclerocyte still highly branched. **b** Fully grown sclerocyte cut lengthwise through a branch which terminates in distal processes (*dp*, cf. Fig. 5b). **c** Sclerocyte with an intranuclear crystalloid (*cr*, cf. Fig. 7a). Explanation in the text

(lamina externa, *le*) which surrounds the cell body as well as its thick branches (*br*) but lacks the distal processes (*dp*) which are attached to the trabeculae (Figs. 4, 5). The boundary layer is separated from the cell surface by a clear halo of inconstant thickness.

The sclerocyte plasm contains clusters of free ribosomes (*r*), Golgi complexes (*G*), and a scarce but distinct rough endoplasmic reticulum (*RER*) which often shows its connection with the nuclear envelope (Fig. 5a). The cytoplasm contains multivesicular bodies (*mvb*) and membrane-bound bodies (*hb*) with moderately dense, homogeneous content which reach up to 1.5 µm in diameter. Coated vesicles (*cv*) which are associated with the Golgi complex are often seen to be fusing with the homogeneous bodies (Fig. 7a).

Inclusions other than multivesicular or homogeneous bodies are rare. The sclerocytes contain many, often very numerous, mitochondria (*m*). In several instances enlarged mitochondria are found (Fig. 6a) which may fuse entirely, reaching a diameter three times that of normal mitochondria.

In sclerocytes that are positioned at the periphery and which are engaged in calcite deposition onto newly formed trabeculae the cytoplasm extends into many contorted branches, whereas it is relatively thin around the nucleus (Fig. 4a). The mitochondria and the homogeneous bodies lie within the branches, and because the branches are often narrow these inclusions cause bulges (Pilkington's 'first order spherical bodies'). The sclerocytes envelop the outermost calcite trabeculae with a thin, continuous cytoplasmic sheath (Fig. 3a *sh*). In the area of calcite apposition there is a space in between sheath and trabecula (in the sections it is filled with the first phase of araldite). The cytoplasmic sheath is devoid of the boundary layer.

Sclerocytes positioned within the fully grown stereom withdraw most of their branches. They have more compact cell bodies which contain the nucleus and many mitochondria etc. There are only few, straight, branches of cytoplasm (*br*) which run towards the trabecle wall. Irregularly arranged microtubules (*mt*) occur in the cell body, whereas they are stretched lengthwise in a few branches. Microtubules are not conspicuous in the highly branched stages mentioned above.

The few branches (*br*) of the sclerocyte terminate in distal processes (*dp*) which are attached to the trabeculae. In contrast to the cell body and its branches the distal processes are devoid of the boundary layer (Fig. 5b, *arrow heads*). The distal processes obviously develop from the plasmatic sheath. They may be flat and attached to the trabeculae as thin layers containing microtubules. In most instances, however, the branches ramify distally into numerous filiform processes. The latter are more or less transversally cut in the sections and appear, therefore, as a string of circular or ovoid bodies (about 100 nm in diameter) accompanying the trabecle wall in full length (Fig. 6b). They correspond to Pilkington's 'second order spherical bodies'. The latter are typical of fully grown echinoderm skeletons. They probably have to maintain the trabeculae in a flawless condition (cf. Pilkington 1969). Normally, stroma cells other than sclerocytes do not touch the walls of the trabeculae. Cell bodies of sclerocytes are rarely found in sections through fully grown skeletons, but there are large areas in which the pore spaces appear empty except for the distal filiform processes mentioned. This indicates that each sclerocyte covers a considerable territory with its distal processes. The calcite trabeculae themselves are tightly clothed with an electron-dense, delicate but continuous trabecle coat (*tc*). This coat is as straight and smooth as the surfaces of the stereom's trabeculae.

The polycrystalline cortex layer is deposited by sclerocytes which lie within the extracortical stroma (below the outer epithelium) and cover the outer, growing face of the cortex with their plasmatic sheaths. The calcite of the cortex is covered by a coat (*ct*) which shows some indentations and other irregularities according to the shape of the crystallites that compose the cortex (Fig. 8). The channels which run through the cortex are likewise

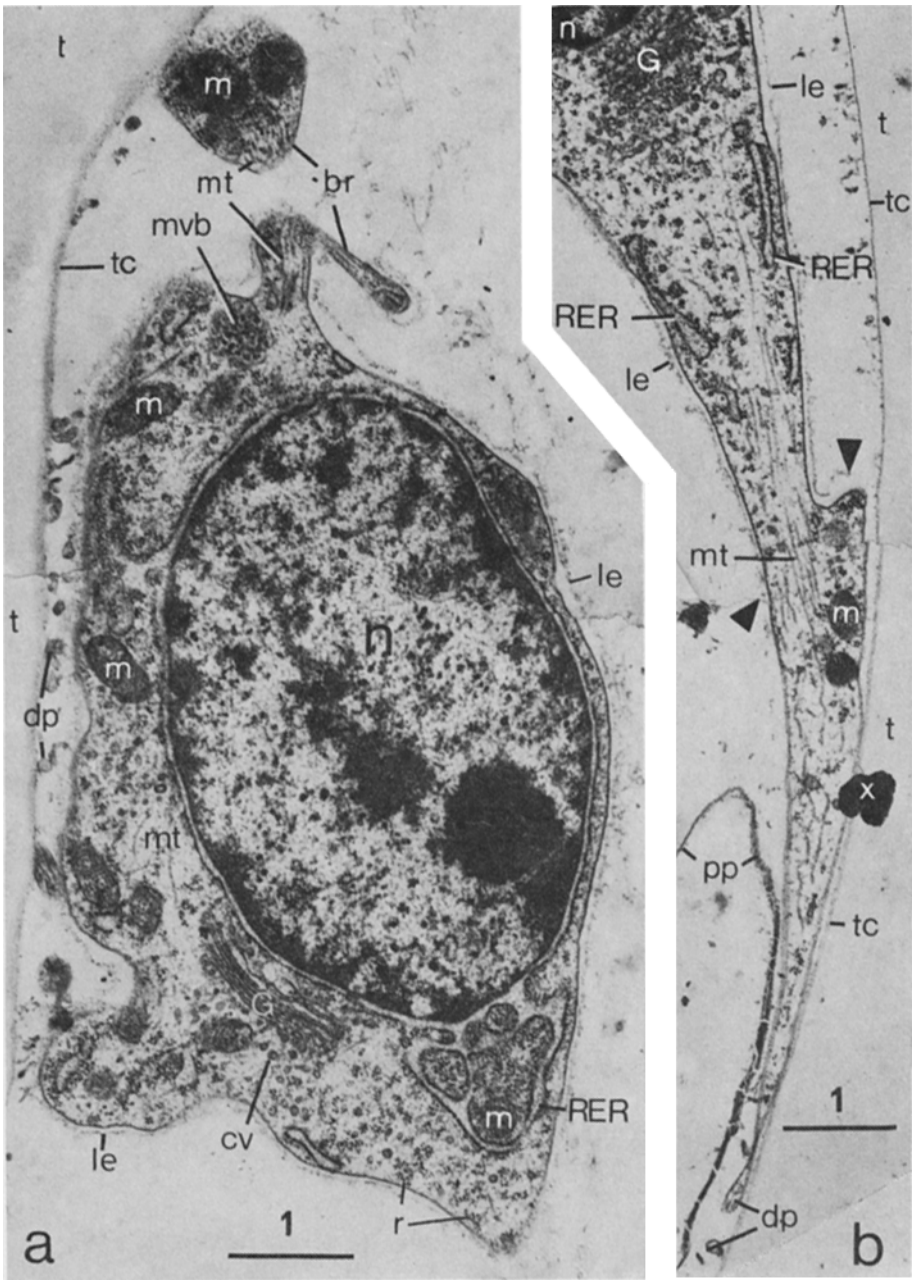

Fig. 5a. Fully grown sclerocyte. **b** Branch of a sclerocyte (cf. Fig. 4b), the boundary layer terminates at the *arrow heads*. *pp* part of a phagocyte. *x* dirt. Measurements in μm

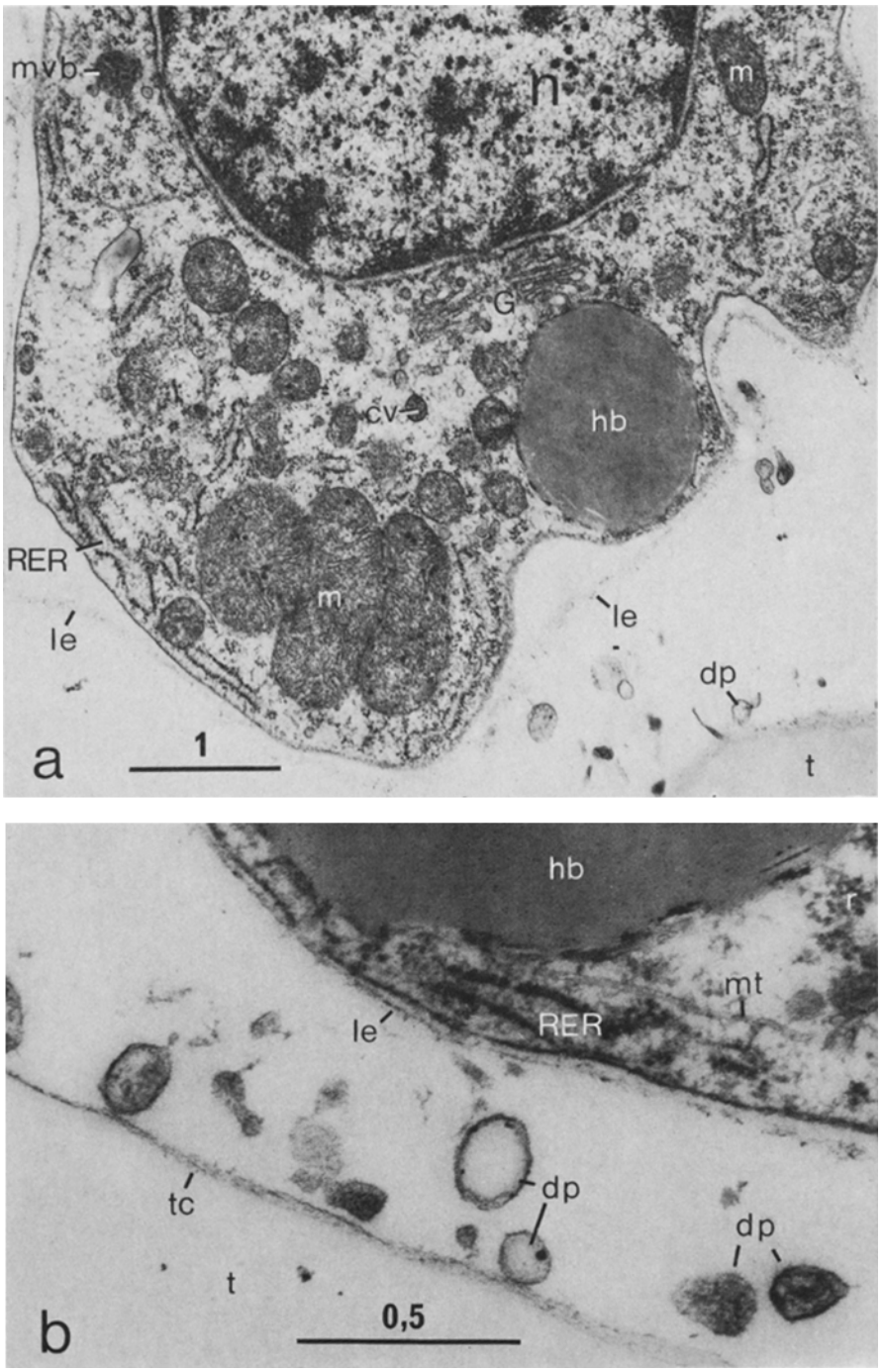

Fig. 6a. Part of a sclerocyte with some unusually big mitochondria beside normal sized ones. **b** Transversally cut distal processes (*dp*). This figure is typical of fully grown echinoid skeletons. Measurements in μm

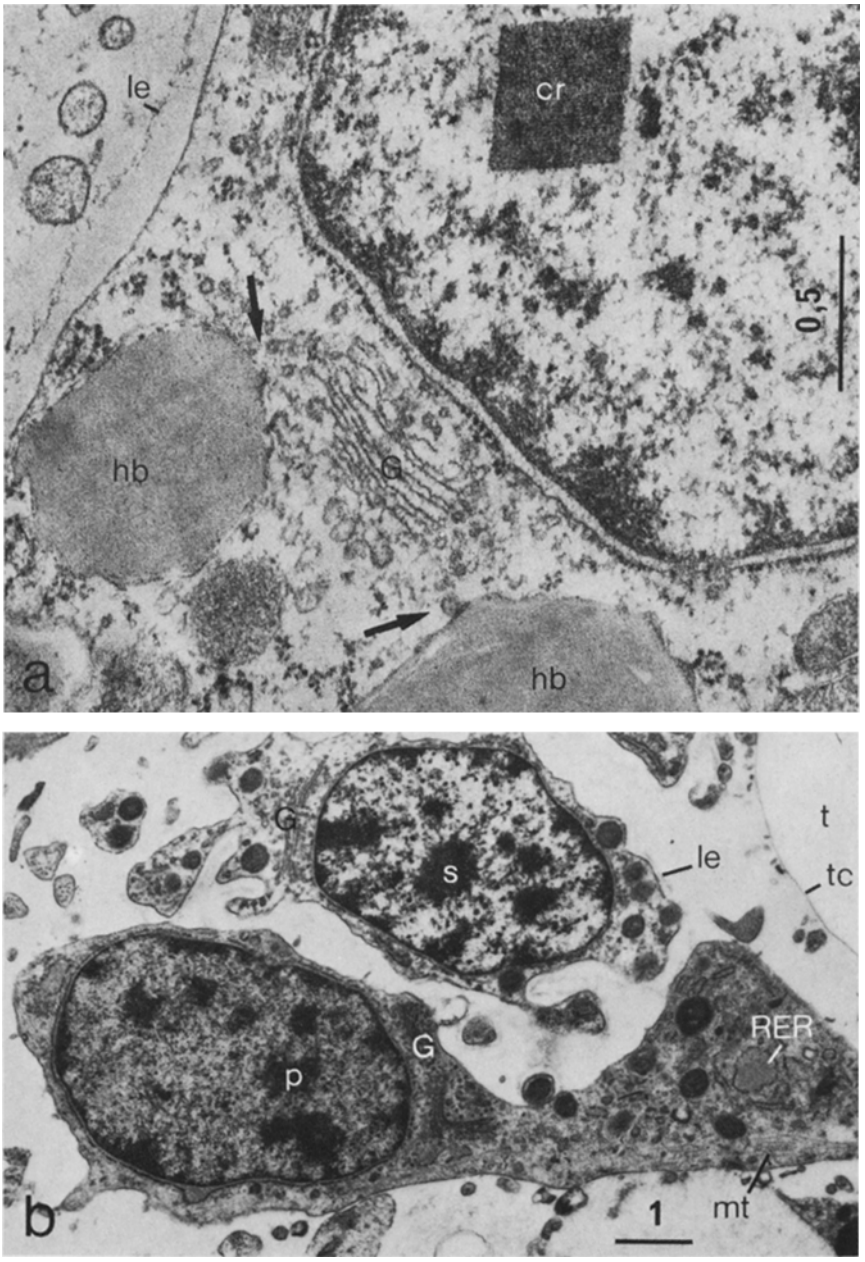

Fig. 7a. Partial view of a sclerocyte (cf. Fig. 4c) with a crystalloid (*cr*) within its nucleus and vesicles fusing with the homogeneous bodies (*arrows*). **b** Phagocyte (*p*) and sclerocyte (*s*). Note the differences in the densities of the cytoplasms. Measurements in μm

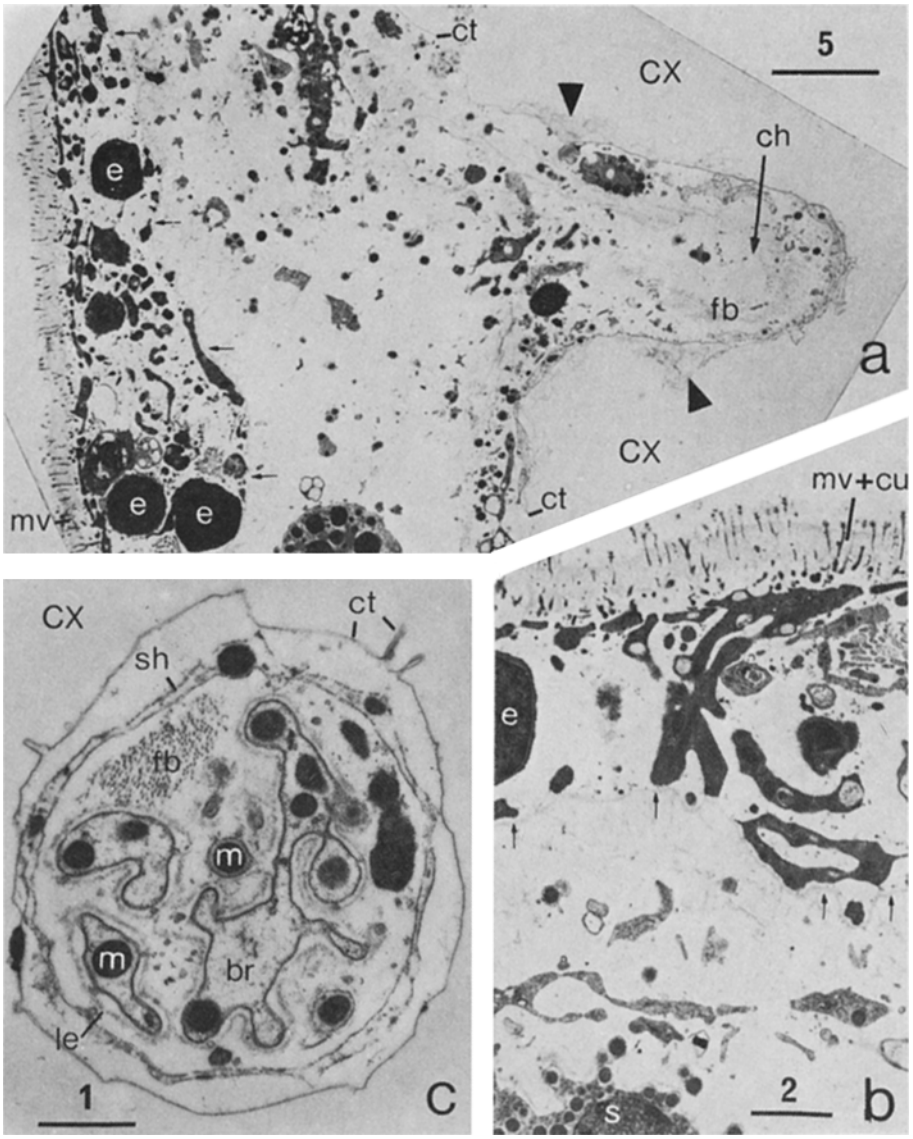

Fig. 8a. Epithelium, extracortical stroma and orifice of a cortex channel (*ch*). *CX* cortex (dissolved), *ct* coat, in places highly split up (*arrow heads*), *fb* bundle of fibres. *Small arrows* indicate the basal lamina. **b** Partial view of a. **c** Transversally cut stroma channel showing the cytoplasmatic sheath (*sh*) and branches (*br*) of a sclerocyte. Measurements in μm

provided with cytoplasmic sheaths or distal processes respectively. Bundles of fibres (*fb*) run through the channels, and in places different types of stroma cells are found (Fig. 8b).

b. The Phagocytes. According to Bertheussen and Seljelid (1978) 64%–70% of the cells floating in the coelomic fluid of echinoids are phagocytes. Phago-

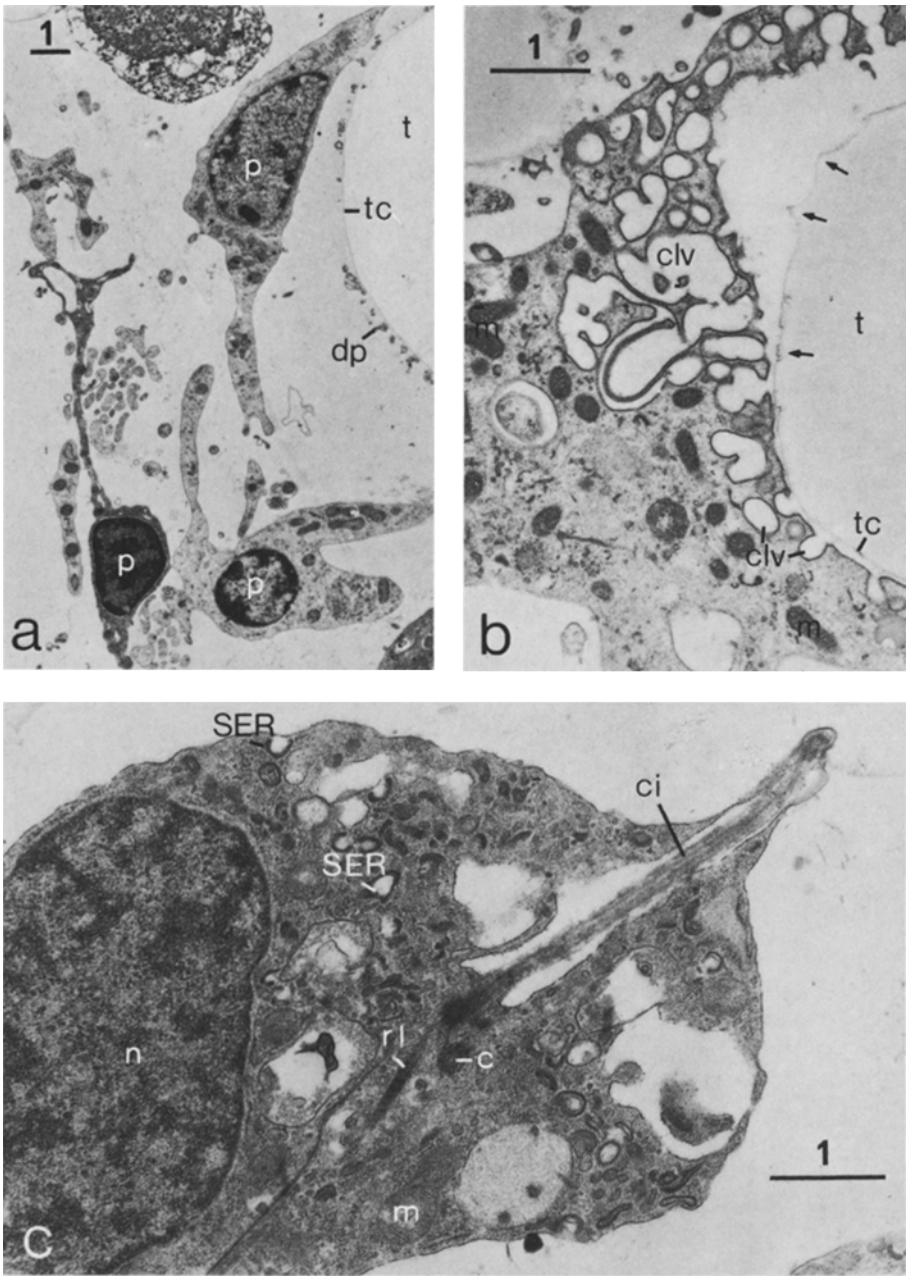

Fig. 9a. Low magnification photograph showing three phagocytes (*p*) with long pseudopodia and different densities of the cytoplasms. **b** Etching face of a phagocyte showing clear vesicles (*clv*). *Arrows* indicate irregularities in the trabecle's etched surface. **c** Vibratile cell with a cilium (*ci*), its rootlet (*rl*) and second centriole (*c*). Note the numerous SER-cisternae. Measurements in μm

cytes are also numerous within the stroma fluid, and besides the growth regions of the stereom they are even more numerous than the sclerocytes are. The cytoplasm of the phagocytes is electron dense (Fig. 7b), though the densities of neighbouring cells may differ. This indicates that the phagocytes do not form a single syncytium (Fig. 9a), although in places several phagocytes may cohere. Most phagocytes protrude straight and often extremely long pseudopodia which extend through the midst of the pore space (Fig. 2) and often contain bundles of microtubules. The RER is filled with a medium dense content and often some storage of its products takes place within distended cisternae. The most striking features are small cisternae of the SER, called 'sausage shaped bodies' by Heatfield and Travis (1975a). They are present in all phagocytes but vary in number. These cisternae are scattered within the whole cytoplasm, whereas Heatfield and Travis (1975a) found these bodies near the cell surface and often associated with pinocytotic vesicles. Cisternae of this sort are also found in the haemocytes of other echinoderms (Fontaine and Hall 1981).

Bertheussen and Seljelid (1978) described vibratile cells in the coelomic fluid. Vibratile cells are occasionally also found within the stroma (Fig. 9c). Their single cilium is provided with a rootlet (*rl*) as well as a second basal body (*c*). The environment of the cilium (which is deeply embedded in a pouch) is crowded with SER-cisternae, i.e., the vibratile cells show the feature of phagocytes.

Normally, phagocytes do not touch the trabeculae, but there are distal processes (*dp*) of sclerocytes in between. In a single instance, however, within a young, still regenerating spine a phagocyte was found which directly touched a trabecula and which obviously did etch its surface (Fig. 9b). Calcite resorption is known to occur in certain places of echinoid skeletons (Lovén 1892), and the etched trabeculae are known to show rough surfaces (Märkel 1981). In the section through the trabecula in question its trabecle coat is irregularly shaped (*arrows*) and the phagocyte itself shows numerous clear vesicles (*clv*) which are not present in normal phagocytes. This accidental finding indicates that the resorption of the calcite skeleton is done by phagocytes. An investigation on phagocytes that are especially active in calcite resorption is published separately (Märkel and Röser 1983).

D. Discussion

Eucidaris tribuloides seems especially suitable for ultrastructural studies. The specimens used in this investigation were kept for months in artificial conditions, and only conventional methods were used. Nevertheless, the ultrastructure of the tissues was well preserved, e.g., the presence of both the microvilli and the cuticle of the epithelium was invariably demonstrated in the sections. Many authors have failed to demonstrate any cuticle at all, although it is probably present in all echinoderms (Holland and Nealson 1978). According to observations on *Diadema* made by Kawaguti and Kamishima (1964) the cuticle is considerably less developed in specimens kept in tanks than in newly collected animals.

Weber and Grosmann (1977) distinguished in the epithelium of *Diadema* supporting cells, ciliated cells, pigment cells, and nerve cells. The epithelium of *Eucidaris* is obviously composed of a single cell type bearing a cilium, tonofilaments, and vacuoles containing pigments (Fig. 3a). The most striking features, however, are the pinocytotic vesicles and the small cisternae of the SER which lie near the base of the cilium. These vesicles and cisternae are also characteristic of phagocytes, which supports the conjecture that they are involved in skin digestion which has been proved to occur in several echinoderms (Péquignat 1966, 1972). On the other hand the epithelium has also an excretory function. The extrusion of excretory products is already known from the gills of *Echinus esculentus* (Cobb and Sneddon 1977).

With respect to the stroma the present investigation verifies the opinion of Heatfield and Travis (1975a, b) that the stroma contains cells of different types. Pilkington (1969) called all these cells 'sclerocytes' which he considered to represent different phases of a single cell type. His diagrammatic representation of a 'sclerocyte' combines features of a sclerocyte in the restricted sense used in this paper (the first and second order spherical bodies) and features of a phagocyte or even a spherulocyte (the vacuolized cell body).

The sclerocyte is the sole cell type which is characteristic of the skeleton. So far too few investigations on the stroma have been made and, therefore, it is impossible to give a general account of the echinoderm sclerocyte. In *Eucidaris* the sclerocytes are clearly characterised by their distinct boundary layer (*le*) which is present without exception from the early stage until it dies and disintegrates, but which is never found in any other stroma cell. Heatfield and Travis (1975a) did not mention a boundary layer in the sclerocytes (calcoblasts) of *Strongylocentrotus*. In general, sclerocytes show an electron-translucent cytoplasm which contains numerous free ribosomes, whereas inclusions other than vesicular bodies or homogeneous bodies are rare. The presence of microtubules is not mentioned by Heatfield and Travis. In the sclerocytes of *Eucidaris*, however, microtubules are present, and they are especially abundant in the stalks or branches which extend towards the trabeculae (Fig. 4b). It is noteworthy that according to Gibbins et al. (1969) microtubules are also abundant in the primary mesenchyme of *Arbacia* which is known to form the larval skeleton. The ultrastructure of the primary mesenchyme is very similar to that of adult sclerocytes: its cytoplasm is electron translucent and contains numerous ribosomes. The mesenchyme cells protrude stalks which are filled with microtubules and fuse distally to form a syncytial 'cable' which obviously corresponds to the plasmatic sheath of young sclerocytes. Enclosed within the cable lies the skeletal rod which is not directly touched by the cytoplasm but surrounded by some electron-opaque material. The latter corresponds to the space which is to be found in between still growing trabecle surfaces and the plasmatic sheath of the sclerocyte.

Shimizu and Yamada (1976) described 'sclerocytes' within the blastema of regenerating tests in *Strongylocentrotus*. These cells are multinucleate, have an electron-dense cytoplasm and contain calcite which is enclosed in

vacuoles. It cannot be excluded that these cells are phagocytes which are still engaged in the elimination of odd calcite fragments broken off in the process of preparation rather than true sclerocytes; at least they have very abnormal properties. Vacuoles containing calcite have never been observed by other authors within true sclerocytes. (Due to the double embedding method the calcite *within* the sclerocytes cannot be dissolved during decalcification, because they are embedded in the first phase of araldite.)

In decalcified sections the space which was formerly occupied by the calcite trabeculae is enveloped by the electron-dense trabecle coat (*tc*). Pilkington (1969) considered this coat to be part of the plasma membrane of the sclerocytes, especially of the second order spherical bodies. Heatfield and Travis (1975a) did not observe this coat in growing surfaces of the trabeculae but around fully grown ones. These authors thought the trabecle coat to be the secretory product of the older sclerocytes. In the cortex (*cx*) of the cidaroid spines a similar coat (*ct*)covers the surface of the calcite. Due to the polycrystalline structure of the cortex it is not smooth but shows edges and indentations which partially reach deeply into the cortex (Fig. 8a, *arrow heads*). Moreover, a sort of trabecle coat is also present in the etched surface of the trabeculae (Fig. 9b) which are not in contact with sclerocytes. These facts indicate that the coat is a precipitation upon the calcite surface, rather than a secretory product of the sclerocytes.

Sclerocytes produce the calcite of the stereom which (at least optically) behaves as a monocrystal as well as the polycrystalline calcite of the cortex. Although their products differ considerably in structure we failed to prove differences in the ultrastructure of both sclerocytes.

Phagocytes of the stroma have been described by Heatfield and Travis (1975a) who found round to ovoid phagocytes below the epidermis of *Strongylocentrotus*. In *Eucidaris* the phagocytes are polymorphic, but they are stretched and drawn out into long pseudopodial processes. Bachmann et al. (1980) observed intranuclear crystalloids in most of the phagocytes of the axial complex in *Sphaerechinus*. In the stroma of *Eucidaris* intranuclear crystalloids occur in sclerocytes as well as in phagocytes, and even in highly active phagocytes they are no more numerous than in other cells.

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