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Occurrence of a microcanalicular system within the ossicles and dermal tissue of *Asterias rubens* and *Marthasterias glacialis* (Echinodermata: Asteroidea)

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Summary. A microcanalicular network is demonstrated within the ossicle stroma and the dermal tissue of two asteroid species. Microcanaliculi are presumed to be mesodermal structures. They consist of convoluted tubular ducts lined by epithelial cells associated with scattered basiepithelial nervous processes. Such a microcanalicular system has not been reported previously from any echinoderm species. Its discovery in asteroids entails some conceptual changes, especially considering the physiology of the body wall.

Key words: Ossicle – Dermis – Epithelium – Mesoderm – Echinodermata

The body wall of most echinoderms consists mainly of a collagen-rich dermal tissue containing rather thick calcareous ossicles. Echinoderm ossicles (viz. plates, spines and pedicellarial valves) have a unique structure being made of a tridimensional network of magnesian calcite, the stereom, the pores of which are filled with a connective-like tissue, the stroma (Cuénot 1948; Nichols 1962). There is no published description of the fine structure of the stroma of asteroid echinoderms.

In the course of an ongoing study of the fine structure of the asteroid body wall, unexpected microcanaliculi were discovered within the ossicle stroma and the uncalcified dermal tissue. The significance of this finding for functional considerations, regarding especially the physiology of the body wall, prompted us to present this short contribution.

Materials and methods

Asterias rubens L. and *Marthasterias glacialis* (L.) were collected at Scharendijk (Zeeland, Netherlands) and Morgat (Brittany, France), respectively. Calcified appendages (adambulacral spines and pedicellarial valves) and pieces of body wall were fixed with 3% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.3), and postfixed with 1% OsO_4 in the same buffer. For light microscopy and TEM study, decalcifications were performed using the ascorbic acid method (Dietrich and Fontaine 1975). Pieces were dehydrated in ethanol and embedded in Spurr's medium. Semithin sections were stained using the method of Humphrey and Pittman (1974). Ultra-thin sections were stained with uranyl acetate and lead citrate, and observed with a Philips EM 300 transmission electron microscope. For SEM study, pieces were dehydrated with ethanol and dried by the critical-point method, using CO_2 as transition fluid. SEM cryofractures were done by adapting the method of Humphreys et al. (1974). Pieces were mounted on aluminium stubs, coated with gold in a sputter coater and observed with a ISI DS-130 scanning electron microscope.

Results

As in other echinoderm groups the ossicles of asteroids are typically made of a tridimensional skeletal network the pores of which are filled with a connective-like tissue or stroma (Figs. 1, 2). The asteroid stroma basically consists of a fibrillar extracellular matrix including both single cells and epithelium-lined microcavities (Fig. 3). Most single cells have elongated cytoplasmic extensions that sometimes are close to the stereomic trabeculae. Some of them could represent sclerocytes on which investigations on their nature are in progress.

Occurrence of epithelialized microcavities is by far the most striking feature of the asteroid stroma. These microcavities are circular to ovoid in shape, measuring from 2.5 to 6.5 μm in diameter (Figs. 3–6). On most TEM sections they are lined only by two to three cells resting upon a basal lamina. Epithelial cells are flat, being thickest at about level of the nucleus. The flattened shape of the cells suggests that they spread over a rather large surface. Most cells bear a well-developed cilium and numerous microvilli that fill the microcavity (Figs. 4, 5). They are attached together by short desmosomes, presumably spot desmosomes (Fig. 5). The cytoplasm of these cells contains numerous small, sometimes distinctly coated, clear vesicles. A well-developed Golgi apparatus generally occurs as well as some mitochondria. A few cells, moreover, appear to have bundles of myofibrillae in their most basal part (Fig. 6). It is noteworthy also that scattered nerve-like structures are seen occasionally in the space between the epithelial cells and their basal lamina. Microcavities occur in many stereomic pores of all ossicles that we observed. Reconstruction of stroma from semi-thin sections suggests that microcavities correspond to either cross or oblique-sections through a

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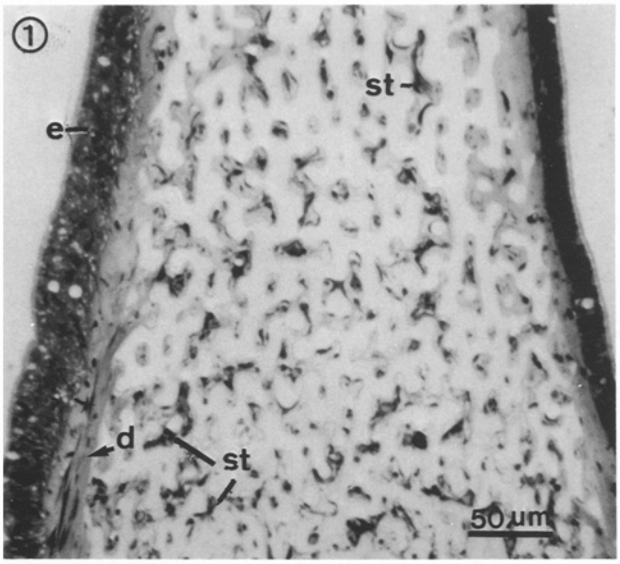
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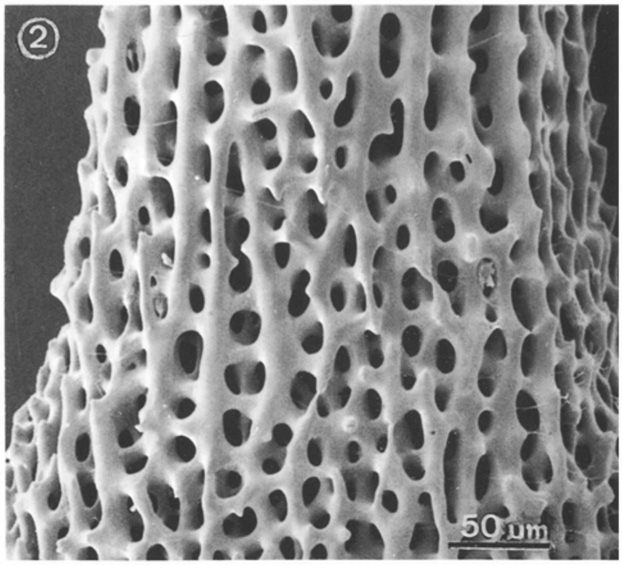
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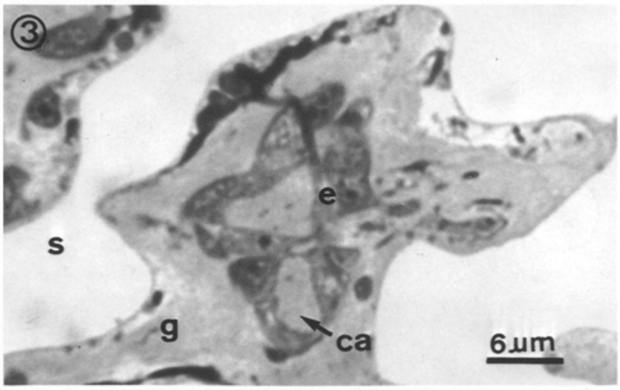
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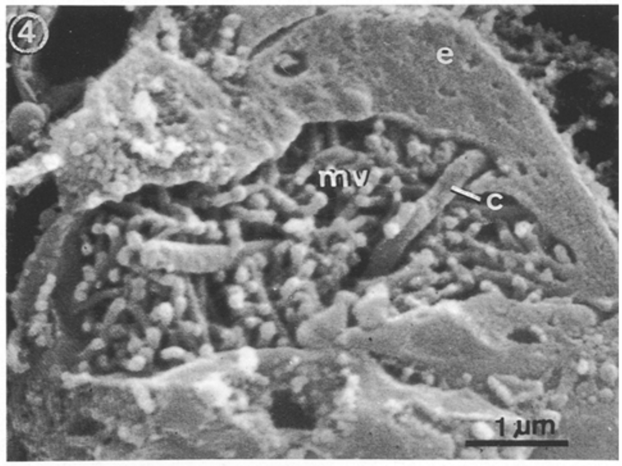
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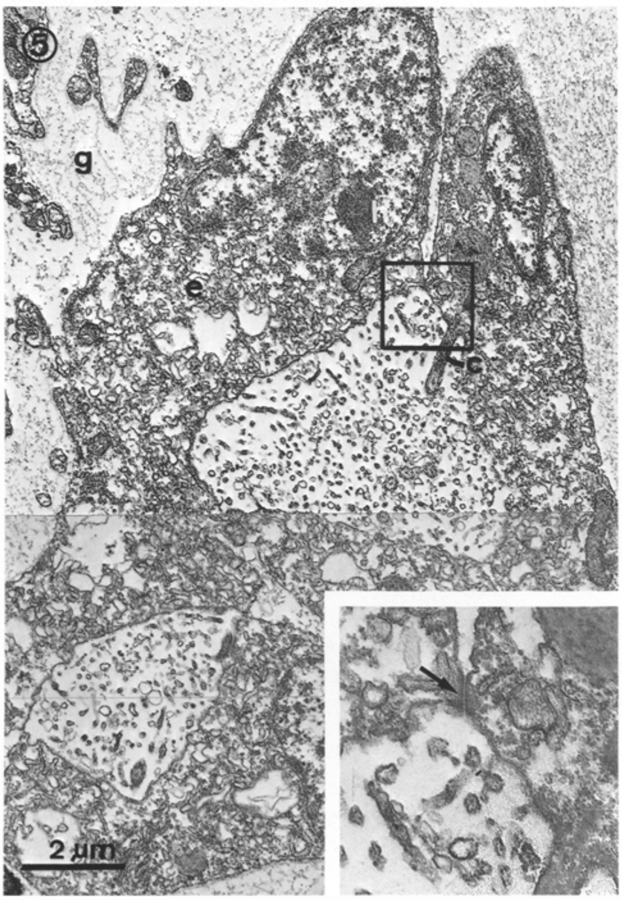
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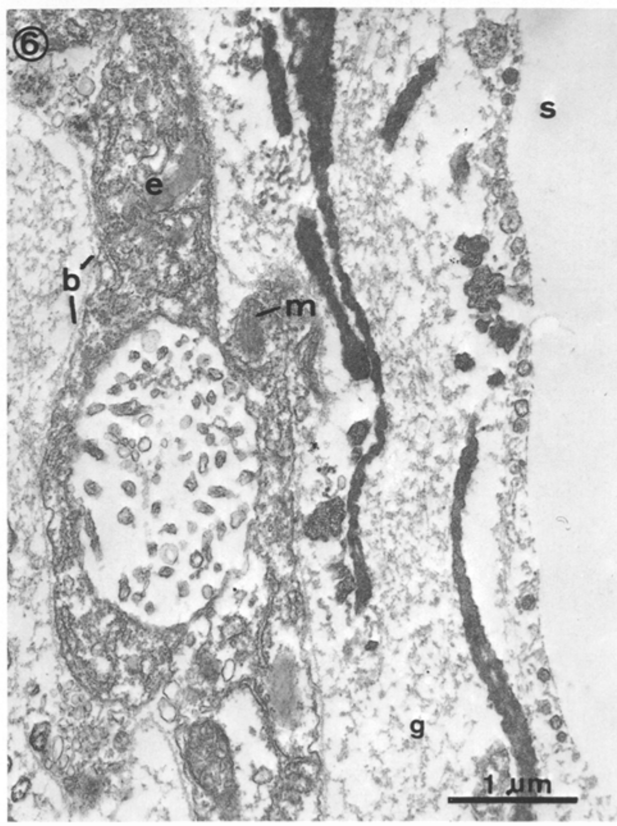
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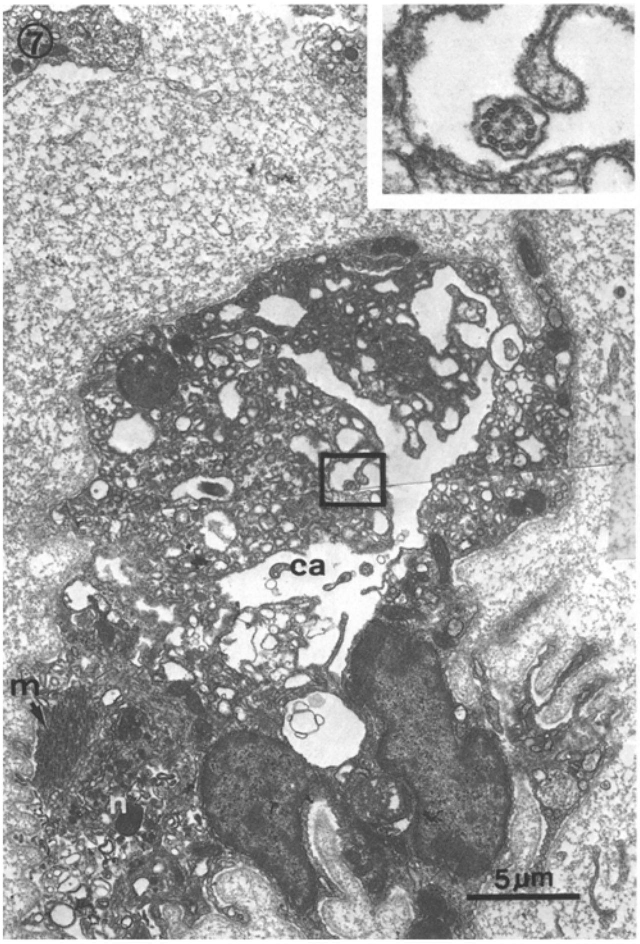

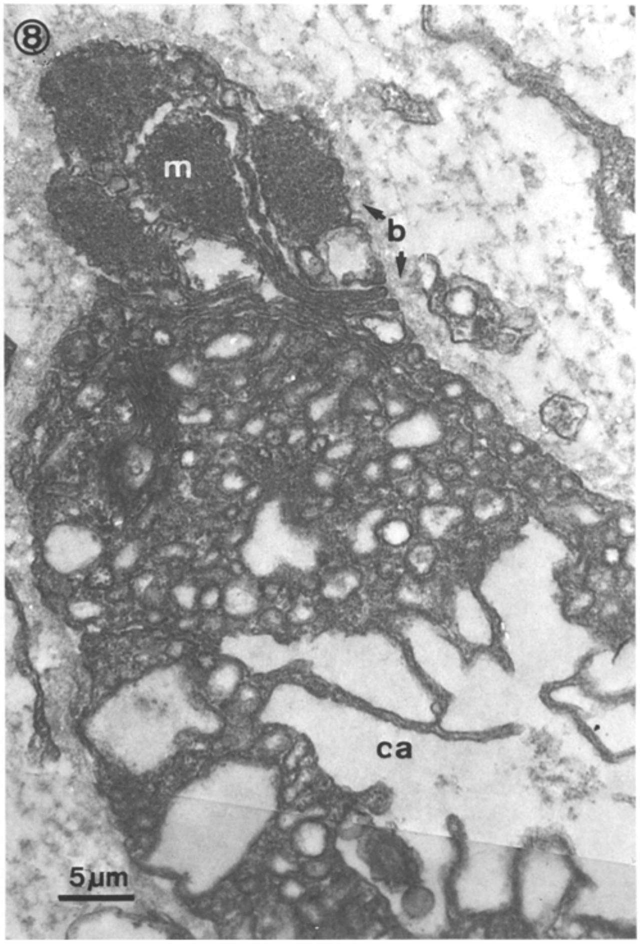

Fig. 7. General aspect of a dermal microcanaliculus showing the irregular outline of the epithelial cells and the occurrence of basiepithelial nervous processes (*n*); *ca* lumen of the microcanaliculus, *m* myofibrillae (the *insert* illustrates the occurrence of cilia)

Fig. 8. Dermal microcanaliculus with well-developed myoepithelial cells (*m*); *ca* lumen of the microcanaliculus

highly convoluted microcanalicular system extending throughout the ossicle stroma.

Canalicular microstructures are seen also outside the ossicle stroma, namely in the dermal connective tissue surrounding and/or separating the ossicles (Fig. 7, 8). The dermal microcanaliculi have a narrower central lumen being lined by enlarged cells with rather irregular outlines. These microcanaliculi are larger (ca. 12 µm in diameter), less numerous and seemingly less convoluted than those in the ossicles. That shape might be linked to the nature of the tissue in which microcanaliculi occur, namely intraossicular microcanaliculi should be necessarily convoluted giving the kind of tridimensional arrangement of the stereomic meshes. It should be emphasized that the epithelium of dermal microcanaliculi rather often harbours cells with well-developed bundles of myofibrillae (Fig. 8). These myoepithelial cells are always associated with a well developed basiepithelial nerve plexus (Fig. 7). Such features occur

Figs. 1–6. Ossicle microcanaliculi of the adambulacral spine (*Asterias rubens*)

Fig. 1. Longitudinal semi-thin section through a decalcified spine; *e* epidermis, *d* peripheral dermal tissue, *st* stroma (white areas correspond to the decalcified stereom)

Fig. 2. SEM-view of a cleaned spine showing the tridimensional arrangement of the stereom

Fig. 3. Enlarged view of a stereomic pore (semi-thin section) with two adjoining microcanaliculi; *ca* (lumen) and *e* (epithelium) of the microcanaliculi, *g* extracellular matrix of the stroma, *s* decalcified stereom

Fig. 4. SEM-cryofracture of a microcanaliculus showing two cilia (*c*) and numerous microvilli (*mv*) filling the lumen; *e* epithelial cell

Figs. 5, 6. TEM-views of spine microcanaliculi (compare Fig. 5 and Fig. 3); *b* basal lamina, *c* cilium, *e* epithelial cell, *g* extracellular matrix of the stroma, *m* myofibrillae, *s* decalcified stereom (the *insert* illustrates the usual aspect of the intercellular junctions) especially in places where appendage and tegumentary muscles develop.

The microcanalicular system has neither openings into the outer medium nor contacts with the epidermal layer. While preliminary observations allowed us to see that enlarged canaliculi occur between the inner surface of plate ossicles and the coelomic side of the body wall, direct communications between the canaliculi and the general coelomic cavity were not observed.

Discussion

The microcanaliculi observed within the dermis of both *Asterias rubens* and *Marthasterias glacialis* form a well developed system entering each ossicle and extending throughout the uncalcified dermal tissue. Microcanaliculi do not occur within the stroma of spines or ossicles of the previously investigated species of echinoids and crinoids (Heatfield and Travis 1975a, b; Märkel and Röser 1983; and Grimmer et al. 1984; respectively). They have never been reported from the dermis of any other echinoderm species. Comparative ultrastructural studies are needed to determine whether or not microcanalicular systems occur in all asteroid groups as well as in non-asteroid asterozoans, namely in ophiuroids.

The microcanalicular system is made of convoluted tubular ducts lined by epithelial cells, which show some resemblance to the mesothelial cells of the coelomic system (see, e.g., Nørrevang and Wingstrand 1970; Walker 1979). Microcanalicular cells are undifferenciated flattened monociliated cells. They bear more or less spaced microvilli and are attached together by rudimentary desmosomes. Moreover, typical myoepithelial cells occur in places within the microcanalicular system. So far this cell type has been recognized only in the mesothelium (e.g., Walker 1979; Wood and Cavey 1981; Jangoux 1982), being absent from both ectodermal and endodermal epithelia, namely the epiderm and the digestive epithelium. The occurence of myoepithelial cells in the microcanalicular system is intriguing. Could these cells be those from which the appendage and tegumentary muscles develop? If this can be demonstrated, it would mean that the asteroid muscle system is structurally unique, namely that its muscles are always made of myoepithelial cells.

The microcanalicular system never contacts the epidermal layer. It develops in the very dermal tissue of the asteroids. This, together with the structure of its lining epithelium, strongly suggests that system should be of mesodermal origin. Whether it would result from secondary epithelialization of mesenchyme or corresponds to particular tegumentary ingrowths of the otherwise well-known coelomic system is not known. Whichever, if one accepts the microcanalicular system to be of mesodermal origin, then it should be properly recognized as a coelomic structure. Until now we have not been able to determine whether or not that presumed intrategumentary coelom connects to one or another coelomic partitions of asteroids.

The discovery of such an intrategumentary network of microcanaliculi naturally leads to some general considerations. It seems obvious that both dermal and ossicle microcanaliculi should have a prominent role in the general turnover of the body wall and thus should be involved in its growth and regression. One may suggest also that ossicle microcanaliculi could be involved in the processes of calcification and resorption of skeletal ossicles. Detailed ultrastructural studies are needed to clarify the overall course of the microcanalicular system within the asteroid tegument and to determine whether or not connections occur with the coelomic system, and from that with the internal organ systems.

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References

Cuénot L (1948) Anatomie, systématique et éthologie des échinodermes. In: Grassé PP (ed), Traité de Zoologie, vol 11. Masson, Paris, pp 3–363

Dietrich HF, Fontaine AR (1975) A decalcification method for ultrastructure of echinoderm tissues. Stain Technol 50:351–354

Grimmer JC, Holland ND, Messing CG (1984) Fine structure of the stalk of the bourgueticrinid sea lily *Democrinus conifer* (Echinoderma: Crinoidea). Mar Biol 81:163–176

Heatfield BM, Travis DF (1975a) Ultrastructural studies of regenerating spines of the sea urchin, *Strongylocentrotus purpuratus*. I. Cell types without spherules. J Morphol 145:13–50

Heatfield BM, Travis DF (1975b) Ultrastructural studies of regenerating spines of the sea urchin, *Strongylocentrotus purpuratus*. II. Cell types with spherules. J Morphol 145:51–72

Humphrey CD, Pittman FE (1974) A simple methylene blue – azure II – basic fuschin stain for epoxy-embedded tissue sections. Stain Technol 49:9–14

Humphreys WJ, Spurlock BO, Johnson JS (1974) Critical point drying of ethanol infiltrated, cryofractured biological specimens for scanning electron microscopy. In: Johari O, Corvin I (eds) Scanning electron microscopy (IITRI Proc.) 7(1):275–282

Jangoux M (1982) Digestive systems: General considerations. In: Jangoux M, Lawrence JM (eds) Echinoderm nutriton. Balkema, Rotterdam, pp 185–186

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

Nichols D (1962) Echinoderms. Hutchinson, London, p 200

Nørrevang A, Wingstrand KG (1970) On the occurence and structure of choanocyte-like cells in some echinoderms. Acta Zool 51:249–270

Walker C (1979) Ultrastructure of the somatic portion of the gonads in asteroids, with emphasis on flagellated-collar cells and nutrient transport. J Morphol 162:127–162

Wood RL, Cavey MJ (1981) Ultrastructure of the coelomic lining in the podium of the starfish *Stylasterias forreri*. Cell Tissue Res 218:449–473

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