

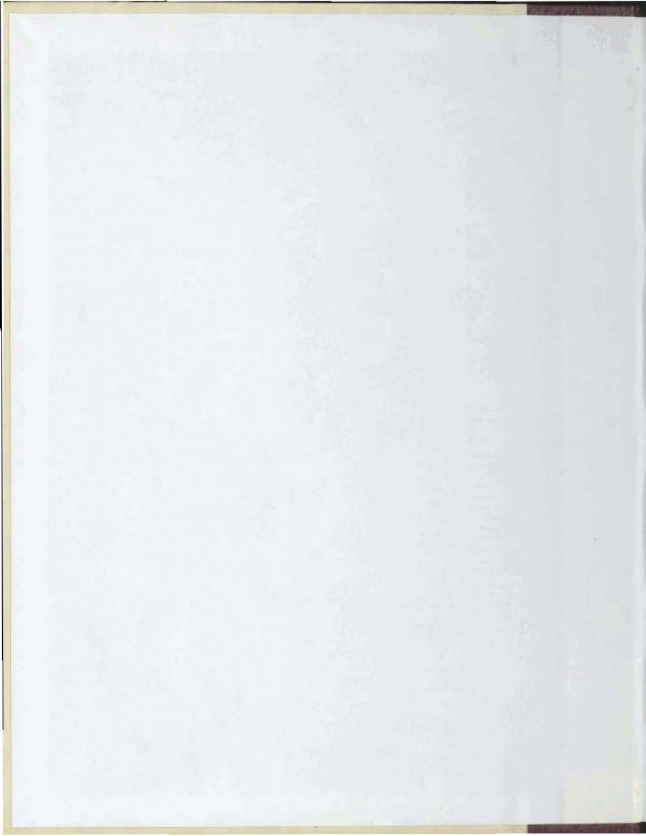
DEVELOPMENTAL STUDIES ON THE GLOBIFEROUS  
PEDICELLARIAE OF THE ECHINOID  
STRONGYLOCENTROTUS DROBACHIENSIS  
O. F. MÜLLER WITH SPECIAL EMPHASIS  
ON THE SKELETON

CENTRE FOR NEWFOUNDLAND STUDIES

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SYLVIA LILLIAN BRODERICK MOORE







DEVELOPMENTAL STUDIES ON THE GLORIFEROUS  
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C. F. MÜLLER WITH SPECIAL  
EMPHASIS ON THE  
SKELETON

by



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## ABSTRACT

Developmental stages of the globiferous pedicellariae of the sea urchin Strongylocentrotus drobachiensis O.F. Müller were investigated with a view to understanding the development and differentiation of the skeletal elements, muscles, sensory areas, nerves and venom glands. Specimens were studied using the light microscope, the transmission electron microscope, the scanning electron microscope, and the analytical electron microscope.

A globiferous pedicellaria begins as a tiny finger-shaped projection on the test of the sea urchin. The tip gradually separates into three jaws which continue to merge with each other at their bases. The skeleton is one of the first components to develop in the appendage. A skeletal element is deposited in each of the three jaws and in the basal stalk region. The skeleton remains surrounded by a cluster of cells throughout development. These cells send out numerous projections which are continuous with thin areas of cytoplasm surrounding, or near the skeleton; mitochondria are numerous in the cytoplasmic projections. X-ray

microprobe analysis with EMMA-4 showed significantly high amounts of calcium in the cells associated with the skeleton, especially in the mitochondria, vesicular structures, and nuclei. The term calcicyte (L. calx, lime) has been proposed to refer to this skeletogenic cell type.

The skeletal valves of the jaws develop from a tri-radiate spicule into a very elaborate ossicle with areas for articulation, muscle insertion, and passage of nerve fibers.

Unlike the mature appendage, the developing pedicellaria possesses an epithelium on the outer sides of the jaws that is several cell layers thick. This has been shown to develop into the venom glands.

The sensory hillock appears as a thickening of the epithelium on the inner side of each jaw. Nerves from the sensory hillocks enter the skeletal valves through a foramen to innervate the muscles which insert on the skeleton.

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## INTRODUCTORY STATEMENT

Globiferous pedicellariae of echinoids are defense organs equipped with venom glands. It has been suggested that these organs function only once, since they autotomize after discharging venom (Chia, 1969, 1970a). It is therefore necessary that these appendages be replaced by regeneration, or by development of new globiferous pedicellariae, during the life of the animal.

Although histological and ultrastructural studies have been done on the globiferous pedicellariae of several species of echinoids, little attention has been focused on these appendages in Strongylocentrotus dröbachiensis Müller. Moreover, the developmental aspects of pedicellariae in most species have been ignored, except for the work of Chia (1970a) in regenerating pedicellariae of Psammechinus miliaris (Gmelin).

Pedicellariae in various stages of development can usually be found on the test of a sea urchin, making specimens for study readily available. In the mature condition globiferous pedicellariae basically consist of skeletal elements made of calcite, muscles, sensory areas, nerves and venom glands.



In recent years much interest has arisen in echinoderm calcite. It has the advantage of being strong and light weight, and its porous nature allows conservation of material (Nichols, 1962). Since globiferous pedicellariae are small, the study of the skeletal development can be made possible by exploiting the depth of focus and the three dimensional images obtained by the scanning electron microscope. The minuteness of the skeletal material in early developmental stages also allows histological and ultrastructural preparations to be made without decalcification. Such material is therefore ideal for the study of distribution of calcium in the cells that are involved in skeletal deposition.

This study was undertaken, with consideration of the above points, to investigate the developmental changes in the globiferous pedicellariae of S. dröbachiensis. An attempt has also been made to relate changes in the morphology of the developing appendage with its function as a highly specialized sensory and defensive organ.

### GENERAL MORPHOLOGY AND FUNCTION

Pedicellariae are appendages found on the tests of echinoids and asteroids. According to Hyman (1955), they are best developed and always present on the former. Four types of echinoid pedicellariae are recognized on the basis of their morphology: tridentate, triphylous, ophiocephalous, and globiferous. All have the same basic structure, consisting of a head region composed of three moveable jaws, and a supporting stalk which articulates with the test of the animal. The common green sea urchin found in Newfoundland littoral waters is S. drobachiensis. It possesses all four types of pedicellariae (Mortensen, 1943). The echinoid globiferous pedicellaria, equipped with venom glands to play a defensive role, is considered by Hyman (1955) to be the most specialized and interesting of all.

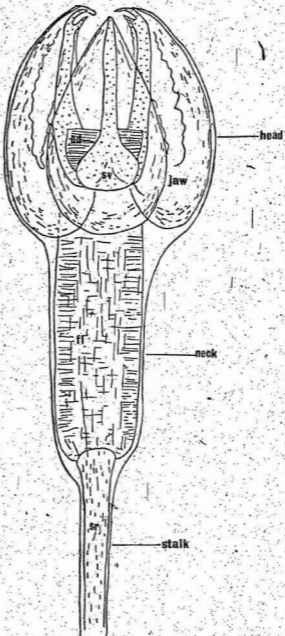
The head region of the globiferous pedicellaria is made up of three moveable jaws which merge with each other at their bases, (Figure 1). Each jaw is supported by an internal skeletal valve or ossicle, to the outer side of which is a glandular sac filled with toxic secretions

Figure 1 - A diagram of a globiferous pedicellaria  
of Strongylocentrotus.

The three regions of the pedicellaria - head,  
neck, and stalk - are shown, as well as the jaws,  
the skeletal valves, the skeletal rod, the adductor  
muscles, and the flexor muscles (after Hyman, 1955).

abbreviations, in alphabetical order:

ad - adductor muscles  
fl - flexor muscles  
sr - skeletal rod  
sv - skeletal valve



(Hyman, 1955). The wall of the glandular sac is muscular and is lined by a secretory epithelium which releases venom into the lumen of the sac. Hamann (1887) and Cuenot (1948) depicted the lumen as an acellular space. However O'Connell (1974) considers this acellular lumen to be a fixation artifact. He found the venom glands in Strongylocentrotus purpuratus (Stimpson), prepared in a variety of fixatives in sea water, to be filled with large vacuolated cells with little cytoplasm, and basally displaced nuclei. This agrees with the condition shown in P. miliaris by Chia (1970a), and in Sphaerechinus granularis (Lamarck) by Foettinger (1881).

The muscles of the glandular sac are involved in venom emission, which has been described in detail in Parachinus angulosus (Leske) by Cannone (1970). Chia (1970a) characterised the discharged venom of P. miliaris as a mucoid, non-sulphated acid mucopolysaccharide. He also reported that a layer of simple epithelium covers the entire appendage. The epithelium of the head consists of cuboidal or squamous cells, except on the inner surface of the jaws, where they are cuboidal or columnar. At the base of the inner surface of each jaw, there is a sensory hillock, which is composed of ciliated columnar epithelial cells. Also present on the inner surface of the jaws are smaller mechanoreceptor and chemoreceptor

areas made of ciliated columnar epithelial cells.

In the family Strongylocentrotidae, all the globiferous pedicellariae have a flexible neck region (Figure 1) with flexor muscles composed of outer longitudinal and inner circular fibers (Hyman, 1955). The neck is capable of extending greatly when the appendage is stimulated, and the head can be bent in all directions (Mortensen, 1943).

The stalk of the pedicellaria is supported by a calcareous skeletal rod (Figure 1) which articulates with a tubercle on the test by means of a ball-and-socket joint (Hyman, 1955).

The response of the globiferous pedicellariae to chemical and tactile stimuli has been recently studied in different species of echinoids by several investigators (Jensen, 1966; Campbell and Laverack, 1968; Chia, 1969). The appendage can respond to chemical stimuli, which causes it to rise and open its jaws, exposing the sensory hillocks on the inner surface. However Campbell and Laverack (1968) found that the jaws will close only on an appropriate chemical, or intense mechanical stimulus. Venom is discharged only when the foreign organism caught by the jaws is soft enough for

the tips of the skeletal valves to pierce it and large enough to prevent complete closure. (Chia, 1969).


A globiferous pedicellaria functions only once. After the venom is discharged the appendage autotomizes, or remains attached to the tissue it closed on (Chia, 1969, 1970a). Therefore, the pedicellariae must be replaced as they are lost, and new ones must develop as the animal grows. For these reasons globiferous pedicellariae in various stages of development can usually be found on a sea urchin.

Basically the internal calcareous skeletons of all four types of pedicellariae are similar, as shown by Mortensen (1913), although they are modified to perform specialized functions.

The echinoderm skeleton is mesodermal in origin and consists of a number of plates, or ossicles, each of which is believed to be a single crystal of calcite (Nichols, 1969). However, the skeleton is typically not solid, but has fenestrations, giving it a reticulate structure; such a characteristic makes it light-weight, as well as strong, and also prevents fractures along natural cleavage planes (Nichols, 1969). The pores provide areas for the insertion of muscles and connective

tissue, as well as for the passage of nerves. Cellular and extracellular material may also be found in these interconnecting spaces.

The skeleton of an echinoid globiferous pedicellaria consists of four ossicles, and has been described by numerous researchers, such as Herapath (1865), Perrier (1876), Sladen (1880), Mortensen (1927, 1943), Hyman (1955), Chia (1970a), Cannone (1970) and Campbell (1972).



The globiferous type, with venom glands, has the tip of each skeletal valve modified to form a grooved tooth for piercing and injecting the venom. The tooth is the distal region of a central tubular keel which strengthens the valve, and receives nerves from the mechanoreceptor and chemoreceptor areas on the inner surface of the jaw. The nerve from the sensory hillock enters the valve by the middle foramen (Campbell, 1972).

The proximal region of the valve is wider, with numerous ridges, folds and perforations for articulation of the valves with each other, and for the insertion of the muscles that control the jaws (Campbell, 1972). The adductor muscles, which close the jaws upon contraction, are inserted on either side of the keel. The abductors, which open the jaws, are



smaller than the adductors. Each abductor muscle stretches from the base of one valve to the adjacent one. Both the adductors and abductors form a triangle in cross sections of the pedicellaris. The point of insertion of the flexor muscles, which originate on the distal region of the skeletal rod of the stalk, is also on the base of the valve, but below the insertion of the abductors. The flexors are responsible for bending the head and extending the neck of the pedicellaris.

According to Cobb (1968a), projections from the muscle cells pass through the pores of the skeleton, and are innervated inside the keel.

The valves articulate with each other by cog-teeth, which according to Campbell (1972), insure coordinated movements of the jaws. The central area is ridged and grooved on either side, so that each valve articulates with the other two. Cog-teeth are also found laterally, so that the left side of one valve makes contact with the right side of the adjacent valve. The articulation is so precise that the jaws meet perfectly at their tips, and muscular contractions result in perfectly coordinated movements. Campbell (1972) also suggested that the teeth prevent vertical movement of

the valves relative to each other.

The base of the skeletal rod forms a socket, which articulates with a tubercle on the test (Hyman, 1955). The distal region is in the form of a rounded head, at the base of which are perforations for the insertion of the flexor muscles and connective tissue.

LITERATURE REVIEW

According to Herapath (1865) O. F. Müller, who first named pedicellariae, believed them to be parasites, creating the genus Pedicellaria. To be more specific, we learn from Sladen (1880) that Müller established or recognized three species - P. globifera, P. triphylla, and P. tridens. The differences were based on morphological variation.

Herapath (1865) described the basic morphology of pedicellariae and recognized their defensive role. He also realized that these appendages are useful in classification of the animal. A method of isolating the skeleton, similar to that used in the present study, was also reported.

Perrier (1870) dealt with the basic structure of pedicellariae and their skeletal parts, and described in detail those from a large number of species. He also recognized the fact that pedicellariae can be useful in the classification of sea urchins. Four types of pedicellariae were identified - pédicellaires gemmiformes, pédicellaires ophicéphales, pédicellaires tridactyles, and pédicellaires trifolies.

Sladen (1880), in his study of the globiferous pedicellariae of S. granularis, contributed to our understanding of the histology and function of these appendages. He considered their main function to be the discharge of mucus for the removal of offensive matter from the surface of the animal, being aided by the spines and water currents.

Foettinger (1881) expanded our knowledge of the histology of the stalk glands of the gemmiforme (globiferous) pedicellariae of S. granularis, the epithelium and the glands of the head. He found the jaws to be made up of an outer epithelial layer, underneath which is a layer of connective tissue surrounding the muscular wall of the venom sac, which contains the glandular secretions. These secretions were believed to be formed by the transformation of cells into a mucous substance, and not by secretion of the cells.

Hamann (1887) described the sensory areas of pedicellariae and traced nerve fibers from three main nerve cords in the head to the musculature, sensory epithelium, and "sense organs".

Prouho (1890), in studying the globiferous pedicellariae, attempted to remove doubts that these

organs are used for defense, even though they cannot bend sufficiently to protect the test from small animals crawling over it. Another reason for doubt was that large animals would be protected from the pedicellariae by the spines. Prouho discovered that they are important weapons against starfish in that the pedicellariae attack the enemy's tube feet. He also recognized the fact that these appendages are used only once.

The behaviour and physiology of pedicellariae were described by von Uexküll (1899), who found that the secretion of the globiferous pedicellariae is toxic. Much of our knowledge of the behaviour of pedicellariae comes from this author.

The development of the skeleton of the ophiocephalous pedicellariae was described by Gordon (1926) in the pluteus larva of Psammechinus miliaris (Gmelin) (designated Echinus miliaris Müller by Gordon). She traced the development of the valves from the calcareous rudiments to the fully formed stage.

Great systematic importance was attributed to pedicellariae by Mortensen (1927) who described their skeletal valves in detail for many groups of echinoids.

Fujiwara (1935) described the toxic effect of pedicellariae of Toxopneustes pileolus (Lamarck) on humans and mice. He found the results to be a relaxation of muscles, difficulty of respiration and a drop in body temperature. The structure of the pedicellaria was also described.

Péres (1950) gave a detailed account of the histology of the stalk glands and glands of the head in the globiferous pedicellariae of S. granularis. He also reported on their numbers, distribution, autotomy, regeneration, and pharmacology of the venom.

Jyman (1955) gave a thorough account of echinoid pedicellariae based mainly on the works given above.

Lewis and Saluja (1967) described the stalk glands of the ophiocephalous pedicellariae of Diadema antillarum Phillipi.

Campbell and Laverack (1968) devised a method of recording activities of the jaws of pedicellariae, and their closing and opening following stimulation.

Cobb (1968a) was the first to study the fine structure of the muscles of pedicellariae. His work dealt with the tridentate pedicellariae of Echinus esculentus Linnaeus.

The muscle fibrils which open and close the jaws break up into finger-like projections at each end, some of which attach to the skeletons of the jaws, while others pass through the pores in the skeleton into the nerve fibers contained within.

The fine structure of the sensory system was investigated by Cobb (1968b) in the tridentate and globiferous pedicellariae of E. esculentus. He stated that all epithelial cells have a sensory function since they apparently send axonic processes to the nerve tracts of the jaws. The sensory hillock was described as an area of laterally compressed epithelial cells believed to be tactile and chemosensory in function.

Chia (1969) discovered the globiferous pedicellariae of E. esculentus, P. miliaris and S. dröbachiensis react to a number of inorganic salts in a manner similar to the response to predatory starfish. Chia (1970b) also described the escape response of S. dröbachiensis to a number of inorganic salts.

Chia (1970a) made significant contributions to the histology of the globiferous pedicellariae of P. miliaris, and included a brief report on their regeneration. This research confirmed his earlier hypothesis that the groove

of the terminal tooth is sealed, enabling it to act as a hypodermic needle to inject venom into the foreign organism. He showed that the venom sac terminates distally in a duct, which lies within the groove of the tooth.

A detailed account of the anatomy and mechanism of venom emission of globiferous pedicellariae of P. angulosus was given by Cannone (1970). Longitudinal sections showed that at the opening of the venom duct a ring of duct tissue encloses the terminal tooth and duct. It is this ring, and the arrangement of the muscle fibers of the wall of the venom sac, that allow the secretory duct to open upon contraction of the gland lumen. This results in the venom being injected into the puncture made by the terminal tooth. The role of the sensory and nervous systems in pedicellarial activity was also discussed.

Campbell (1972) was the first to study the functional morphology of the skeleton of pedicellariae using the scanning electron microscope. He was concerned with the four types of pedicellariae from E. esculentus.

Larrain (1972) described the histology of the globiferous pedicellariae of Loxechinus albus (Molina).



its behaviour, and also the morphology of its skeleton. The anatomy of this appendage was used as a basis for classification.

The venom gland cells in globiferous pedicellariae from S. purpuratus were studied at both the light microscopic and electron microscopic levels by O'Connell (1971, 1974). Preliminary light microscopic, histochemical analysis was also done on the gland cells by O'Connell (1971).

Numerous researchers have been concerned with the process of calcification in echinoderms. Although relatively little is known about the process, and much disagreement exists among researchers, there is general belief that calcite deposition is under the control of mesenchyme cells, or an organic matrix produced by them. Most of our knowledge of skeletal development has come from studies on sea urchin larvae. Among the most important works are those of Selenka (1879), Semon (1887), Théel (1892), Woodland (1906), Prenant (1926), Onoda (1931), Runnström (1931), von Ubbish (1937, 1950, 1957), Okazaki (1960), Bevelander and Nakahara (1960), Wolpert and Gustafson (1961), and Raup (1966).

Gibbins et al (1969) in an electron microscopic study of the larva of Arbacia punctulata Gray found the

membrane-bound skeleton to be surrounded by a continuous cytoplasmic skeletal sheath. From light microscopic observations Okazaki (1960) and others had previously reported the larval skeleton to be surrounded by a thin sheath of protoplasm, formed by the fusion of pseudopodia from primary mesenchyme cells.

Pilkington (1969) described the skeletal tissues of the spines of E. esculentus. He called the cells occupying the skeletal spaces sclerocytes, and proposed that cytoplasmic processes extend from the cell bodies to the mineral surface. He interpreted the membrane surrounding the skeleton to be continuous with that of the cytoplasmic processes, and the sclerocyte cell membrane. The skeleton would therefore be intracellular, and in direct contact with the cytoplasm only in areas where the cytoplasmic processes meet the skeleton. Swollen regions of the processes, containing mitochondria and various inclusions, were termed first order spherical bodies. They are connected with smaller second order spherical bodies near the calcite surface.

Kniprath (1974) stated that in the sea urchin tooth from the lantern of Paracentrotus lividus (Lamarck) the skeleton grows intracellularly within a membrane bound space, directly onto the inner coating of the surrounding

membrane. Each calcified part was found to be surrounded by three sheaths - cellular membrane, skeletal sheath, and the inner coating of the sheath.

Heatfield and Travis (1975) described the ultrastructure of regenerating spines of S. purpuratus. Three types of cells were found in the dermis of the regenerating tips. Precalcoblasts appeared to be oriented in the general direction of skeletal growth. The cell body was somewhat elongated and found to be ultrastructurally similar to calcoblasts, of which they were considered precursors.

Calcoblasts were discovered as the active skeletogenic cell type. They extend a thin cytoplasmic sheath around the growing tip of micro-spines, however the cell membrane is not adjacent to the calcite surface. At points where the cytoplasmic sheath is discontinuous, the space between it and the skeleton becomes continuous with the extracellular space. Each microspine is therefore extracellular. Few mitochondria and no microtubules were found in the sheaths. The older region of the skeleton was found to be enclosed by a membrane-like structure. The authors interpreted the function of the cells in this area to be maintenance of the integrity of the mineral, possibly to inhibit dissolution of the

skeleton, or further mineralization.

The third cell type was the phagocyte. These cells contained vacuoles of various sizes. Materials of various electron densities were found in these vacuoles.

Several workers have suggested that mitochondria participate in calcification processes. Vasington and Murphy (1961, 1962) and DeLuca and Engstrom (1961) discovered that isolated mitochondria can accumulate large net amounts of  $Ca^{2+}$  from the suspending medium during electron transport. Large electron dense deposits of calcium phosphate were shown in electron micrographs of rat liver mitochondria by Lehninger et al (1963), Greenwalt et al (1964), Brierley and Slautterback (1964), and Weinbach and von Brand (1965). A working hypothesis on the possible role of mitochondria in calcification was proposed by Lehninger (1970). It was pointed out that the ability to form hard tissues is present in a wide variety of plants and animals, both in normal and pathological conditions. He speculated that calcium is accumulated and released by mitochondria, thereby regulating and controlling the process of deposition.

#### MATERIALS AND METHODS

Sea urchins (*S. dröbachiensis*) were collected at Logy Bay, Newfoundland, and maintained in an aquarium. Globiferous pedicellariae in various stages of development were removed from the tests of sea urchins having a test diameter of approximately 3 cm. Fine forceps were used to remove the pedicellariae, as well as a small area of the surrounding epidermis of the test, to prevent damage to the appendage. All specimens, except those used for scanning electron microscopy, were fixed for one hour in one of the following fixatives:

- a. 2.5% glutaraldehyde in Millonig's Buffer, pH 7.0-7.2
- b. 2.5% glutaraldehyde in Sorensen's Buffer, pH 7.0-7.2
- c. 2.5% glutaraldehyde in Sorensen's Buffer, pH 6.8-6.9
- d. 1% osmium tetroxide in sea water, pH 7.0-7.2

No significant difference in tissue preservation was found among the various fixatives, except where the pH was allowed to drop to 6.8-6.9. This slightly acidic condition resulted in a partial decalcification of the skeleton.

After fixation, tissues were rinsed several times in buffer, and then treated with 1% osmium tetroxide in phosphate buffer, pH 7.2.

The calcareous skeleton of the pedicellariae was often found to make sectioning difficult, this being most pronounced in later stages where more hard material had been deposited. In some cases, the specimens were left in a saturated solution in water of diamino-ethane-tetra-acetic acid (EDTA) for two to three days, after osmication. This treatment decalcified the tissue without greatly affecting the fine structure of the soft tissues.

In all cases the specimens were dehydrated in a graded series of water-ethanol mixtures to absolute alcohol, where they remained for one hour with three or more changes. Tissues were infiltrated as described by Luft (1961). Samples were transferred to propylene oxide for one hour with three or more changes, then to a propylene oxide-epon mixture for two to three hours. The pedicellariae were then allowed to infiltrate in epon for one to two hours at room temperature, after which curing was done at controlled temperatures for two days.

For histological studies the tissues were sectioned at 1  $\mu$ m on an LKB ultramicrotome, using glass knives.

Sections were then stained with 1% alkaline toluidine blue, using standard procedures (Hayat, 1970).

Ultrathin sections for transmission electron microscopy were cut on a Porter-Blum ultramicrotome, using a diamond knife. The sections were stained for one minute in lead citrate, followed by one minute in uranyl acetate (Hayat, 1970).

For X-ray microanalysis of calcium, it was necessary to use specimens that had not been treated with EDTA. Care was taken to insure that at no time during the preparation did the pH drop below 7.0, since even slightly acid solutions were found to remove some calcite. Sections were cut at 130 nm using glass knives, supported on unfilmed grids, and left unstained.

Microprobe analysis was done at the Physiological Laboratory, Cambridge, England, where the electron microscope micro-analyser (EMMA-4) facilities were made available for a limited period.

The EMMA-4 has been developed from the standard electron microscope, with certain modifications and additions, which make possible examination of specimens at high resolution, and their elemental analysis. The illumination system, with

the mini-lens, can produce a probe of diameter 100-200 nm or greater. This can be positioned over any area of interest in the specimen. When the area is bombarded with electrons, X-rays are emitted, which are detected by the two crystal spectrometers, and dispersed into the characteristic wavelengths for the elements present. The non-dispersive detector measures the white count, which can be related to the characteristic count for the element to determine mass. When analysing for elements known or believed to be present in the specimen, the optimum crystal is selected in each spectrometer. For a more complete account of the instrument and its uses see Weavers (1971) and Appleton (1974).

Additional data on microprobe analysis was obtained through the courtesy of JEOL Ltd., Medford, Massachusetts, United States of America. Ultrathin sections were analysed in JXA-50A electron probe microanalyser. The use of various attachments allows transmitted electron images to be obtained, as well as X-ray images.

In preparing the skeleton for examination with the scanning electron microscope, it was first necessary to remove the soft tissues. After removal from the test, pedicellariae were rinsed in distilled water and immersed in Javex (sodium hypochlorite). The bleach removed the soft tissues from the skeleton, which was then rinsed twice in



distilled water to remove debris and bleach. Specimens were pipetted onto stubs coated with partially wet household cement. The cement was allowed to harden, thus securing the ossicles to the stubs. While working with the earlier stages of development, it was necessary to carry out the entire procedure of removing organic material and rinsing the skeleton on a cover slip. This permitted continuous observation through a dissecting microscope, thus preventing loss of specimens during transfer from one solution to another. When the ossicles were sufficiently rinsed, a piece of the cover slip bearing the specimens was coated with gold in a vacuum evaporator, and examined in a Cambridge Scanning Electron Microscope.

An attempt was made to assign a specific time value to the developmental stages of the pedicellariae. This was done by removing several mature globiferous pedicellariae from a sea urchin, and examining the animal every day until the appendages had reached full size. However, there was so much variation in the rate of growth of the appendages, even on the same individual, that only the sequence of events could be described. This is in agreement with a regeneration experiment done by Chia (1970a) on the globiferous pedicellariae of P. miliaris.

## RESULTS AND DISCUSSION

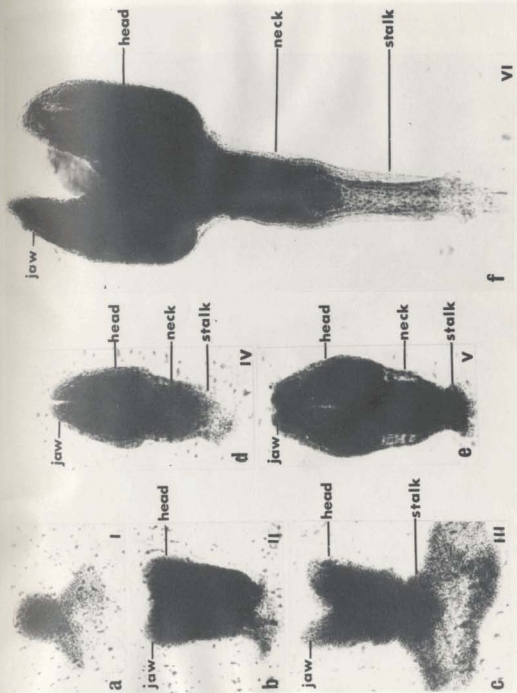
### Developmental Changes in External Features

In this study of the globiferous pedicellariae six morphologically distinct developmental stages have been recognized, (Figure 2). A new pedicellaria is first visible as a protrusion of the epidermis which covers the test of the sea urchin. The specimen shown in Figure 2a has been placed in Stage I, and measures approximately 110  $\mu\text{m}$  in diameter. In this stage of development there is no division of the tip into three separate jaws externally, however the size of the appendages considered to be in Stage I may vary somewhat, depending on the exact age of the developing pedicellaria. Towards the end of Stage I a slight change in shape occurs, the distal region (head) becoming slightly greater in diameter than the basal and middle regions of the appendage.

The specimen shown in Figure 2b is from Stage II, and measures approximately 270  $\mu\text{m}$  in length, with the diameter of the head region being approximately 180  $\mu\text{m}$ . Besides the obvious increase in size over those in Stage

Figure 2 - Whole mounts of developing globiferous pedicellariae of Strongylocentrotus dröbachiensis.

- a ..... Pedicellaria in Stage I; 130X.
- b ..... Pedicellaria in Stage II, showing the separation of the tip into three jaws; 130X.
- c ..... Pedicellaria in Stage III; note the further elongation of the three jaws; 130X.
- d ..... Pedicellaria in Stage IV, showing head, neck, and stalk regions; note the closed position of the jaws; 130X.
- e ..... Pedicellaria in Stage V, showing the rounded shape of the jaws at their bases. The flexor muscles of the neck are visible through the somewhat transparent epithelium; 130X.
- f ..... Pedicellaria in Stage VI; besides the large increase in the size of the appendage, the major change has been the elongation of the stalk; 130X.



I, there is also a change in shape. The tip of the appendage has clearly formed three projections; each one is the beginning of a moveable jaw. It is also clearly shown that the pedicellaria is slightly narrowed proximally, having a diameter of approximately 170  $\mu$ m. Thus the finger-shaped projection is shown to be differentiating gradually into a specialized appendage.

The third stage of development is represented by Figure 2c. The three distal projections, which are the separated tips of the jaws, have become more distinct externally. The length of the pedicellaria has increased to approximately 320  $\mu$ m, however there has been no comparable increase in cross sectional area. This fact is explained by a change in shape of the appendage resulting from a change in the rates of development of the three regions (head, neck, stalk) relative to each other. This changes the proportion of the total length contributed by each region. The increase in length of the appendage has been mainly due to the elongation of the jaws. There is now clearly a narrowing of the appendage towards the base. The neck region is not distinct externally, but is the area between the head and the stalk.

Stage IV shows a further differentiation of the three regions of the pedicellaria. The specimen shown in Figure 2d has a length of approximately 445  $\mu\text{m}$ , however there has been no further increase in cross sectional area. Although the stalk is still short, it is the area which is smaller in diameter. The neck region has elongated and has become more distinct from the head and stalk regions. The most obvious change in the head region is an elongation of the jaws. It is in this stage that the three jaws first come to meet each other at their tips. This is the closed position. It is uncertain whether or not the jaws have the ability to open at this state of development. During the course of this study none has been observed to show any appreciable movement.

The next major change, that is evident in Stage V, is an increase in the cross sectional area of the head region. Figure 2e shows the rounded bases of the jaws. Little change in the neck and stalk regions has taken place, although the flexor muscles can now be seen through the somewhat transparent epithelium. The diameter of the head region of the specimen in Figure 2e is approximately 235  $\mu\text{m}$  compared to Figure 2d which is

of equal length but has a diameter of 200  $\mu$ m. This is the first major increase in cross-sectional area since growth has taken place during Stage I.

Stage VI is represented by a pedicellaria with all essential components well formed. Each of the three regions is distinct, although the stalk is at first short. The flexor muscles in the neck have been observed in both the extended and relaxed conditions, indicating that the appendage is capable of bending its head on the stalk. The pedicellaria in Figure 2f has changed little in shape from that in Figure 2e, although there has been a relatively large increase in size, especially in the head region. It is only when the other components of the appendage are well formed that the stalk begins to elongate. The specimen in Figure 2f has reached a length of approximately 1.2 mm and a diameter through the base of the head of approximately 430  $\mu$ m.

#### Histology and Ultrastructure of the Epithelium

The epithelial layer covering the pedicellaria remains continuous with that of the test. The mature appendage possesses an epithelium consisting of a single layer of cells (Hyman, 1955). This is true in later

developmental stages (Figures 3 and 4), however, in earlier stages the epithelium is several cell layers thick (Figure 5).

The external surface of the outermost epithelial cells is shown in electron micrographs to possess numerous microvilli, measuring approximately 0.1  $\mu$ m in diameter and 1.0  $\mu$ m in length, as shown in Figures 3, 4, 5 and 6. Occasionally the microvilli are shown to branch distally, terminating in an electron-dense tip. A layer of fine filamentous material covers the surface of the pedicellaria, interconnecting the distal halves of the microvilli (Figure 6).

Mitochondria and Golgi bodies are common in epithelial cells of the developing pedicellaria, together with numerous vacuoles and vesicles. The fine structure of the epithelium of pedicellariae of E. esculentus was described by Cobb (1968b) who reported that the microvilli are connected to vacuoles found throughout the epithelial cells. In the present study vacuoles were common near the microvillar surface, but they were not shown to continue into the microvilli.

Cobb (1968b) also found the epithelial cells of E. esculentus pedicellariae to be joined by desmosomes for



Figure 3 - Electron micrograph of the epithelium of a globiferous pedicellaria (Stage IV or V) of Strongylocentrotus dröbachiensis, showing two types of vacuolated cells.

The cell labelled vc contains electron lucent vacuoles, with traces of granular material. One vacuole has several rounded inclusions. The cell labelled spc contains several dark vacuoles, or spherules, sl, and a large amount of irregularly distributed granular material; 6000X.

abbreviations, in alphabetical order:

ds - desmosome  
ep - epithelial cell  
G1 - Golgi body  
mv - microvilli  
n - nucleus  
sl - spherule  
spc - spherulated cell  
v - vacuole  
vc - vacuolated cell

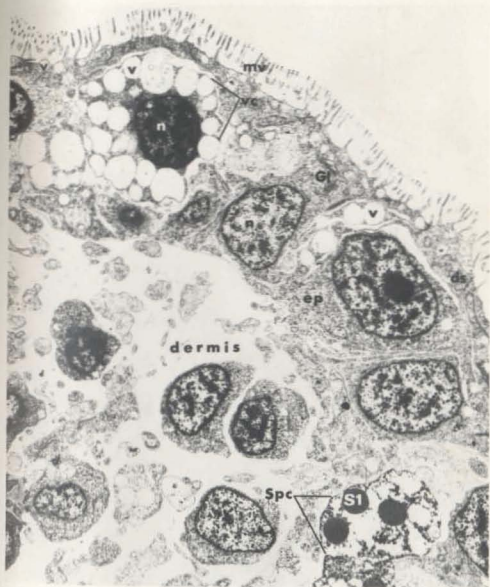


Figure 4 - Electron micrograph of a globiferous pedicellaria (Stage IV or V) of Strongylocentrotus dröbachiensis, showing vacuolated cells in the dermis and in the epidermis.

The vacuolated cell, vc, in the epithelium, contains electron lucent vacuoles, while those in the dermis, Spc, contain vacuoles, or spherules, with extremely electron dense material (S1), with moderately dense granular material (S2), and with only small amounts of granular material (S3); 6000X.

abbreviations, in alphabetical order:

ad - adductor muscle  
bb - basal body  
ds - desmosome  
ep - epithelial cell  
G1 - Golgi body  
mv - microvilli  
n - nucleus  
S1 - spherule containing extremely dense material  
S2 - spherule containing moderately dense material  
S3 - spherule containing scattered granular material  
Spc - spherulated cell  
v - vacuole  
vc - vacuolated cell



Figure 5 - Electron micrograph of a globiferous pedicellaria (Stage I or II) of Strongylocentrotus dröbachiensis, showing an epithelium that is several cell layers thick.

Note the columnar epithelial cells with large electron dense granules concentrated near the external surface. Two vacuolated cells containing electron lucent vacuoles can be seen; 6000X.

abbreviations, in alphabetical order:

ds - desmosome  
ep - epithelial cell  
g - granule  
Gl - Golgi body  
mv - microvilli  
n - nucleus  
v - vacuole  
vc - vacuolated cell



Figure 6 - Electron micrograph of the epithelium of a developing globiferous pedicellaria of Strongylocentrotus dröbachiensis.

Note the presence of microvilli which end in electron dense tips (c), and the layer of fibrillar material (f). Also shown are desmosomes, mitochondria, and numerous vacuoles and vesicles; 24,600X.

abbreviations, in alphabetical order:

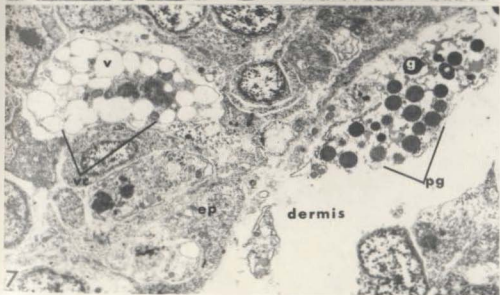
c - terminal cap of microvilli  
ds - desmosome  
f - fibrillar material  
mit - mitochondrion  
mv - microvilli  
v - vacuole

Figure 7 - Electron micrograph showing a packet of granules in the dermis of a developing globiferous pedicellaria of Strongylocentrotus dröbachiensis.

Note also the presence of a vacuolated cell in the epidermis; 6000X.

abbreviations, in alphabetical order:

ep - epithelial cell  
g - granule  
pg - packet of granules  
v - vacuole  
vc - vacuolated cell





a distance of several micrometers from the surface, these being septate at the surface and at the termination of the desmosomal regions. Figures 3, 4, and 6 show the epithelial cells of developing pedicellariae of S. dröbachiensis to have intercellular junctions similar to those described by Coleman (1969) for the tube feet of the regular echinoid D. antillarum. There is an indentation or "cup" formed by adjacent cell membranes near the external surface. This has an electron dense coating and leads to an area where adjacent membranes are closely connected by a septate desmosome.

Cobb (1968b) found each general epithelial cell of E. esculentus pedicellariae to possess a single cilium enclosed by a ring of microvilli. Although the micrographs presented here do not show cilia, basal bodies have been found in the epithelial cells (Figure 4).

Each cell of the epithelium contains a large nucleus, and one or two nucleoli can usually be seen.

Also present in the epidermis are distinct vacuolated cells (Figures 3, 4, 5, 8a and 8b). In histological sections these appear as large, rounded cells, containing numerous large, apparently empty vacuoles. They are probably pigment or mucous cells. The contents of the

Figure 8 - Light micrographs of globiferous pedicellariae (Stage I) of Strongylocentrotus dröbachiensis.

a ..... Longitudinal section showing the distal head region, and the basal stalk region (the skeleton is indicated by arrows); note the adductor muscles, and the calcicytes surrounding the skeletal elements; 500X.

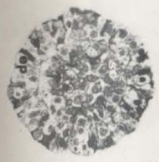
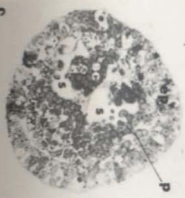
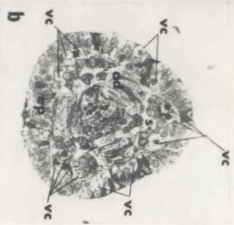
b ..... Cross section through the head region, showing the skeletal valves, calcicytes and adductor muscles; note the triangular shape; 500X.

c ..... Cross section through the base of the head region, showing the basal layers of the skeletal valves, and the cells (calcicytes) which surround the skeleton and fill its pores; 500X.

d ..... Cross section of the stalk region showing the skeletal rod (arrows) and the calcicytes; note the round shape; 500X.

abbreviations, in alphabetical order:

ad - adductor muscle  
ep - epithelium  
lsc - layer of skeletal cells (calcicytes)  
p - pore  
s - skeleton  
sc - skeletal cells (calcicytes)  
vc - vacuolated cell



vacuoles may have been lost during the fixation process. The deeply staining nucleus is usually located near the periphery of the cell.

In electron micrographs the nucleus is shown to be smaller and more rounded than the nuclei of the other epidermal cells (Figures 3 and 5). Both types have irregularly distributed clumps of chromatin, however in the vacuolated cells there is usually a thicker area of perinuclear chromatin.

The vacuolated cells described above are found mainly in the epidermis, although they occasionally appear in the dermis. This is specially true in the early stages of development where the vacuolated cells in the dermis may outnumber those in the epidermis (Figure 8b). They may be found among the mesenchyme cells, near centers of calcification, or even between muscle fibers (Figures 8b and 19a). Such a location is less frequent in later stages of development, where they may be found in the dermis, although not closely associated with any tissue (Figure 19b).

Packets of granules have been observed in the dermis of pedicellariae in various stages of development (Figures 7, 10a, 15b and 16a). In both histological studies and

electron micrographs, the granules stain heavily, and probably contain pigment. These packets have been observed in the dermis, in the epidermis, and occasionally on the boundaries separating the two, but most frequently in the dermis. They possibly are formed in the dermis and migrate outwards.

Figure 7 also shows a vacuolated cell in the epithelial layer. It is similar in size and shape to the packet of granules described above. The most obvious difference is that most of the vacuoles are electron lucent. Some vacuoles appear to be somewhat broken up, with no distinct membrane in certain areas. It is possible that these are morphologically different phases of one cell type, possibly a pigment cell.

Another morphologically different vacuolated, or spherulated cell, has been observed in electron micrographs, both in the epidermis, and in the dermis (Figures 3 and 4). The chromatin material is concentrated around the periphery of the nucleus, with only scattered clumps in the center. Some vacuoles or spherules contain extremely electron dense material (S1), others are less dense (S2), while others contain only small amounts of granular material (S3).

Developmental Changes in Internal Anatomy

Stage I. Figure 8a is a longitudinal section of a pedicellaria at the stage where it is a finger shaped outgrowth with no jaws yet separated at the tip. The dermis narrows towards the proximal end. The compensating increased thickness of the epithelial layer, at the base of the appendage, obscures this shape externally, giving the pedicellaria an almost uniform diameter throughout the length.

The proximal part of the appendage represents the stalk region, while the neck region cannot be distinguished clearly from the stalk. These areas are round in cross section (Figure 8d). The head is the distal region, and cross sections show that this area is triangular in shape (Figures 8b and 8c). Each apex of the triangular shaped head region corresponds to one of the developing jaws. The skeleton appears very early in the development of a globiferous pedicellaria. The earliest indication of this in histological sections is a clustering of cells around the developing skeletal elements. This takes place in four areas - each of the three differentiating jaws, and to a lesser extent in the stalk, see Figures 8a, 8b, 8c and 8d.

Each of the skeletal elements is presumably deposited by these clusters of cells that differ slightly in appearance from the other mesenchymal cells of the dermis. They have been referred to as sclerocytes, in sea urchin spines, by Pilkington (1969) while Heatfield and Travis (1975) termed the active skeletogenic cells in regenerating spines calcoblasts. The cells associated with the developing pedicellariar skeleton are termed calcicytes in this thesis. The reasons for adopting this term are discussed on page 121.

In light microscope studies the cytoplasm of the calcicytes is small and indistinct. The nuclei appear more rounded than those of the other cells, and one or more nucleoli can usually be seen. The nuclei of the calcicytes stain differently with toluidine blue from those of the other dermal cells. There is more clumping of chromatin, with a concentration around the nuclear membrane, giving the nuclei a sharp outline in these histological sections.

In longitudinal sections the neck and stalk regions can be seen to be separated from the head region by a layer of cells, as described by Chia (1970a). However, this layer, shown in Figure 8a, conforms with the shape of the skeletal valves, each of which at this stage has a flattened base, as seen in scanning electron micro-

graphs of the isolated skeleton (Figures 32a and 32b). The layer of cells is composed of calcicytes underlying the skeletal valves, which are oriented with their basal layers along the horizontal plane of the pedicellaria (Figure 8a). When fully developed the base of a skeletal valve articulates laterally with the other two adjacent valves, and all three meet in the center of the appendage, but in the very early developmental stages, as shown in Figures 2a and 8a, before the three jaws differentiate and divide distally, there is no articulation.

Echinoderm calcite is typically porous or fenestrated, (Raup, 1966). These pores in life are filled with cells and other organic materials, most likely connective tissue. This is evident in histological preparations (Figure 8c). Since the material has been decalcified to enable sectioning, the skeleton is represented by "empty" spaces. The location of the calcicytes in histological sections corresponds closely with the pores of the valves studied with the scanning electron microscope, compare Figures 8c and 32a, and Figures 10b and 33a. Examination of sections of pedicellariae in various stages of development shows that these cells continue to surround, and presumably determine the shape of the skeleton.



Neither the three basal layers of the skeletal valves, nor the underlying cellular layer, has been demonstrated in any one cross section. This is explained by the fact that these areas would be approximately 3-4  $\mu\text{m}$  thick, making it necessary to orient the specimens very precisely while sectioning to get an exactly transverse section through the required region.

Examination of the skeletal valves in the scanning electron microscope shows that the base is the widest part, and above this there is a gradual narrowing to the tubular keel (Figures 32a and 32b). The dermis at the bases of the jaws is almost entirely composed of the inorganic calcite and its organic matrix. Distally, however, the skeleton occupies a much smaller area in cross section, leaving the spaces between the three skeletal valves free for the development of the adductor muscles which close the jaws in the mature appendage. The cells on either side of each jaw are early developmental stages of the adductor muscles. In cross section (Figure 8b) they can be seen to span the distance between adjacent valves. The cells in these three areas gradually elongate, becoming spindle shaped. Electron micrographs show that this change in shape is

true of both the nuclei and the cytoplasm, and in Figure 9 scattered fibrils can be seen in the cytoplasm. In light micrographs the cells are shown to be at first rather loosely arranged, (Figures 8a and 8b), but as more cells differentiate a true muscle bundle is formed. The individual cells elongate such that they appear to completely span the distance between adjacent valves in histological sections, but in electron micrographs they do not appear to have inserted on the skeletal valves at this stage (Figure 9), and it is not known how early they do so.

The adductor muscles appear even before the three jaws separate at the distal end (Figures 8a and 8b). Therefore, by the time the jaws are sufficiently differentiated, the adductors are well developed.

Stage II. One important difference from the previous stage is that the tip of the pedicellaria has now formed three separate jaws, as shown in Figure 2b. No new tissues have appeared, but the muscles and skeletal elements have increased in size to correlate with the growth of the appendage (Figure 10a). There is also some indication, from cross sections through the head region, that the simple triangular shape seen

Figure 9 - Electron micrograph of globiferous pedicellaria (Stage I or II) of Strongylocentrotus dröbachiensis, showing the developing adductor muscles.

In this stage the muscles are not inserted on the skeletal valves. Note the presence of scattered fibrils in the cytoplasm; 4200X.

abbreviations, in alphabetical order:

c - cytoplasm  
ep - epithelial cell  
f - fibrils  
g - granule  
n - nucleus  
s - skeleton  
sc - skeletal cell (calcicyte)

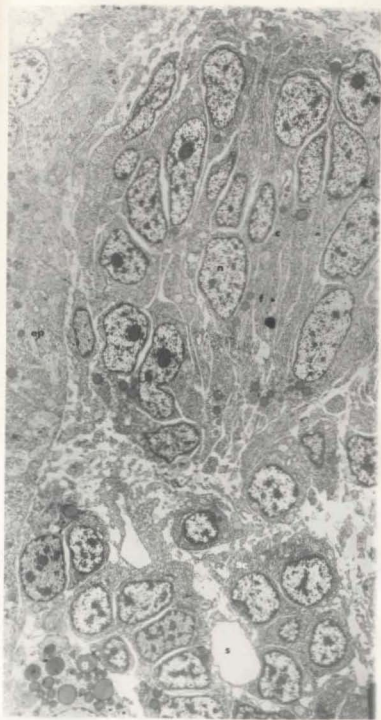


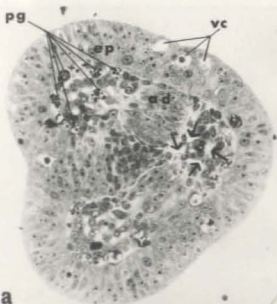
Figure 10 - Light micrographs of developing globiferous pedicellariae of Strongylocentrotus dröbachiensis.

a . . . . . Cross section of a pedicellaria in Stage II; note the change in shape from the cross section of the pedicellaria (Stage I) shown in Figure 8b. Numerous packets of granules are found in the dermis, near the skeletal valves. Several vacuolated cells are shown in the epithelium. Skeletal valves are indicated by arrows; 500X.

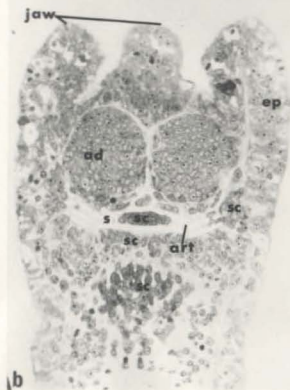
b . . . . . Longitudinal section of a pedicellaria in Stage III, showing the adductor muscles (cut in cross section) as well as the three skeletal valves and their means of articulation. The skeletal rod and its organic matrix can also be seen; 500X.

abbreviations, in alphabetical order:

ad - adductor muscle  
art - articulation area  
ep - epithelium  
pg - packet of granules  
s - skeleton  
sc - skeletal cells (calcicytes)  
vc - vacuolated cell



a



b

in Figure 8b will be gradually lost. As the jaws increase in cross sectional area, they protrude outwards, but continue to merge at their bases. The slightly rounded outer side of the jaws is evident in Figure 10a.

Stage III The three jaws have further differentiated in Figure 12a, and it is clear that the simple triangular shape of the head region has been lost. As elongation of the jaws proceeds, histological sections show spaces between the three separated jaws (Figures 11a and 11b). The dermis contains mainly the skeleton and its organic matrix. The cells which are presumably responsible for skeletal deposition, the calcicytes, are still observed in association with the ossicles (Figures 10b, 11a, 11b, 12a, 12b, 13a and 13b). A relatively large number of calcicytes are found at the tips of the jaws where growth is occurring to lengthen the valves (Figure 11a). They are also common throughout the length of the keels where calcite deposition is making the structures stronger and tubular. Calcicytes are most numerous at the widened basal areas of the skeletal valves (Figure 12b). Besides strengthening the valve, growth here results in the formation of numerous ridges and grooves characteristic of the fully formed skeletal valve. These ridges and grooves function in articulation of the valves, attach-

Figure 11 - Light micrographs of a globiferous pedicellaria (Stage III) of Strongylocentrotus dröbachiensis.

a ..... Cross section through the separated tips of the jaws, at the level of the developing sensory hillocks, showing the skeletal valves (arrows) which are open on their inner sides; 500X.

b ..... Cross section through a more proximal area of the separated jaws; note that the epithelium of the inner surface of each jaw gradually increases in thickness towards the center, forming the sensory hillock. Arrows indicate a skeletal valve; 500X.

abbreviations, in alphabetical order:

ep - epithelium  
sc - skeletal cells (calciocytes)  
sh - sensory hillock





a



b

Figure 12 - Light micrographs of a globiferous  
pedicellariae (Stage III) of  
Strongylocentrotus dröbachiensis.

a . . . . . Cross section of the area immediately below the point of separation of the jaws; the skeletal valve is indicated by arrows; 500X.

b . . . . . Cross section of the base of the head region, showing parts of the basal layers of the skeletal valves and the calcicytes surrounding them; 500X.

abbreviations, in alphabetical order:

ad - adductor muscle  
ep - epithelium  
s - skeleton  
sc - skeletal cells (calcicytes)

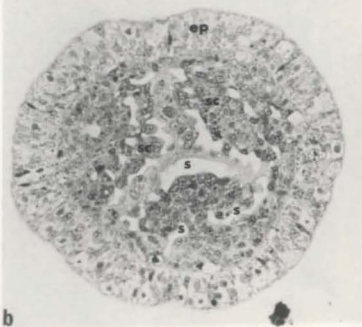
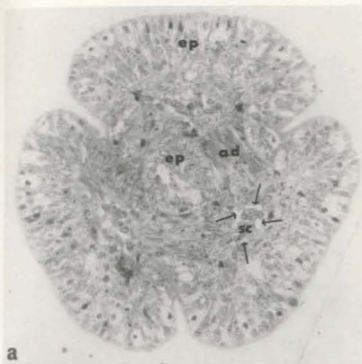


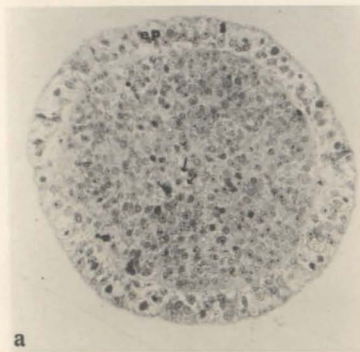
Figure 13 - Light micrographs of a globiferous pedicellaria (Stage III) of Strongylocentrotus dröbachiensis.

a ..... Cross section of the neck region showing the closely packed cells, and the tip of the skeletal rod (indicated by arrows); 500X.

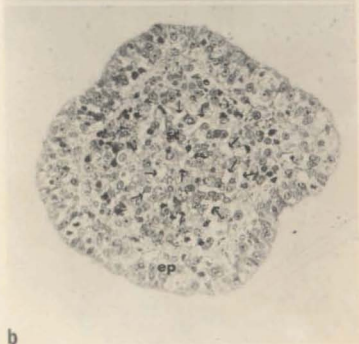
b ..... Cross section of the stalk region showing the skeletal rod (indicated by arrows) and the calcicytes which are less compactly clustered than those in the head region; 500X.

abbreviations, in alphabetical order:

ep - epithelium  
sc - skeletal cells (calcicytes)



a



b

ment of muscles and connective tissue, and passage of nerve fibers. This is described in detail in a later section.

It is not known if the jaws are moveable at this stage, however in Figure 10b the valves are shown to "articulate" with each other. The adductor muscles, which are responsible for closing the jaws in the mature pedicellaria, are well developed. The abductors, which open the jaws, and the flexors, which allow the head to bend on its stalk, do not appear to have formed at this stage.

The cells which are depositing the skeletal rod of the stalk tend to be less compactly clustered than those which are forming the skeletal valves of the jaws (Figures 10b and 13b). This is in agreement with the form of this part of the skeleton, which is less complex, more reticulate, and is being deposited at a slower rate in the stalk than in the head region. Examination of the whole mounts in Figure 2 demonstrates that elongation of the stalk occurs only after the components of the head region are well formed.

The area between the stalk and the bases of the skeletal valves is the neck region of the pedicellaria.

It is not well differentiated at this stage, but in Figure 13a it is shown to contain closely packed cells. In the center, the tip of the skeletal rod and its organic matrix can be seen.

Longitudinal sections (Figure 10b) show that there is little distance between the tip of the skeletal rod and the bases of the skeletal valves of the jaws. This is also true of the fully developed pedicellaria when it is in the relaxed state, but when stimulated the neck region elongates so that the head extends toward the source of stimulation. Therefore the distance between the tip of the skeletal rod and the bases of the skeletal valves is then greatly increased. However, at the stage of development shown in Figure 10b, the necessary muscles and connective tissues which allow elongation and flexure of the neck are not yet formed.

It now becomes evident that the neck region has increased in diameter and thickness in relation to the stalk (Figures 13a and 13b). This was not the case in earlier stages where only the head region was larger in diameter.

Cross sections clearly show that the epithelial layer of the pedicellaria is several cell layers thick in

the head region (Figures 11a, 11b, 12a and 12b). Sections through a pedicellaria, above the point of separation of the jaws, show that the thickest areas are on the outer surfaces of the jaws, and on the middle of the inner surfaces (Figures 11a and 11b). There is a gradual increase in the thickness of the epithelial layer on either side of the inner surface of the jaw, with the greatest width in the center. This thickening is shown to form a distinct hillock, and is the beginning of the sensory hillock of the mature appendage (Figure 11a).

In distal areas of the jaw, (Figures 11a and 11b), it is shown that the dermis is represented by only a small area in the center of each jaw, containing the keel of the valve, its organic matrix, and a few undifferentiated mesenchyme cells. From the outline of the decalcified skeleton it can be seen that the keel is proximally a tubular structure (Figure 11b). Figure 11a, which is a transverse section through a more distal region, at the level of the developing sensory hillock, shows that here the inner surface of the keel is still open. This condition allows for the formation of the foramen through which the nerves from the sensory hillocks pass.

Stage IV. The skeletal valves have increased greatly in length, and in complexity at the bases where



intricate grooves for articulation are formed (Figures 14a, 35a and 35b). This development correlates with the enlargement of the adductor muscles, and the appearance of the abductors and flexors, the latter of which are represented by muscle fibers found in the neck region, running from the bases of the skeletal valves to the tip of the skeletal rod of the stalk.

It is in this stage of development that the explanation for the thickened epithelial layer covering the outer surfaces of the jaws become evident, as it is shown to gradually differentiate into the venom glands.

The epithelial layer in the stalk and neck regions is only one cell layer thick, see Figure 14a. This is the condition characteristic of the mature pedicellaria. However, the epithelium on the outer surface of each jaw is several cell layers thick, see Figures 14a, 15a, 15b, 16a and 16b. At the level of the adductor muscles, it is clear that the epithelium of the remaining two sides of each jaw has become one cell layer thick, as shown in Figure 15a. This area appears as an infolding in the wall of the pedicellaria.

Apparently, there is a rapid division of cells in the epithelium, causing the layer to project inwards into

Figure 14 - Light micrographs of a globiferous pedicellaria (Stage IV) of Strongylocentrotus dröbachiensis.

a . . . . . Longitudinal section showing the skeletal valves, skeletal rod, calcicytes, adductor muscles, and flexor muscles. Note that the epithelium in the stalk and neck regions is only one cell layer thick, while that in the head region is several cell layers thick; 320X.

b . . . . . Cross section of the base of the head region, showing the bases of the skeletal valves, the cells and organic material (most likely connective tissue) surrounding the valves, and the abductor muscles. The epithelium gradually thickens distal to the base of the jaws, as shown in Figure 14a. A slightly tangential section would explain the presence of the thickened epithelial layer in this micrograph; 500X.

abbreviations, in alphabetical order:

- ab - abductor muscle
- ad - adductor muscle
- art - articulation area
- ct - connective tissue
- ep - epithelium
- fl - flexor muscle
- s - skeleton
- sc - skeletal cells (calcicytes)

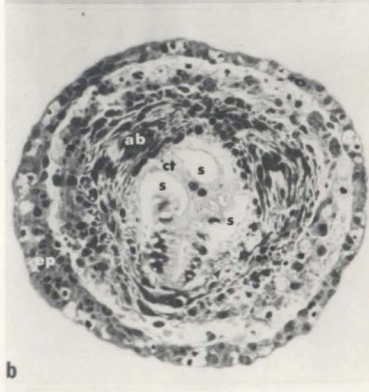


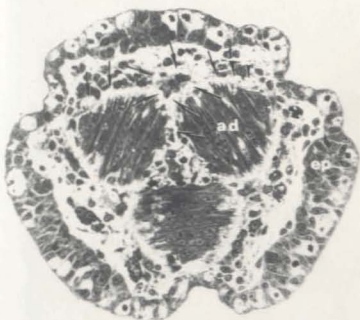
Figure 15 - Light micrographs of a globiferous pedicellaria (Stage IV) of Strongylocentrotus dröbachiensis.

a . . . . Cross section of the base of the jaws at the level of the adductor muscles, showing the thickened epithelium found only on the outer side of each jaw. Note the areas of adductor muscle insertion on the skeletal valves, which are indicated by arrows; 500X.

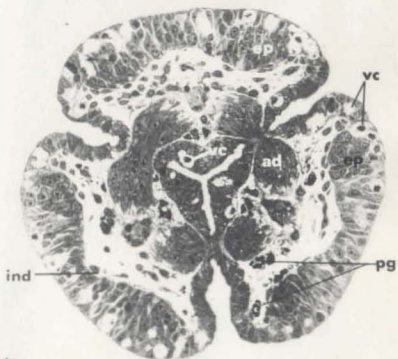
b . . . . Cross section at the level where the three jaws begin to separate; packets of granules can be seen in the dermis; 500X.

abbreviations, in alphabetical order:

ad - adductor muscle  
ep - epithelium  
ind - indentation  
pg - packet of granules  
vc - vacuolated cell



a



b

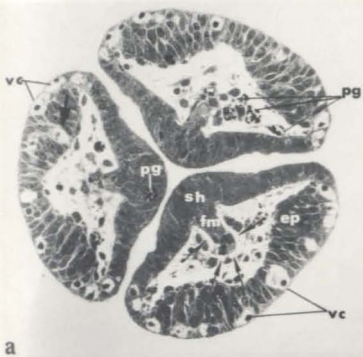
Figure 16 - Light micrographs of a globiferous  
pedicellaria (Stage IV) of  
Strongylocentrotus dröbachiensis.

a ..... Cross section of the three separated  
jaws showing the sensory hillocks, packets of  
granules and a vacuolated cell (indicated by large  
black arrow). Note the bi-lobed thickening of the  
epithelium, the skeletal valve (indicated by  
smaller arrows) and the nerve foramen; 500X.

b ..... Cross section of a distal region of  
the pedicellaria; note that the valves are open on  
their inner sides and are surrounded by relatively  
large numbers of calcicytes, indicating skeletal  
growth; 500X.

abbreviations, in alphabetical order:

ep - epithelium  
fm - nerve foramen  
pg - packet of granules  
s - skeleton  
sc - skeletal cells (calcicytes)  
sh - sensory hillock  
vc - vacuolated cell



b

the dermis. There has been little increase in cross sectional area since the previous stage. However, comparison of histological features shows a marked re-organization of tissues. The adductor muscles have increased in size by the differentiation of more cells, and by the formation of numerous fibrils. The cells occupying the dermis have become less compact, and there is a relatively large space immediately inside the thickened epithelium of the outer surface. Distal to this, at the level where the three jaws begin to separate in the center of the pedicellaria (Figure 15B), the epithelium is observed to be thickest on either side in the outer surface of each jaw. This results in a bi-lobed thickening, with an indentation in the layer at the center, directly outside the keel of the skeletal valve. A cluster of cells is thus formed on either side. However, the jaw does not take on this shape externally, indicating that the layer of cells grows inward. To accommodate this there must be an increase in the area enclosed by the epithelium to make sufficient space for these cells inside the jaw. This is suggested as the reason for the space between the epithelial layer and the skeleton and adductor muscles, for the loose arrangement of the cells occupying the dermis, as well as for the decrease in thickness of the epithelium of the



inner sides of the jaws. These developing venom glands give the head region a rounded appearance which is most pronounced in the mature pedicellaria, see Figures 2d, 2e and 2f.

The cells of the epithelial layer are similar to those described in a previous section (page 32). The vacuolated cells are usually found in the outermost region of the epithelium of the outer sides of the jaws. They are occasionally found in the epithelium of the inner sides of the jaws, especially in the region of the developing sensory hillock (Figure 15b). Like the vacuolated cells, the packets of granules are occasionally found in the dermis (Figures 15b and 16a). Vacuolated cells have also been found in the thickened lobes of the epithelium, (indicated by arrow, Figure 16a), suggesting that as yet the tissue has not differentiated into a true glandular area, but is merely a cluster of cells which allows wandering cells to move through.

Figure 16a is a cross section of the pedicellaria through the developing sensory hillocks. The nerve foramens of the skeletal valves can also be seen. The center of each valve is filled with a material that is probably nervous tissue. It appears to run from the sensory hillock to enter the valve through the foramen.

Figure 17 shows a cross section of the keel of the skeletal valve at the level of the nerve foramen. The developing nerve found inside the keel is also shown in cross section. Cobb (1968a) described the innervation of the muscles of the tridentate pedicellariae of *E. esculentus* at the ultrastructural level. He found the mass of nerve fibers that fills the cavity of the basal regions of the skeletal valves to contain between one and two thousand axons, with occasional cell bodies. The axons varied in size between 0.1  $\mu\text{m}$  and 2  $\mu\text{m}$  in diameter. Apart from the larger, less dense areas, the developing fibers shown in Figures 17 and 18a vary in size between 0.8  $\mu\text{m}$  and 6  $\mu\text{m}$  in diameter. The smaller processes are similar in size and shape to those shown by Cobb (1968a), however, those shown here are more electron dense. Also, no large areas containing scattered amounts of cytoplasm, (as shown in Figure 18a), were demonstrated in the previous study. It is suggested that these are areas of cytoplasm that have not yet differentiated. In later developmental stages, the axons have been observed to be separated by larger spaces, making them less closely packed together (Figure 18b).

Cross sections through the pedicellaria near the tip show that the bi-lobed condition of the epithelial

Figure 17 - Electron micrograph of a developing globiferous pedicellaria of Strongylocentrotus drobachiensis, showing the skeletal valve enclosing the developing nerve.

Note the position of the nerve foramen, and the adductor muscles which insert on the skeleton; 6000X.

abbreviations, in alphabetical order:

ad - adductor muscle  
fm - nerve foramen  
g - granule (lipid?)  
n - nucleus  
nt - nervous tissue  
s - skeleton

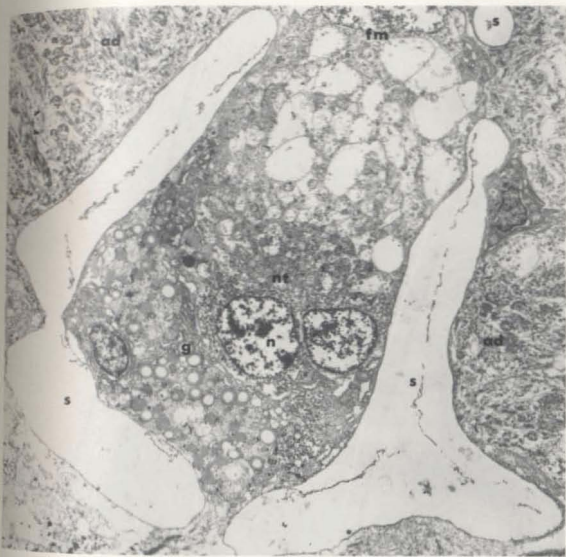


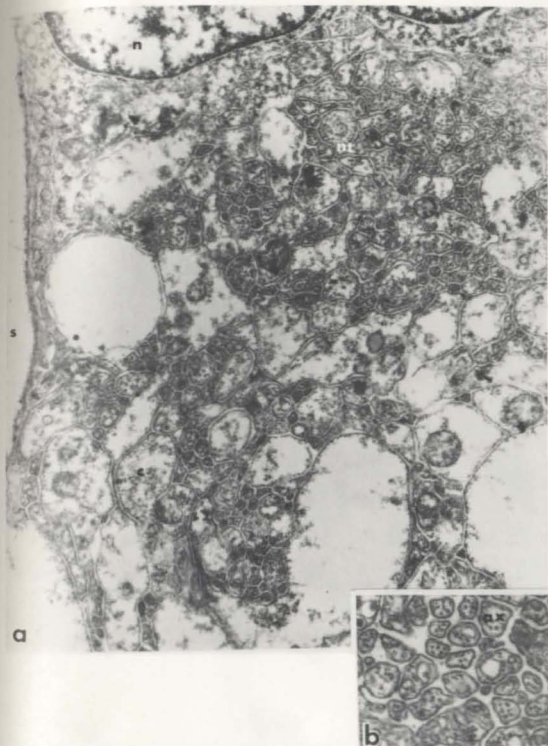
Figure 18 - Electron micrographs of a developing globiferous pedicellaria of Strongylocentrotus dröbachiensis showing developing nervous tissue.

a ..... Early developmental stage of the differentiating nerve; note the presence of cytoplasmic areas and the closely packed axons; 24,600X.

b ..... Later developmental stage showing axons separated by larger spaces; 24,600X.

abbreviations, in alphabetical order:

ax - axon  
c - cytoplasm  
n - nucleus  
nt - nervous tissue  
s - skeleton



layer of the outer surface becomes reduced distally (Figure 16b). No duct is yet formed.

The calcicytes, which are presumably depositing the calcite, are still found to be associated with the skeleton, although now in fewer numbers. This is because the skeleton is already well developed in comparison with the other components of the pedicellaria. Growth is still taking place, but at a much slower rate, relative to the other components. The main change is reflected in the filling in of pores in certain areas, and the calcite becoming more compact. The organic matrix remains, even in the mature condition, presumably to maintain the calcite in good condition.

At this time the necessary areas for muscle attachment have formed. The adductor muscles are well developed and are attached to the skeleton (Figures 15a and 15b). The abductors have also appeared, and are attached to the bases of the valves (Figure 14a).

The process of differentiation of mesenchyme cells into muscle fibers is similar to that already described for the adductor muscles. At the same time other cells of the neck form longitudinal fibers, which run from the bases of the skeletal valves to the tip of

the skeletal rod (Figure 14a). Inside these are fibers running circularly. Together they form the flexor muscles which allow the head to bend and extend towards a source of stimulation. No pedicellaria, earlier than, or at this stage in its development, has been observed to move in this study. This is true of the jaws, and of the appendage as a whole, which in the mature condition is equipped with a moveable stalk.

Stage V. Important changes occur internally in this stage of development. The venom glands become a tissue distinct from the epithelium, as shown in Figure 19b. At its proximal end the developing gland is seen as a single, compact group of cells (Figure 19a), somewhat thicker than the corresponding area in the previous stage. Although it has a distinct boundary on its inner side, it is still merged with the covering epithelium on the outer side (Figure 19a). It also contains vacuolated cells which are presumably making their way to the outermost cell layer. The cells seen at the inner edge of this tissue appear to be somewhat elongated. These cells are differentiating into the muscles which surround the venom gland in the mature pedicellaria. The muscle cells are probably formed from the loosely arranged cells which occupy the dermis.



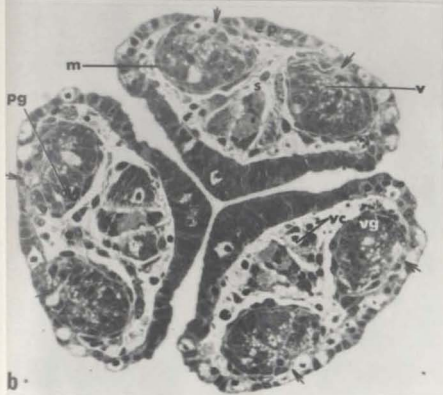
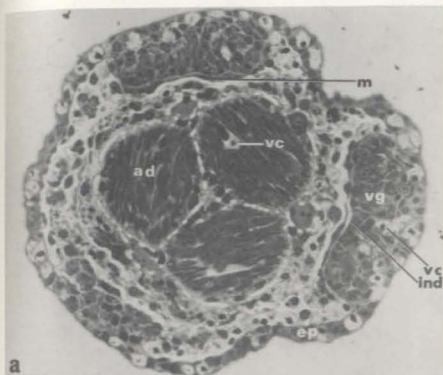
Figure 19 - Light micrographs of a globiferous pedicellaria (Stage V) of Strongylocentrotus dröbachiensis.

a . . . . Cross section through the base of the head region, showing the developing venom glands. Note the muscle cells outside the inner boundary of the glands, and the slight indentation in the glands. Vacuolated cells can be seen among the adductor muscle fibers, in the dermis, in the developing glands, and in the epithelium; 500X.

b . . . . Cross section showing the bifurcation of the venom gland of each jaw. Muscle cells surround the glands, except for occasional areas where the glands merge with the epithelium (arrows). Note the clear vacuoles in the cells comprising the venom glands, as well as a packet of granules; 500X.

abbreviations, in alphabetical order:

ep - epithelium  
ind - indentation  
m - muscle  
pg - packet of granules  
s - skeleton  
v - vacuole  
vc - vacuolated cell  
vg - venom gland



The bi-lobed condition at the base is seen as a slight indentation in the inner boundary of the developing gland (Figure 19a). The muscle cells follow this path also. At the level where the three jaws separate, the bi-lobed condition of the developing gland is complete, (Figure 19b), showing a rounded cluster of cells inside the epithelium on either side of the jaw. These are completely enclosed by muscle cells, except for occasional areas where the cells merge with those of the epithelium, (indicated by arrows in Figure 19b). The muscle cells gradually encircle the cell clusters, completely cutting them off from the covering epithelium, which is now clearly the single layer of cells typical of the mature appendage. Complete separation of the glands from the epithelium is first found approximately half-way up the length of the jaw. Distally, the cells merge again with the outer epithelium, eventually reaching the condition of the previous stage of development, where the glands are represented only by thickenings of the epithelial layer (Figure 20a). No muscle cells are formed here at this stage.

The histology of the glandular areas of the jaws has changed considerably since the last stage. Several tiny vacuoles are observed in the basal areas, and these

are larger and more numerous in further developed areas, where the glands have separated from the epithelial layer (Figure 19b). The "secretory" vacuoles decrease in number toward the tip where the glands are less well developed. However, the presence of these vacuoles in this specimen indicates that some of the cells of the venom glands are already playing a secretory role, and the cells have begun to take their characteristic position, that of a layer of secretory epithelium inside the muscular wall of the venom sac.

Stage VI. . . . In this stage glandular material is found at the level of the bases of the skeletal valves, as shown in Figure 20b, or even proximal to this, and the bi-lobed condition is complete just below the level at which the inner surfaces of the jaws separate (Figure 21a). The space between the two lobes of each gland increases distally until they are separated by the width of the keel of the skeleton, which due to its curvature and position in the jaws, comes to lie directly between the two lobes of the venom sac (Figure 21b). In basal areas the skeletal valve remains just inside the venom sac, although the space separating the two has decreased from the previous stage until the two are touching. The venom glands decrease in size distally

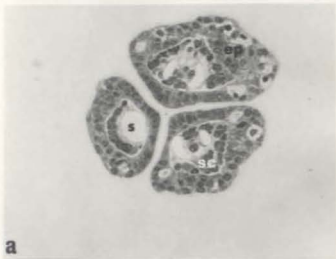
Figure 20 - Light micrographs of developing globiferous pedicellariae of Strongylocentrotus dröbachiensis.

a . . . . . Cross section through the distal region of a pedicellaria in Stage V, showing that the glands are not a distinct tissue at this level, but are represented by thickenings of the epithelium. Note that the bi-lobed condition is reduced; 500X.

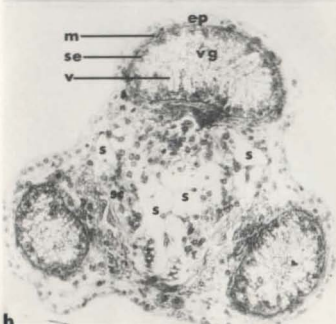
b . . . . . Cross section through the base of the head region of a pedicellaria in Stage VI, showing the bases of the skeletal valves, and the venom glands. A muscular wall has completely surrounded the venom glands so that it no longer merges with the epithelium. The nuclei and cytoplasm of the secretory cells are concentrated inside the muscular wall, while the remainder of the gland is filled with apparently empty vacuoles; 320X.

abbreviations, in alphabetical order:

ep - epithelium  
m - muscular wall  
s - skeleton  
sc - skeletal cells (calcicytes)  
se - secretory epithelium  
v - vacuoles  
vg - venom gland



**a**



**b**

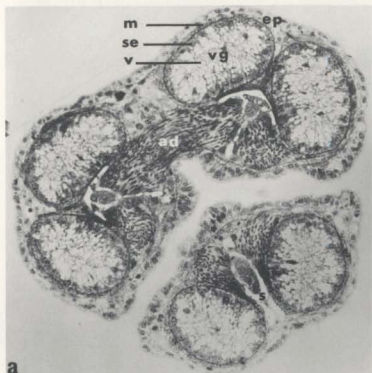
Figure 21 - Light micrographs of a globiferous  
pedicellariae\* (Stage VI) of  
Strongylocentrotus dröbachiensis.

a ...). A cross section showing that the  
venom glands have bifurcated at the level of the  
adductor muscles; 320X.

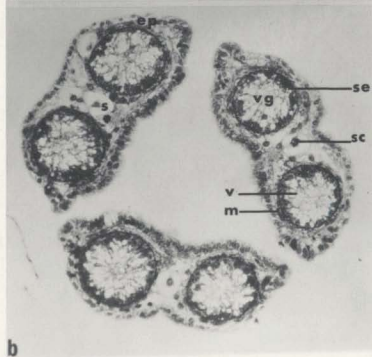
b ..... A cross section through the separated  
jaws, showing that the skeletal valve comes to lie  
between the two lobes of the venom gland. Note  
the tubular shape of the skeletal valves, and the  
enclosed cells; 320X.

abbreviations, in alphabetical order:

ad - adductor muscle  
ep - epithelium  
m - muscular wall  
s - skeleton  
sc - skeletal cells (calcocytes)  
v - vacuoles  
vg - venom gland



a



b



until they unite to form a common tube, or duct, running over the curved tip of the valve which forms the terminal tooth.

Further change has taken place in the histology of the pedicellaria since the previous stage. The venom gland has completely separated from the outer epithelium. Inside the muscular wall of the sac, there is a layer of simple secretory epithelium, while empty vacuoles occupy the remainder of the sac. The venom has probably been washed out in the fixation procedure.

Figures 20b, 21a and 21b correspond closely with the histology of the mature appendage, as shown by Chia (1970a) in the globiferous pedicellariae of P. miliaris and by O'Connell (1974) in the globiferous pedicellariae of S. purpuratus. The major change that takes place in the further development of the pedicellaria is only an increase in size, especially in the length of the stalk. This is in agreement with the suggestion of O'Connell (1971) that maturation of gland cells is a rapid process which occurs after the basic structures of the head are well developed. This equips the pedicellaria to assume its defensive role.

### Ultrastructure of the Developing Venom Glands

In electron micrographs, the early stages of the venom gland development are seen as rounded clusters of cells in the jaws. In Figure 22 the muscular wall has not yet formed, although there is some indication that the cells along the inner edge of the gland are beginning to elongate. Presumably, they differentiate into muscle cells. An intercellular space is shown to separate the outer side of the developing gland from the epithelium. Laterally the gland has not yet separated from the epithelial layer.

The intercellular space, separating the outer side of the developing gland from the epithelium, appears to protrude into the cluster of cells. Some amorphous material, along with some electron dense granules, are found in this space (Figure 22).

Large electron lucent vacuoles form in the cells of the developing gland, as can be seen in Figure 23. While some appear to be empty, others contain small amounts of granular or fibrillar material, similar to that shown by O'Connell (1971, 1974) in the vacuoles of the venom gland of mature globiferous pedicellariae of S. purpuratus. He found the cells to possess an extensive system of vacuoles dominating the apical nine-tenths of each cell.

Figure 22 - Electron micrograph of the venom gland from a globiferous pedicellaria (Stage V) of Strongylocentrotus dröbachiensis.

The cells along the inner edge of the gland are apparently differentiating into muscle cells. Note the area where the gland merges with the epithelium. Several vacuoles have appeared in the glandular cells; #200X.

abbreviations, in alphabetical order:

ep - epithelium  
gc - glandular cell  
ics - intercellular space  
s - skeleton  
v - vacuole  
vc - vacuolated cell

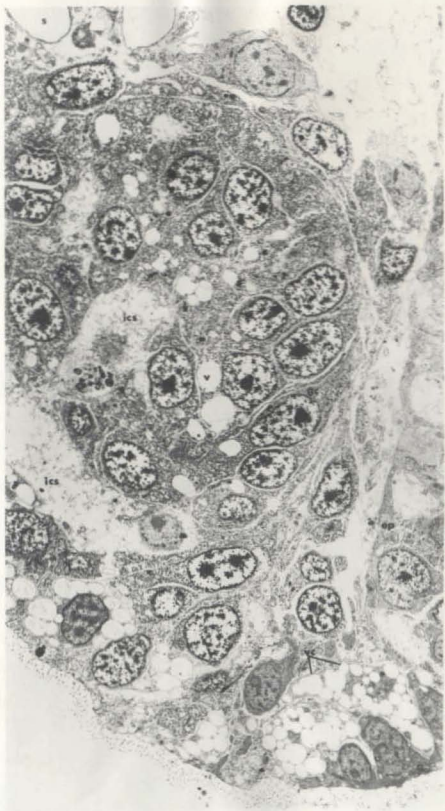
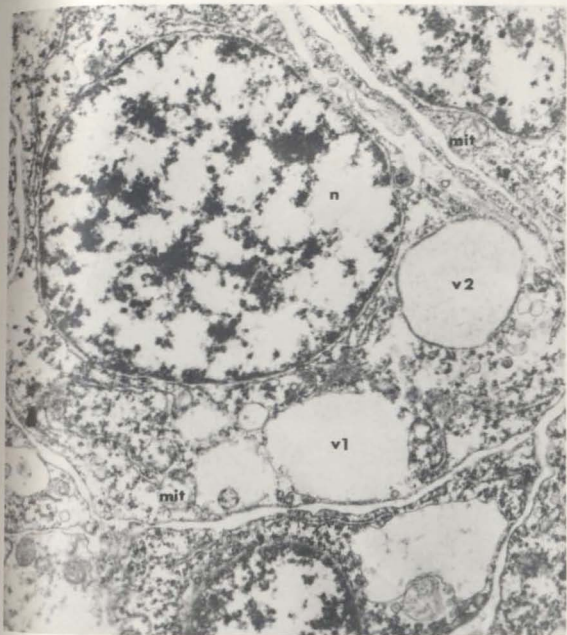


Figure 23 - Electron micrograph of the glandular cells of a globiferous pedicellaria (Stage V) of Strongylocentrotus drbbachiensis.

Some vacuoles (v1) appear to be empty, while others contain small amounts of granular, or fibrillar material (v2); 24,600X.

abbreviations, in alphabetical order:

mit - mitochondrion  
n - nucleus  
v1 - vacuole  
v2 - vacuole



the nuclei and the cytoplasm being basally displaced. The vacuoles from all the secretory cells were found to fill the center of the venom sac. This was found to be the case in Stage VI, (Figures 20b, 21a and 21b).

The striking increase in the degree of vacuolation of the venom glands, from Stage V to Stage VI, is in agreement with O'Connell's (1971) suggestion that, vacuolation and toxin biosynthesis occur rapidly, after the basic supportive jaw components have developed. He found no correlation between the size of the appendage, and the developmental state of the venom gland cells. Although the size range of the appendages was not given, animals ranging from 3 mm to 47 mm in test diameter were used. No developing pedicellariae were studied.

Further detailed studies on developing venom glands, using electron microscopic, histochemical or autoradiographic techniques may prove useful in determining the activities of the glandular cells. Numerous membrane systems were described by O'Connell (1971), indicating much synthetic activity. However, he stated that some functional relationships between membrane complexes and vacuolar networks remain obscure.

The cells associated with the developing skeleton are shown in electron micrographs (Figure 24) to contain large nuclei, surrounded by cytoplasm from which projections, or pseudopodia extend. The micrographs in this study give no definite evidence that pseudopodia from different cells fuse to form a syncytium, as suggested by several researchers working on echinoderm skeleton formation, such as Theel (1892), Prenant (1926), von Ubsch (1937), Bevelander and Nakahara (1960), Okazaki (1960), Nichols and Currey (1968), Gibbins et al (1969), Plikington (1969), and Kniprath (1974). Figures 25 and 26 show that at least some of the pseudopodia are continuous with thin areas of cytoplasm surrounding, or near the skeleton. These electron micrographs reveal the presence of large electron dense granules in the cytoplasm, as well as vacuoles, vesicles, Golgi bodies, polyribosomes, and an abundance of mitochondria. The pseudopodia contain microtubules oriented parallel to their longitudinal axis. They can be seen running from the main body of the cell to cytoplasmic areas close to the skeleton (Figure 26).

#### Skeleton and Associated Cells

#### Ultrastructural and X-ray Microprobe Studies of the



Figure 24 - Electron micrograph of a developing globiferous pedicellaria of Strongylocentrotus dröbachiensis, showing the skeletal areas and associated calcicytes.

Note the projections, or pseudopodia, of the calcicytes, and the cytoplasm surrounding the skeletal areas; 6000X.

abbreviations, in alphabetical order:

c - cytoplasm  
ct - connective tissue  
g - granule  
ics - intercellular space  
mit - mitochondria  
n - nucleus  
s - skeleton  
sc - skeletal cells (calcicytes)

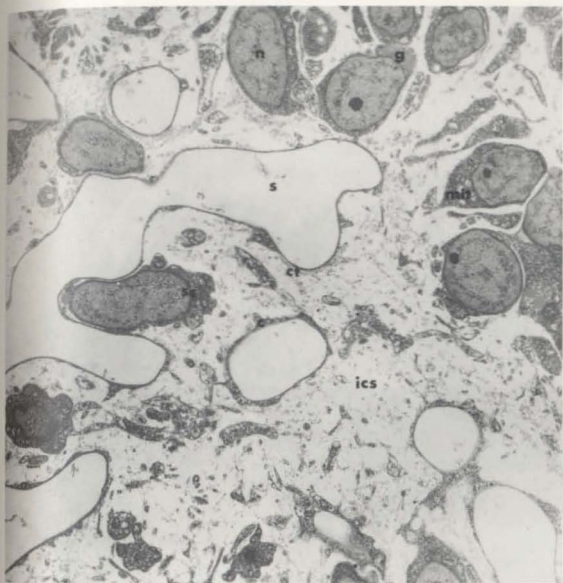


Figure 25 - Electron micrograph of a developing globiferous pedicellaria of Strongylocentrotus dröbachiensis showing skeletal areas surrounded by cytoplasmic extensions, or pseudopodia, of the calcicytes; 24,600X.

abbreviations, in alphabetical order:

ct - connective tissue  
G1 - Golgi body  
ics - intercellular space  
mit - mitochondrion  
s - skeleton

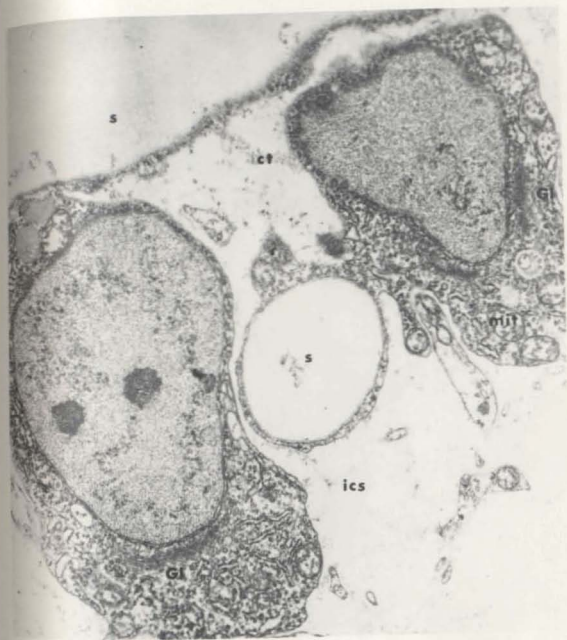


Figure 26 - Electron micrograph of a developing  
globiferous pedicellaria of  
Strongylocentrotus dröbachiensis.

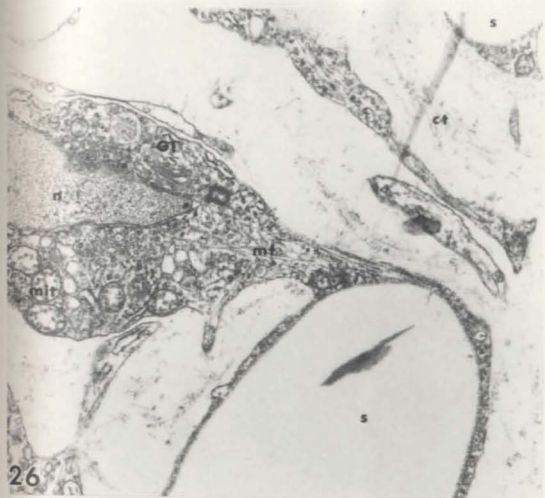
Note the microtubules extending from the  
main body of the cell to the thin cytoplasmic area  
associated with the skeleton; 24,600X.

abbreviations, in alphabetical order:

c - cytoplasm  
ct - connective tissue  
G1 - Golgi body  
mit - mitochondrion  
mt - microtubules  
n - nucleus  
s - skeleton

Figure 27 - Electron micrographs showing stages of morphological change of mitochondria from calcicytes of developing globiferous pedicellariae of Strongylocentrotus dröbachiensis.

- a ..... Typical mitochondrion; 24,600X.
- b ..... Mitochondrion showing loss of some matrix material; 24,600X.
- c ..... Mitochondrion showing one of the boundary membranes being broken down; 24,600X.
- d ..... Mitochondrion containing remnants of cristae; 24,600X.
- e ..... Mitochondrion containing remnants of cristae; 24,600X.
- f ..... Vesicles, or mitochondria that have lost cristae; 24,600X.



The pseudopodia are usually filled with mitochondria, many of which appear to be in various stages of change in morphology (Figure 27). Typical mitochondrial structures showing the two parallel boundary membranes can be seen; such mitochondria contain a number of tubular cristae, and a moderately electron-dense matrix. The first recognizable change is demonstrated by the loss of matrix material, although the boundary membranes and cristae remain intact. The next morphological change is the gradual breakdown of the cristae, and one of the boundary membranes, most likely the inner one. In later stages of modification they can be identified as mitochondria by their size, shape, and the presence of a few remnants of cristae. Membrane bound vesicles of similar size and shape containing only traces of less electron dense material are common in the cytoplasm of the calcicytes. These are found in association with the mitochondria, and because of their comparable size and shape, it is suggested that these vesicles are modified or transformed mitochondria.

Electron micrographs through the developing skeleton often show sections through pseudopodia which are filled with clusters of mitochondria (Figure 28a). They have also been found in the thin cytoplasmic strand in close



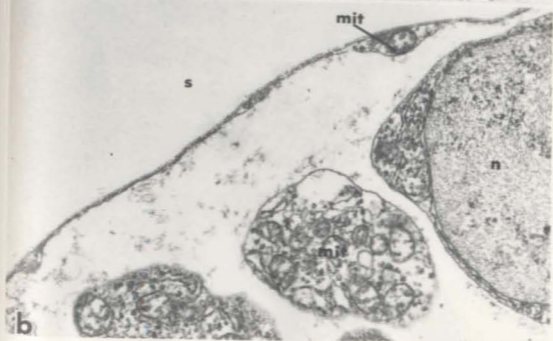
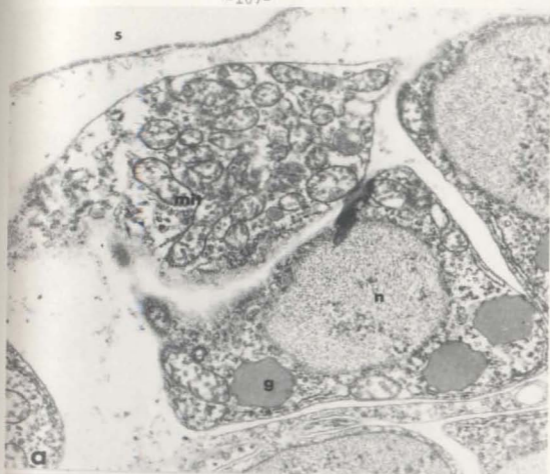
Figure 28 - Electron micrographs of a developing globiferous pedicellaria of Strongylocentrotus dröbathiensis, showing clusters of mitochondria near skeletal areas.

a . . . . . Note the large cytoplasmic area containing numerous mitochondria; 24,600X.

b . . . . . Note the cluster of mitochondria, and the presence of a mitochondrion in the cytoplasm surrounding the skeleton; 24,600X.

abbreviations, in alphabetical order:

g - granule  
mit - mitochondria  
n - nucleus  
s - skeleton



proximity of the skeleton (Figure 28b).

In histological sections and electron micrographs of pedicellariae that were decalcified by treatment with EDTA, the skeleton is represented by epon-infiltrated spaces which are apparently empty, except for remnants of scattered amorphous material (possibly organic), as shown in Figures 24, 25, 26, 28a and 28b. In micrographs of non-decalcified tissue, the skeleton is represented by holes which are comparable in size, shape, and position to the epon-infiltrated spaces of decalcified tissue (Figure 30b). Both conform closely with observations made on the morphology of the isolated skeleton, to be described in a later section. Apparently the calcite in tissues that had not been treated with EDTA falls out during ultrathin sectioning, leaving empty spaces. A similar explanation was made by Gibbons et al (1969). There is no evidence that EDTA treatment after fixation in aldehyde alters greatly the arrangement or histology of the soft tissues.

In micrographs of tissues prepared in fixative with pH 6.8-6.9, the skeleton is represented by large holes surrounded by narrow epon-infiltrated areas (Figure 29). Dense granules representing skeletal fragments can be

Figure 29 - Electron micrograph of a developing globiferous pedicellaria of Strongylocentrotus dröbachiensis, showing skeletal areas that had been partially decalcified during fixation.

The holes, h1, and h2, may represent two vertical struts that became joined by the formation of a horizontal bridge. The newer calcite possibly dissolves in slightly acidic conditions more easily; 6000X.

abbreviations, in alphabetical order:

h1 - hole  
h2 - hole  
g - granule  
s - skeleton

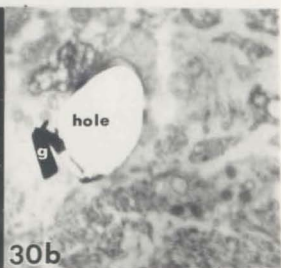
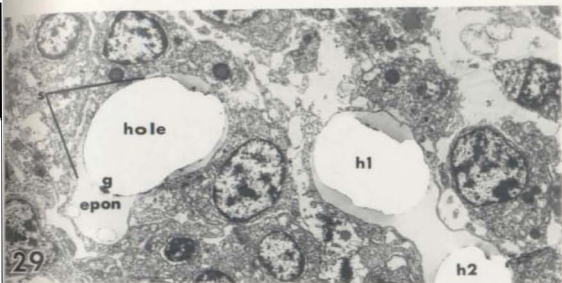
Figure 30. - A calcium K $\alpha$  X-ray image of an area of a developing globiferous pedicellaria of Strongylocentrotus dröbachiensis.

a ..... X-ray map of a skeletal area and the surrounding cells;

b ..... A transmitted electron image of the same area; note the hole representing the skeleton, and the large dark granule that is most likely a fragment of the calcite that was left behind after fracturing.

abbreviations, in alphabetical order:

g - granule



seen along the edges of the holes. Apparently part of the skeleton is dissolved out by slightly acidic conditions, resulting in an epon infiltrated area with the surrounding cells, membranes, and organic material undisturbed. When the pH is kept above 7.0, the skeleton remains in the tissue, but falls out during sectioning.

Gibbons et al (1969), suggested that mineralization in embryos of A. punctulata begins centrally in a membrane bound space. They interpreted holes which were surrounded by embedding material as solid calcite which had fallen out of the section. However Kniprath (1974), "studying the tooth of P. lividus", believed mineralization to begin on the inner coat of the skeletal sheath, with growth of the crystal progressing centripetally.

From the micrographs in the present study it could not be determined if calcification begins centrally, or directly on the surrounding membrane. It is possible that the epon-infiltrated areas in partially decalcified tissues represent new calcite that is added at the periphery, but is less resistant to decalcifying agents. In Figure 29, two holes (h1 and h2), representing areas of solid calcite, are separated by an area of embedding material, representing dissolved, and possibly newer

calcite. This is possibly due to the formation of a bridge between the two vertical struts. This is a common event in the development of the skeleton, as seen in scanning electron microscope studies.

The readings obtained when 130 nm sections of pedicellariae were microanalysed for calcium, using EMMA-4, are given in Tables 1, 2 and 3. The data readings were taken over the  $\text{CaCO}_3$  standard, the cytoplasm of epithelial cells, the cytoplasm and various organelles of the cells near skeletal areas, the holes believed to represent the location of the skeleton, dense objects near the holes, and areas of the sections containing only resin.

Any area giving a peak to background ratio (p/b) of 1.5 to 2.0 or over was considered to contain significant amounts of calcium. The epithelial cells gave negligible readings, while significant amounts of calcium were found in all areas of the cells in close proximity of the holes representing the skeleton. Although the sections were 130 nm thick and unstained, the fine structure of the cells could be interpreted reasonably well when compared with ultrathin sections. A p/b ratio of 2.76 was obtained from mitochondria near areas of skeletal deposition. A relatively high p/b ratio



TABLE 1

X-ray microanalysis of calcium in developing globiferous  
pedicellariae of Strongylocentrotus drobachiensis  
using EMMA-4. Run 1.

Area	Peak (p)	Background (b)	p/b	$\frac{p-b}{w^*} \times 100$
cytoplasm of epithelial cells	19 ± 3	18 ± 4	0.7	0.006 ± 0.007
cytoplasm of cells near holes representing skeleton	35 ± 12	9 ± 1	2.3	0.073 ± 0.039
CaCO <sub>3</sub> standard	149	15	9.0	0.948

\*The white count (w) is measured by a non-dispersive detector, and is used as a measure of mass. The values for  $\frac{p-b}{w} \times 100$  have been corrected for section thickness.

TABLE II

X-ray microanalysis of calcium in developing globiferous  
pedicellariae of Strongylocentrotus dröbachiensis

using EMMA-4. Run 2.

Area	Peak (p)	Background (b)	p/b	$\frac{p-b}{w^*} \times 100$
vesicular cytoplasm near holes represent- ing skeleton	48 ± 11	20 ± 3	1.6	0.101 ± 0.043
non-vesicular cytoplasm near holes represent- ing skeleton	31 ± 9	15 ± 0	1.5	0.077 ± 0.044
dense object or granule near hole representing skeleton	15810	44	359.3	34.089
cytoplasm adjacent to dense objects or granule	674	16	42.1	2.426

Table II Continued

Area	Peak (p)	Background (b)	p/b	$\frac{p-b}{w} \times 100$
CaCO <sub>3</sub> standard	663	12	55.25	4.755
center of hole	8	8	1.0	0.000
resin only	16	18	0.89	0.000

\*The white count (w) is measured by a non-dispersive detector, and is used as a measure of mass. The values for  $\frac{p-b}{w} \times 100$  have been corrected for section thickness.

TABLE III

X-ray microanalysis of calcium in developing globiferous  
pedicellariae of Strongylocentrotus dröbachiensis  
using EMMA-4. Run 3.

Area	Peak (p)	Background (b)	p/b	$\frac{p-b}{w^*} \times 100$
dense objects or granules near holes represent- ing skeleton	35790 ± 32685	68 ± 37	29.6	72.707 ± 7.070
nuclei of cells near holes representing skeleton	84 ± 31	22 ± 5	1.96	0.298 ± 0.082
cytoplasm of cells near holes representing skeleton	53 ± 11	21 ± 7	1.50	0.174 ± 0.045
mitochondria near holes representing skeleton	87 ± 18	22 ± 3	2.76	0.309 ± 0.085

Table III Continued

Area	Peak (p)	Background (b)	p/b	$\frac{p-b}{w^*} \times 100$
vesicles near holes representing skeleton	80 ± 10	20 ± 3	3.04	0.300 ± 0.047
CaCO <sub>3</sub> standard	2567	29	88.5	20.532
hole	11	10	1.1	0.029
resin only	20 <sup>o</sup>	18	1.1	0.011

\*The white count (w) is measured by a non-dispersive detector, and is used as a measure of mass. The value for  $\frac{p-b}{w} \times 100$  has been corrected for section thickness.

(3.04) came from "vesicular" areas. These vesicles were comparable in size, shape, and location with the mitochondria, but their morphology in the sections microanalyzed was not clearly defined. They could possibly be mitochondria, or modified mitochondria, but were not recognizable, clearly beyond doubt as such.

Analysis of dense objects, or granules, near the holes believed to represent the skeleton gave readings comparable to that of the calcium standard ( $\text{CaCO}_3$ ) used. This is further evidence that the skeleton was fractured during sectioning. It is suggested that the dense objects are remnants of the skeleton still attached to the section. Readings taken over the resin only, and over the holes were negligible.

By relating the characteristic count for the element (p-b) to the white count (w), the differences in mass, or section thickness, are taken into account. This value ( $\frac{p-b}{w} \times 100$ ) was calculated for each region of the section analysed. Where more than one set of counts was made for the areas indicated in the tables, the average values and standard deviations were calculated.

The cytoplasm of the epithelial cells gave a considerably lower average reading than the cytoplasm of

the secretory cells. The average value ( $\frac{P-b}{w} \times 100$ ) for the epithelial cells was  $0.006 \pm 0.007$ . The average values for the cytoplasm of the secretory cells were  $0.073 \pm 0.039$ ,  $0.101 \pm 0.043$ ,  $0.077 \pm 0.044$ ,  $2.426$ , and  $0.174 \pm 0.045$ . Considerably higher values came from mitochondria ( $0.309 \pm 0.085$ ), vesicles ( $0.0300 \pm 0.047$ ), and nuclei ( $0.298 \pm 0.082$ ). The nucleus is known to be rich in divalent cations, such as  $Ca^{2+}$  (Alfrey, 1968). The dense objects, or granules, near the holes gave extremely high values ( $34.089$  and  $72.707 \pm 7.070$ ), indicating that they are fragments of the skeleton.

X-ray mapping showed a concentration of calcium over the dense objects, and little or none in the holes. Figure 30a is the X-ray map of the section shown in Figure 30b. A comparison of the two shows the high concentration of grains corresponds closely in shape, size and position with the dense object, or granule. The hole can be identified in the map as an area of similar size and shape, containing an extremely low number of grains.

The ultrastructural and X-ray microprobe studies indicate that the cells associated with the skeleton are involved in skeletogenesis. Pilkington (1969), studying the spines of *E. esculentus*, used the term sclerocyte (Gk. scleros, hard) to represent several morphol-

ogically distinct cells which are possibly different phases of a single cell type. He stated that some, or all, of the sclerocytes may be amoebocytes of the coelomic fluid. Heatfield and Travis (1975), studying the regenerating spines of *S. purpuratus*, adopted the term calcoblast to refer to a specific cell type, considered to be the active skeletogenic cell. This was based solely on the association of the cells with the regenerating microspines. Since the cells which permeate the spaces of the developing pedicellarial skeleton have been shown, in the present study, to contain significant amounts of calcium, the term calcocyte (L. calx, lime) has been proposed to refer to this skelētogenic cell type.

Large numbers of mitochondria, containing significant amounts of calcium, are often found in these cells. Mitochondria may function in the calcification process, as suggested by Lehninger (1974) and others.

From the micrographs of the present study, it could not be definitely determined, if the skeleton is intracellular, or extracellular. Heatfield and Travis (1975) found developing microspines to be extracellular. The calcoblasts were shown to extend a thin cytoplasmic sheath around the growing tip. However, unlike the condition found in the present study, the cell membrane was not



adjacent to the calcite surface, but separated from it by a relatively large, extracellular space; few mitochondria, and no microtubules were found in the sheaths. The microspines shown by Heatfield and Travis (1975) grow mainly in one direction, vertically, whereas the skeletal elements of the pedicellariae do not have this simple orientation. This may explain why the cytoplasmic processes are not simply oriented along the vertical axis in pedicellariae.

Heatfield and Travis (1975) described precalcioblasts, which were considered to be precursors of calcioblasts; the third cell type found in the dermis was the phagocyte. Neither of these was found in developing pedicellariae.

The second order spherical bodies of the sclerocytes, described in Pilkington (1969) as lying close to the calcite surface, were not found in the present study.

Early developmental stages of pedicellariae are ideal tissues for investigating the process of calcification. Autoradiographic techniques using  $\text{Ca}^{45}$  may give information on how calcium is incorporated in the cells, and where it becomes localized. The preliminary X-ray microprobe analysis of the present study may be followed up by the use of ultrathin "dry" frozen sections. The procedure would prevent the removal of soluble components that may

be lost in conventional fixation, dehydration, and embedding techniques. Histochemical techniques may also prove valuable in the study of calcification.

A Scanning Electron Microscope Study of the Calcite Skeleton

The ossicle in Figure 31a is a developmental stage of a skeletal valve from a jaw of a pedicellaria in the early part of Stage I. It is suggested that from the original granule, g, three branches (1, 2, 3) grow in the horizontal plane of the pedicellaria, and one branch, 4, grows vertically, leading to a three dimensional framework. A comparison with Figure 31b, which shows a valve from another jaw of the same pedicellaria, demonstrates that one ossicle may be at a slightly different stage of development from the other two. The arrows in Figure 31b indicate the possible directions of growth. Branches 1 and 2 are shown to have branched dichotomously, although no such change has occurred in branch 3. The valve in Figure 31c is from a pedicellaria in a slightly later part of Stage I than previous examples. Branch 3 is unchanged, although there has been much rebranching in the horizontal plane from branches 1 and 2. A comparison of left and right sides

Figure 31 - Light micrographs of skeletal valves from globiferous pedicellariae (Stage I) of Strongylocentrotus dröbachiensis.

a . . . . . An ossicle from a jaw of a pedicellariae in early Stage I. Branches 1, 2, 3 (in the horizontal plane of the pedicellaria) and Branch 4 (in the vertical plane) have apparently grown from the central area, or first formed granule; 500X.

b . . . . . An ossicle slightly further developed than that in Figure 1a, although they are from the same pedicellaria; arrows show possible directions of growth; 500X.

c . . . . . A further developed ossicle from another pedicellaria in Stage I. Branch 3 is unchanged; branch 1 has bifurcated to form branches 5 and 7, branch 2 has bifurcated to form branches 6 and 8. Newly formed branches in turn bifurcate; branch 7 forms branches 11 and 9; branch 8 forms branches 10 and 12. An incomplete pore, P1, is formed as branches 1, 7 and 9 curve towards branches 2, 8 and 10. Other pores are formed in a similar manner, as the basal layer of the valve continues to develop in the horizontal plane; 500X.

abbreviations, in alphabetical order:

g - original granule



shows a striking symmetry and the beginnings of a very elaborate structure. In the living appendage this valve would be oriented with branch 3 pointing away from the center of the pedicellaria and branch 4 extending toward the tip.

It is by the repeated bifurcation and joining of projections that the base of the developing skeletal valve is formed. In Figure 31c branches 1, 7 and 9 and branches 2, 8 and 10 can be seen to form an incomplete pore, P1. The branches from either side grow towards each other in a circle, until only a short distance separates their tips. They are shown to have fused in Figure 32a. All the other pores of the basal layer appear to be formed in a similar manner to that of pore P1. A comparison of Figure 31c with Figure 32a (late State I) shows that the location of the complete pores in Figure 32a corresponds with that of the incomplete pores of the less well developed valve in Figure 31c. The process of branching and fusing of projections results in the reticulate, or fenestrate structure of the calcite.

The developmental stages described above are in agreement with the work of Gordon (1926) on the ophiocephalous pedicellariae of the pluteus larvae of P. miliaris.

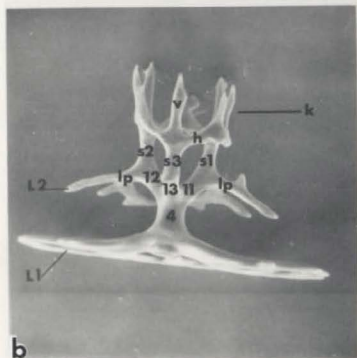
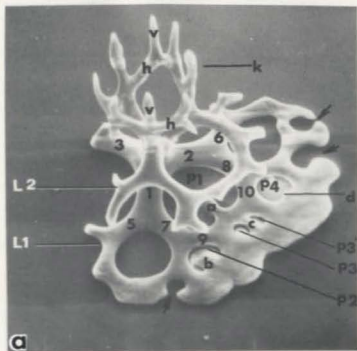
Figure 32 - Scanning electron micrographs of skeletal valves from a globiferous pedicellaria (Stage I) of Strongylocentrotus dröbachiensis.

a . . . . . A skeletal valve seen from its inner side, note that the location of the complete pores in the basal layer corresponds with that of the incomplete pores in Figure 31c. Projections a, b and d are apparently partitioning pores P1, P2 and P4, respectively, while projection c has divided a pore completely, forming P3 and P3'. Incomplete pores are indicated by arrows; 1775X.

b . . . . . A skeletal valve seen from the outer side, showing the growth which has taken place in the vertical plane from branch 4. Branches 11, 12 and 13 arise from branch 4. A lateral process, lp, is given off by both branch 11 and branch 12. These processes branch repeatedly to form a second horizontal layer. The vertical components s1, s2 and s3, which arise from branches 11, 12 and 13, extend for 5-7  $\mu$ m, at which point they send out horizontal processes, h, which fuse with those from adjacent struts. From the circle of calcite formed, eight or nine projections, v, grow vertically; 1700X.

abbreviations, in alphabetical order:

- |                     |                       |
|---------------------|-----------------------|
| a, b, d, c          | - projections         |
| h                   | - horizontal process  |
| k                   | - keel                |
| L1                  | - first layer (basal) |
| L2                  | - second layer        |
| lp                  | - lateral process     |
| P1, P2, P3, P3', P4 | - pores               |
| v                   | - vertical process    |



Although the globiferous pedicellariae, to which this study is restricted, possess a skeleton that is basically of a similar structure, it is modified at early and later developmental stages to perform a specialized function.

In Figure 32a, a projection, a, can be seen arising from the inner edge of pore P1. A similar projection, b, is seen in pore P2, while pore P4 has only a small bump, d, on its inner side. An area of calcite, c, separates two smaller pores, P3 and P3'. It appears that some of the pores become partitioned by the formation of projections which grow from one side of a pore to fuse with the opposite side. Such a process makes the calcite stronger and more compact, while still retaining the fenestrate structure.

Growth occurs at the periphery of the basal plate, and in Figure 32a three incomplete pores can be seen in areas where the projections have not yet fused, but are approaching each other (arrows). This happens on the lateral areas, and on the inner edge of the plate only. No new projections are formed along the outer edge from branches 3, 5 or 6. This area can easily be distinguished throughout all developmental stages, since it is from the area g that the main vertical branch 4, extends. A zone



of more compact calcite remains along the inner edge of the valve where newly forming pores are smaller than those found laterally.

In Figure 32b (late Stage I), the valve is seen from its outer side, showing the projections which have formed from the vertical branch 4. In addition to providing for elongation of the skeletal valve to correspond to the elongating jaw which it is to support, this vertical growth also allows the formation of a second layer of calcite, L2, in the horizontal plane, approximately 8-9  $\mu\text{m}$  distal to and parallel with the first formed, or basal layer, L1. After extending 5-6  $\mu\text{m}$  vertically, branch 4 gives rise to branches 11, 12 and 13 (Figure 32b). Both branch 11 and branch 12 give off a lateral process, 1p. Repeated branching in the horizontal plane results in the formation of a second layer in a similar manner to the development of the basal layer described above.

Growth continues in the vertical plane, as strut-like projections elongate, and increase in number by branching. Distal to the second horizontal layer, there are three vertical components, labelled as s1, s2 and s3 (Figure 32b). Two of these, s1 and s2 are the vertical branches formed by the bifurcation of branches 11 and 12, while s3 is a

continuation of branch 13. The struts, s1, s2 and s3 extend for approximately 5-7  $\mu\text{m}$ , at which point they branch. Each sends out a horizontal process, h, which grows in a slightly curved direction to fuse with a similar process from the adjacent vertical branch, (Figure 32b), thus forming a complete circle, see Figure 32a. As shown in Figures 32a, 32b and 33a, eight or nine strut-like projections, v, grow vertically from this circle. These vertical struts form the basis for the development of a hollow, tube-like structure, which becomes the central keel of the skeletal valve. This configuration is made more sturdy, and less open by the formation of horizontal bridges which connect adjacent struts as they elongate. One such developing bridge, or lateral process, is indicated on a strut in Figure 33a. This valve is from a pedicellaria in Stage II. In more advanced stages of development, where the struts forming the tubular keel have increased in length, both complete and incomplete horizontal bridges can be seen (Figure 34a).

A comparison of Figure 32a (Stage I) with Figure 33b (Stage II) shows that growth has also taken place in the horizontal plane in the basal layer of the valve. The width from the center of one side to the opposite edge has increased from approximately 50  $\mu\text{m}$  to 70  $\mu\text{m}$ , while

Figure 33 - Scanning electron micrographs of skeletal valves from a globiferous pedicellaria (Stage II) of Strongylocentrotus dröbachiensis.

a ..... Valve seen from outer side, showing an increase in the length of the strut-like projections forming the tubular keel. Note the developing lateral process that will eventually link adjacent struts. The projections indicated by arrows mark the beginning of the blade of the valve; 1360X.

b ..... Valve showing the basal layer. Newly forming pores are indicated by arrows. Note the change in size of the basal layer, and the increase in the number of pores since earlier stages; 825X.

abbreviations, in alphabetical order:

h	- horizontal process
k	- keel
L1	- first layer (basal)
L2	- second layer
lp	- lateral process
s1, s2, s3	- struts
v	- vertical process

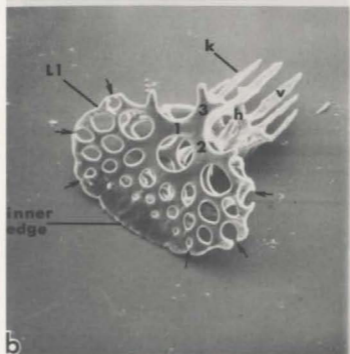
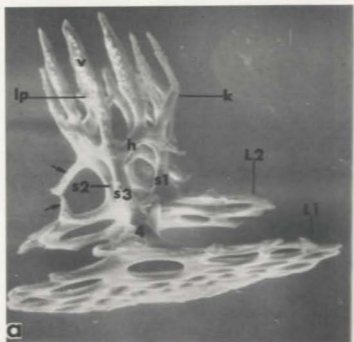


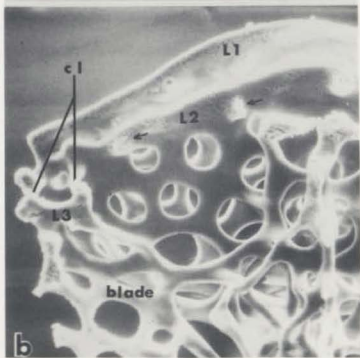
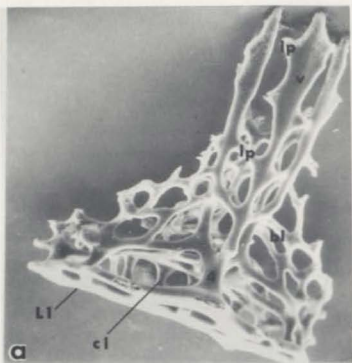
Figure 34 - Scanning electron micrographs of skeletal valves from a globiferous pedicellaria (Stage II or III) of Strongylocentrotus gröbachiensis.

a ..... Valve seen from the outer side, showing columns which have connected the first two basal layers. Note the complete and incomplete lateral processes on the struts of the keel, and the increase in the size of the blade; 1600X.

b ..... Inner side of the base of the valve, showing the formation laterally of a third horizontal layer. Two columns have formed from the second layer, and have become linked by horizontal projections. Note the smaller columns indicated by arrows; 1600X.

abbreviations, in alphabetical order:

- bl - blade
- cl - column
- L1 - First layer (basal)
- L2 - second layer
- L3 - third layer
- lp - lateral process
- v - vertical strut



the distance from the outer most point to the inner edge has increased from approximately 35  $\mu\text{m}$  to 45  $\mu\text{m}$ . The number of pores in the layer has more than tripled by the formation and joining of new projections at the periphery, and by the partitioning of some of the earliest formed pores. The area along the inner edge remains relatively compact, with only tiny pores. In Figure 33b several newly formed pores (indicated by arrows) can be seen along the inner edge where the projections have not completely fused. Unlike those which are seen on the periphery in lateral areas, and in the earliest stages of development, the projections on the inner edge are not long and slender, but are short and thick, and often are recognized only by slight indentations in the periphery of the calcite plate. According to Raup (1966) pores are coarser in zones of more rapid calcite deposition. By this process the calcite is made stronger and more compact, as the surface area is increased. The outer edge of the basal plate, between branches 5 and 6, shows no change in shape, and little change in length, if any.

Similarly, there has been an increase in the surface area of the second horizontal layer that is distal to and parallel with the basal layer described above, however the second layer remains slightly smaller than the basal layer.

Figure 33a shows projections that are growing distally from the second horizontal layer to fuse with other projections from the central keel which are growing toward the base. This is the beginning of the blade of the valve. Much further branching and joining of projections results in a complex meshwork, with new calcite being added at the periphery as the pedicellaria continues to develop.

In Figure 34a (Stage II-III), lateral processes have completely linked adjacent struts of the central keel, while others are incomplete. As the struts elongate, other bridges or lateral processes form at intervals along the outer side of the keel.

It can be clearly seen in Figure 34a that columns have developed, connecting the first two horizontal layers of the base. These columns later become areas for attachment of muscles.

The beginning of a third horizontal layer can be seen on lateral areas of the valve, shown in Figure 34b (Stage III). Columns grow distally from the second horizontal layer and apparently send out horizontal projections which fuse with those from adjacent columns. The small projections, indicated by arrows, in Figure



34b, gradually develop into columns, as shown in Figures 35a and 35b (early Stage IV). A third basal layer is thus formed by the linking of adjacent columns from the upper side of the second horizontal layer. Projections also arise from the blade to fuse with the basal layers, thus strengthening the base of the valve.

In Figure 35b a column, c1, has grown from the third layer. From the tip of the column, horizontal branches form in a similar manner to that encountered in the formation of the third basal layer described above. However, the process in this case remains confined to the lateral areas. It is in this manner that the lateral cog-teeth by which the valves articulate with each other are formed.

In the meantime the central teeth are also developing, in a similar manner to that described above, except for the fact that each succeeding horizontal layer is narrower (Figures 35b, 36b (Stage V) and 37a (Stage VI)). They gradually taper to the thickness of the median wall above the keel (Figure 37a).

Figure 35a shows that the median wall has formed from the inner side of the central keel. It is evident that the outer side of the keel is lengthening at a faster rate than the inner side. Although the keel is a complete hollow tube at its base, the distal end remains open on

Figure 35 - Scanning electron micrographs of  
a skeletal valve from a  
globiferous pedicellaria (Stage  
IV) of Strongylocentrotus  
dröbachiensis.

a ..... Inner side of the valve, showing  
the columns which have formed from the second  
horizontal layer, and the horizontal processes  
which are linking them to form a third layer.  
The central keel has elongated but remains open  
distally; 430X.

b ..... A column, c1, has developed from  
the third horizontal layer as the lateral cog-  
teeth develop. The developing central teeth  
also shown; 875X.

abbreviations, in alphabetical order:

bl - blade  
cl - column  
ct - central cog-teeth  
h - horizontal process  
k - keel  
L1 - first layer (basal)  
L2 - second layer  
L3 - third layer  
lt - lateral cog-teeth  
w - median wall

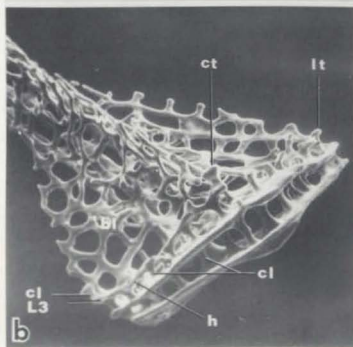
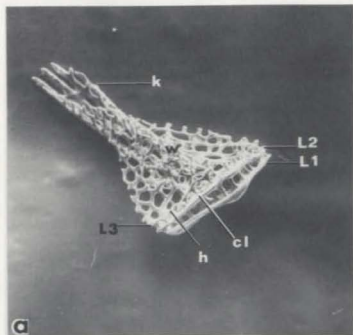


Figure 36 -- Scanning electron micrographs of skeletal valves from glóbfiferous pedicellariae (Stage V) of Strongylocentrotus dróbbachiensis.

a ..... The outer side of a skeletal valve; note that the basal layer and the distal half of the keel has become less porous. To allow articulation with the other two valves, both laterally and centrally, the inner edge of the basal layer has become pointed; 420X.

b ..... The inner side of a valve, showing the nerve foramen and tubular keel; 175X.

abbreviations, in alphabetical order:

ad - adductor muscle insertion  
bl - blade  
ct - central cog-teeth  
fm - foramen  
k - keel  
L1 - first layer (basal)  
lt - lateral cog-teeth

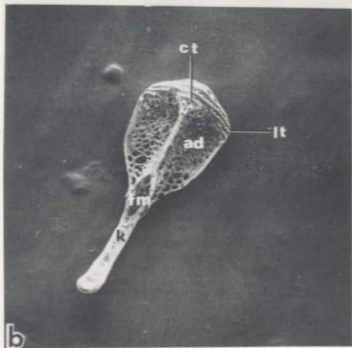
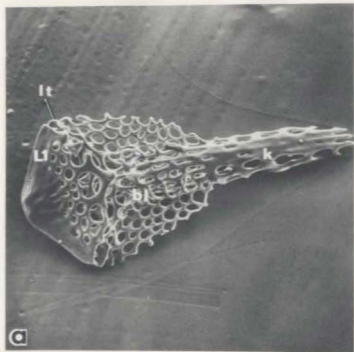


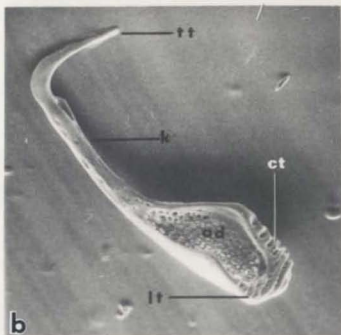
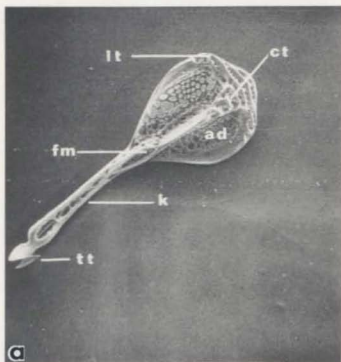
Figure 37 - Scanning electron micrographs of skeletal valves from the globiferous pedicellariae (Stage VI) of Strongylocentrotus dröbachiensis.

a ..... Inner side of the valve, showing a well formed valve, with lateral cog-teeth, central cog-teeth, and areas for muscle insertion. The distal end of the valve has become modified into a grooved tooth for piercing; 195X.

b ..... Lateral view of the valve, showing the terminal tooth for piercing, and the articulation areas of the base. Note that the calcite has become less porous than in earlier stages; 170X.

abbreviations, in alphabetical order:

ad - adductor muscle insertion  
ct - central cog-teeth  
fm - nerve foramen  
k - keel  
lt - lateral cog-teeth  
tt - terminal tooth



the inner side, until later stages of development. In this way preparation is made for the middle foramen, which receives the valve nerve from receptor areas on the inner side of the jaw.

Changes have also taken place in the outer side of the valve. A comparison of Figure 34a with Figure 36a (Stage V) shows a widening of the blade and an elongation of the keel, which also becomes less porous. The porous area of the basal layer is becoming relatively smaller, as those pores toward the inner edge apparently become filled in by calcite deposition, often leaving slight indentations (Figure 36a). The inner edges of the basal layers have become pointed, to allow the three valves to articulate with each other, both centrally and laterally.

In Figure 35a, the distal region of the keel is shown to be open on its inner side. In Figure 36b (Stage V), horizontal bridges have linked the vertical struts, making the distal part a complete, although porous, tube. However the middle part of the valve remains open, and this is the important nerve foramen, through which the nerves from the receptor areas on the inner surface of the jaw enter the keel to innervate the muscles which insert upon it. Smaller nerves from sensory papillae may also enter the pores distal to the foramen.



At first the central keel is rounded distally, as in Figure 36b, but later an extension grows upward and inward to form the grooved tooth which is used for piercing (Figure 37a). The groove forms the floor of the venom duct. Although the valve itself lies outside the venom gland in the living appendage, the groove of the terminal tooth is sealed by a ring of duct tissue which allows the tooth to act as a hypodermic needle (Chia, 1970a).

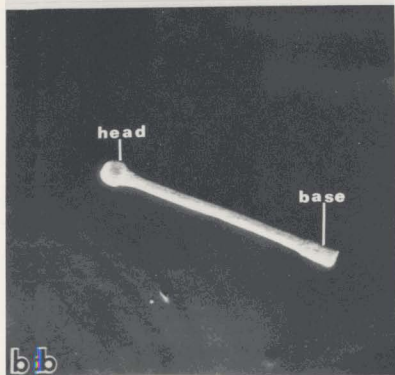
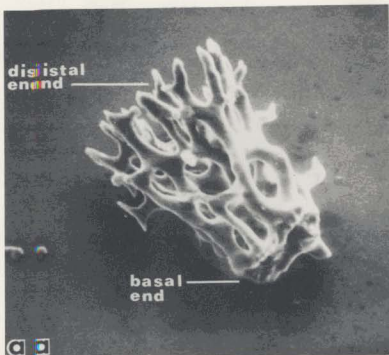
The only further change to take place in the valve is the strengthening of the structure by further calcite deposition. In Figure 37a the spaces between the layers of the base have filled in somewhat, forming ridges with pores between them for muscle insertion. A comparison of Figures 35a, 36b, 37a and 37b shows the calcite becomes less porous in all areas.

The skeletal rod of the stalk (Figure 38a) is less complex than the valves. Strut-like trabeculae bifurcate, grow in length, and become linked by cross-bridges, forming the typical fenestrate pattern. The process is similar to the development of the central keel of the valve, although the rod remains more highly reticulated and less modified. The process of development has not been investigated in detail, however examination

Figure 38 - Scanning electron micrographs of  
the skeletal rod of globiferous  
pedicellariae of  
Strongylocentrotus dröbachiensis.

a . . . . . Skeletal rod from a developing  
pedicellaria; 1600X.

b . . . . . Skeletal rod from a mature  
pedicellaria; 80X.



of Figure 38b, which is a fully developed skeletal rod, shows that it becomes a cylindrical structure with the base somewhat enlarged. According to Hyman (1955) the base is concave to articulate with a tubercle on the test. The proximal end is rounded, and of greater diameter than the rest of the rod. The pores at the base of this rounded head provide areas for insertion of the flexor muscles and connective tissue.

#### SUMMARY OF DISCUSSION

A globiferous pedicellaria begins as a tiny finger shaped appendage. As it increases in length and diameter, three projections form at the tip, each one representing a jaw. The distal region becomes larger in diameter than the basal region. Further increase in length in early developmental stages is mainly due to elongation of the jaws. The head, neck, and stalk regions gradually become easily recognizable externally. However, the stalk remains short until the head and neck regions are well developed. The head region increases in diameter as the jaws become rounded at their bases. The neck becomes more distinct as the flexor muscles develop. The result is a globiferous pedicellaria with the shape of the mature appendage, except for the shorter stalk. The only further change externally is an increase in size.

The skeleton is one of the first structures to be formed in a developing pedicellaria. The stalk and its supporting skeletal rod grow at a slow rate, until later developmental stages, when

all the components of the head are well formed. The early appearance of the skeletal valves may be explained by the fact that they are necessary for the support and movement of the jaws. The adductor muscles begin to differentiate early, but they cannot function until the necessary insertion areas on the skeletal valves are formed. The articulation areas, and areas for insertion of abductor muscles, flexor muscles, and connective tissue must develop before the jaws are capable of opening and closing. It is after these areas form on the bases of the skeletal valves that the smaller abductors and flexors develop. Due to the small size of these muscles at this stage, it is likely that the jaws are not capable of any extensive movements. This is in agreement with the fact that the pedicellaria is not equipped to play a defensive role at this time because the venom glands, sensory areas, and terminal teeth for piercing have not formed.

The sensory hillock first appears as a thickening of the epithelium on the inner surface of each jaw. The inner side of the keel of the skeletal valve remains open to permit the development of the nerve foramen, through which nerves from the sensory hillock pass to

enter the skeletal valve. At the time when all the muscles have formed and the venom glands are beginning to differentiate, the sensory hillocks are distinct rounded areas, and nervous tissue can be seen running from it to enter the valve via the nerve foramen. Ultrastructural studies indicate these are developing axons. The inner surface of the keel then closes distally by the formation of cross bridges, leaving the foramen open. However, the tip of the valve at this time is still rounded.

The mature pedicellaria possesses an epithelium that is one cell layer thick. In early developmental stages it is several cell layers thick, but gradually narrows in all areas except the outer surfaces of the jaws and the sensory hillocks. It is from the epithelium of the outer surfaces of the jaws that the venom glands differentiate. The first indication of this is the formation of an indentation in the inner boundary of the thickened epithelium, distal to the point of separation of the jaws. In the mature condition the venom gland is single at its base, but then bifurcates, uniting again at the tip. As development proceeds muscle cells can be seen in the process of forming a wall outside the inner boundary. The gland remains single at its base, but distal to this it separates

completely into two lobes which gradually become enclosed by muscle cells. As the muscular wall becomes complete, the entire venom gland is separated from the epithelium, which is then one cell layer thick. Vacuoles develop in the cells of the venom glands. They are at first few in number, but increase rapidly. When the venom glands are completely enclosed by a muscular wall, vacuoles almost fill the glands. The nuclei and cytoplasm of the secretory cells are basally displaced, and line the venom glands. By the time the venom glands are well developed, the tip of the skeletal valve has become modified to form the terminal tooth for piercing and injecting the venom.

The first structures to be formed are the skeletal valves and the muscles which are involved in opening and closing the jaws. When these are fairly well developed the sensory areas, nerves, venom glands and terminal teeth develop. These structures make the pedicellaria a very specialized appendage which plays an important defensive role. Judging from the large changes in the histology of the glandular areas from Stage V to Stage VI, vacuolation and toxin biosynthesis is apparently a rapid process, occurring after the basic supportive jaw components have developed. This suggestion



was made by O'Connell (1971), who found the venom glands of globiferous pedicellariae of young S. purpuratus (3 mm test diameter) to be well developed, however he did not study developing pedicellariae.

Each of the three skeletal valves is believed to begin as a tiny granule of calcite from which three rays grow in the horizontal planes of the pedicellaria, and one in the vertical plane. From further branching and joining of projections, layers form in the horizontal plane, representing the base of the valve, while vertically the central keel develops. The elongation of the keels of the skeletal valves correlates with the elongation of the jaws. Elaborate structures for articulation of the valves with each other, for muscle and connective tissue attachment, and for the passage of nerve fibers, develop at the bases of the skeletal valves. Examination of developing skeletal valves shows that these ridges, grooves, and pores are shaped by the formation and joining of projections, which is the basic method of skeletal growth in echinoderms. The process is not only important in providing the reticulate, or fenestrate structure typical of echinoderm calcite, but is also the means by which the skeletal valves become shaped to play a very specialized role.

The skeleton is surrounded by clusters of cells throughout all developmental stages. In electron micrographs these cells are shown to give off projections, which are continuous with areas of cytoplasm surrounding, or near the skeleton. These projections often contain microtubules oriented parallel to their longitudinal axis.

Mitochondria are numerous in the projections. Many appear to be in various stages of change in morphology. It is suggested that many become modified or transformed into vesicle-like structures.

Electron micrographs through the developing skeleton often show cytoplasmic projections which are filled with clusters of mitochondria. They have also been found in the thin cytoplasmic strands in close proximity of the skeleton. It is possible that mitochondria function in the calcification process, as suggested by Lehninger (1970) and others.

In sections of *Pedicellariae* that were decalcified by treatment with EDTA, the skeleton is represented by epon infiltrated areas that correspond closely with the shape and size of the skeleton studied with the scanning electron microscope. In nondecalcified tissues the skeleton is represented by holes which are

comparable in size, shape and position to the epon-infiltrated spaces of decalcified tissues.

Apparently the calcite falls out during sectioning. In specimens subjected to very slightly acidic conditions, the holes representing the skeleton are surrounded by thin areas of embedding material, suggesting that the peripheral, or possibly newly deposited calcite, becomes dissolved by the slightly acidic conditions.

Microanalysis for calcium using EMMA-4 showed no significant amounts of calcium in the epithelial cells, significant amounts in all areas of the cells in close proximity of the holes representing the skeleton and negligible readings over the holes. Analysis of dense objects or granules near the holes gave extremely high readings, suggesting that these were fragments of the skeleton that were left behind. Confirmation to this effect was also obtained by X-ray mapping. The areas of the cells that showed the greatest amounts of calcium were mitochondria, and vesicles that were similar in size, shape and location to the mitochondria, but could not be recognized clearly beyond doubt as such.

Ultrastructural and X-ray microprobe studies

indicate that the cells associated with the skeleton are involved in skeletogenesis. Since they have been shown to contain significant amounts of calcium, the term calcicyte (L. calx, lime) has been proposed to refer to this skeletogenic cell type.

### CONCLUSIONS

The following conclusions regarding the development of globiferous pedicellariae and their skeletal formation in Strongylocentrotus dröbachiensis can be drawn from the present study.

A globiferous pedicellaria begins as a tiny finger-shaped projection which as it grows, gradually differentiates into three regions - a head composed of three jaws, a neck, and a stalk.

Each of the skeletal valves of the jaws develops from a tri-radiate spicule by the formation and joining of projections, resulting in a porous structure. By the same process the ridges and pores necessary for articulation of the valves, muscles and connective tissue attachment, and passage of nerve fibers are formed.

The venom glands develop from the epithelial layer covering the outer surfaces of the jaws. The glands become enclosed by muscular walls. Vacuoles appear in the cells of the venom glands and increase rapidly in number until they almost fill the glands. The nuclei and cytoplasm of the secretory cells become basally displaced and line the venom glands.

Throughout their development the skeletal elements are surrounded by cells which are responsible for calcite deposition and maintenance. These cells have cytoplasmic projections surrounding the skeleton, and clusters of mitochondria have been located in close proximity of the skeleton. Significant amounts of calcium could be detected in these cells as well as in mitochondria, and vesicles that were interpreted as modified mitochondria. It has been suggested that calcite deposition in sea urchin skeleton is a mitochondria-mediated process, which is in keeping with the hypothesis put forward for hard tissue formation in a wide variety of plants and animals.

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