A Scanning Electron Microscope Study of Sensory Tentacles on the Mantle Margin of the Gastropod *Acmaea (Notoacmea) scutum*

BY

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(3 Plates)

INTRODUCTION

THE ARCHEOGASTROPOD *Acmaea (Notoacmea) scutum* Eschscholtz, 1833, is a common inhabitant of the intertidal zone along much of the Pacific coast of North America (McLEAN, 1969). To survive in this intertidal zone, *A. scutum* must maintain a delicate vertical balance between the hazards that are posed by predatory sea stars from below and the hazards posed by physical factors, such as desiccation, from above. An avoidance response, which is given to distant sea stars, is an important part of this limpet's successful behavioral strategy (PHILLIPS, 1975a, 1976). Considerable additional information on the behavioral and physiological ecology of *A. scutum* is also available (*e.g.*, FEDER, 1963; MARGOLIN, 1964; KINGSTON, 1968; ROGERS, 1968; WEBBER, 1970; MENGE, 1972; WOLCOTT, 1973).

The avoidance response that *Acmaea scutum* gives to distant predatory sea stars consists primarily of increased upward movement. This avoidance response can be observed in the field as well as in the laboratory; it specific for particular predatory sea stars; and it can be modulated by various physical factors such as light (PHILLIPS, 1975a, 1976). The distance chemoreceptors that trigger the avoidance response are located on the limpet's mantle margin along with several other types of receptors that may directly influence the animal's integrated behavior; these include photoreceptors, tactile receptors, and contact chemoreceptors (PHILLIPS, 1975b).

The mantle margin of *Acmaea scutum* clearly functions as an important and versatile sensory structure. The following is a general description of structures found on the mantle margin of *A. scutum* by light and scanning electron microscopy. Emphasis is placed on the mantle tentacles and on the sensory endings found on these tentacles. The present study is intended to provide a basis for a more restricted and detailed investigation that will deal specifically with the receptors that mediate avoidance behavior.

MATERIALS AND METHODS

Acmaea scutum were collected intertidally near Bodega Bay, California, and maintained in recirculating aquaria at 12 - 14° C. Limpets ranging in shell length from 13 to 36 mm were studied. Living intact animals were examined for general patterns of ciliary activity using a dissecting microscope. For compound light microscopy, portions of fresh mantle margin containing several tentacles were excised and examined either in seawater or in a solution consisting of equal parts of seawater (34‰) and isotonic $MgCl_2 \cdot 6H_2O$ (7.2 g/100 m*l*).

Mantle margins were prepared for scanning electron microscopy by standard methods employing a variety of fixatives. The best results (*i. e.*, least distortion of cilia and microvilli) were obtained using the following procedure. Sections of mantle margin were excised and relaxed for 2 hours in a 1:1 solution of seawater and isotonic magnesium chloride. The tissue was then fixed for 2 hours in cold Parducz' fixative (6 parts 2% osmium tetroxide: 1 part saturated, aqueous mercuric chloride), dehydrated in increasing concentrations of ethanol, and transferred to 100% amyl acetate. Several fixatives in addition to Parducz' were also tried, including 2% and 4% glutaraldehyde in seawater and 2% and 4% formaldehyde in seawater. Fixation with these aldehydes, however, even when followed by a post-fixation with 2% osmium tetroxide, was generally less satisfactory than direct fixation with Parducz'. Fixed, dehydrated specimens were dried from amyl acetate by the CO_2 critical-point method of ANDERSON (1951). They were attached to stubs with silver paint and coated with gold-palladium. Observations were made using a Cambridge Stereoscan electron microscope.

RESULTS

Organization of the Mantle Margin

The mantle of *Acmaea scutum* is a fold of body wall that encircles the animal. As in other limpet-shaped gastropods, the mantle of *A. scutum* encloses a mantle cavity consisting of a nuchal cavity anteriorly and a pallial groove surrounding the body. The edge of the mantle is covered with small, sensory tentacles, and it projects beyond the periphery of the shell when fully expanded (Figure *1*). The entire mantle is highly vascularized, and scattered clumps of motile cilia create water currents that run dorsally up the mantle wall. Motile cilia are largely restricted to this wall; there is little or no ciliary activity on the mantle edge in the region of the sensory tentacles.

The sensory mantle tentacles are arranged in a small number of irregular rows around the mantle margin. Tentacles within each row are of uniform length, but the lengths vary between rows. The longest tentacles, which are about 0.8 mm long when fully extended, occur proximally. These tentacles project ventrally and laterally and may contact the substratum. Shorter tentacles occur in the next rows, and new tentacles arise near the distal edge of the mantle margin. The width of the margin and the total number of tentacles increase with the size of the limpet: a limpet with a shell length of 20 mm may have 120 tentacles whereas a limpet that is 35 mm long may have 190 tentacles. Each mantle tentacle is capable of a full range of bending and lengthening movements, and each can be completely retracted into slit-like pits in the mantle margin.

Mantle Tentacles:

Light microscopy on fresh preparations

Details of tentacle anatomy are apparent in fresh preparations of mantle margin viewed through the compound light microscope. Most of the surface of the sensory tentacles is covered with a pigmented epithelium. Pigment granules can be detected within the epithelial cells, and the intracellular distribution of granules allows approximate cell boundaries to be seen as relatively clear areas. The ventral surface of the mantle wall and the region between the tentacles are also covered with an epithelium similar in appearance to that covering the sensory tentacles.

The tip of each mantle tentacle bears a conspicuous cap of cilia (Figure *2*), regardless of the size of the tentacle or its position on the mantle edge. In addition to the densely ciliated cap, smaller clusters of cilia erupt from between pigmented epithelial cells all along the length of the tentacle. In contrast to the large numbers of cilia present on the sensory tentacles, cilia are rarely seen on the mantle margin immediately surrounding the tentacles. The exact density of ciliation on the tentacles varies considerably between limpets and between tentacles on the same limpet.

The clusters of cilia on the tentacles are varied in size and appearance. Observations of fresh preparations showed that large tufts containing many cilia were always stiff and non-motile, whereas some smaller clusters consisting of only a few cilia waved passively in a water current. On rare occasions, a few isolated cilia were observed to twitch repeatedly; however, in these cases, twitching became increasingly pronounced as the preparation aged and may have been artifactual. In any event, none of the cilia on the tentacles exhibited activity comparable to that of most motile cilia, such as those found on the mantle wall or on the ctenidium. Since the cilia on the mantle tentacles are clearly not used for water movement or particle movement, they are all presumed to be sensory in function.

Mantle Tentacles:

Scanning electron microscopy

The surface features of a mantle tentacle are prominent in low-magnification scanning electron micrographs (Figure *3*). The pigmented epithelial cells form a background that is relatively smooth and dark in appearance, and the tip of each tentacle is completely covered by a dense crown of sensory cilia. Additional clumps of cilia can be seen scattered across the surface of the tentacle.

The free surface of the epithelial cells consists of a dense packing of microvilli; at high magnification, the tips of the microvilli give a cobble-stone appearance to the surface of the cells (Figure *4*). Occasionally during preparative procedures, splits occurred between adjacent epithelial cells, allowing the microvilli to be viewed in perspective (Figure *5*). Most of the microvilli are 2 - 3 μm long and 0.15 - 0.20 μm in diameter.

The tip of each tentacle is covered with erect sensory cilia, some of which may be organized into separate clusters (Figure *6*). This prominent cap of cilia varies in size between individual limpets and also between tentacles on the same limpet, but it is usually 32 - 40 μm across at the widest point and may contain several hundred cilia. Most of the cilia in the cap are 6 - 8 μm long and approximately 0.3 μm in diameter.

The additional clumps of cilia, which are scattered along the tentacle, contain a variable number of cilia and may differ greatly in appearance. In Figure *7*, 2 large, erect tufts of cilia can be seen along with several smaller tufts. The larger tufts on a tentacle may be as much as 15 μm across, and they undoubtedly consist of cilia from many sensory cells. Most of the cilia in these scattered tufts, like those on the tip, are 6 - 8 μm long; however, even within a clump, the length of the cilia may vary considerably (Figure *8*).

Clusters of cilia with lengths that differ markedly from the average are also present in small numbers on most tentacles. Rows of extremely short cilia that barely extend above the tips of the microvilli are often seen (Figure *7*). In addition, a number of extremely long, flexible cilia are usually present (Figure *9*). These cilia are frequently as much as 20 μm long. Most often they arise in small groups consisting of fewer than 6 cilia each. These groups are often widely separated, but because of the great length of the cilia, as many as 30 - 40 of these long cilia may be intertwined distally.

The surface of the mantle tentacles is normally coated with a thin layer of mucus (Figure *9*). This thin coat of mucus routinely veiled some of the finer features of the tentacles, and, in some preparations, the mucous coat was so thick that it completely obscured the tentacle surface. As might be expected, preparations with as little mucus as possible were usually selected for detailed study and micrography. The presence of a thin mucous coat, however, should be considered as part of the most accurate representation of the "natural" condition.

The results presented in Figures *3* - *9* were obtained using mantle margins that were first relaxed in a solution consisting of equal parts of seawater and isotonic magnesium chloride and then fixed directly in Parducz' fixative. Of the several methods that were tried during the course of this study, this procedure was considered to give a preparation that most accurately reflected the living condition. When other commonly used procedures were followed, some significant differences in appearance were found; these are also reported here for completeness. Primary fixation with aldehydes (primarily 4% glutaraldehyde in seawater) consistently produced cilia that differed from those fixed in Parducz'. Cilia fixed with aldehydes were usually bent over instead of erect, and many

Explanation of Figures *1* to *3*

Figure *1*: Ventral view of *Acmaea (Notoacmea) scutum* showing the fringe of sensory, mantle tentacles encircling the limpet. As many as 200 tentacles may be present along the mantle margin of a limpet 35 mm in shell length × 15

Figure *2*: Dense cap of non-motile, sensory cilia covering the tip of a mantle tentacle. Unstained, fresh preparation viewed through the compound light microscope × 430

Figure *3*: Scanning electron micrograph of mantle tentacle. Note the dense crown of sensory cilia covering the tip and the smaller clumps of cilia scattered along the length of the tentacle. Parducz' fixative × 290

Explanation of Figures *4* to *7*

Figure *4*: Surface of the epithelial cells that cover the mantle tentacles; shows the round tips of numerous microvilli forming a continuous array. Parducz' fixative. Scale equals approximately 5 μm × 6 300

Figure *5*: Layer of microvilli viewed in perspective. Parducz' fixative. Scale equals approximately 5 μm × 6 500

Figure *6*: Crown of erect sensory cilia covering the tentacle tip. Parducz' fixative. Scale equals approximately 15 μm × 1 400

Figure *7*: Clusters of sensory cilia scattered along the shaft of a tentacle. Note the large cohesive tufts of cilia, the smaller clusters of cilia, and the row of truncated cilia that barely extend above the level of the microvilli (arrow). Parducz' fixative. Scale equals approximately 10 μm × 2 800

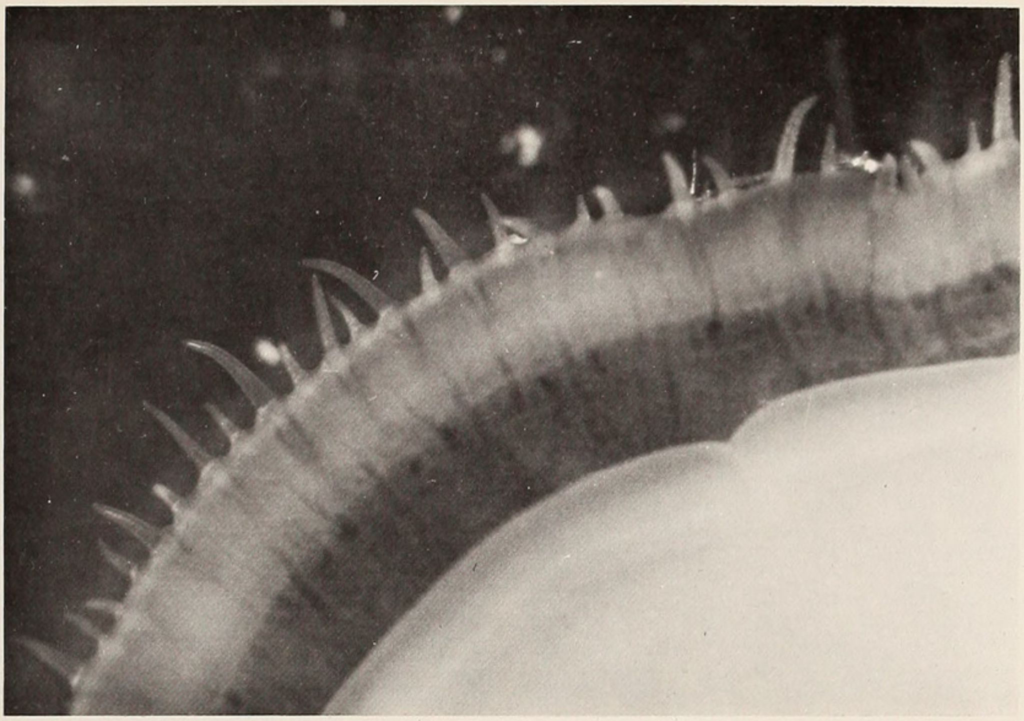


Figure *1*

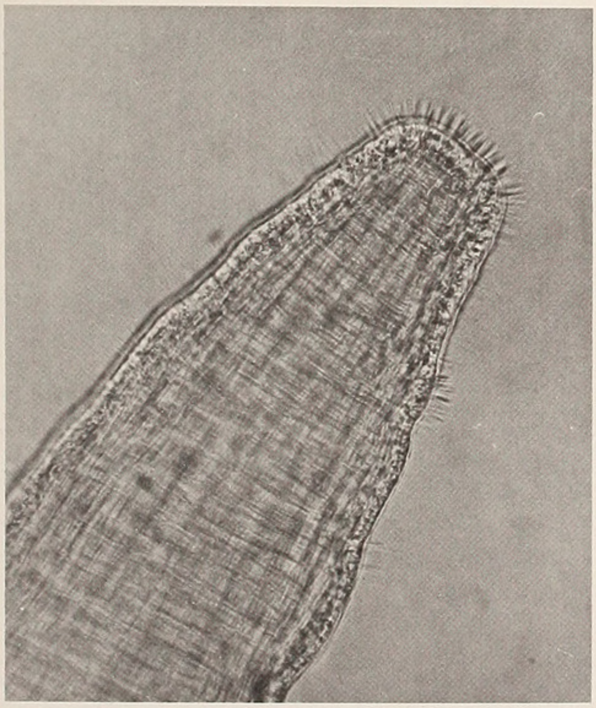


Figure *2*

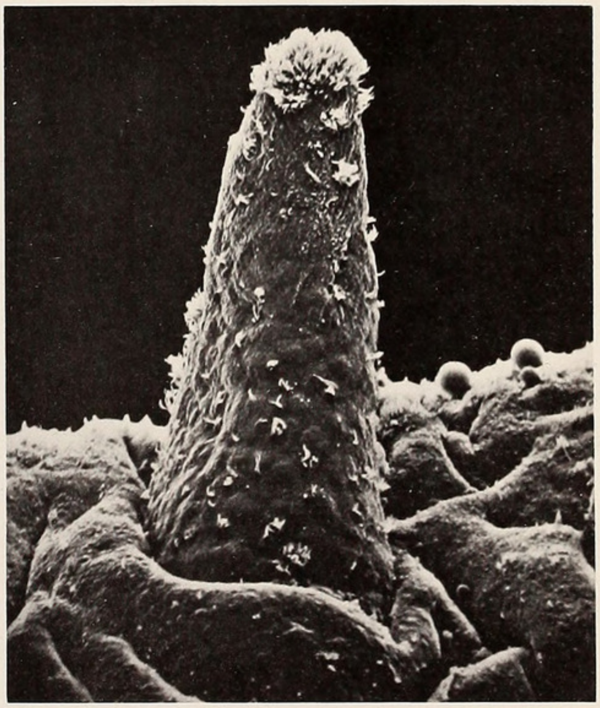


Figure *3*

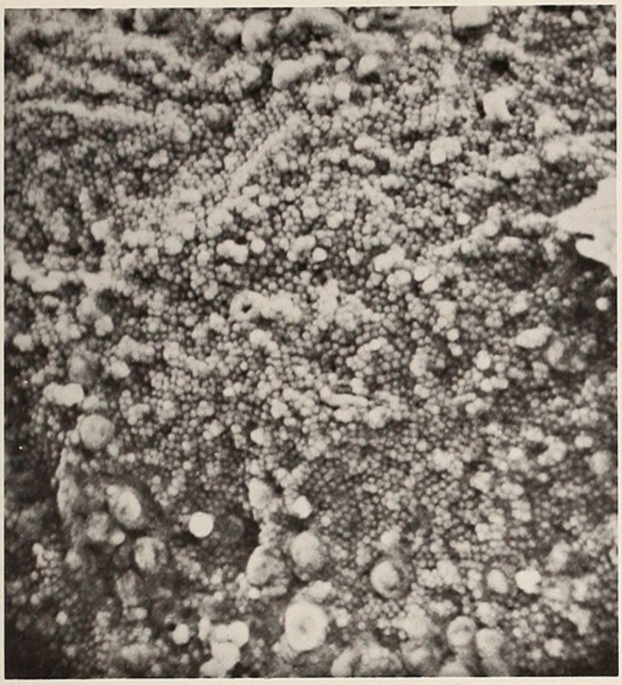
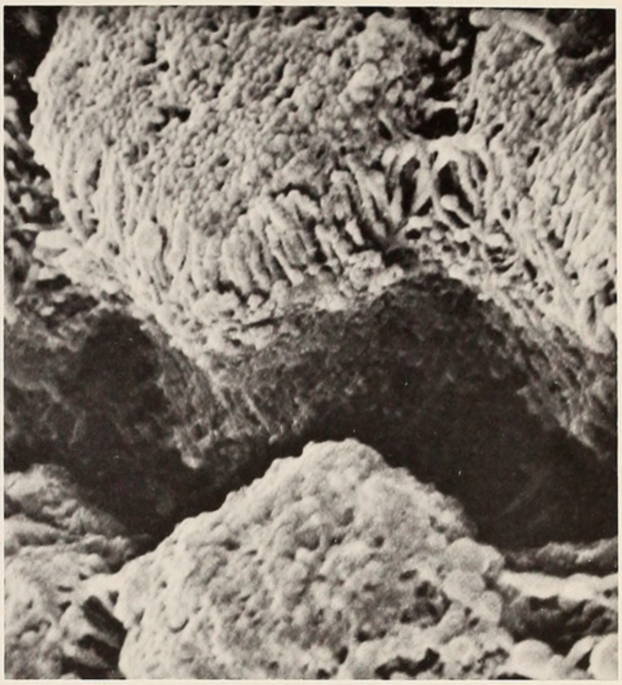


Figure 4



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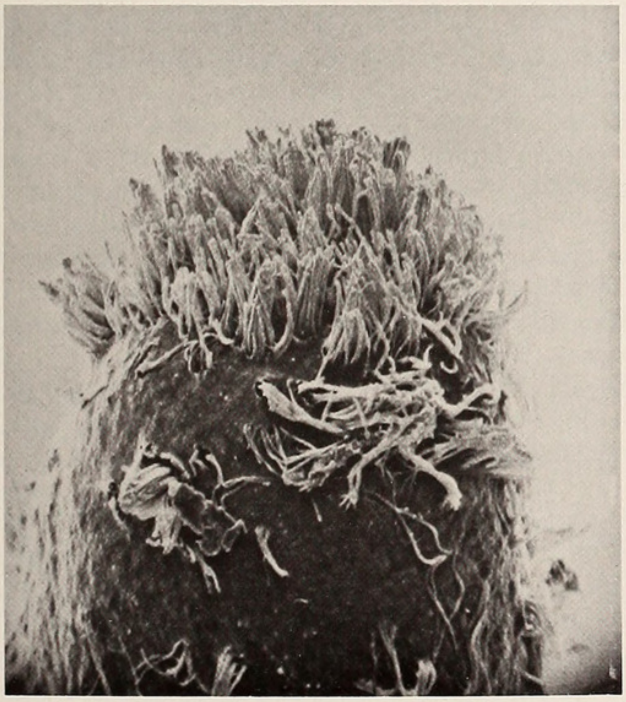

Figure 6

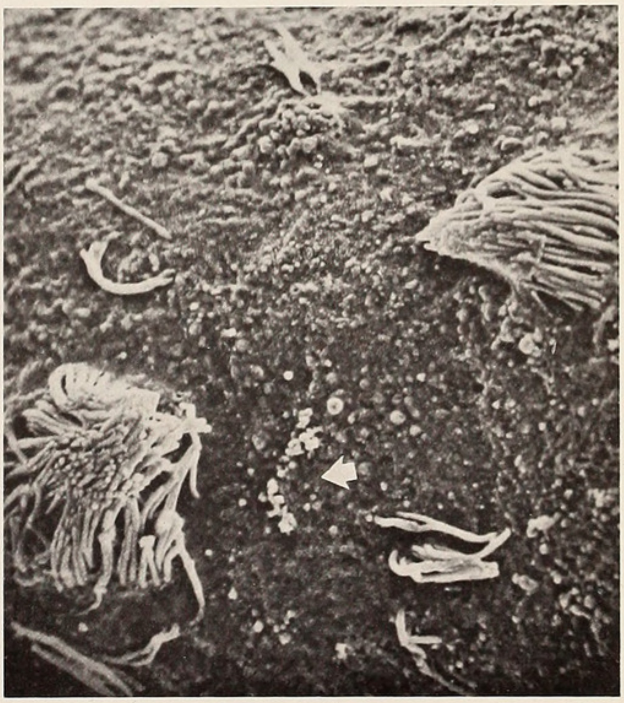

Figure 7