EJECTION OF THE WART CONTENT INCLUDING WHEEL-SHAPED OSSICLES FROM THE BODY WALL OF THE APODID SEA CUCUMBER POLYCHEIRA FUSCA (RUFESCENS) INDUCED BY MECHANICAL STIMULATION

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ABSTRACT

The apodid sea cucumber *Polycheira fusca* that was pushed hard on its body surface ejected the wart contents including the wheel-shaped ossicles from the body wall. The ejection always occurred quickly in a small circular region around the press point, indicating that pushing caused a nervous signal, which was diffusely conducted to the surrounding area and induced rupture of the warts and ejection of their contents. All ejected ossicles dispersed but were still attached to the wall by a branched string connecting the ossicles to the wall. The selective advantage of ejecting the wart contents was discussed.

Keywords : Ossicle, Wart, Sea cucumber, Echinoderm, Ejection

INTRODUCTION

During the course of studying the reproduction of the apodid *Polycheira fusca* (Kubota et Tomari, 1998), one of the authors observed that when the body wall was slowly cut with scissors, wheel-like ossicles were ejected from the warts around the cutting site. Externalizing of the ossicles from the holothurian body wall has been briefly mentioned only by Hyman (1955). According to Hyman, the apodid family Synaptidae has an anchor-shaped ossicle in the body wall, whose pointed tip strongly sticks to one's fingers when the synaptidae is handled. As for the synaptid anchors, Östergren (1897) has pointed out that they may serve as anti-predatory devices which hold the animals in its burrow during attack by a predator.

In this paper, the wart contents and their ejection processes of *P. fusca* were observed in a living or sectioned specimen under a dissecting or high-powered light microscope, and the details of the ejection in freeze-dried samples under a scanning electron microscope. Furthermore, the influence of the wart contents on the swimming movement of the echinopluteus was observed.

MATERIALS and METHODS

In this study the apodid sea cucumber *Polycheira fusca* (Quoy et Gaimard) [synonym: *P. rufescens* (Bradt)] was used. Sea cucumbers of 8 to 10 cm in body length were collected in full and new moons at Iso Beach in Kagoshima, Japan (for details of the collection site, refer to Kubota & Tomari, 1998), and maintained in aerated seawater at temperatures of 16 to 27 ℃ for a maximum of two weeks before use.

To mechanically induce ejection of the wart content, a sea cucumber was removed from a bowl, placed on a

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thick, flat rubber sheet in air, and a spot on the body wall was sharply pinched or given a hard push with tweezers for 2 to 3 sec. To observe the warts that responded, immediately after stimulation, 10% neutralized formalin was injected into the body cavity. After 2 to 3 hr, the body wall of about 2 cm square around the region that responded was excised, rinsed in water for 1 to 2 hr, transferred to a 70% ethanol solution, separated from both the muscle layer and the connective tissue layer except for the latter upper part with tweezers, and immersed in 0.01% Methyl Green for 6 hr. The stained specimen was then embedded in glycerin and observed under a microscope. Similar processes were used to observe the wart of the unstimulated animal. Furthermore, the following solutions were used for examining the wart inclusions: Ca, Mg-free seawater with 10 mM EDTA (pH 7 to 8); normal seawater with 1% Triton X-100 detergent; a solution of 20 mM EDTA and 2% Triton X-100; a solution of 1% phosphotungstic acid, 1% Aniline Blue, and 10% formalin.

To test the toxicity of the wart content, five warts with a small amount of the surrounding tissue were cut off, transferred to a few drops of seawater placed in a dish, and ruptured with needles. About 15 ml of seawater containing the echinopluteus larva reared in the laboratory was added to the dish, and the influence of the wart content on the larva was checked under a dissecting microscope.

For observing the body wall and the wart in sections, Bouin fluid was injected into a sea cucumber just after stimulation. After 2 to 3 hr, the wall was separated from the body, dipped three times in 80% ethanol every half hour, and placed in the same solution overnight. The wall around a stimulated site was then cut into a piece of about 5 mm square under a dissecting microscope, dehydrated with an ethanol series, embedded in paraffin after immersion in xylol, sectioned into a 12 μ thickness, and stained with Azan using Azocarmine, Aniline Blue, and Orange G.

For scanning electron microscopy, sea cucumbers were stimulated, injected with 2.5% glutaraldehyde seawater, and dipped in the same fixative for 2 to 3 hr. The body wall was separated, and fixed for additional one day. After fixation, the wall was trimmed, dehydrated in ethanol, and dried by the critical point method (using isoamyl acetate and CO_2 as the transition fluids), mounted on aluminum stubs, coated with gold, and observed using HITACHI S-450 scanning electron microscope.

RESULTS

The warts with an elliptical outline and no appreciable surface protuberance were widely scattered in the body wall. In living animals, the major and minor axes of the wart changed in size together with extension and contraction of the wall following the body movement. Formalin-fixed warts had a major axis of 0.4 to 0.8 mm and a minor of 0.2 to 0.4 mm. Each wart was observed through the epidermis to be packed with many wheel-like ossicles. Isolated ossicles measured 50 to 100 μ in diameter. Furthermore, in the wart, there was a Methyl Green-stained fibrous structure.

Ejection processes Sea cucumbers pinched or pushed hard with tweezers always ejected the wart contents. Ejection occurred 1 to 2 sec after pushing, in a circular area with a diameter of about 1 cm around the push point. The ejected ossicles were connected together by a whitish string, which was attached to the ruptured wart for at least 1 hr. In its epidermis, a hole was formed (Fig. 1A). The occurrence of the ejection could be seen easily with the naked eye by emergence of highly refractive ossicles on the dark brown body wall.

Histological observations The body wall is composed of an epidermis covered with cuticle, the dermis, and muscles (Smiley, 1994; Fig. 1C). The dermis of *P. fusca* was thin. In the upper dermis built mainly of sparse fibers, the wart cavity was found, but ossicles had dissolved out during immersion in a acid fixative, i.e., Bouin fluid (Fig. 1C). The histological observations showed that before the beginning of ejection, the wart cavity increased in volume, the epidermis overlying the cavity decreased in thickness, and the outmost cuticle layer disappeared (Fig.1D). Thinning of the epidermis was most significant near the top of the cavity, where the epidermis ruptured

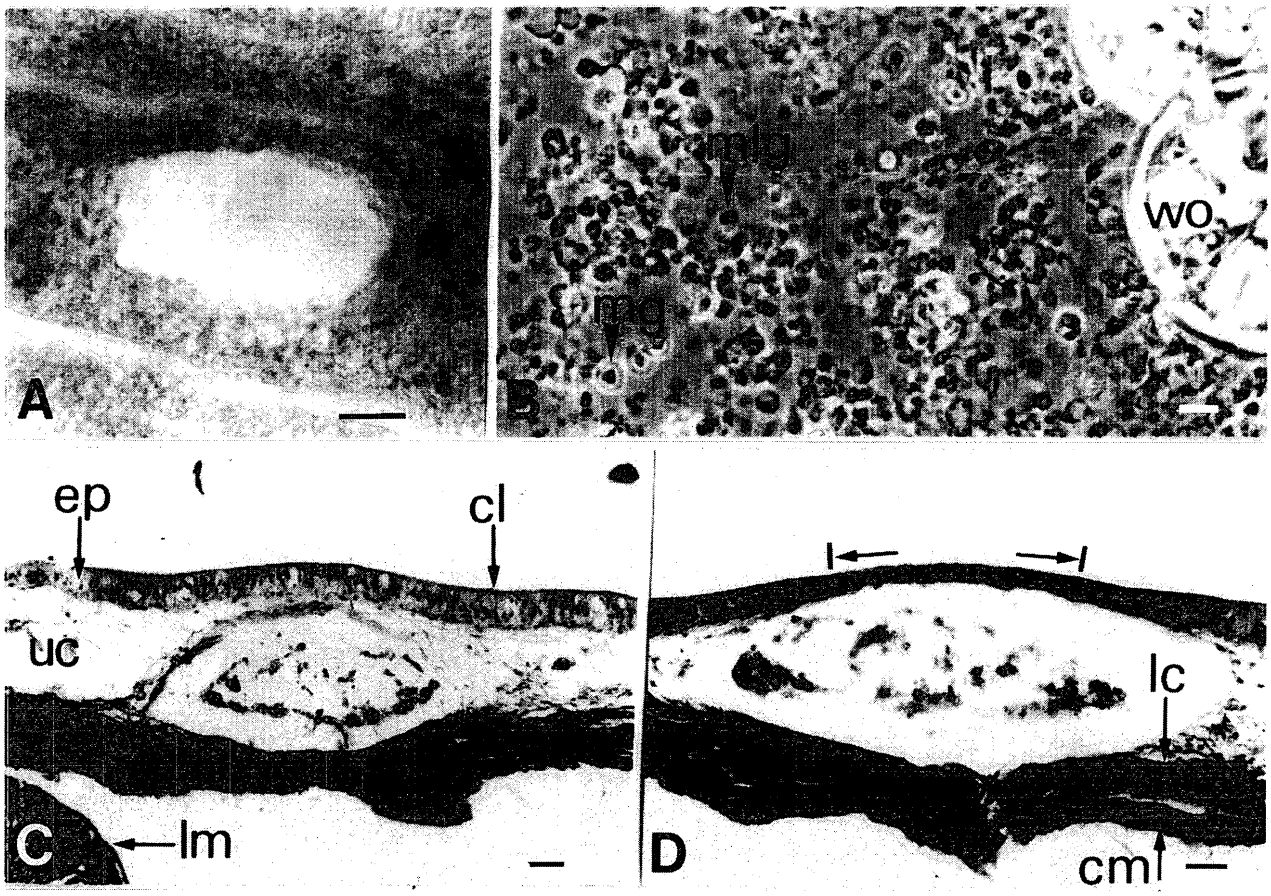

Fig.1. Body wall, wart, and wart contents. A: the hole seen in a wart after injection (surface view). Ossicles and the string were removed. B: granular contents from the isolated, broken wart (with a phase microscope). C: the body wall of the unstimulated animal (section). D. the body wall just before injection (section). The area between the arrows shows the region where the presence of a cuticle layer was not detected. Note the change in the cuticle layer, local thinning of the epidermis, and swelling of the wart cavity. In sections (C,D), no ossicle is seen in the cavity because of dissolution. cl: cuticle layer; cm: circular muscle; ep: epidermis; lc: lower connective tissue; lm: longitudinal muscle; mg: membrane-enclosed granules; mlg; menbraneless granules; uc: upper connective tissue; wo: wheel-like ossicles. Scale bars: A, 100 μ; B, 10 μ; C & D, 100 μ.

and ejection occurred.

Scanning electron microscopical observations The micrographs showed that the wart surface swelled up (Fig. 2A) and tore, and a compacted mass of ossicles was extruded from the opening (Fig. 2B). They then dispersed, but were connected together by a string that branched like the trunk of a tree (Fig. 2C). The string corresponds to the Methyl Green-stained fibrous structure observed under a light microscope. The string appeared to be made of many fibrils (Fig. 2D). The branched part of a string was further branched at the end into six lines. Their tips were fastened to the base of forks of a round-shaped wheel (Fig. 2E).

Wart inclusions In addition to the ossicles and the string, two sorts of granules were found in the cavity fluid of the isolated, broken warts (Fig. 1B). One of them was enclosed by a membrane, and dissolved in a Triton-X solution within 1 hr but not in an EDTA solution after 3 hr. The string also showed the same results and the ossicle the reverse. Another item was membraneless, globular in shape, insoluble in a solutions of Triton-X and EDTA, and faintly stained with Aniline Blue.

Toxicity of the wart content The addition of the wart content to the seawater in which echinopluteus larva were suspended exerted no influence on their movement during a 20 min-observation. The 20 sec-stay of a sea cucumber

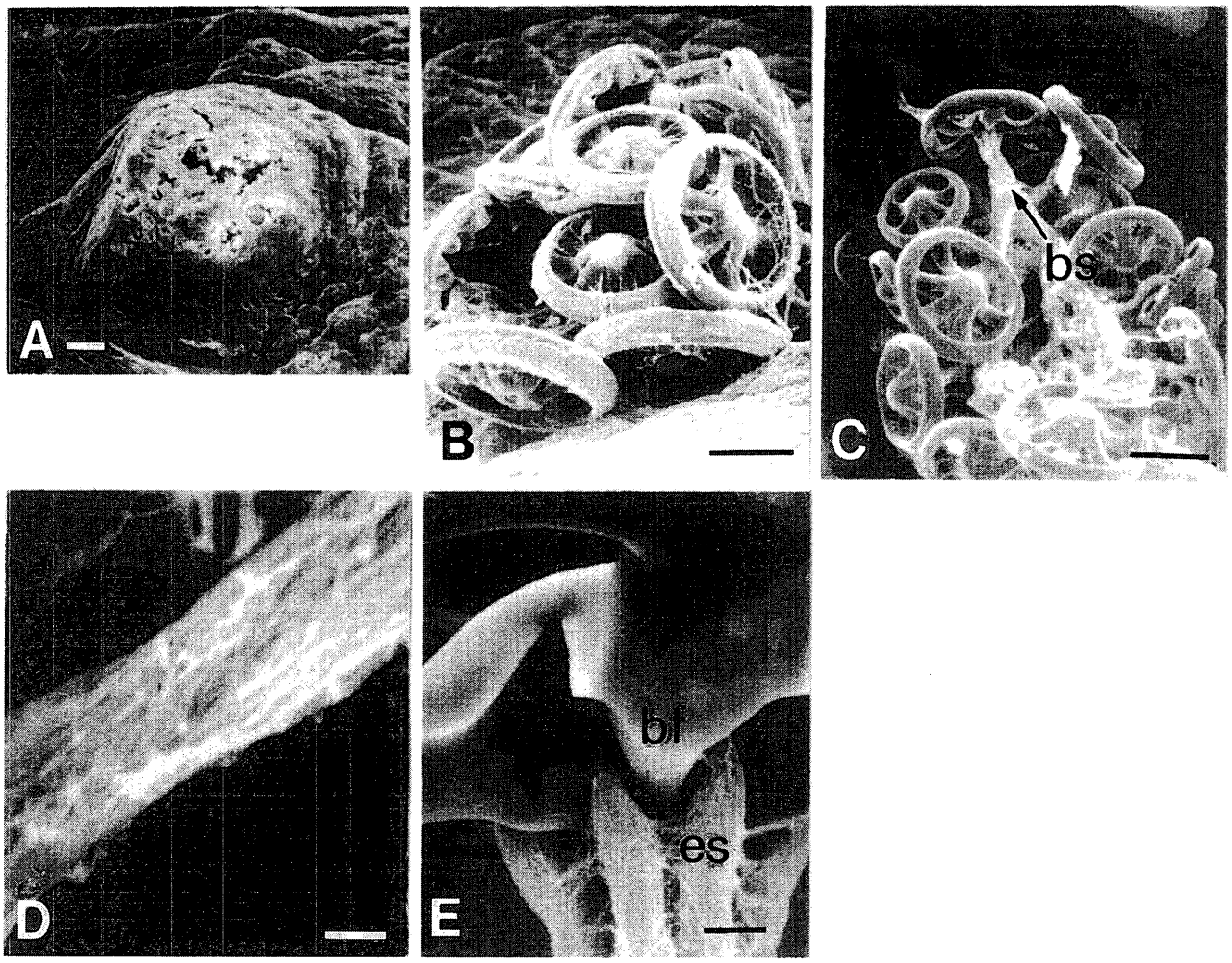

Fig. 2. Ejection observed with a scanning electron microscope. A: swelling up of the wart surface before the beginning of ejection. B: a compact mass of ossicles exposed on the wart. C: ossicles which dispersed but were connected together by a tree-like string. D: partially enlarged string, showing a fibrous appearance. E: enlargement of the part connecting the ends of the branched string with the base of the ossicle forks. bf: base of ossicle forks; bs: branched string; es: ends of six lines of a string. Scale bars: A, 10 μ; B & C, 50 μ; D & E, 5 μ.

in about 15 ml of the larva-suspending seawater also had no influence, but for the 30 min-stay, the immobile larva increased in number with time. When a hard push was given to the quiet sea cucumber, all swimming larva quickly ceased to swim and sank.

DISCUSSION

A hard point press on the wall surface provoked ejection of the ossicles and the other wart contents in a small circular region around the stimulated site. This fact indicates that an ejection-inducing signal was produced and diffusely conducted with decreasing intensity, along the epidermal plexus (Cobb, 1989; after Smiley, 1994), but not along the radial and transverse nerves. The latter nerves were found to be concerned with the conduction of an autotomy-inducing signal caused by repeated taps (Kubota et al, 2001). Thus, ejection of the wart contents occurred as the result of a sequence of reactions; namely, production of a nerve signal by pushing, its diffuse conduction to the surrounding area, in response to the signal swelling of the wart cavity and disappearance of the cuticular layer, and the eventual rupture of the cavity leading to ejection. When ejected, all the ossicles dispersed but were still

attached to the wall by a branched string connecting each ossicle to the wall.

Based on the complexities of the reactions and structures participating in the ejection, it was suspected that the ejection has some selective advantage, especially a defense function (reviewd in Bingham & Braithwite, 1986; Fracour, 1997). In this study, no influence of the wart contents on larval swimming was detected. However, since the swimming instantly ceased by hard pushing the sea cucumber which was put together, ejected twinkling ossicles may produce a visual warning against predators that began attacking. Another possible case is that a soluble inclusion released from the wart is effective in deterring predators (about chemical deterrence to predation in holothurians, e.g., refer to Bakus, 1974; Bryan et al., 1997).

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