terestingly, proteolytic activity of the pharyngeal juice of *P. bachei* reaches similar levels of activity to the gastric juice of zooplankton-feeding septibranch bivalves[9]. Though the occurrence of acid proteases has been described as rare[10], this is probably a reflection of the rarity of investigations and the predelictions of comparative enzymologists to concentrate upon the more familiar vertebrate enzyme homologues.

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2 We are grateful to Betsy Sweeney for her technical assistance.
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Self-suturing by a synaptid sea cucumber (Holothuroidea: Echinodermata)[1,2]

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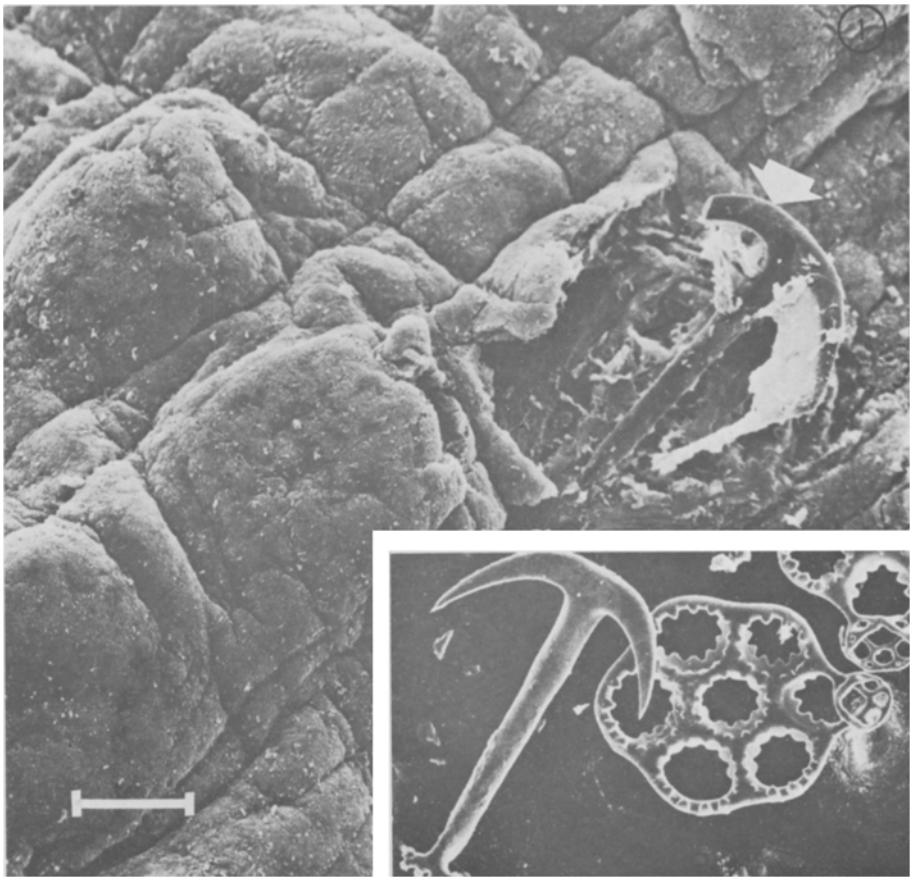
Summary. The synaptid sea cucumber *Opheodesoma spectabilis* responds uniquely to wounding by closing the wound channel via its own 'sticky' anchor ossicles.

The Hawaiian sea cucumber *Opheodesoma spectabilis* Fisher is a conspicuous inhabitant in the quiet protected waters of Kaneohe Bay, Oahu Island, where it browses upon the surfaces of the brown alga *Sargassum echinocarpus*[3] and coral rubble. Lacking tube feet, *O. spectabilis* adhers to the substratum by means of thousands of minute, calcareous ossicles which protrude from the integument of its lengthy body[4,5]. *O. spectabilis* lives openly on the reef flat and would appear vulnerable to predation by fishes, and indeed, attacks do occur[3]. Interestingly, *O. spectabilis* responds uniquely to wounding by rapid closure and sealing of the wound channel by means of 'sticky' dermal ossicles until healing can occur.

Anchor ossicles are a characteristic feature of synaptid sea cucumbers[5] and give the body surface its unpleasant, sticky feeling when handled. In *O. spectabilis*, anchor ossicles arise



SEM preparation of the outer body wall of *Opheodesoma spectabilis*. A thin 300 µm diameter nodular cap of epithelium has been dissected away to expose a single anchor ossicle in its natural posture (white point). Inset. Isolated button ossicle and anchor ossicle. During self-suturing, the pointed hooks of the anchor ossicles are protruded through the thin epithelium to stick together the edges of the wound channel (white bar equals 100 µm).

from the edges of dermal plate ossicles and extend outwards from the central axis of the holothurian at about a 45° angle (figure). This posture permits the pointed hooks of the anchors to be brought to bear against the substratum or, in the case of wounding, set into the sea cucumber's own flesh.

When wounded either in the field or the laboratory, *O. spectabilis* immediately folds its torso towards the wound opening so that the anterior and posterior edges of the wound channel are pressed against one another. Next, peristaltic muscle waves arising from anterior and posterior ends of the animal apply additional force on the appressed sides of the wound closure and set the pointed hooks of anchor ossicles from both edges of the wound into adjacent flesh in a fashion similar to a 'Velcro' clothing fastener. Following completion of self-suturing, *O. spectabilis* resumes normal posture and feeding activity.

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Damage by ozone to the mechanical integrity of the protoplast plasmalemma[1]

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Summary. Ozone acts on the plasmalemma as to weaken its mechanical properties. This results in the bursting of protoplasts.

Pinpointing the action of ozone on cell constituents and cell organelles was started in 1954 when Geise and Christensen[2] demonstrated lipid peroxidation in yeast. However, in order to find the first cell organelle attacked by ozone, attention must be focussed on the plasmalemma, which is considered as the first essential living barrier for ozone to traverse in order to penetrate the cell. Work along this line was only begun in 1963 when oxidative damage to cell plasmalemma of *E. coli* was demonstrated[3]. More specific exploration of the primary target of ozone action on the plasmalemma was achieved by studies on permeability[4-12]. No mention is made in the literature of the action of ozone on the mechanical properties of the plasmalemma, beyond a vague statement that ozone modification of both critical sulfhydryl groups and fatty residues may cause changes in the membrane's fluidity[9]. Thus it has been felt by the authors that investigation of the effect of ozone on the mechanical properties of the plasmalemma might give a new approach to the knowledge of what actually happens.

Materials and methods. Protoplasts from the radish tuber (cultivar 'longs à feuilles courtes') are selected because of the ease with which they are obtained[13], and because they are non-photosynthetic, a feature which eliminates one variable in laboratory light. The stability of protoplasts immediately after washing in T_0 according to Bourgin et al.[14] was also checked. 10-ml suspensions of freshly washed protoplasts from 4 different harvests were placed in 6-cm Petri dishes. Counting of cells was done immediately and thereafter every 30 min for a period of 3 h. In order to work with stable populations (results shown in figure 1), treatment with ozone was always done at least 3 h after harvesting and washing. However, ozone fumigation was always done on the same day, because protoplasts rapidly start regenerating their cell walls and after 3 days provoke a characteristic folding of the external membrane[15].

The classical method of fumigating cell suspensions by a bubbling gas flow, as used for example on *Chlorella*[6], cannot be used with radish protoplasts because preliminary experiments showed that mechanical agitation resulting from the passage of air bubbles was in itself a cause of disruption of the plasmalemma of protoplasts. We therefore spread 10-ml aliquots of the protoplast suspension in T_0 with mannitol as the osmoticum in 6-cm Petri dishes. Prior to fumigation, the number of protoplasts present in the suspension is evaluated by counting them with a Levy-Neubauer hematocimeter. 10 separate counts are made per Petri dish, and the figures are averaged. Half of Petri dishes are then placed in the ozone fumigation chamber while the other half are left exposed to laboratory air. After the fumigation period, the protoplasts are again counted both in the experimental and the control dishes. Ozone is

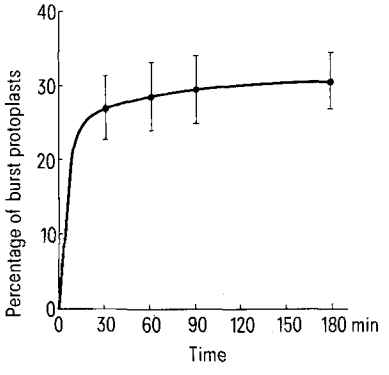

Fig. 1. Rate of bursting of protoplasts immediately after harvesting and washing.

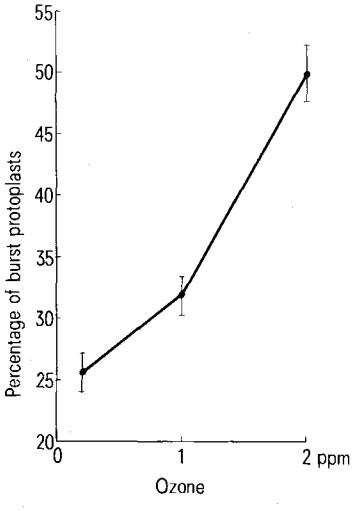

Fig. 2. Percentage of burst protoplasts resulting from a 30-min ozone fumigation at 0.2, 1 and 2 ppm in air.