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Inner Structures of the Cerebral Vesicle in the Ascidian Larva, *Styela plicata*: A SEM Study

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ABSTRACT—The inner structures of the cerebral vesicle in larvae of the ascidian, *Styela plicata*, were examined by scanning electron microscopy. The ocellus of this species is situated in the postero-dorsal wall of the cerebral vesicle. The ocellus is as degenerate as that of the other species of *Styela* which consists of only a single cell containing a small pigment body. The cell body of the statocyte was found to be attached to the ventral floor of the cerebral vesicle by two junctions. The cell body is hollowed between the junctions, forming an inverted pigment cup. Four types of protuberance were recognized in the cerebral vesicle. Three of these project from the ventral floor under the cell body of the statocyte. The distribution of these three types is restricted to the floor on the right side of the junctions of the statocyte. The fourth type of protuberance was always found as a pair on the left part of the postero-dorsal wall of the cerebral vesicle. Probable functions of these protuberances are discussed.

INTRODUCTION

Three types of sensory receptor (statocyte, ocellus and hydrostatic pressure receptor) have been reported in the cerebral vesicle of ascidian larvae after observations by transmission electron microscopy (TEM) [1–12].

The larva *Styela plicata* has a large statocyte, which protrudes from the mid-ventral wall of the cerebral vesicle and a considerably degenerate, small ocellus, which is situated in the postero-dorsal wall of the vesicle [13] like that of the other species of *Styela* [14–19]. Torrence ['80, Am. Zool., 20, 886. Abstract] has reported that the statocyte of *S. plicata* is a photolith, an organ sensitive to both light and gravity, similar to that found in the larvae of species belonging to the Botryllinae (colonial species of Styelidae). He reported that the statocyte in *S. plicata* forms an inverted pigment cup, which is invaded by globular protuberances arising from the ventral wall of the cerebral vesicle. The present author has confirmed his observation by light microscopy with serial paraffin sections [20]. Torrence [11] has also reported that in the cerebral vesicle of *S. plicata* two sensory endings, from which many narrow processes extend against the surface of the cell body of the statocyte, protrode from the left floor to the junction of the statocyte. He suggested that the sensory endings function to detect the movement of the statocyte. According to Reverberi, the hydrostatic pressure organs in *Styela* sp. are rudimentary [9] or absent [10].

Scanning electron microscopy (SEM) is an excellent means of studying three-dimensional fine structure. However, there has apparently been no SEM study on the structures of the sensory organs in the cerebral vesicle of ascidian larvae. The present paper describes the structures within the cerebral vesicle of *S. plicata* shown by SEM and their probable functions are discussed.

MATERIALS AND METHODS

Fertilized eggs of *Styela plicata* (Lesueur) were obtained by natural shedding of gametes from mature adults (4–7 individuals) in a plastic dish containing aerated natural seawater. The eggs were washed once with filtered seawater by settling and decantation. Hatching occurred about 12 hr after shedding at 20°C. Free-swimming larvae

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were fixed in 2% glutaraldehyde in 77% seawater [21] for 20–24 hr. The fixed larvae were soaked briefly in distrilled water, dehydrated in a graded ethanol series and stored in 80% ethanol below 4°C until used.

The stored materials were dehydrated and the ethanol-infiltrated larvae were cryofractured at liquid nitrogen temperature. The larvae, which were cut through their cerebral vesicle, were collected under a stereoscopic microscope and rinsed with M/30 phosphate buffer (pH 7.4) to remove precipitated debris in the cerebral vesicle with ethanol. The rinsed larvae were dehydrated again and dried with a critical-point dryer (Hitachi HPC-2) using CO_2 with isoamyl acetate as the transition fluid. The dried materials were mounted on electron-conductive tape (Sumitomo-3M) attached to an aluminium stub. They were coated by platinum-palladium usting an ion sputterer (Hitachi, E 102). All dried and coated materials were examined with a scanning electron microscope (Hitachi S-800).

RESULTS

The cerebral vesicle in the swimming larvae of *Styela plicata* was situated in the right side of the dorsal half of the trunk. Figures 1 and 2 show the structures in the right, posterior half of the cerebral vesicle exposed by a sagittal cut through the right side to the median plane of the larval trunk of *S. plicata*. An ocellar pigment body was seen in the postero-dorsal wall of the cerebral vesicle (Fig. 2). It consisted of a mass of small grains. Neither lens cells nor retinal cells were found around the pigment body.

The cell body of the statocyte was attached to the ventral wall of the cerebral vesicle, where it occupied a large space in the lumen (Figs. 1–3). The cell body of the statocyte was joined to the floor of the cerebral vesicle by two junctions (a and b in Figs. 3–6). When the cell body of the statocyte was accidentally lost during the preparation procedure, two scars were made by the cell body being torn off at its junctions (a and b in Figs. 4, 5). The two junctions of the statocyte were confirmed by an oblique section, which fortunately cut the statocyte near the junctions (Fig. 6). The anterior junction looked like a conical stalk (2 μm height, 2 μm base diameter), which extended some fibrous projections from its base to the surface of the floor (a in Figs. 4–6 and 9). The posterior junction (0.7 μm height) was shorter than the anterior one (b in Fig. 6).

Figure 6 also shows that the cell body of the statocyte developed an invagination to form an inverted pigment cup at its ventral surface between the two junctions.

Many protuberances projected from the ventral floor under the cell body of the statocyte (Figs. 2, 3). The protuberances were distributed on the ventral floor in the right-hand region of the junctions of the statocyte (Figs. 4, 5). These protuberances could be classified into three types (1–3 in Figs. 4 and 5); 1: globular (0.7–2.0 μm diameter), 2: tapered band (0.15–0.4 μm proximal width, 0.6–1.7 μm length), and 3: small tubular (0.13 μm diameter, 0.1–0.5 μm length).

The protuberances of type 1 were arranged in a cluster at the anterior position right of the anterior junction of the statocyte. Those of type 2 were distributed in a crescent-shaped area just right of the cluster of type 1. The type 3 protuberances were mostly limited in their distribution around the anterior and posterior junction of the statocyte. A network structure was seen beneath the ventral floor from which the protuberances extended (Figs. 2, 3).

The fourth type of protuberance (2 μm diameter) was always present in pairs on the left part of the postero-dorsal wall of the cerebral vesicle (4 in Figs. 6–8). They extended some fibrous projections to the inner surface of the cerebral wall and were loosely surrounded by small protuberances (Fig. 8) like those of type 3 on the ventral floor.

DISCUSSION

It has been generally accepted that the statocyte of ascidian larvae is unicellular and that the cell body of the statocyte is joined by its foot-piece in the ventral wall of the cerebral vesicle by only a single narrow junction [22]. This paper is the first report of a statocyte having two junctions. In the developing embryos of *Styela plicata*, the pigment mass of the statocyte was seen to consist of two

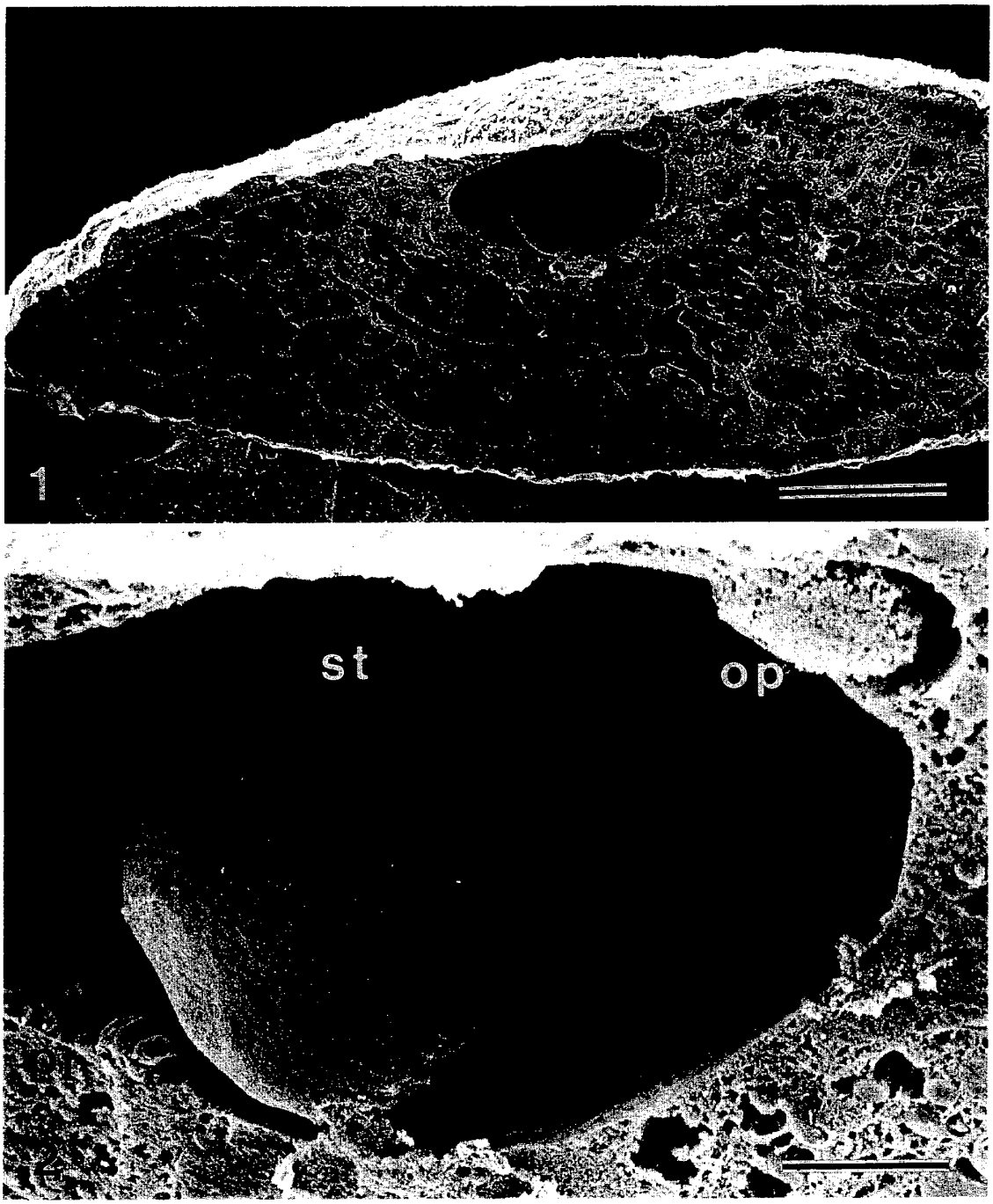

FIG. 1. Scanning electron micrograph of the larval trunk of *Styela plicata*, cut longitudinally through the right side to its median plane. Scale: 25 μm.

FIG. 2. Enlargement of Fig. 1, showing inner structures of the cerebral vesicle viewed from the left, slightly postero-dersal direction. op: ocellar pigment, pt: protuberances on the ventral floor, st: statocyte. Scale: 5 μm.

pigment blocks joined together from an early period of their pigmentation. The pigment blocks retain their conection during their growth period in subsequent development up to the swimming tadpole stage [20]. These observations suggest the possibility that the statocyte of the species consists of two cells. Another possibility is that one of the two junctions may be a sensory ending as reported by Torrence, since sensors for perception of statocyte movement [11] cannot be discarded. However, unlike his report, no narrow prosesses extended from the surface of both junctions, though many protuberances on the floor (type 3) encircled the base of each junction (Figs. 4, 5).

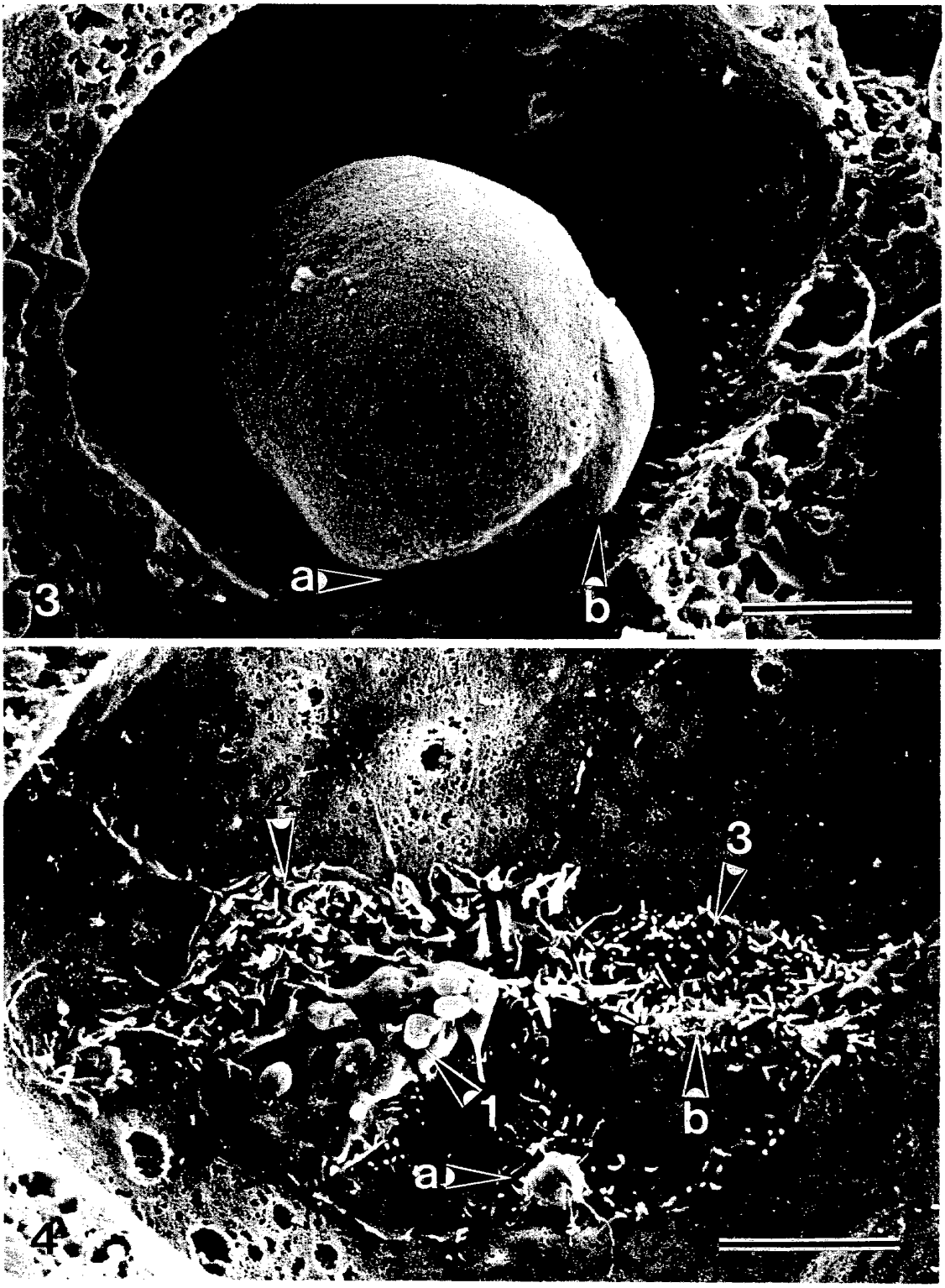

FIG. 3. Scanning electron micrograph of the cerebral vesicle, its left, dorsal wall having been cut off. Arrows (a) and (b) show the anterior and posterior junctions of the cell body of the statocyte, respectively. Scale: 5 μm.

FIG. 4. Scanning electron micrograph of the cerebral vesicle, its dorsal hemisphere having been cut off. Arrows (a) and (b) show scars of the anterior and posterior junctions of the statocyte at which its cell body was torn off. 1–3: type 1- type 3 protuberances on the ventral floor of the cerebral vesicle. Scale: 5 μm.

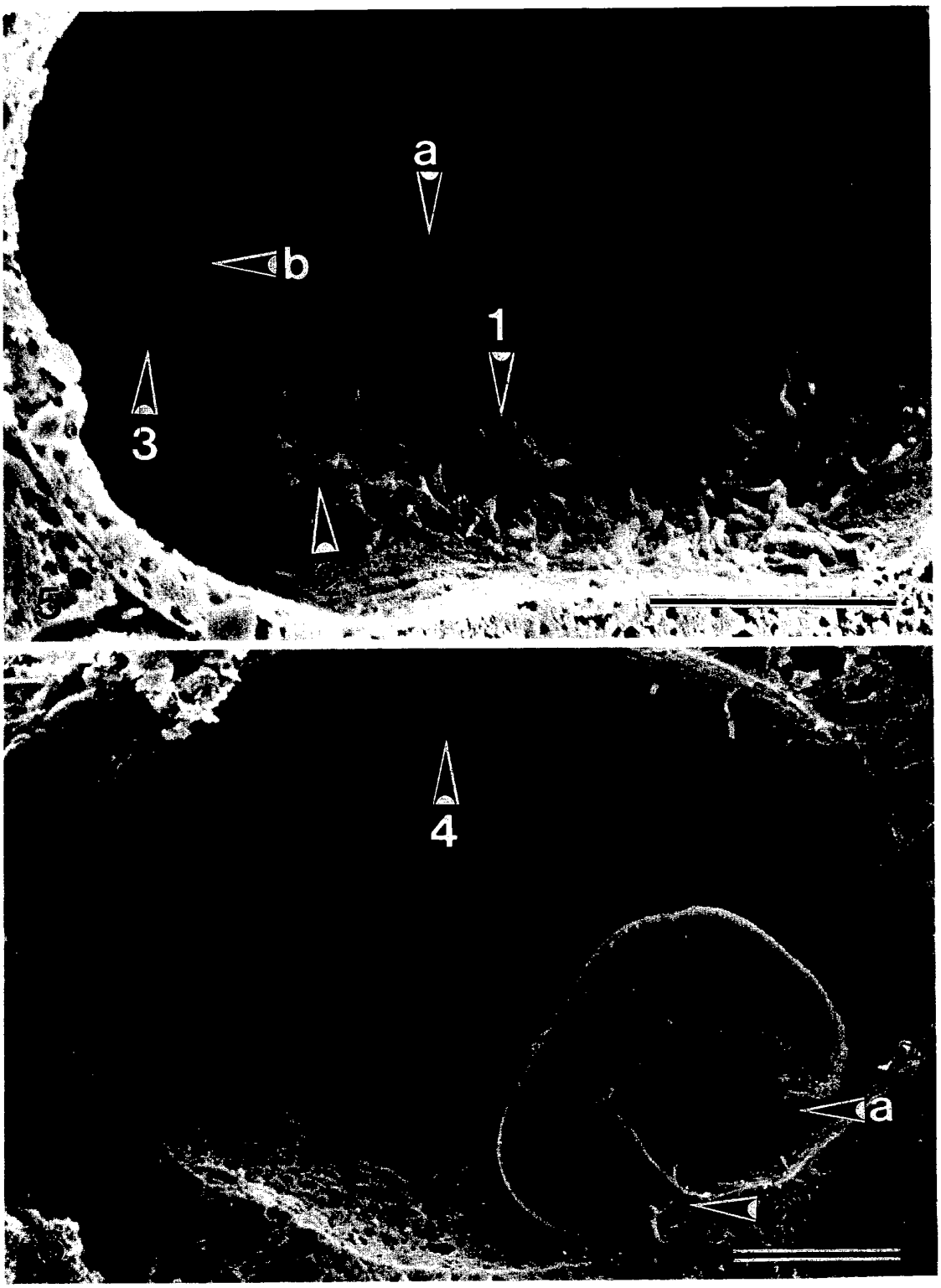

FIG. 5. Scanning electorn micrograph of the cerebral vesicle, its right, anterior part having been cut off. Arrows show scars of anterior (a) and posterior (b) junctions at which the cell body of the statocyte was torn off. Three types of protuberance (1–3) kare shown to the right of the junctions of the statocyte. Scale: 5 μm.

FIG. 6. Scanning electron micrograph of the cerebral vesicle, its right anterior half having been cut off near the anterior junction (a) and through the poster ior one (b). A pair of protuberances (4) are present on the left part of the postero-dorsal wall of the vesicle. Scale: 5 μm.

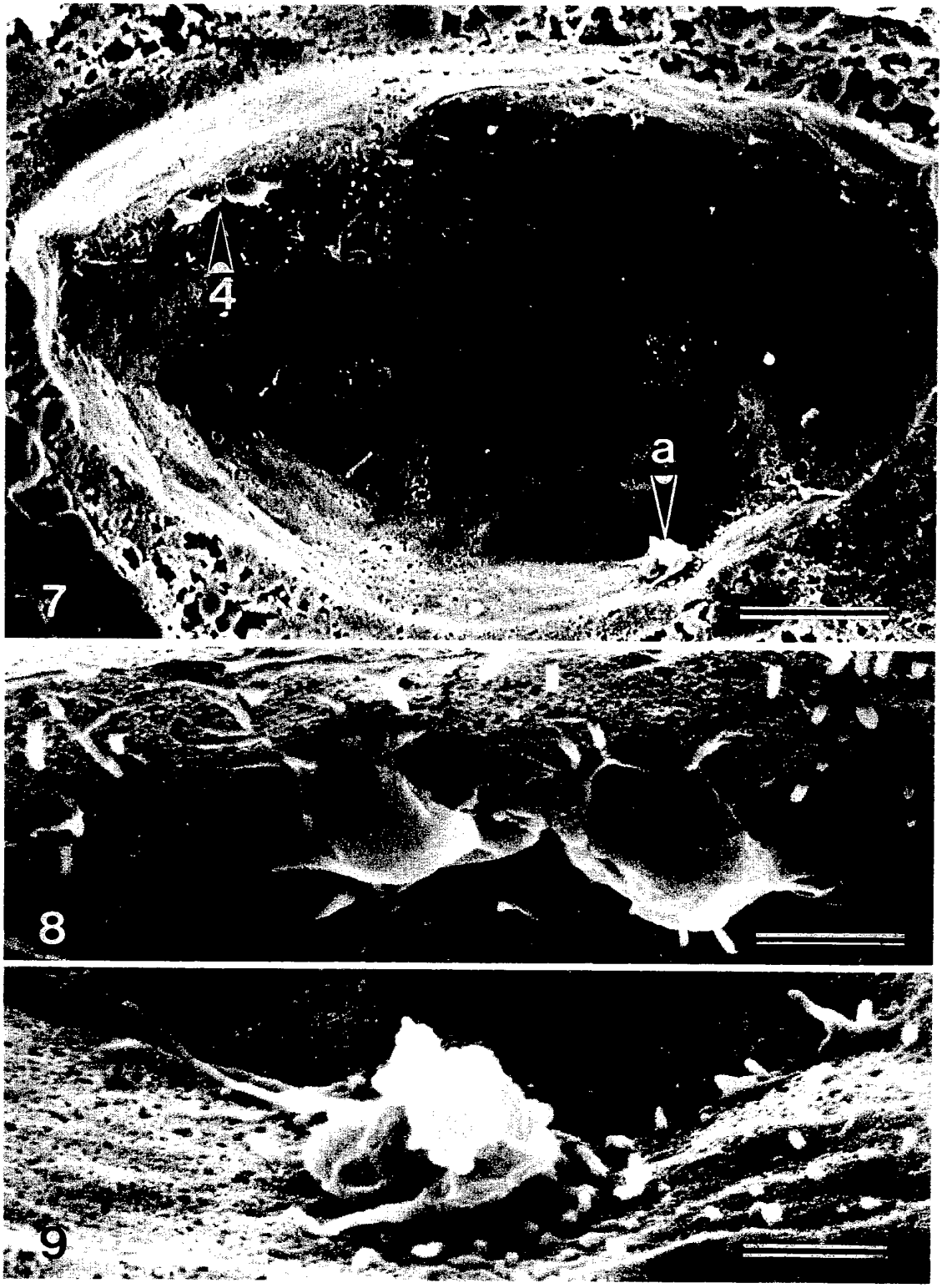

FIG. 7. Scanning electron micrograph of the cerebral vesicle, its right half having been cut off. a: anterior junction at which the cell body of the statocyte was torn off. 4: a pair of protuberances on the left part of the postero-dorsal wall of the vesicle. Scale: 5 μm.

FIG. 8. Enlargement of 4) in Fig. 7. Scale: 1 μm.

FIG. 9. Enlargement of a) in Fig. 7. Scale: 1 μm.

It was confirmed in this work that the ocellus of *S. plicata* is degenerate and consists of only a cell containing a tiny pigment body [13, 20] similar to that of the other species of the same genus [14–19]. In some other genera of Styelidae (Botryllinae), the larvae completely lose their ocellus. In those species, the statocyte changes to a "photolith", an organ sensitive to both light and gravity [14, 22–26]. The statocyte of *S. plicata* forms a hollow cup like the photolith (Fig. 6). In the photolith and the typical ocellus (consisting of lens cells, retinal cells and a pigment cell), light-sensitive cells project their fibrous endings into the pigment cup.

Globular protuberances extending from the ventral floor of the cerebral vesicle into the pigment cup of the statocyte have been shown in serial paraffin sections of the larvae of *S. plicata* [20]. The globular-type protuberances (type 1) in *S. plicata* are different from the sensory endings of the photoreceptive cells in either the photolith [12] or the typical ocellus [1, 2, 4, 6, 7] in that the type 1 protuberances lack fine processes at their apical surface. In the photolith of *Stolonica socialis* [14] and *Botryllus schlosseri* [25], photosensitive processes arise from the posterior wall of the cerebral vesicle where the retinal cells in the ocellus of Enterogona and Pyuridae are located. In contrast, the type 1 protuberances in *S. plicata* project from slightly anterior to the middle position of the ventral wall of the cerebral vesicle (Figs. 2–5). It remains unclear if the type 1 protuberances are really photosensitive.

The other types of protuberance (type 2–4) are newly found structures in the cerebral vesicle of *S. plicata*. The type 2 protuberances are arranged like many rows of fences making a right angle with the stream of fluid which may occur when the statocyte is bent at the two junctions by the rotation of the larva. It is possible that they play a role in the perception of the stream on the ventral floor of the cerebral vesicle.

The type 3 protuberances encircle the bases of both junctions of the statocyte. They may be identical with the fine processes protruding from dendrites which, as Torrence suggested, are responsible for sensing movements of the statocyte [11]. The present author agrees with him on the function of the type 3 protuberances, although unlike his report, they extend directly from the ventral floor of the cerebral vesicle.

The fourth type of protuberance (type 4) projects from the left, postero-dorsal wall of the cerebral vesicle in pairs (4 in Figs. 6–8). At an identical position in the cerebral vesicle, the larvae of *Ciona intestinalis* have a cluster of globular structures. These were first reported by Dilly [5] as a second type of photoreceptor in ascidian larvae. Eakin and Kuda [6] and Reverberi [9, 10] considered them to be sensitive to hydrostatic pressure, because their structures closely resemble those of the globular body of the cornet cells in other Chordates. According to Svane [8] and Reverberi [9], such globular structures are found at the dorsal wall of the cerebral vesicle in all species of Phlebobranchia (Enterogona). The latter author also found the globular structures at an auxiliary brain vesicle in two species of Pyuridae (Stolidobranchia, Pleurogona), *Pyura tessellata* and *Boltenia echinata*. He recognized the structural resemblance between the globular structures in the cerebral vesicle of the ascidian larvae and those of coronet cells, but he doubted their function as a pressure receptor.

The protuberances (type 4) in *S. plicata* (Styelidae, Stolidobranchia) and the globular structures in the other ascidian larvae are almost identical in size (2 μm in diameter), while the former is not so globular and does not have a stalk like the latter. The type 4 protuberances may thus be rudimentary structures of the globular bodies found in other species [9].

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REFERENCES

1 Barnes, S. N. (1971) Fine structure of the photoreceptor and cerebral ganglion of the tadpole larva of *Amaroucium constellatum* (Verrill). (Subphylum: Urochordata; Class: Ascidiacea). Z. Zellforsch., **117**: 1–16.

2 Dilly, N. (1961) Electron microscope observations of the receptors in the sensory vesicle of the ascidian tadpole. Nature, **191**: 786–787.

3 Dilly, N. (1962) Studies on the receptors in the cerebral vesicle of the ascidian tadpole. 1. The otolith. Quart. J. Micr. Sci., **103**: 393–398.
4 Dilly, N. (1964) Studies on the receptors in the cerebral vesicle of the ascidian tadpole. 2. The ocellus. Quart. J. Micr. Sci., **105**: 13–20.
5 Dilly, N. (1969) Studies on the receptors in *Ciona intestinalis*. III. A second type of photoreceptor in the tadpole larva of *Ciona intestinalis*. Z. Zellforsch., **96**: 63 –65.
6 Eakin, R. M. and Kuda, A. (1971) Ultrastructure of sensory receptors in ascidian tadpoles. Z. Zellforsch., **112**: 287–312.
7 Gorman, A. L. F., McReynolds, J. S. and Barnes, S. N. (1971) Photoreceptors in primitive chordates: Fine structure, hyperpolarizing receptor potentials, and evolution. Science, **172**: 1052–1054.
8 Svane, I. (1982) Possible ascidian counterpart to the vertebrate saccus vasculosus with reference to *Pyura tessellata* (Forbes) and *Boltenia echinata* (L.). Acta Zoo. Stockh., **63**: 85–89.
9 Reverberi, G. (1975) On the "third" sensory receptor of the ascidian tadpoles. Rend. Acc. Naz. Lincei, **58**: 945–948.
10 Reverberi, G. (1979) On the third sensorial organ of ascidian tadpoles. Acta Embryol. Exp., **1**: 91–99.
11 Torrence, S. A. (1986) Sensory endings of the ascidian static organ (Chordata, Ascidiacea). Zoomorph., **106**: 61–66.
12 Vorontsova, M. N. and Malakhov, V. V. (1984) Anatomy and fine structure of larvae in *Cnemidocarpa finmarkiensis* (Ascidia). 2. Sense organs. Zool. Zh., **63**: 1036–1045.
13 Hirai, E. (1958) Morphological observations of ascidian larvae. 4. *Styela plicata* (Lesueur). Bull. Mar. Biol. St. Asamushi, **9**: 21–22.
14 Berrill, N. J. (1948) The nature of the ascidian tadpole, with reference to *Boltenia echinata*. J. Morphol., **82**: 269–285.
15 Grave, C. (1944) The larva of *Styela* (*Cynthia*) *partita*: Structure, activities and duration of life. J. Morphol., **75**: 173–191.
16 Wallace, H. (1961) The breeding and development of *Styela mammiculata* Carlisle. J. Mar. Biol. Assoc. U.K., **41**: 187–190.
17 Whittaker, J. R. (1966) An analysis of melanogenesis in differentiating pigment cells of ascidian embryos. Develop. Biol., **14**: 1–39.
18 Whittaker, J. R. (1973) Tyrosinase in the presumptive pigment cells of ascidian embryos: Tyrosine accessibility may initiate melanin synthesis. Develop. Biol., **30**: 441–454.
19 Lützen, J. (1960) The reproductive cycle and larval anatomy of the ascidian *Styela rustica* (L.). Vidensk. Medd. Dansk. Naturh. Foren. Kbb., **123**: 227–235.
20 Ohtsuki, H. (1990) Statocyte and ocellar pigment cell in embryos and larvae of the ascidian, *Styela plicata* (Lesueur). Develop. Growth Differ. **32**: 85–90.
21 Tegner, M. J. and Epel, D. (1976) Scanning electron microscope studies of sea urchin fertilization. I. Eggs with vitelline layers. J. Exp. Zool., **197**: 31–58.
22 Berrill, N. J. (1975) Chordata: Tunicata. In "Reproduction of Marine Invertebrate II". Ed. by A. C. Giest and J. S. Pease, Academic Press, New York, pp. 241–282.
23 Berrill, N. J. (1949) The gonads, larvae, and budding of the polystyelid ascidians *Stolonica* and *Distomus*. J. Mar. Biol. Assoc. U.K., **28**: 633–650.
24 Abbott, D. P. (1955) Larval structure and activity in the ascidian *Metandrocarpa taylori*. J. Morphol., **97**: 569–593.
25 Grave, C. and Woodbridge, H. (1924) *Botryllus schlosseri* (Pallas): The behavior and morphology of the free-swimming larva. J. Molphol. Physiol., **39**: 207–247.
26 Grave, C. and Riley, G. (1935) Development of the sense organs of the larva of *Botryllus schlosseri*. J. Morphol., **57**: 185–211.