FOOD CAPTURE AND INGESTION IN THE LARGE HELIOZOA,  
ECHINOSPHAERIUM NUCLEOFILUM*

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SUMMARY

Using its microtubule-containing axopodia, a heliozoan Echinospaerium nucleofilum feeds on  
various kinds of protozoans and small metazoans. The present study revealed that food capture  
and ingestion were carried out in 2 different ways or by a combination of them. The first one  
was by the rapid contraction of axopodia, by which the food organism was conveyed directly  
toward the body surface. After such a contraction, many of the microtubules which had been  
present inside the axopodia degraded and were replaced by C-shaped microtubules. Bundles of  
tubular bodies were also detected alongside the axonemal microtubules, especially following  
the use of glutaraldehyde fixative containing ruthenium red. The second method was by means  
of axopodial flow, by which a food organism attached to an axopodium was conveyed to the  
body surface along the axopodial surface without accompanying axopodial degradation or  
contraction. Subsequently the food organism was surrounded by several small pseudopodia to  
form a food vacuole; many filamentous structures (5-10 nm in diameter) were observed inside  
the pseudopodia. During the ingestion process many cytoplasmic extensions, including rosary-  
like filaments, were observed to protrude from the contracted axopodia and the cell body.  
Mottled dense granules were observed to be discharged from the axopodial surface just when  
the prey was captured.

INTRODUCTION

The large actinophryid Echinospaerium nucleofilum usually feeds on various types of protozoans and small metazoans. During the process of food capture and subsequent ingestion, a large number of axopodia radiating from the spherical cell body have been considered to play an important role. However, there are few available descriptions concerning food capture and the ingestion mechanism in this organism, and in these some different types of feeding behaviour have been reported to occur (Tilney & Porter, 1965). In the related heliozoan Actinophrys sol, a rapid contraction of axopodia (more than 100 μm/s) was observed to occur when small flagellates were captured for ingestion (Ockleford & Tucker, 1973). Almost the same observation as in Actinophrys was made by Bardele (1975) in a centrohelid Heterophrys marina. The present study was undertaken, therefore, to examine the feeding behaviour of Echinospaerium, with particular reference to the function of axopodia in food capture and ingestion.

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A large number of observations have been made on the degradation and reformation of axonemal microtubules in heliozoans, revealing that the microtubules are conspicuously labile to various environmental factors (Roth, Pihlaja & Shigenaka, 1970; Shigenaka, Roth & Pihlaja, 1971; Tilney, Hiramoto & Marsland, 1966; Toyohara, Shigenaka & Mohri, 1978), and that the microtubules are involved in several fundamental functions such as cell locomotion (Suzaki, Shigenaka & Takeda, 1978a; Watters, 1968), cell division (Suzaki et al. 1978a), cell-to-substrate adhesion (Suzaki et al. 1978a) and cell fusion (Shigenaka & Kaneda, 1979). In this connexion, it was found here that food capture in heliozoans often resulted from rapid shortening or contraction of axopodia. A part of the present study, therefore, deals with the problem of axopodial contraction and the concomitant structural changes of microtubules during the process of food capture and ingestion. In addition to axopodial contraction, food ingestion was found to be accompanied by streaming of axopodial cytoplasm toward the cell body surface. This phenomenon is regarded as closely related to the saltatory movement of intra-axopodial particles, which has been shown to be one of the characteristic types of cell motility in heliozoan cells, though not hitherto discussed in relation to food ingestion (Edds, 1975a; Bardele, 1976). The possible mechanisms of both axopodial contraction and particle movement are also discussed in the present paper.

MATERIALS AND METHODS

Organisms

Live samples of *Echinosphaerium nucleofilum* were originally purchased from the Carolina Biological Supply Company (Burlington, North Carolina, U.S.A.) and cultured together with small protozoans using 0.01 % Knop solution as culture medium. Subculturing was carried out every 1 or 2 weeks. The food organisms were *Chilomonas paramecium* cultured in wheat infusion and *Tetrahymena thermophila* cultured axenically in aqueous solution of 2 % proteose peptone, 1 % yeast extract, and 0.6 % glucose.

Light microscopy

Prior to each experiment, the cells were put into a ring mounted on a slide (14 mm inner diameter and 2 mm high) filled with fresh culture medium and allowed to settle for at least 1 h. Light-microscopical observations were carried out with Nomarski differential interference optics. Photographs were taken with 35-mm film (Kodak, Panatomic-X). Axopodial contraction was recorded on 16-mm cine films (Kodak, 4-X) with a Bolex camera driven at 10 frames/s.

Electron microscopy

For transmission electron microscopy, the cells were fixed with glutaraldehyde and OsO4 by the method of Shigenaka et al. (1971), dehydrated through a graded ethanol series, and embedded in Spurr's low viscosity embedding medium (Spurr, 1969). In some experiments, the routine glutaraldehyde fixative was replaced by glutaraldehyde fixative containing ruthenium red (0.5 mg/ml).

Ultrathin sections were stained with 3 % uranyl acetate aqueous solution for 10 min and lead citrate stain (Reynolds, 1963) for 5 min, and examined under a JEOL JEM-100S electron microscope operating at 100 kV. For scanning electron microscopy, the cells were fixed as described above and then freeze-dried by the method of Suzuki, Shigenaka, Toyohara & Otsuji (1978b). In heliozoan cells, such a freeze-drying procedure was found to preserve them much better than the critical-point drying technique. The freeze-dried specimens were coated
with carbon and gold, and examined with a JEOL JSM-T-20 scanning electron microscope operating at 20 kV.

RESULTS

In *Echinosphaerium*, food capture and ingestion are divided into the 3 successive stages: (i) adhesion of a prey organism to the axopodial surface, (ii) conveyance of the prey toward the body surface by means of axopodial contraction or flow as described below, and finally (iii) enclosure of the prey by a newly formed food vacuole derived from small pseudopodia. These are schematically shown in Fig. 1.

Fig. 1. A schematic drawing of food capture and the ingestion process in *Echinosphaerium nucleofilum* when fed with *Tetrahymena thermophila*. The prey attached to the axopodial surface is conveyed to the cell body either by axopodial contraction (A–D) or by axopodial flow (A′–D′), being enclosed by newly formed pseudopodia which are transformed finally into a food vacuole.

Adhesion of the prey organism to axopodial surface

The prey organisms were found to adhere to any region of the cell surface which they touched. In consequence, the ability to catch prey is considered not to be a special characteristic of the axopodial tip. However, ciliates adhering to the heliozoan cell
Fig. 2. A series of light-micrographs showing the heliozoan cell which feeds on *Tetrahymena*. A, B, C, D, E, F, were taken at 0, 10, 30, 40, 50, and 140 s, respectively. See text for details. x 110.
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surface seemed not to be paralysed but continued to move actively by ciliary beating; at this time, they occasionally succeeded in escaping and swimming away from the predator in a normal swimming manner. Prey organisms, furthermore, were observed to maintain their motility for a while even after they were taken into newly formed food vacuoles, suggesting that they were not yet paralysed.

Fig. 3. A series of light-micrographs taken from a 16-mm cine film (10 frames/s), showing a food organism being conveyed to the proximal region of an axopodium by axopodial contraction (arrows in b and c). x 80.

Conveyance of prey to the body surface

The prey organism adhering to an axopodium was found to be conveyed to the proximal region of the axopodium by one of the following 2 ways or by a combination of them. The first one is termed 'axopodial contraction' and is characterized by rapid contraction of the axopodium to convey the prey to the cell body surface (Fig. 1A-D; Fig. 2A, arrow). This phenomenon was usually observed to occur instantaneously when the prey attached to the tip of an axopodium. In this case, the axopodia always contracted to less than one third of their initial length, which drew the prey toward the body surface. Complete contraction of axopodia was observed only when the axopodia had fully previously elongated to their full length. When a heliozoan was transferred by pipetting, it took more than 30 min before the axopodia became capable of contracting, since they became tangled and/or were partially degraded by pipetting. At the end of the recovery process, all of the axopodia elongated to their full extent and thereafter spontaneous axopodial contraction occurred at random intervals. To measure the velocity of axopodial contraction, food-capture was recorded with a 16-mm cine camera driven at 10 frames/s. Fig. 3 represents a series of frames, revealing that the whole process of axopodial contraction occurred within 100 ms. Since the full length of an axopodium varies from 200 to 300 μm, the velocity of axopodial contraction is more than 2 mm/s.

When the axopodium was touched with the tip of a glass or metal needle, such axopodial contraction could not be induced. On the other hand, carmine particles coated with albumen were found to induce axopodial contraction just as with living prey organisms, and they were subsequently ingested into the newly formed food
Figs. 4, 5. Scanning electron micrographs of heliozoans.

Fig. 4. A non-feeding organism with fully extended axopodia. $\times 300$; bar, 100 $\mu$m.

Fig. 5. A fully fed organism in which the supporting axopodia are indicated with arrows. $\times 420$; bar, 100 $\mu$m.
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vacuoles. From these results, it appears that certain chemical receptors might be present on the axopodial membranes which recognize prey organisms. Scanning electron micrographs shown in Figs. 4 and 5 represent a control organism and a fully-fed one surrounded by some tens of food organisms (*Tetrahymena thermophila*).

The second method of food conveyance is termed ‘axopodial flow’, by which the prey attached to an axopodium is transported toward the cell surface by means other than axopodial contraction (Fig. 1A–D; Fig. 2, arrows in D–F). Unlike the first way, this process was observed mainly when the prey organisms attached to the middle or

![Fig. 6. Light micrograph showing formation of several swellings or beads of axopodial cytoplasm (arrows). This was taken about 30 s after the prey, *Tetrahymena* (t), attached to the axopodium. × 280; bar, 100 μm.](image)

nearly proximal regions of axopodia. Furthermore, this was also found to occur just after the axopodia were disturbed by pipetting. Some 10 s after the prey adhered to an axopodium, the axopodial cytoplasm just beneath the point of prey attachment started to stream toward the cell body together with the prey organism; the small intra-axopodial particles in this region, which had previously been moving up and down in a saltatory manner, began to move simultaneously in the same direction and continued to do so for a few minutes. On the other hand, the particles located between the axopodial tip and the point of prey attachment continued to move up and down for a while; they accumulated gradually at different points, resulting in several swellings or beadings along the length of the axopodia (Fig. 6, arrows). These swellings appeared to be formed with relatively definite spacings between them and they continued to exist for about 1 min, exhibiting little particle movement inside them.
After that, the intra-axopodial particles began to move again in saltatory fashion and the swellings disappeared. If the axopodia contracted partially just when the prey organism was caught, the prey was found subsequently to be conveyed toward the cell surface by means of axopodial flow. The 2 ways of food-capture may thus be combined.

Food vacuole formation

The prey organisms conveyed to the cell surface by axopodial contraction and/or flow were found to be surrounded by petal-like pseudopodia (Fig. 2C, arrows) protruding from the proximal region of a contracted axopodium or the cell body surface. These pseudopodia subsequently formed a food vacuole. The inclusions of these pseudopodia were observed to be derived directly from the contracted axopodium whose contents flowed down toward the body surface together with the captured prey organisms. Furthermore, it was found that prey organisms initially attached to the proximal regions of axopodia were not always surrounded by such pseudopodia but could be ingested directly into food vacuoles (asterisks in Fig. 2B–E). This is probably due to the fact that the plasma membrane and the cytoplasmic materials were not sufficient to construct the new pseudopodia. If the heliozoan was supplied with sufficient food organisms, food vacuoles were formed in rapid succession; the heliozoan was finally covered with many food vacuoles, leaving few axopodia, as many of them had contracted to ingest the prey (Figs. 2F, 5). If the prey was captured by axopodial flow, on the other hand, the number and length of axopodia remained as they were even after many food vacuoles had been formed. Under these circumstances, the axopodia were observed not to be immediately able to catch further prey.

Within 1 h after the prey was captured, the newly formed food vacuole moved from ectoplasm to endoplasm, followed by gradual re-extension of a new axopodium. After that, it appeared to take about 2–3 h to acquire the capacity for food capturing.

The above-mentioned results relate to food capture and ingestion when organisms were supplied with *Tetrahymena* as food. However, the method of food-capture was found to vary according to the species of prey: *Chilomonas, Paramecium,* and *Stentor* were captured only by axopodial flow. On the other hand, *Chlamydomonas* and *Tetrahymena* were captured mainly by axopodial contraction but occasionally by axopodial flow.

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**Figs. 7–9.** Electron-micrographs of cross- and longitudinal sections through heliozoan axopodia.

Fig. 7. Cross-sectioned axopodium of an organism that was fixed with glutaraldehyde containing ruthenium red and subsequently with OsO₄. × 93,000; bar, 200 nm.

Fig. 8. Longitudinally sectioned axopodium of the same organism as in Fig. 7. × 114,000; bar, 500 nm.

Fig. 9. Cross-sections through 2 axopodia of the organism fixed during the food ingestion process, which is here occurring by means of axopodial flow. × 114,000; bar, 200 nm. ax, axoneme; mg, mottled dense granule; tx, tubular X body; x, X-body.
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7

mg

8

tx

ax

9

ax
Ultrastructural changes accompanied by food capture and ingestion

By addition of ruthenium red, the glutaraldehyde fixative enabled us to detect another type of fibrous structure, different from microtubules, inside the axopodia (in Figs. 7, 8). These were tubular filaments (about 10 nm thick) arranged in bundles. Since these filaments were found to be continuous with the so-called X body reported by Shigenaka & Kaneda (1979), especially at the region of the axopodial base, the 2 are collectively termed here the ‘tubular X body’; if the fixative contained no ruthenium red, they were not preserved or detected. It is not clear why this was so. Because other cell organelles are well preserved, however, this newly found structure is considered not to be the artefact caused by the presence of ruthenium red in the fixative. As described elsewhere, a layer staining with ruthenium red is present just outside the axopodial membrane (Figs. 7, 8), which is known to be digested by trypsin treatment (300 µg/ml for 4 h at pH 7.5 and 30 °C) (Shigenaka, Maruoka, Toyohara & Suzaki, 1979). It was also observed here that food organisms were not captured and ingested by cells that had been treated with trypsin (300 µg/ml) for 3 h. Fig. 9 is an electron micrograph showing cross-sections through 2 axopodia, one of which is at the level of the previously mentioned bead-like structure formed during food ingestion. Inside the bead there are a number of X bodies, in addition to electron-dense granules, vesicles and other inclusions.

In axopodia that had contracted following food capture, the axonemal microtubules were found to have largely disassembled; they were fewer in number and as a consequence the characteristic double-helical arrays were disturbed (Fig. 10, ax). Furthermore, C-shaped microtubules, intermediate products of microtubule degradation, were always observed inside the contracted axopodia and cell body (Fig. 11, arrows). However, no other intermediate structures (e.g. macrotubules or tubular filaments (Toyohara et al. 1978)) were detected around them.

Many protuberances (Fig. 10, pr) were observed to extrude from the plasma membranes of contracted axopodia; they varied from 10 to 100 nm in diameter and typically resembled a rosary (string of beads). They were limited by a unit membrane which was continuous with the plasma membrane of the axopodia and cell body. In negatively stained specimens, the rosary-like protuberances appeared to display a periodicity of about 30 nm along their length (Fig. 12). The total length of individual protuberances was hard to measure because of their complicated tangling, but it seemed to be more than 3 µm.

Just under the plasma membrane of both axopodia and cell body, many granules (electron-dense or electron-opaque granules) are already known to be present (Tilney & Porter, 1965). These granules, which are membrane-bound, were found to vary in size and shape and to be of 2 types. The first one was 65 to 380 nm in diameter and uniformly electron-dense (eg in Figs. 10, 13, 14); the second was larger, varying from 200 to 600 nm in diameter, less electron-dense than the other, and sometimes mottled with electron-opaque and electron-lucent areas (mg in Figs. 13, 14). The latter mottled granules were found to expel their contents into the surrounding medium when food organisms were captured; this phenomenon was observed on both axopodia and cell
Figs. 10-12. Electron micrographs of feeding organisms. 

Fig. 10. Cross-section through the axopodium. × 32000; bar, 1 μm.

Fig. 11. Highly magnified picture of the area shown in Fig. 10, in which arrows indicate C-shaped microtubules. × 167000; bar, 100 nm.

Fig. 12. Negatively stained specimen showing rosary-like protuberances. × 130000; bar, 200 nm.

(ax: axoneme; cg: condensed dense granule; pr: protuberance.)
Figs. 13, 14. Electron micrographs of longitudinal and cross-sections through axopodia which have captured prey (*Tetrahymena*). *ax*, axoneme; *c*, cilia of *Tetrahymena*; *cg*, condensed dense granule; *mg*, mottled dense granule; *mt*, mitochondrion. × 60000; bar, 200 nm, in both figures.
Figs. 15, 16. Electron micrographs of sections through a newly forming food vacuole (Fig. 15) and the pseudopodium protruded from the proximal region of a contracted axopodium (Fig. 16). cg, condensed dense granule; f, fluffy filaments; pr, protuberance; ps, pseudopodium; t, Tetrahymena. Fig. 15. × 4400; bar, 5 µm. Fig. 16. × 42500; bar, 1 µm.
body. On the other hand, the former condensed granules were never observed to be discharged to the surrounding medium.

Figs. 15 and 16 are electron micrographs of the petal-like pseudopodia which surround the food organism and subsequently form food vacuoles. Below the plasma membranes of pseudopodia, there could be observed many electron-dense granules, mitochondria, and small vesicles just as in the axopodia and the cortex of the cell body. In addition, many fluffy filaments, 5-10 nm in diameter, and oriented at random were always observed inside the pseudopodia (Fig. 16, f). A number of protuberances were also observed to extend from the plasma membrane of pseudopodia.

**DISCUSSION**

**Adhesion of prey organisms to axopodia**

The present study demonstrated that food capturing was initiated by attachment of a prey organism to the distal or middle region of an axopodium, followed by formation of cytoplasmic protuberances and discharge of the mottled dense granules. The functions of the electron-dense granules are still unknown, but it has been suggested by Tilney & Porter (1965) that they contain enzymes and/or substances which assist in quieting the prey organism or fastening it to the axopodia. In the axopodia of other heliozoans, conicysts in *Heterophrys* and muciferous bodies in *Ciliophrys* are thought to be indispensable for prey-capturing since the number of these granules decreases conspicuously during feeding (Davidson, 1976). From the fine-structural viewpoints, the condensed or mottled granules in *Echinosphaerium* appear to be very similar to mature or nearly mature muciferous bodies in *Ciliophrys*, respectively. It is probable, therefore, that the condensed or mottled granules also function in food-capturing. Since the prey organisms stop their flagellar movement and appear to be paralysed within a few seconds of becoming attached to the axopodia of *Heterophrys*, it is assumed that the conicysts and the muciferous bodies contain certain kinds of paralysing substances (Davidson, 1976). In *Echinosphaerium*, on the other hand, prey organisms adhering to an axopodium or ingested into a food vacuole did not appear to be paralysed but continued their active ciliary beating. Hence, the contents of the mottled granules may contain not a paralysing but an adhesive substance.

In the present study, it was demonstrated that protuberances were formed from the surface of axopodia and cell body. Although their mode of functioning remains uncertain, it is most likely that they act by catching or twining around some parts of the prey organism (e.g. cilia). Similar protuberances, including rosary-like ones, have been reported in the same species to be protruded from the degrading axopodia of fusing organisms (Shigenaka & Kaneda, 1979). In this case, there are 2 possible explanations of the formation of protuberances: either the axopodial membrane, loosened as a result of axopodial degradation, might protrude passively, or the protuberances might be formed actively during cell fusion. Since similar protuberances were observed even when the prey was captured without axopodial degradation, it is considered that they were not formed as the result of membrane loosening but were produced actively to some extent. In membranelles of *Tetrahymena* and *Stentor*, many
protuberances are known to extend from the ciliary surface (Shigenaka, 1963; Randall & Jackson, 1958); they are limited by unit membranes which are continuous with the ciliary membrane and exhibit a rosary-like appearance, with a maximum diameter of about 30 nm (Watanabe et al. in preparation). These structures are thought to play an important role in integrating the cilia composing each membranelle, to make it a functional entity (Shigenaka, 1963). Likewise, the protuberances in *Echinosphearia* may play some role in intertwining with the partner's axopodia during cell fusion or with the cilia of a prey organism upon food capture.

**Axopodial contraction**

It is known that the axopodia of other heliozoan species such as *Heterophrys*, *Ciliophrys*, and *Actinophrys* are contractile. Especially in *Heterophrys*, axopodial contraction can be induced by physical and chemical stimuli, and also by exposure to a microflash. In food capture single axopodia contract individually when a prey organism is captured. Upon mechanical stimulation, the axopodia (about 50 μm in length) contract in about 20 ms. This can be inhibited with Ca²⁺-free seawater but not by treatment with cytochalasin B (Davidson, 1973). In *Ciliophrys*, all axopodia contract simultaneously within a fraction of second when the organism receives a mechanical shock. Axopodial contraction does not occur in Ca²⁺-free seawater (Davidson, 1969). In *Actinophrys*, axopodial contraction involved in capture and ingestion of certain small flagellates takes place within a second from a normal length of 120–170 μm to a length of 20–50 μm. In this case, axopodial contraction is known not to be induced by mechanical stimuli (Ockleford & Tucker, 1973). It was demonstrated here that *Echinosphearia* also exhibited complete axopodial contraction within 100 ms on food capture. This axopodial contraction appears to be similar in nature to that in *Actinophrys*, rather than in *Heterophrys* and *Ciliophrys*, because the contractions were not induced by any mechanical stimuli. In all of these species, however, the axopodial contraction always appears to be related closely to food capture.

Although axopodial contraction is evoked by various environmental factors other than food organisms, the similarity in contraction strongly suggests that it may be produced by identical mechanisms. With regard to the contractile elements in other protozoan cells, myonemes and spasmonemes are known to be present, in which the velocities of contraction are reported to be 10–20 cm/s for the former (Newman, 1972) and 23 mm/s for the latter (Jones, Jahn & Fonseca, 1970). Although Stephens & Edds (1976) suggested the possibility that the contractile heliozoan axopodia might also contain structures similar to the contractile elements in ciliates, no special structures have so far been reported which could be candidates for them. It was suggested by Davidson (1974) that neither depolymerization of microtubules nor alteration of surface tension produced the contractile force in *Heterophrys*, since cold- or chemically-induced microtubule depolymerization caused a loss of axopodial rigidity but did not result in contraction. In *Echinosphearia*, the slow shortening induced by cold or colchicine treatment is not regarded as contraction at all, and the 2 processes are regarded as having different character and mechanism. Both processes must involve microtubule disassembly; one quick, one slow. But the fast process could be caused by
a contractile event involving an associated structure. When treated with low temperature (2 °C), it takes about 3 h to attain one tenth of the initial axopodial length in *Echinosphaerium* (Toyohara et al. 1978). Although these results do not exclude the possibility that axopodial contraction is caused by microtubule degradation or alteration of surface tension, it seems reasonable to consider other mechanisms which cause axopodial contraction for the following reasons. (i) As demonstrated by Edds (1975b), the isolated cytoplasm of *Echinosphaerium* is contractile and contains many filaments, including actin filaments. (ii) By observing whole-mounted and negatively stained specimens, many randomly oriented filamentous structures are detected inside the cytoplasmic projections protruding from the axopodia of *Echinosphaerium* (Toyohara, Suzaki, Watanabe & Shigenaka, 1979). (iii) The possibility that actin filaments might disintegrate with routine osmium tetroxide fixation has been suggested by Maupin-Szamier & Pollard (1978). (iv) As shown in the present study, the tubular X body might be a strong candidate for inducing axopodial contraction.

**Axopodial flow**

The streaming of axopodial cytoplasm with a captured food organism is related closely to the movement of intra-axopodial particles; such particles, including mitochondria, dense granules, and many small vesicles, usually move up and down in a saltatory manner just beneath the axopodial membrane (Bloodgood, 1978; Edds, 1975a; Shigenaka, Watanabe & Kaneda, 1974). The axopodial flow that results in conveying the prey to the cell body is considered to be a result of unidirectional movement of axopodial material initiated by attachment of the prey organism. The beads formed in the distal region of the axopodium are also considered to be the result of this unidirectional particle movement.

Saltatory movement of axopodial particles, which has been also reported in *Heterophrys*, *Raphidiophrys*, and *Acanthocystis* (Bardele, 1976; Troyer, 1975), is generally thought not to be related to the axonemal microtubules for the following reasons. (i) In artificial axopodia formed with glass needles, bidirectional particle motions occur which are similar to those in normal axopodia (Edds, 1975a). (ii) The motions of particles within either normal or artificial axopodia are not interrupted by treatment with colchicine (Edds, 1975a; Tilney, 1968). (iii) Particle movements are also observed in colchicine-induced knobs of cytoplasm (Tilney, 1968). (iv) In *Heterophrys*, the movements of axopodial particles, called conicysts, appear to depend upon a specialized fibrillar structure attached to the outer surface of the axopodial membrane (Davidson, 1976). In addition, some extracellular markers such as adherent bacteria and gold particles also undergo salination, as well as the intracellular conicysts (Troyer, 1975). From these observations, the plasma membrane, rather than the axonemal microtubules, is thought to be involved in particle saltation. It has been postulated by Bardele (1976) that an actomyosin-like system might be involved in the saltatory movement of centrohelidian conicysts. He also has noted that the difficulty of detecting such a system in the axopodium does not seem to be a question of adequate fixation but may rather be a question of the aggregate size of the contractile protein. In the present study, many fluffy filaments were observed inside the food-
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Vacuole-forming pseudopodia. These filaments appear to resemble the fibres that were reported inside the cell body following treatment with hydrostatic pressure (Tilney et al. 1966). However, the fluffy filaments reported here are thought not to be concerned with microtubule degradation, because they were observed even if food ingestion was carried out without any axopodial shortening. As the pseudopodia move quite actively, these filaments are most likely to correspond to some contractile elements (probably actin filaments). They are similar to those reported by Edds (1975b) in the isolated cytoplasm of Echinosphaerium.

In the present study, we found another kind of filamentous structure (tubular X body) inside the axopodia. In addition to the axopodial membrane itself and the unidentified actomyosin system, this may be another candidate for both particle movement and axopodial contraction. A similar structure has been reported by Tilney & Porter (1965) who termed it an 'excretion granule' because of a close resemblance to the excretion product of insects (brochosomes). There is no direct evidence, however, to indicate that the X body is involved in excretion. Indeed, judging by its morphology and distribution, it seems unlikely that it is involved in excretion.

Microtubule degradation accompanied by food-capturing

Since Kitching (1964) demonstrated that the axonemes of heliozoan axopodia were composed of many microtubules arranged in a characteristic double-helical manner, many investigations have been carried out on the degradation and reformation of axonemal microtubules. In the present study, axopodial shortening was shown to occur during the process of food capture and ingestion. Electron-microscopical survey of partially degraded axonemes revealed that many C-shaped microtubules appeared, just as after treatment with heavy metal ions (Roth & Shigenaka, 1970), dilute urea (Shigenaka et al. 1971), or higher concentrations of calcium ions (Schliwa, 1976; Shigenaka, Tadokoro & Kaneda, 1975). During the cell fusion process, on the other hand, another type of intermediate structure (tubular filament) has been observed in axopodia which are just breaking down, located between the 2 participant organisms (Shigenaka & Kaneda, 1979). The velocities of axopodial shortening in these 2 different cases are distinctly different: more than 2 × 10^5 μm/s in axopodial contraction associated with food capture, but about 6 × 10^{-2} μm/s during the cell fusion process (Toyohara, Maruoka & Shigenaka, 1977). From these results, it is presumed that the degradation mechanism of microtubules might be different in the two cases, resulting in the appearance of different degradation products. In addition, it was found that microtubule degradation occurred even inside the cell body. This might imply that the effect of certain intracellular signals which cause microtubule degradation may not be restricted to the intra-axopodial cytoplasm but extend to the region of the cell body.

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