THE FINE STRUCTURE AND FUNCTION OF THE TENTACLE IN *TOKOPHRYA INFUSIONUM*

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ABSTRACT

The feeding apparatus of Suctoria consists of long, thin, stiff tubes called tentacles. When a swimming prey attaches to the tip of the tentacle a number of events follow in rapid succession. The tentacle broadens, a stream of tiny granules starts to move upward at its periphery to the tip, the prey becomes immobilized and shortly thereafter the cytoplasm of the still living prey begins to flow through the center of the tentacle to the body of the predator. An electron microscope study of the tentacle in *Tokophrya infusionum*, a protozoan of the subclass Suctoria, has disclosed a number of structural details which help to clarify some of the mechanisms involved in this unusual way of feeding. Each tentacle is composed of two concentric tubes. The lumen of the inner tube is surrounded by 49 tubular fibrils most probably of contractile nature. In the inner tube the cytoplasm of the prey is present during feeding, and in the outer tube are small dense bodies. It was found that the dense bodies originate in the cytoplasm of *Tokophrya*. They have an elongate, missile-like appearance, pointed at one end, rounded at the other, and are composed of several distinct segments. At the tip of the tentacle they penetrate the plasma membrane, with their pointed ends sticking out. It is assumed that the missile-like bodies play a major role in the feeding process. Their composite structure suggests that they might contain a number of enzymes which most probably are responsible for the various events preceding the actual food intake.

INTRODUCTION

One of the most difficult and at the same time most interesting and intriguing problems in protozoology is the feeding mechanism in Suctoria. The name of the group stems from suction, the supposed way of feeding through extremely fine, stiff, and long tubes called tentacles. To the tips of the tentacles the living prey attaches and its content then passes through the tentacles into the body of the predator. This peculiar way of feeding attracted wide attention among early students of Protozoa. In recent years Kitching (17), Canella (2) and Hull (13, 14) have attacked this problem again and tried to explain it by experiments and calculations. In spite of repeated efforts, the problem of how the prey gets attached to the tentacles and how its content is drawn into the body of the predator has remained still far from being fully explained. Most investigators agree that suction actually takes place in feeding, and a number of diverse hypotheses have been proposed to account for the processes creating it. The oldest hypothesis ascribed suction to a pumping action of the tentacle (12). Others have attributed it to: an increase of the vacuolar output (5); peristaltic waves of contraction along the lumen of the tentacle (18); an increase of body volume of predator at the start of feeding and resulting in lowering of its internal pressure, as first postulated by Penard (25) and supported later by others (2, 14, 17). Also, one hypothesis rejected suction in the physical sense and accepted instead an increase in prey's pressure as the driving force for feeding (15). All the hypotheses are assembled and reviewed in Canella's extensive monograph (2).

Feeding in *Tokophrya* and most Suctoria proceeds in two stages. The first could be described as preparatory and precedes food intake, the second as the actual flow of prey's cytoplasm into the body of the predator. It is the latter that has attracted most attention. Both stages are equally important, for proper understanding of the first is a prerequisite for understanding the second.

It is the purpose of this paper to elucidate further the preparatory stage in feeding. The small dimension of the tentacle, on the average 2 μ in diameter, has limited the observations on its behavior during feeding. Information pertaining to the structure of the tentacle and particularly of its tip, since this is the only part where attachment to prey occurs, was obviously essential for this task. It was contemplated that the fine structure of the feeding and non-feeding tentacle might provide clues to a better understanding of the mechanisms involved.

MATERIAL AND METHODS

Tokophrya infusionum is a small, sessile, fresh water protozoan (20 to 50 μ in diameter). It belongs to the subclass Suctoria (46), a group of Protozoa possessing highly specialized functions and apparently closely related to the holotrichous ciliates. The most characteristic features of the whole group are the manners of feeding and reproduction. *Tokophrya* feeds only on living ciliates, the content of which is drawn in by tentacles. It reproduces by endogenous budding, forming a succession of ciliated embryos. After leaving the parent body, they swim in the surrounding medium for several minutes to several hours, then finally stop moving and become attached to the substrate by the formation of a disc and stalk. The cilia disappear, tentacles start to grow, and in 5 to 10 minutes the embryo undergoes a complete metamorphosis from a motile to a sessile form showing all the characteristics of the adult organism (37).

Tokophrya infusionum has been kept in bacteria-free cultures in the laboratory since 1948. The first strains were obtained through the courtesy of Dr. Daniel M. Lilly. Most of the present work was performed on a strain found in 1959 in a pool at The Rockefeller Institute. The cultures have been maintained in screw-capped tubes in 0.5 per cent yeast medium (20). The food organism, *Tetrahymena pyriformis*, grown axenically in a proteose-peptone agar medium was introduced into cultures of *Tokophrya* twice a week and subcultures of both organisms were made once a week.

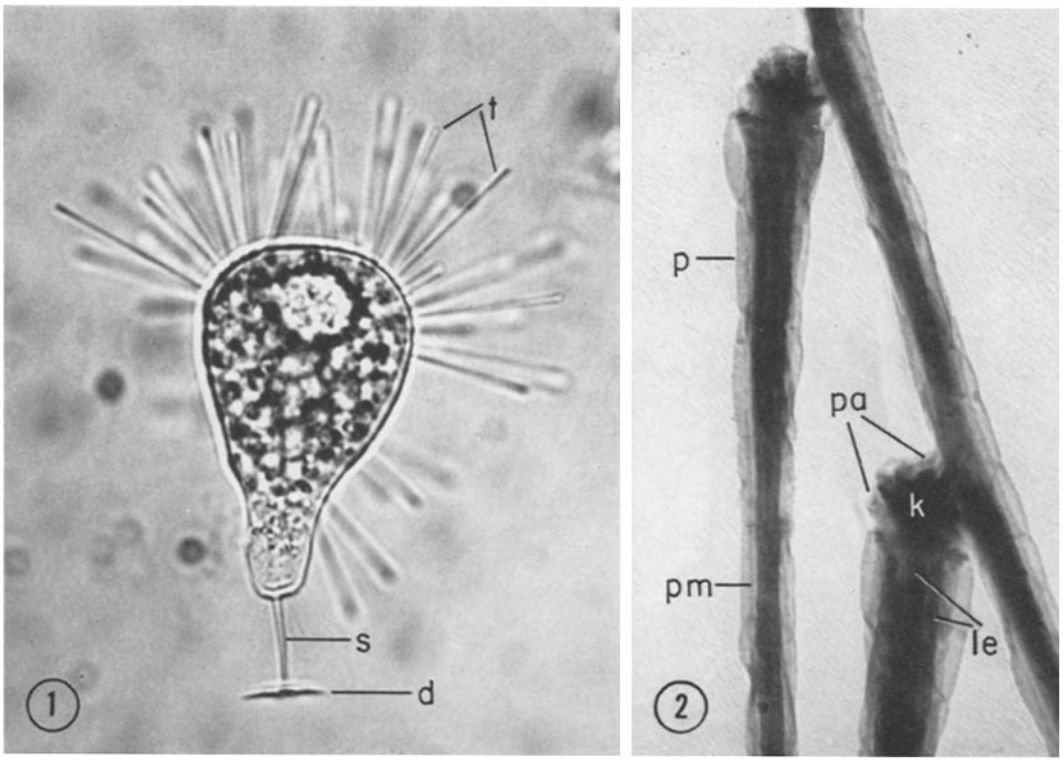
To observe live *Tokophrya* while feeding, the organisms were maintained in hanging drops (37). For the electron microscope study of whole unsectioned tentacles, *Tokophrya* was grown on formvar-coated grids or coverslips, as described previously in detail (42). After fixation with vapor of 2 per cent OsO_4, they were washed and dried (30), and shadowed with gold, or gold manganin. The study of sectioned tentacles required embedding of great amounts of young organisms possessing many tentacles. A 3- to 4-day-old culture of *Tokophrya* met these requirements (37). Since *Tokophrya* is sessile in the adult state and firmly attached to the walls of the culture tubes, the organisms were scraped from the walls and then collected by centrifugation at the bottom of the culture tube. They were thereafter fixed for 1 hour in 1 per cent OsO_4 buffered with Veronal-acetate (24) at a pH ranging from 7.3 to 8.5. To get information on the behavior of the tentacle during feeding, a somewhat different method was used. Food was introduced while *Tokophrya* was still attached to the walls of the tube. At desired times after the start of feeding the medium was poured off and replaced by the fixative. Then the *Tokophrya* were scraped from the walls and collected by centrifugation. Dehydration was done in a graded series of ethyl alcohols, and embedding in a mixture of butyl and methyl methacrylates (4:1) or in Epon (21). The organisms were mildly centrifuged before each change of fluid or handled as a pellet (35). Thin sections were cut with a Porter-Blum microtome, stained with uranyl acetate in 40 per cent alcohol, or with Karnovsky's stain (16), or with both stains by the double staining technique. Electron micrographs were taken with an RCA EMU-2c or EMU-3E or a Siemens Elmiskop I microscope at original magnifications of 5,000 to 20,700, and enlarged further photographically.

OBSERVATIONS

Light Microscopy

The tentacles of *Tokophrya infusionum* are arranged in two bundles extending from both sides of the apical part of the body (Fig. 1). The number of tentacles varies from 10 to 60, depending on the age of the organism (37). Each tentacle is 20 to 50 μ long and less than 1 μ thick and, considering its dimensions, is surprisingly straight, stiff, and rigid. The distal end of the tentacle terminates in a knob about 2 μ in diameter. The knob is the only part of the tentacle to which the swimming prey attaches, suggesting the presence of a sticky substance at its surface.

Tokophrya feeds only on living ciliates and is unable to feed on flagellates or amebae. It will not feed on dead organisms. Since *Tokophrya* is sessile and firmly attached to the substrate by a disc (42), the prey, *Tetrahymena*, is captured by



Abbreviations for Figures

b, dense body
d, disc
ep, end of pellicle
it, inner tube
k, knob
le, longitudinal elements
m, membrane
mi, mitochondrion
ms, missile-like bodies
ot, outer tube
p, pellicle of *Tokophrya*
p_1, pellicle of *Tetrahymena*
pa, papillae
pf, peripheral fibrils
pm, plasma membrane of *Tokophrya*
pm_1, plasma membrane of *Tetrahymena*
s, stalk
t, tentacles

FIGURE 1 Photomicrograph of living *Tokophrya* showing numerous tentacles (*t*), the stalk (*s*), and the disc (*d*). × 800.

FIGURE 2 Electron micrograph of distal part of unsectioned tentacles. Note that the knob (*k*) is composed of several papillae (*pa*). The shaft is covered by two membranes, the plasma membrane (*pm*) and the pellicle (*p*); the latter ends at the base of the knob. Below the plasma membrane are longitudinal elements (*le*). × 11,000.

chance contact with the tip of the tentacles. Immediately after attachment, the prey tries to free itself from the tentacles by sharp movements. The firmness of attachment to the thin thread-like tentacles is remarkable, for *Tetrahymena* although several times the size of *Tokophrya* is seldom able to liberate itself. In a matter of seconds the prey becomes motionless but still living as evidenced by the continued functioning of the contractile vacuole. It seems likely that the prey is paralyzed by the introduction of a toxic substance from the captor. A paralytic effect on the prey induced by Suctoria has been observed by a number of investigators (6, 11, 15, 25, 32). A *Tetrahymena* freed from tentacles by means of a micromanipulator needle remains motionless from several

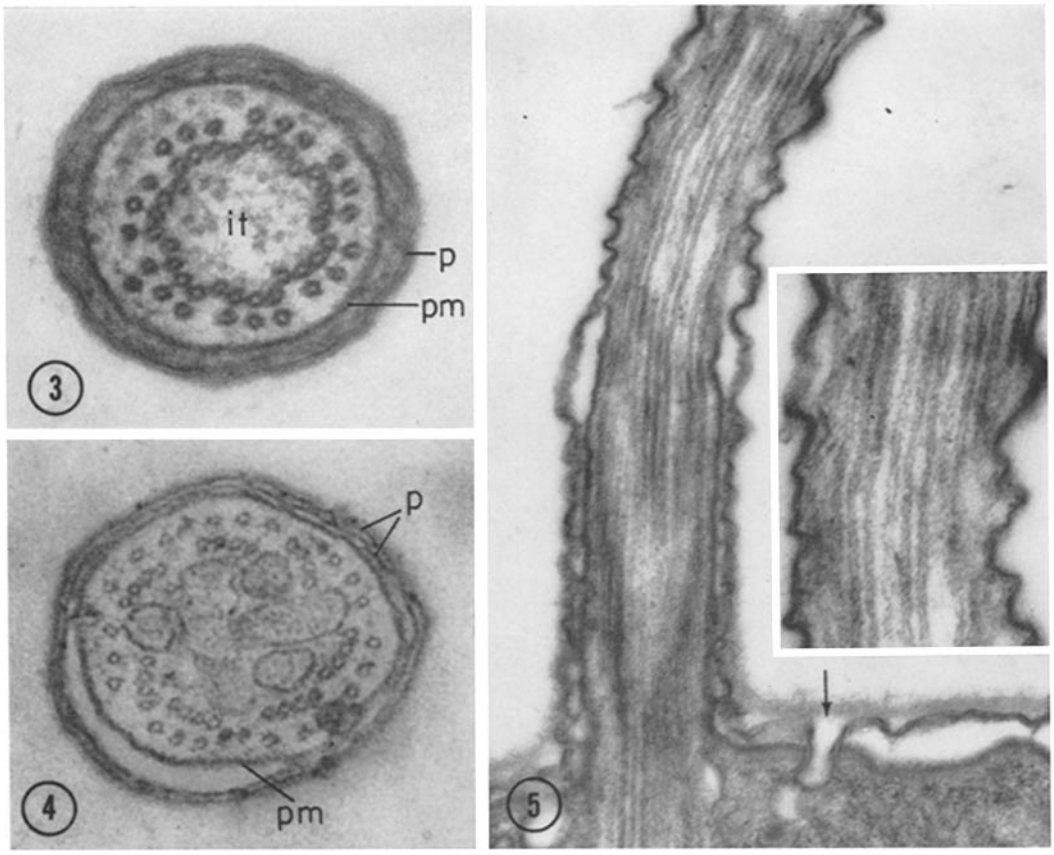


FIGURE 3 Cross-section of non-feeding tentacle showing the pellicle (*p*), plasma membrane (*pm*), the scalloped lumen of the inner tube (*it*) and its wall composed of 49 tubular fibrils arranged in seven bundles, each containing seven fibrils in two rows.

FIGURE 4 Cross-section of feeding tentacle. Note that the fibrils are pushed to the periphery and that those in the inner row do not form a closed scallop-shaped ring. × 68,000.

FIGURE 5 Longitudinal section through tentacle in the region of the fibrils to show their longitudinal appearance. At arrow the pellicle of the body forms a projection protruding into the cytoplasm together with the plasma membrane. × 38,500. The insert is a higher magnification of part of the tentacle. × 57,500.

minutes to several hours, depending on the length of time of attachment to the tentacles, and in most instances it regains its normal mobility.

After attachment of the prey the tentacles broaden and shorten and at the same time a stream of tiny granules, visible in dark field illumination, starts to flow rapidly centrifugally at the periphery of the tentacle. Soon the centrifugal flow is replaced by a centripetal stream of larger granules from the prey to predator, indicating that the tentacle is a tube and that a connection between both organisms has been established. A centrifugal current in the tentacle preceding a centripetal one at the start of feeding was first observed by Maupas in 1881 (22) in two species of Suctoria. It was thereafter reported by several investigators (2, 4, 11, 13, 15, 18).

Electron Microscopy

WHOLE MOUNT PREPARATIONS

The unsectioned tentacle is thin enough to show some details of its fine structure (Fig. 2). Each tentacle is covered by two sheaths, the

plasma membrane and the pellicle; the latter appears to be folded. The folds represent most probably the peculiar relationship between the two membranes as found in thin sections of the organism (37). The distance between the pellicle and plasma membrane is considerable (250 to 630 A); at intervals, however, both sheaths come closely together and a projection protrudes from the pellicle into the plasma membrane (Fig. 5), resulting in the folded appearance of the former.

While the plasma membrane covers the whole tentacle, the pellicle ends at the base of the knob and thus leaves the latter with only the plasma membrane as its external coat (41, 42). This seems to be significant since the knob is the only part of the tentacle to which the prey attaches. It is therefore reasonable to assume that the plasma membrane representing the external membrane of the knob might be sticky. Stickiness of the plasma membrane has been observed in protozoa in which the pellicle was removed by digestion (1). In addition, electron micrographs have disclosed that the knob has a complicated structure (42). It is composed of a tuft of papillae, which greatly enlarge the surface of the knob, and apparently of the "adhesive" area. This might be of some importance in catching the prey.

In the shaft of the tentacle longitudinal elements can be seen below the plasma membrane. They represent the internal wall of the tentacular tube; their fine structure was resolved in cross- and longitudinal sections.

Thin-Sectioned Tentacles

THE SHAFT: Sections perpendicular to the long axis of the tentacle (Figs. 3 and 4) show that below the pellicle and plasma membrane are numerous fibrils surrounding the lumen of the tentacle (38–41). The fibrils are about 230 A thick and have the appearance of fine tubules with a lumen about 110 A in diameter and a wall about 60 A thick. Figs. 3 and 4 show the tubules in cross- and Fig. 5 in longitudinal sections. Their arrangement around the tentacular lumen displays a regular pattern. They are grouped in seven bundles (Fig. 3) each composed of seven fibrils, the total number being 49. The fibrils in each bundle are arranged in two rows, an external row of three fibrils and an internal row of four fibrils. The fourth fibril in the internal row is pushed somewhat inward toward the lumen and it comes to lie between two adjacent bundles; thus the internal row acquires a curved semicircular shape. Since the fibrils of the internal row lie close to each other they form an almost closed scalloped ring composed of seven folds. The folds correspond most probably to the longitudinal elements seen in whole mount preparations (Fig. 2).

The arrangement of fibrils just described is characteristic for a non-feeding tentacle. During feeding the configuration of the fibrils changes. This is illustrated in Fig. 4, which shows that the inner row of fibrils is somewhat dislocated by the passing food particles. In electron micrographs showing cross-sections of tentacles containing a mitochondrion from the prey, the fibrils appear to be arranged in a single row, as if pushed away to the periphery by the traversing mitochondrion for which the lumen of the already broadened tentacle is apparently still too tight. The lumen of the non-feeding tentacle is about 230 mμ wide; during feeding it increases greatly in width to about 700 mμ. It might be assumed that the fibrils are contractile and responsible for broadening of the lumen.

Longitudinal sections through tentacle and body disclosed that the tentacle originates deep within the cytoplasm (Fig. 6). Thus each tentacle is composed of an extra- and intracellular part. This was somewhat anticipated from light microscope observations of feeding organisms, in which granules of prey cytoplasm were found to be moving in a straight line inside the cytoplasm of the predator, as if enclosed in a tube continuous with that of the tentacle (2, 3, 9, 11, 12, 23). Not all the components of the extracellular tentacle extend into the cytoplasm. The pellicle and plasma membrane cover the extracellular part of the tentacle only to its base where they become continuous with the membranes surrounding the body (Fig. 6). It is only the tentacular lumen and its wall composed of fibrils that protrudes into the cytoplasm (Fig. 6).

The electron microscope study showed in addition that the extracellular part of the tentacle is not a single tube but is composed of two concentric tubes, an inner one surrounded by the fibrils, and an outer one between the fibrils and the plasma membrane. Observations on feeding tentacles have suggested the presence of two tubes (2, 3, 14, 18). Fig. 7 which is a longitudinal section of a feeding tentacle shows that in the inner tube a mitochondrion is present, and in the outer tube a small dense oval body (about 110

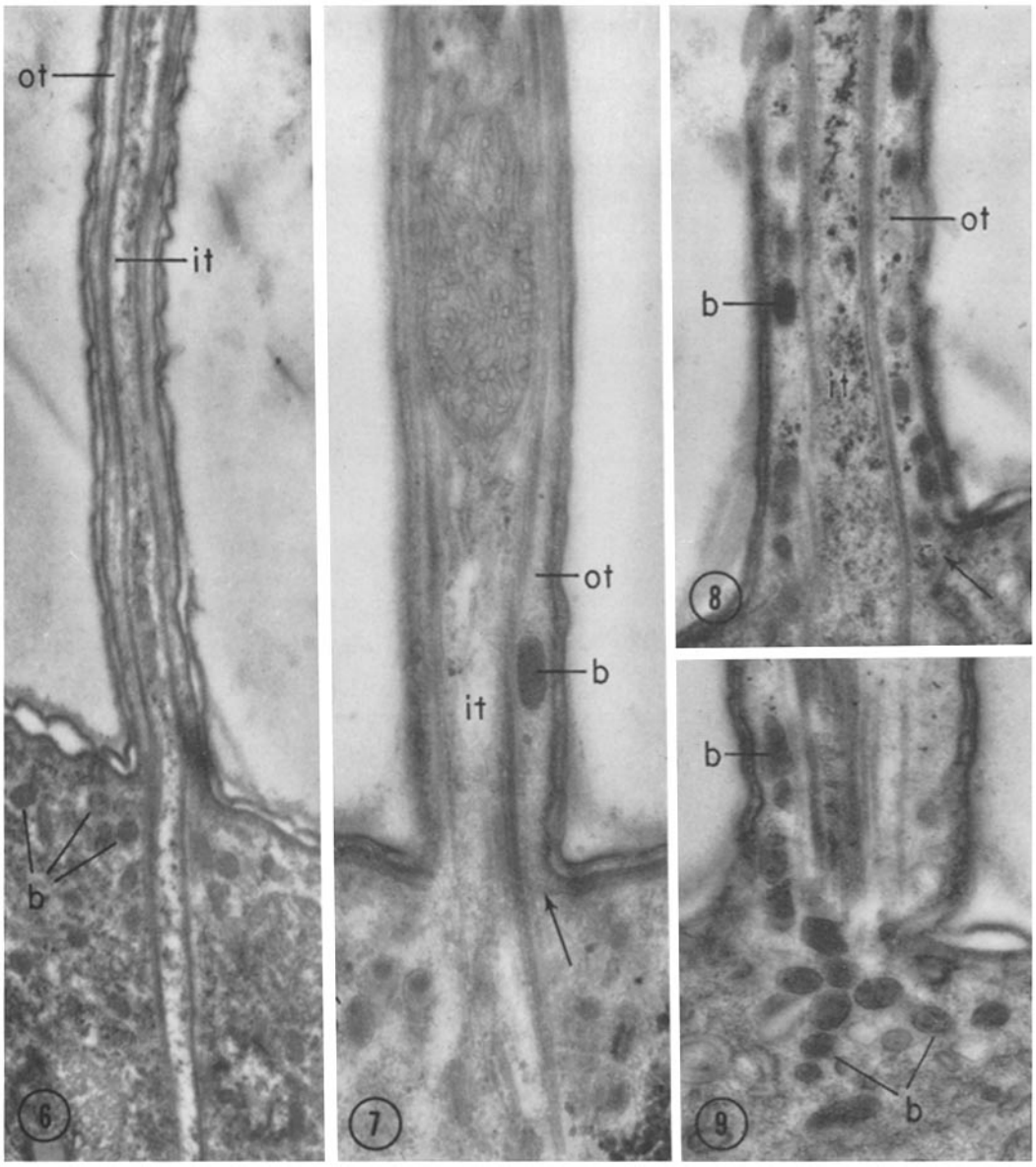


FIGURE 6 Longitudinal section through extra- and intracellular parts of a non-feeding tentacle. Note that the pellicle and plasma membrane of the tentacle are continuous with the pellicle and plasma membrane covering the body of *Tokophrya*. It is only the lumen and its wall composed of fibrils that extends into the cytoplasm and forms the intracellular part of the tentacle. Small dense bodies (*b*) are seen in the cytoplasm in the nearest vicinity of the tentacle. × 22,000.

FIGURE 7 Longitudinal section through a feeding tentacle. Two concentric tubes, the inner (*it*) and outer tube (*ot*), of the extracellular part of the tentacle can be well distinguished. In the inner tube a mitochondrion from the prey is present, in the outer a small dense body (*b*). The continuity between the outer tube and cytoplasm is clearly seen at arrow. × 37,500.

FIGURE 8 Longitudinal section of tentacle probably at the start of feeding. Note numerous dense small bodies (*b*) in the outer tube and continuity between outer tube and cytoplasm at arrow. × 28,000.

FIGURE 9 Longitudinal section of tentacle to show the dense bodies (*b*) in the cytoplasm and in the outer tube. × 35,000.

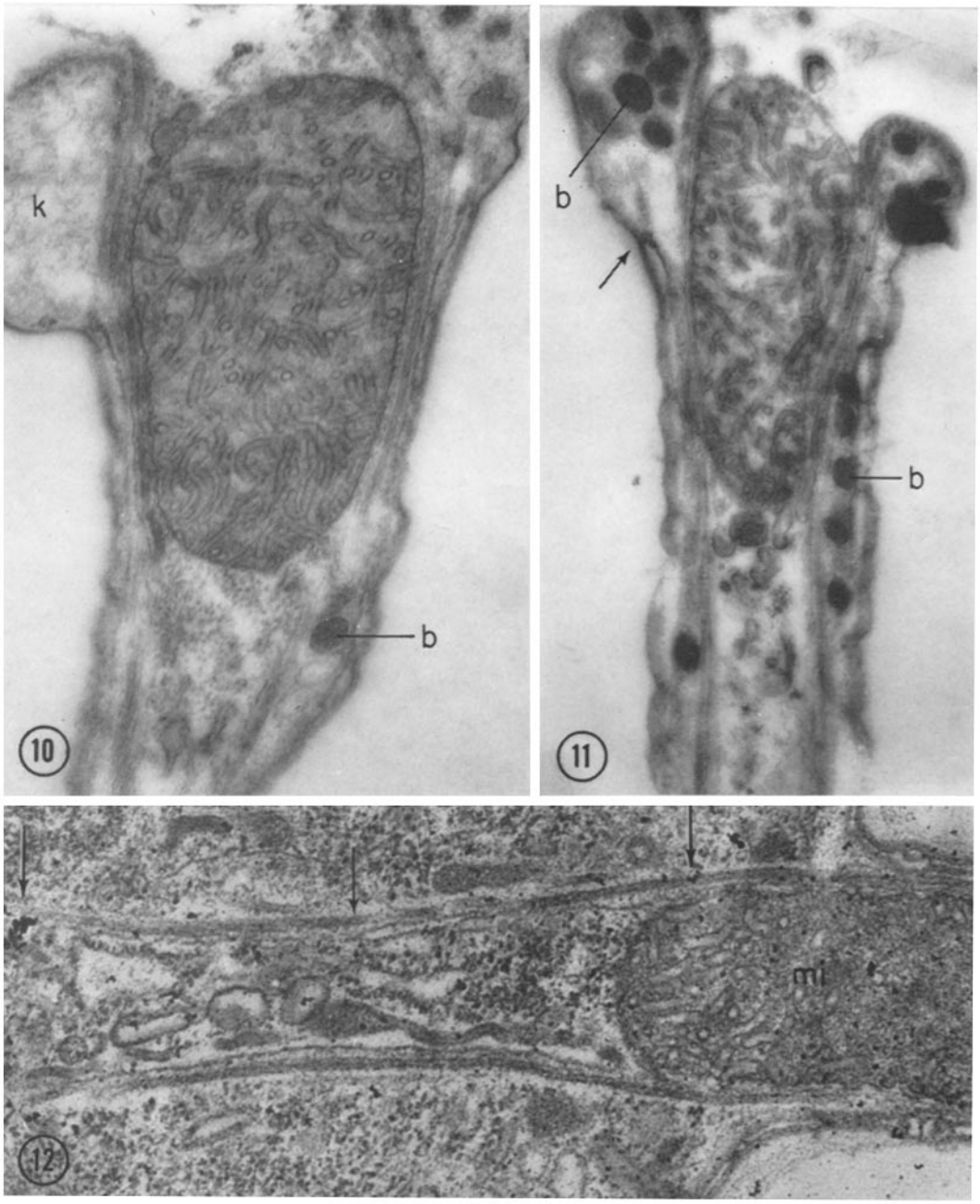


FIGURE 10 Longitudinal section through distal part of the tentacle during feeding. In the inner tube a large mitochondrion from the prey is present, in the outer tube a small dense body (*b*). The outer tube is greatly inflated in the part forming the knob (*k*). × 41,000.

FIGURE 11 Longitudinal section of feeding tentacle showing a mitochondrion from the prey in the inner tube and some dense bodies (*b*) in the outer tube including the part forming the knob. The arrow points to the end of the pellicular sheath at the base of the knob. × 28,000.

FIGURE 12 Longitudinal section of feeding tentacle. Note the narrow (black arrow) and broad sections (black and white arrows) of the inner tube of the intracellular part of the tentacle. × 47,000.

mμ). It is of interest that such oval bodies were never found in the inner tube, but were frequently present in the outer tube of feeding tentacles, as seen in Figs. 8 and 11. Small dense bodies of similar size and structure may be found in the cytoplasm in the vicinity of the tentacle, as illustrated in Figs. 6 and 9. Figs. 6 to 9 show also that there is no boundary between the outer tube and the cytoplasm; this uninterrupted continuity permits a free communication and flow of matter from one place to the other, and explains the presence of the dense oval bodies in both places.

It should be stressed that the small dense bodies were found in the cytoplasm of both feeding (Fig. 9) and non-feeding organisms (Fig. 6) but that their presence in the outer tube is closely associated with feeding (Figs. 7 to 11). Mitochondria, on the other hand, are found exclusively in feeding tentacles where they are present in the inner tube as seen in Figs. 7, 10, and 11. All the figures are longitudinal sections of tentacles during feeding and they show mitochondria in the inner tube and small dense bodies in the outer tube. Since the small dense bodies are present in the cytoplasm of *Tokophrya* in the vicinity of the tentacle not only during feeding (Fig. 9) but also in non-feeding organisms (Fig. 6), it is reasonable to assume that they originate in the cytoplasm of the predator and migrate to the outer tube. Since mitochondria, on the other hand, are present in the tentacle only during feeding and are found exclusively in the inner tube, and since they are large enough to be seen in tentacles of living organisms during feeding as they stream centripetally from prey to predator, no doubt exists as to their origin.

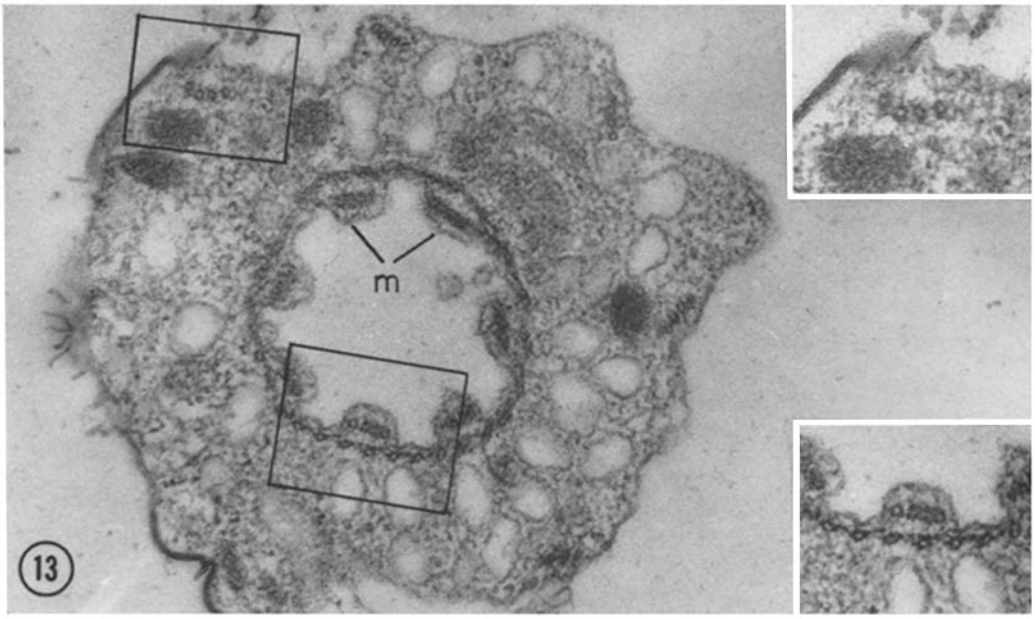
It is remarkable that the mitochondria found within the tentacle are excellently preserved in shape and structure (Figs. 7, 10, and 11), as are the cilia, basal bodies, and the endoplasmic reticulum deriving from *Tetrahymena*. They appear to be in perfect condition, with no sign of even the slightest change in their fine structure despite their having been removed from their own environment into a narrow tube of a foreign organism.

The luminal wall of a feeding tentacle is not a straight and stiff tube. It appears constricted at intervals, as if composed of alternating broad and narrow sections (Fig. 12). This suggests that during feeding some kind of peristaltic contractions of the wall surrounding the inner tube might take place.

THE KNOB: Since the tip of the tentacle is the only place to which the prey attaches, its fine structure is of special interest and importance. Longitudinal sections show that both the inner and outer tubes extend into the tip (Figs. 10 and 11). Its knob-like shape is due to the inflation of the outer tube, which represents the major constituent of the tip. Some investigators have assumed that the knob is composed of the inner tube only (2, 18), and that the outer tube terminates at the base of the knob. The size relationship between the two tubes is well illustrated in Figs. 10 and 11. Below the knob the width of the outer tube is about ⅓ to ¼ of that of the inner tube; in the knob itself both tubes show the same diameter. This is the situation in feeding tentacles when the inner tube is 2 to 3 times enlarged. In the non-feeding tentacle the diameter of the outer tube in the knob far surpasses that of the inner tube. The electron microscope study showed clearly that the outer tube is closed at its tip (Figs. 10 and 11).

Although the knob is a continuation of both tubes its fine structure differs considerably from that of the shaft. The wall of the inner tube in the knob is lined by a thin membrane (Fig. 13) and is composed of more fibrils than in the shaft. The seven bundles are more pronounced and so is the scalloped shape of the lumen. The groups of 4 fibrils in the inner row are far apart, and in the outer row all fibrils are interconnected by fine fibrils about 40 A thick and arranged in a circle, a situation the reverse of that in the shaft. The number of fibrils in the outer row is 28, 7 more than in the shaft (Figs. 13 and 14). In addition, fibrils are present also at the periphery of the knob just below the plasma membrane. They are arranged in seven bundles each containing four fibrils (Figs. 13 and 14). The fibrils are located at indentations of the scalloped plasma membrane in the form of "ribs" running lengthwise (Fig. 14). They serve most probably as a skeleton stiffening the knob which is covered only by the soft plasma membrane, and deprived of the tough pellicular cover supplied to the rest of the body including the shaft of the tentacle. The scalloped form of the knob corresponds most probably to the tuft of papillae seen in electron micrographs of unsectioned tentacles as described earlier (Fig. 2).

Other structures present in the knob are dense bodies of the same size, structure and density as



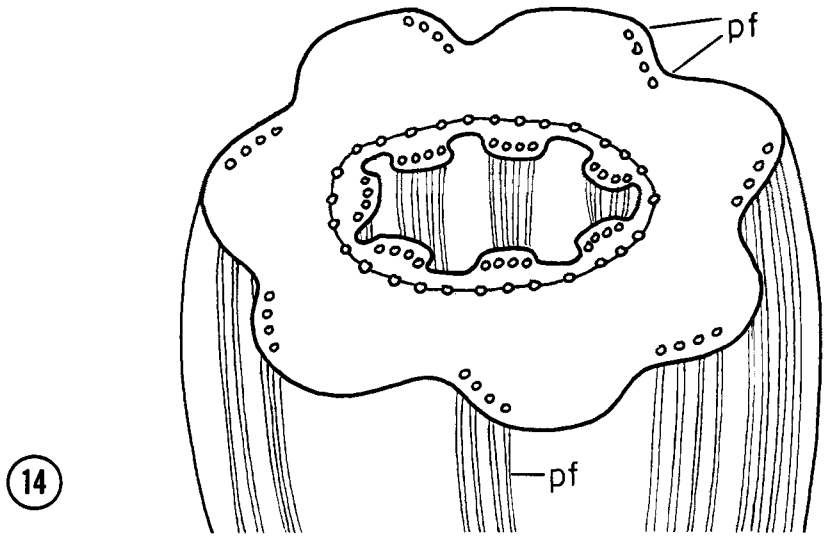


FIGURE 13 Cross-section through knob. Note the membrane (*m*) lining the scallop-shaped lumen. Also note the seven bundles of fibrils and outside of them the closed ring of fibrils. At the periphery at intervals are seven bundles of fibrils. The insert at the upper right is a higher magnification of the outlined area at the upper left to show that each peripheral bundle is composed of four fibrils. The insert at the lower right is a higher magnification of the outlined area of the wall of the inner tube to show that each of the seven bundles of the inner wall contains four fibrils, and that the outside fibrils are interconnected. × 47,000; upper insert, × 70,500; lower insert, × 59,000.

FIGURE 14 Diagram of knob at the level of the cross-section in Fig. 13 to show the arrangement of fibrils; *pf*, peripheral fibrils.

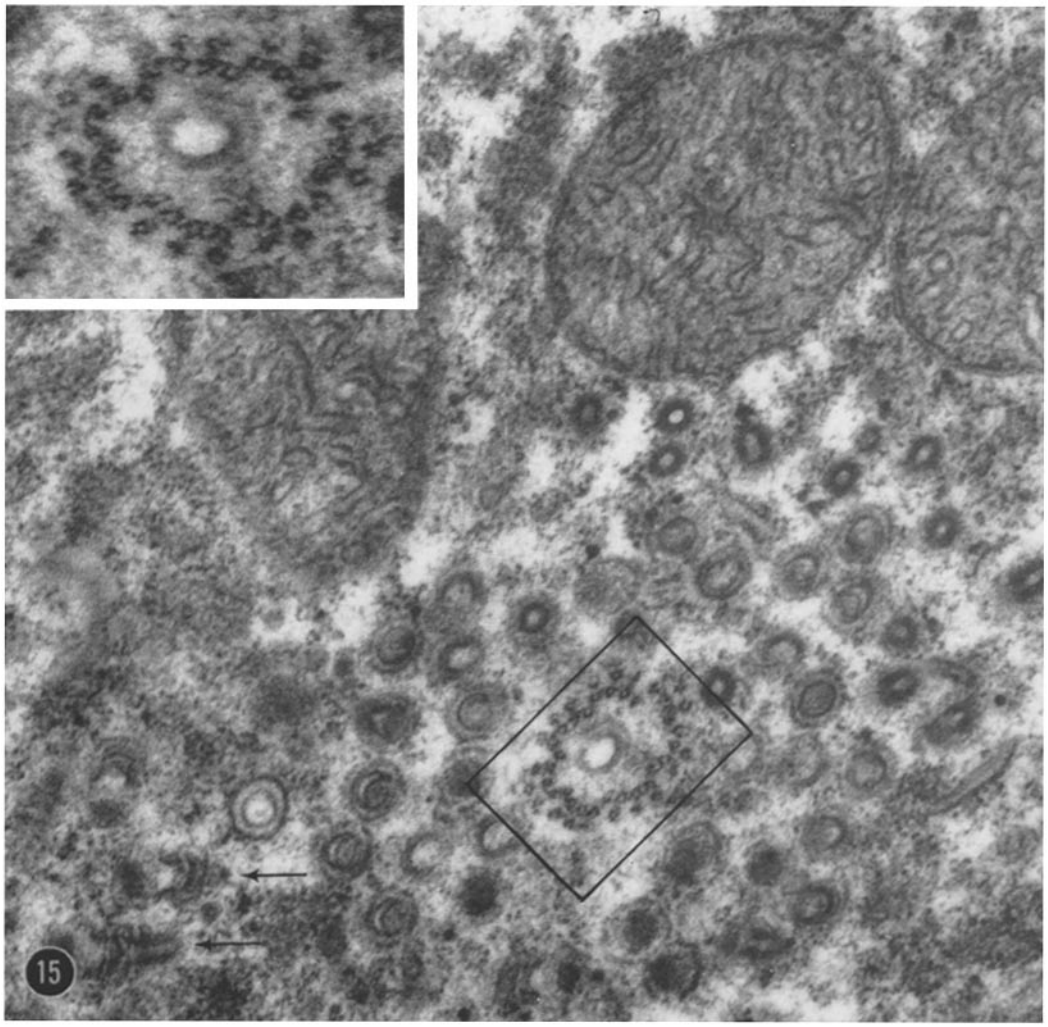


FIGURE 15 Section through the body of *Tokophrya*. The outlined area which is magnified in the insert at the upper left is a cross-section of the intracellular part of the tentacle. The 49 fibrils arranged in seven bundles form the wall of the inner tube. The whole area around the tentacular wall is filled with round and oval bodies of different size, density and structure. It is assumed that they represent cross, oblique and tangential sections of missile-like bodies (at arrows) and seen to better advantage in Fig. 16. × 57,500; insert, × 96,000.

those found in the outer tube and in the cytoplasm in the vicinity of the tentacle. The dense bodies will be described and discussed in detail below.

MISSILE-LIKE BODIES: The dense bodies found in the outer tube of the tentacle and in the cytoplasm in the vicinity of the tentacles are not uniform as to their structure, density, shape and size. This is particularly well illustrated in Fig. 15 which shows numerous dense bodies assembled around the intracellular part of the tentacle seen in cross-section. Some have a less dense core surrounded by a much denser rim, or a dense core and less dense rim; some are larger, some smaller, round or oval, with a clear or fuzzy outline; others display the same degree of density throughout (Figs. 6 to 11). In addition, others were found which have a complicated structure, are elongate and composed of several parts unequal in density, shape, and size (Fig. 16). It is not known whether all the enumerated forms represent different planes of

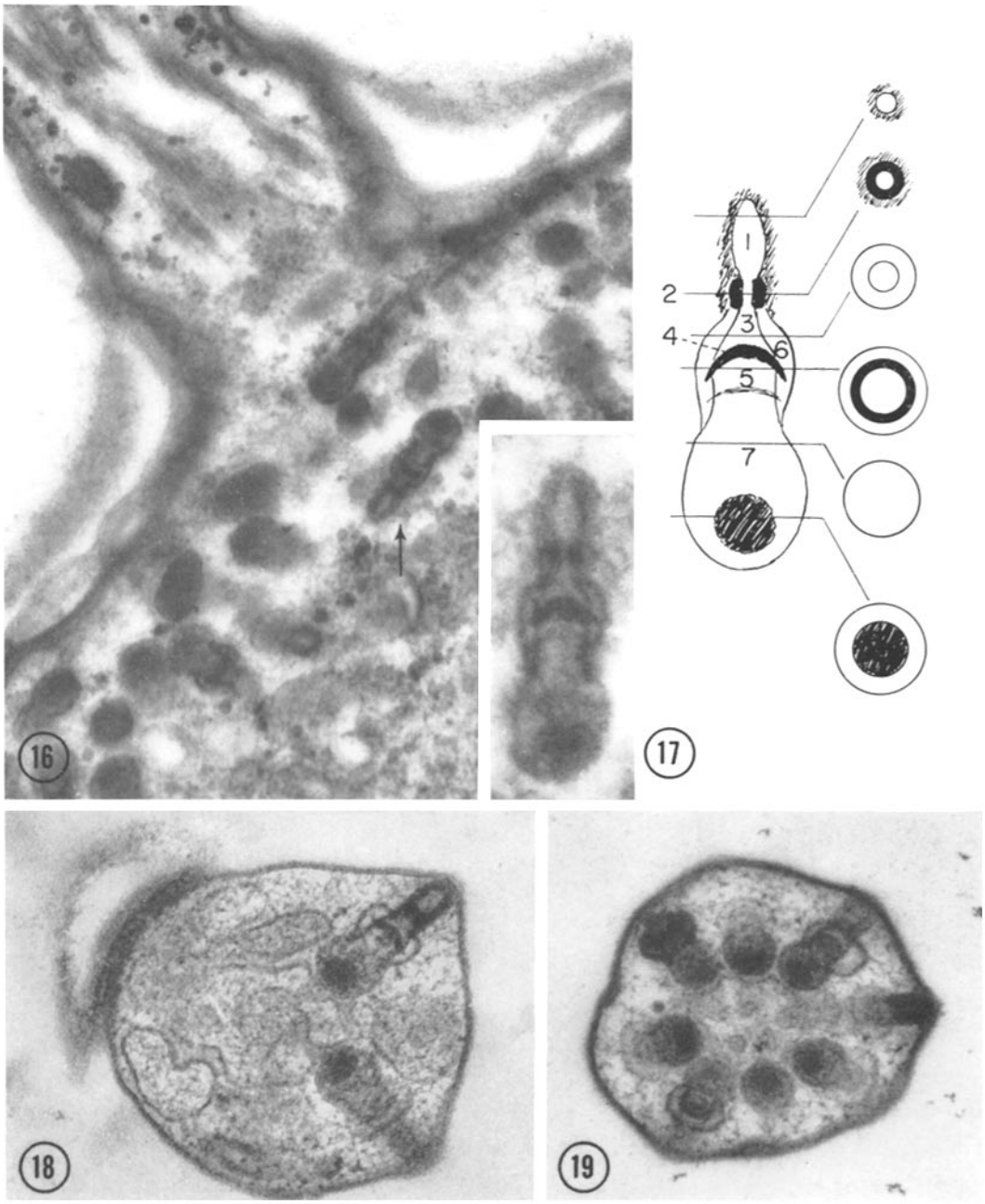


FIGURE 16 Section through proximal part of tentacle near its connection with the body. Several missile-like bodies are present in the cytoplasm in the vicinity of the tentacle. × 51,500. The insert shows one of the missiles (at arrow) at higher magnification. × 124,000.

FIGURE 17 Diagram of a missile. Note the seven distinct segments (*1–7*) of the missile and the cross-sections at different levels.

FIGURE 18 Transverse section through knob with two missiles to show that they have the same structure as those found in the cytoplasm. Note that only the plasma membrane covers the knob. × 65,000.

FIGURE 19 Section through knob with eight missiles cut at different angles. × 47,000.

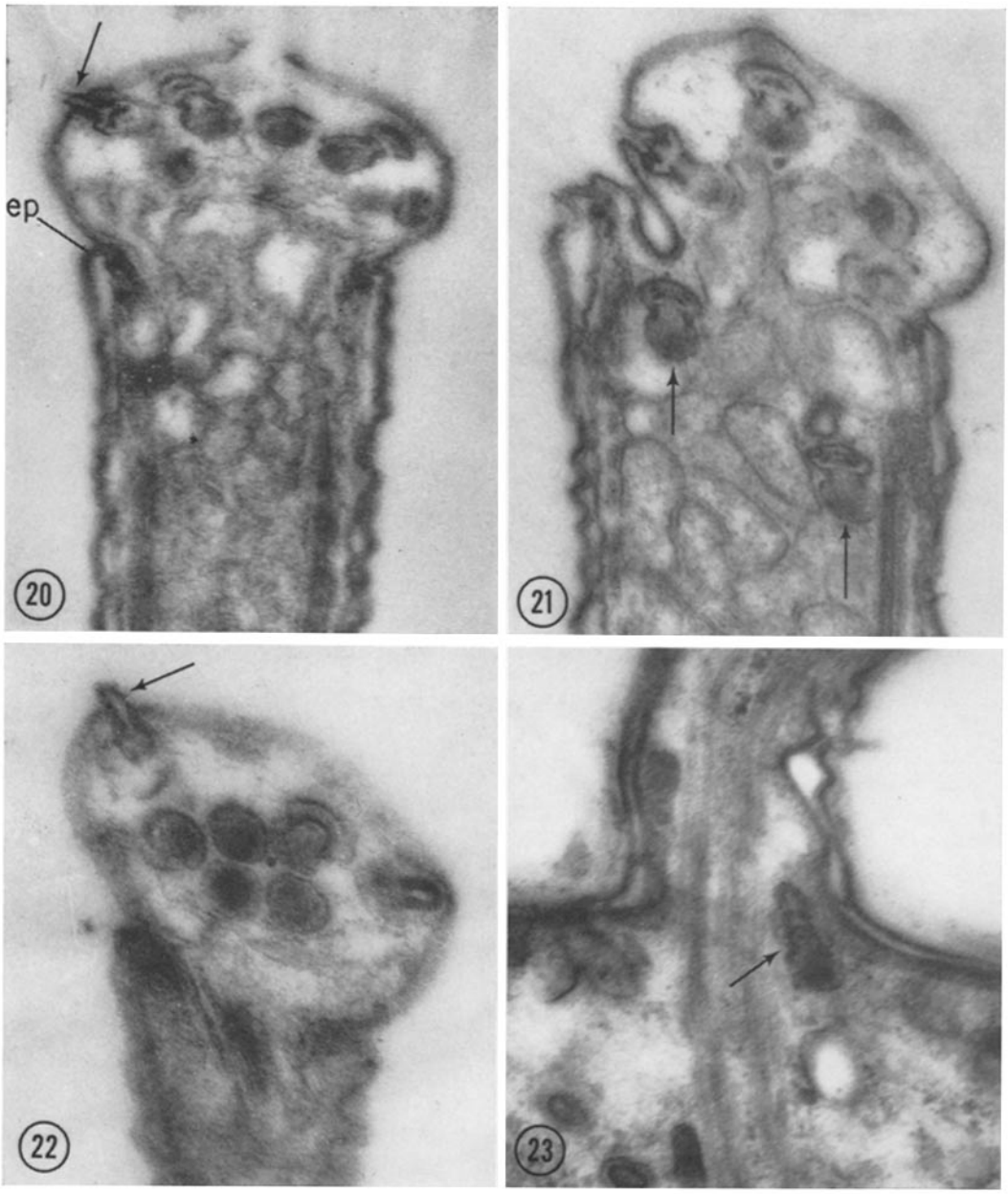


FIGURE 20 Longitudinal section through distal part of a non-feeding tentacle. The pointed end of one of the missiles (at arrow) is sticking out through the plasma membrane of the knob. The pellicle of the tentacle ends at the base of the knob (*ep*). × 48,500.

FIGURE 21 Longitudinal section through distal part of tentacle. Two missiles (at arrow) are on the way to the knob. × 55,000.

FIGURE 22 Oblique section through knob with several missiles. The pointed end of one of the missiles (at arrow) is seen sticking out through the plasma membrane. × 62,000.

FIGURE 23 Longitudinal section of tentacle at its base. A missile (at arrow) is entering the outer tube. × 55,000.

section of one organelle or whether several different structures are involved. The former seems to be more likely, and a detailed analysis favors the hypothesis that all the forms described are sections of the elongate bodies of complicated structure.

These elongate bodies are about 380 mμ long and have a missile-like appearance, pointed at one end, rounded at the other (Figs. 16, 17, 18). They are composed of at least seven different parts which may be easily distinguished. The pointed segment is about 100 mμ long and 75 mμ wide with a tip 35 mμ in diameter, has an interior of low density and a broad dense fuzzy rim. This segment could, in cross-sections, correspond to the smallest round or oval bodies of low density with a fuzzy dense outline seen in Fig. 15. Fig. 17 is a diagram of the missile. The rounded end-part of the missile is about 130 mμ in diameter at its broadest part, has a bulb-like shape, and is of medium density with a dense sphere near the terminal part. Cross-sections at various angles to the long axis could produce round or oval forms of medium density; forms that are dense inside and less dense outside would occur in sections near the terminal part (Figs. 15 and 17). Between the end-parts (*1* and *7* in Fig. 17) are a number of distinct segments as diagrammed in Fig. 17. Behind end-part (*1*) is a small dense ring with less dense material at intervals, (*2*), followed by a kind of neck, (*3*), a dense crescent (*4*), and a ring of lower density, (*5*). A halo of low density, (*6*), extends from the neck, (*3*), to the upper part of the last segment, (*7*). Sections through these segments could give all the other varieties seen in Figs. 15 and 17.

The greatest accumulation of missiles was found around the intracellular part of the tentacle (Fig. 15) and in its knob. Figs. 18 to 22, which are sections of the tip at different angles, show the location of the missiles in this part of the tentacle. In Figs. 20 and 22 the pointed end of one of the missiles sticks out through the plasma membrane; all other missiles no doubt penetrate the membrane in the same way, although the plane of section does not show it. In Fig. 23 a missile is entering the outer tube of the tentacle and is oriented with its narrow end upwards. In Fig. 21 two missiles are approaching the knob. It could be concluded from all these electron micrographs that the missile-like bodies travel from the cytoplasm of *Tokophrya* through the outer tube of the tentacle to its knob where they pierce the plasma membrane with their pointed ends. The knob's being covered only by the plasma membrane (Figs. 18 to 22) may facilitate the penetration of the missiles. The missiles were found in the knobs of both feeding and non-feeding tentacles. It may be assumed, therefore, that once they reach the knob and penetrate the plasma membrane they remain there stationary. The electron micrographs do not provide information as to the way the missiles work, however. It is not known whether they are ejected from the tentacle like nematocysts and injected into the prey, or whether they remain at the tip of the tentacle and are used up in succession. The latter seems to be more likely since so far no missiles have been found in *Tetrahymena* at the site of tentacle attachment, nor in other parts of its body during feeding.

It seems likely that the fuzzy material at the protruding end of the missile is adhesive and may be responsible for attachment of prey to the tips of the tentacles. As described earlier, the prey does not attach to any other part of the tentacle.

This may be one of the functions performed by the missiles, but not the only one. The composite structure of the missile suggests that it might contain a number of enzymes responsible for the numerous events following attachment of prey and leading to ingestion of the prey's cytoplasm.

RELATIONSHIP BETWEEN TENTACLE AND PREY DURING FEEDING: Electron micrographs show that the knob of a feeding tentacle is entirely embedded in the cytoplasm of *Tetrahymena* as seen in Figs. 24 to 26. This apparently is achieved not only by dissolution of the pellicle of the prey but also by some forceful pushing of the tentacle as evidenced by the infolding of the pellicle of *Tetrahymena* around the knob. The figures show also that the pellicle of the tentacle is continuous with the pellicle of *Tetrahymena*. This is a remarkable phenomenon, for the pellicle of the tentacle which usually ends at the base of the knob apparently merges with the pellicle of the prey. In this way the two organisms become united by a continuous common membrane enveloping both prey and predator. This complete union of *Tokophrya* and *Tetrahymena* explains why it is so difficult to separate them once the connection has been made.

DISCUSSION

The process of food ingestion in *Tokophrya* is preceded by a series of events which start at the moment the tentacle makes contact with the

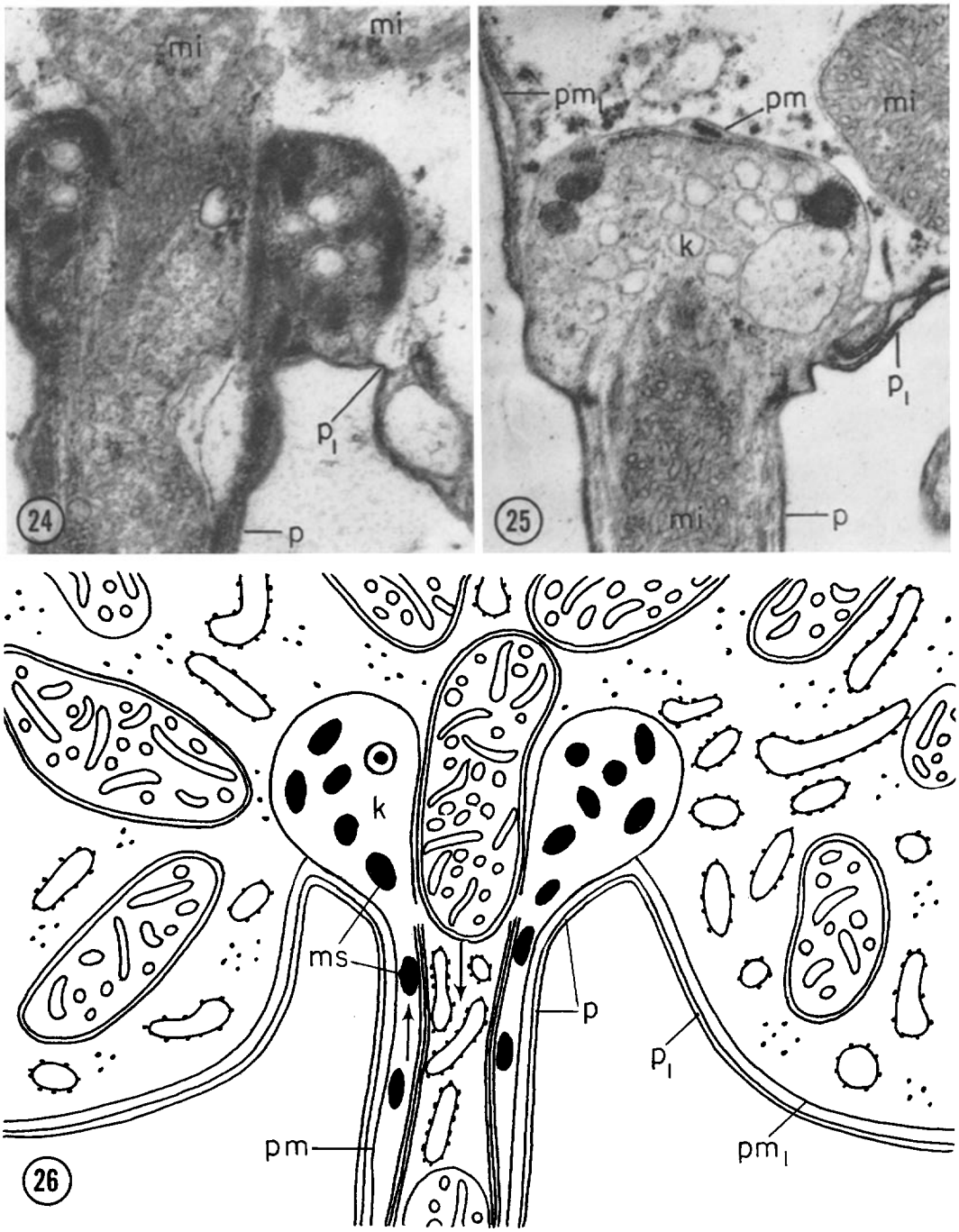


FIGURE 24 Longitudinal section through knob embedded inside the prey *Tetrahymena* during feeding. Note that the pellicle of the tentacular shaft (*p*) is in continuity with the pellicle of *Tetrahymena* (p_1) and that the latter is pushed inward around the knob. × 27,500.

FIGURE 25 Side view of the knob embedded in the cytoplasm of *Tetrahymena* during feeding. The pellicle of the tentacle shaft (*p*) is continuous with the pellicle of *Tetrahymena* (p_1). The latter appears to be infolded around the knob. × 27,000.

FIGURE 26 Schematic drawing showing the relationship between tentacle and *Tetrahymena* during feeding. The pellicle of the tentacle (*p*) is continuous with the pellicle of *Tetrahymena* (p_1). The latter forms an invagination around the knob. The arrows show the direction of movement, during feeding, in the inner and outer tubes. In the former, mitochondria and vesicles of the endoplasmic reticulum deriving from the prey can be seen; in the latter, missile-like bodies (*ms*) are present.

prey. Fine granules seen at the limits of resolution by light microscopy and identified by electron microscopy in this work as missile-like bodies begin to move rapidly in the outer tube of the tentacle toward its tip; at the same time the tentacle shortens and broadens considerably (2 to 3 times); the prey becomes immobilized but is still living and as soon as the connection is made between tentacle and prey a stream of prey's cytoplasm starts to flow centripetally through the lumen of the inner tube of the tentacle into the body of the predator. The sequence of these events is so fast that most of them seem to occur almost simultaneously.

The electron microscopy of the tentacle before and during feeding revealed numerous structures which help to clarify some of these processes and the mechanisms involved. The study showed that the wall of the lumen of the tentacle is composed of fine tubular fibrils about 230 A in diameter arranged in seven bundles each containing seven fibrils (39, 40). In two other suctorians, *Ephelota gemmipara* (34) and *Discophrya piriformis* (31), fibrils of similar dimensions, although differing in numbers and arrangement, were found in the wall of the lumen of the tentacle.

It is of interest to point out that the fibrils are of the same thickness and structure as those in cilia and flagella (8, 28, 29), suggesting a possible relationship between these structures and tentacles. Such a relationship was suggested also by morphogenetic changes during matamorphosis in Suctoria, where the ciliated embryo loses its cilia and concomitantly tentacles are formed. It has been assumed in the past that the basal bodies of the lost cilia might give rise to tentacles. However, silver impregnation methods (11) and electron microscopy (36) have provided evidence that the basal bodies persist after metamorphosis in the adult organism and that tentacles do not originate from them. The present study shows conclusively that there is no relationship between tentacles and cilia. The pattern of 9 peripheral fibrils or its multiple, characteristic for cilia and flagella, does not occur in tentacles.

However, the other feature of the tentacular fibrils, namely their tubularity, has some general significance and seems to offer some clues to the mechanism of food intake. Electron microscope studies have disclosed that tubular fibrils are present not only in cilia, flagella and tentacles, but appear to be a common component in the cytoplasm of a great variety of cells (44). For example, the mitotic spindle has been shown to be composed of tubular fibrils (19, 33); they were found in the cortex of plant cells where they are allegedly responsible for cytoplasmic streaming (19); in dissociated cells in tissue culture they seem to be connected with the formation of pseudopods (45); they are present below the plasma membrane in flagellates of the families Trypanosomatidae (43, 47) and Bodonidae (27), where they most probably influence the changes in body shape; they are part of the structure of the canal of the contractile vacuole in *Tokophrya* (36) where, it is assumed, they are responsible for opening and closing the pore during systole and diastole, respectively. It appears that tubular fibrils perform a variety of functions, most of which are related to different forms of movements within the cell and of the cell itself. It is important that ATPase activity has been detected in cilia and localized in the nearest vicinity of the peripheral tubular fibrils (10). It seems therefore reasonable to assume that the presence of numerous tubular fibrils in the tentacles of *Tokophrya* provides them with what may be considered contractile elements which may be responsible for broadening of the inner tube of the tentacle at the start of feeding. Such broadening of the lumen of feeding tentacles has been observed in a number of Suctoria (3, 9, 12, 14, 15, 23). The sudden and considerable enlargement of the lumen of the inner tube of the tentacle could create conditions favorable for the initial flow of cytoplasm from prey to the tentacle (2, 14).

It is conceivable also that the fibrils represent a structural basis for microwaves of contraction which in moving down the tentacle would carry the food from the prey to *Tokophrya*. Longitudinal sections through some feeding tentacles suggest the existence of such waves. Collin (3) has observed peristaltic waves of contraction along the lumen of the inner tube of the tentacle in a number of feeding suctorians. He considered that such waves could be responsible for suction in Suctoria.

The tentacle consists of two concentric tubes. Outside the wall of the inner tube is a narrow space enclosed between the fibrils and the plasma membrane. This space is the outer or external tube. The presence of two tubes in the tentacle is of particular importance. Each of them appears to perform a different function. In the inner tube, the cytoplasm of the prey moves centripetally; in the outer tube the small granules or missile-like

bodies stream centrifugally during feeding. Each of the tubes has a one-way traffic but in opposite directions; each of them specializes in forwarding a specific material. The origin of the material content within the inner tube is obvious. The origin of the small granules or missile-like bodies in the outer tube is less obvious, however. This study of *Tokophrya* showed that the missile-like bodies are present in the cytoplasm around the intracellular part of the tentacle in feeding and non-feeding organisms as well as in the outer tube of feeding tentacles. It showed also that there is uninterrupted continuity between the outer tube and the cytoplasm, enabling a free communication between both places. It could be concluded therefore that the missile-like bodies found in the outer tube during feeding derive from the cytoplasm of *Tokophrya*. The direction of their movement is in favor of such a conclusion. An additional piece of evidence comes from the recent light microscope observations of Canella (2) and Hull (14) who have found that at the onset of feeding there is, in addition to an upward rush of tiny granules in the outer tube, a "disappearance" of other granules residing in the cytoplasm just below the plasma membrane. The electron microscope study leaves no doubt that the cytoplasmic granules are identical with the granules in the outer tube of the tentacle and that they do not "disappear," as claimed by both these investigators, but apparently move to the outer tube of the tentacle in which they stream upwards.

These granules or missile-like bodies appear to play a very important role. Their upward onrush through the outer tube invariably precedes the start of the downward movement of prey's cytoplasm through the inner tube. This time sequence suggests strongly that the missile-like bodies are involved in the preparatory steps preceding food intake and actually trigger the whole feeding process.

A complex structure characterizes the missile-like bodies. They are composed of several distinct parts differing in shape, size, density, and smoothness of outline. The great accumulation of missiles at the periphery of the knob in non-feeding tentacles makes it clear that once they reach the knob they remain there. They eventually penetrate the plasma membrane with their pointed ends and the surface of the knob thus becomes covered with short stubs. Since these projecting stubs are the first structures that contact the cilia of the prey, they may be responsible for making the prey stick to the tentacle. The fuzzy appearance of their outline suggests that they may be coated with a sticky substance of high specificity since it adheres only to cilia and not to flagella. In his experiments with *Podophrya*, Hull found that adherence to tentacles may be influenced by changes in the medium, or by pretreatment of the food organism (13). These findings also point to the specificity of the substance covering the projecting stubs of the missiles.

After the prey attaches to the tentacle, its cilia slow down in movement and finally stop beating, suggesting that a toxic substance has been released to the prey. Shortly thereafter connection is made between the tentacle and prey, since the cytoplasm from *Tetrahymena* starts to stream down through the inner tube of the tentacle to the body of *Tokophrya*. To make this connection the pellicle of the prey must be pierced or dissolved. Electron micrographs of tentacles of *Tokophrya* to which *Tetrahymena* are attached suggest that pellicle dissolution takes place, for during feeding the whole knob of the tentacle is embedded in the cytoplasm of the prey. The pellicle of the tentacle shaft becomes continuous with the pellicle of *Tetrahymena*. This is one of the most unexpected findings. It appears that pellicles of two unrelated organisms are able to merge. This unusual phenomenon is of great importance, for it prevents any leakage of cytoplasm from the prey, which would obviously result in its fast disintegration, and it also unites prey and predator into one organism.

It is assumed that all these elaborate changes which follow in a remarkably rapid and precise succession are produced by highly specific and potent enzymes. The composite structure of the missile-like bodies suggests that they may contain an assortment of such enzymes: one may be responsible for attachment of prey, another for dissolving the pellicle of the prey in order to establish the connection between prey and predator, and still another for contraction of the fibrils resulting in broadening the tentacle. A part of the missile may also contain a toxic substance which is released to paralyze the prey, and possibly also an enzyme responsible for the change in viscosity of prey cytoplasm. Observations by light (14) and electron microscopy (39, 41) have suggested that such a change in viscosity occurs in prey cytoplasm. A less viscous cytoplasm would facilitate flow from prey to predator.

The only experiments that have been carried out to determine the presence of enzymes in Suctoria were performed by Fauré-Fremiet on *Dendrocometes paradoxus* (7). Using Gomori's method he was able to detect acid phosphatase at the tips of the tentacles and at their cytoplasmic base. At both these places the missiles in *Tokophrya* show the greatest accumulation. It may be that one of the enzymes contained in the missiles is acid phosphatase.

The participation of enzyme-like substances in the feeding of Suctoria was first proposed by Kahl (15) in connection with his concept of the feeding mechanism in this group. He rejected suction in the physical sense and postulated that the driving force in feeding of Suctoria is the increased internal pressure in the prey which is produced by the lytic action of enzymes released from the tip of the tentacle through radial pores. Thus, according to his concept, the prey's cytoplasm undergoes "external digestion" before it reaches the suctorian. Kahl's incisive concept of the presence of enzymes in the tip of the tentacle and their role in feeding was strongly rejected by Pestel (26) and Kormos (18). The latter investigator emphasized that nothing flows out of the tentacle to the prey and therefore that no "external digestion" is possible.

The electron microscope results presented in this paper do not support external digestion in Kahl's broad sense, since mitochondria, basal bodies, and cilia from the prey are intact and their fine structure is unchanged when they enter the tentacle. At the same time, however, the study shows that the knob of the tentacle contains missile-like bodies which apparently act on the prey, paralyzing its ciliary movement, dissolving its pellicle, and changing the viscosity of its cytoplasm. Paradoxically, Kormos was the first to report in the tentacles of Suctoria the presence of small granules which undoubtedly are identical with the missile-like bodies described in this paper. He did not, however, attach any significance to this valuable finding.

This study suggests strongly that the missile-like bodies contain most probably a number of enzymes which presumably are of great importance for the preparatory steps preceding feeding. The function of the tentacle could not be understood properly without taking into account the essential role played by the missile-like bodies. It seems that without them feeding cannot start and probably cannot be sustained.

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