MICROTUBULES AND ASSOCIATED MICROFILAMENTs IN THE TENTACLES OF THE SUCTORIAN HELIOPHRYA ERHARDI

MATTHES

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SUMMARY

At the ultrastructural level length changes accompanying linear movements of resting (non-feeding) tentacles of the suctorian Heliophrya involve not only altered microtubule numbers, but also marked changes in the specific microtubule pattern of cross-sectioned tentacles. These changes in number and pattern indicate a sliding between axonemal microtubules. The visualization of microfilaments in the cytoplasm at the tentacle base and in the knob region could shed new light on the problem of whether microtubular sliding is an active or passive process. At the tentacle base, microfilaments are either arranged in a ring-shaped configuration around the axoneme, or they run parallel to the axonemal microtubules, whereas at the tentacle tip during the resting state, microfilaments are closely associated with the plasma membrane of the knob. They form a filamentous reticular layer, which is continuous at the anchorage site of axonemal microtubules with the dense epiplasmic layer of the tentacle shaft. Obviously, this filamentous layer is engaged in positioning the haptocysts at the plasma membrane and in holding the membrane itself under tension. The putative contractile nature of microfilaments and the epiplasmic layer is argued from ATP-sensitive glycerol models of tentacles and from the results of halothane treatment of native tentacles. Halothane treatment of resting tentacles also gave indications of the presence of differentially stable intermicrotubule-bridges. The role of microfilaments and halothane-resistant dynein-like inter-row bridges in tentacle movement is discussed.

As soon as the plasma membrane of the knob is ‘sealed’ with the prey pellicle during feeding, the microtubules of the sleeve region slide into the knob where they bend back and outwards. The microtubules now appear decorated and sometimes cross-connected by microfilaments which adhere closely to the plasma membrane — now acting as a peritrophic membrane — lining the prey cytoplasm against the microtubules of the inner tube. These microfilaments which show a close association with the microtubules of the active knob area, are thought to be engaged in microtubular bending and stretching during feeding. They may also be involved in the transport of the peritrophic membrane in distal tentacle regions. Microcinematographically recorded oscillations in tentacle diameter in these regions are in agreement with the electron-microscopic findings of various states of collapsed tentacle axonemes. These observations, as well as the occurrence of helically twisted tentacles during feeding, suggest microfilament-mediated sequential back and forth movements of sleeve microtubules in the knob region which generate a proximally migrating helical wave.

INTRODUCTION

Suctorian tentacles have attracted the interest of numerous cell biologists concerned with cellular transport phenomena because these structures possess a highly ordered array of microtubules (for literature see Bardele, 1972, 1974). This material offers a unique opportunity to study many aspects of microtubular function, for the
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Microtubules not only provide for the rigidity of the tentacle, but may also be responsible for its contraction and extension during feeding and in response to external stimuli. They may likewise contribute to tentacle bending, for instance during the 'seeking movements' of the 1-mm-long prehensile tentacle of Akinetopsis rarai or in other longer tentacles engaged in prey capture (Hitchen & Butler, 1974). They are thought to be involved in the transport of membranes lining the prey cytoplasm.

Relatively little attention has been paid to tentacle motility during the resting state, because most species studied so far either possess tentacles which move very little or which are too short to exhibit any noticeable alteration at the ultrastructural level. Examination of expanded and contracted resting and feeding tentacles has provided evidence that not only is the microtubule arrangement altered, but also that there are marked differences in their number, indicating that sliding of microtubules accompanies length changes of the tentacle.

Tucker (1974) has recently argued that active sliding of the microtubules may not be involved during feeding in the suctorian Tokophrya. He assumed the site of force generation to be localized in the knob region at the tip of the tentacle and that microtubular sliding could well be a passive process. The present paper reveals by means of a modified fixation procedure the presence of numerous microfilaments - a second possible force-generating element - in association with the tentacle microtubules, supporting Tucker's deduction. Some experimental studies employing glycerol models and the use of halothane provide further evidence for a possible interaction of microtubules and microfilaments in tentacle movement.

MATERIAL AND METHODS

Heliophrya erlatardi Matthes, a large disk-like fresh water suctorian with a variable number of tentacle bundles (4-6) and 10-12 contractile vacuoles at the cell periphery, was cultivated in Petri dishes (10-14 cm diameter) in a soil medium with Paramecium multimicronucleatum as food organism. The suctorian was isolated from pond samples collected in 1969 in the vicinity of Aachen, Germany. At room temperature (18-23 °C) mass-conjugation occurs in some Petri dishes periodically every 4-6 weeks.

Electron microscopy

For electron microscopy of tentacle structure, cells were either fixed in 2.5-4% glutaraldehyde buffered with 0.15 M cacodylate or 0.05 M s-collidine adjusted to pH 6.8-7.0. A second fixation procedure was carried out with a recently developed polymeric Schiff-base, a glutaraldehyde-amino reaction product, which acts as fast as OsO4 and gives fixation of quality comparable to that with pure glutaraldehyde (Hauser, in preparation).

After a fixation time of 1 h, cells were washed at 4 °C overnight in the buffers used. Following this, cells were postfixed in a 1% OsO4/cacodylate solution for 30 min followed by 2 repeated washing procedures with cacodylate buffer during 1 h. After routine dehydration in a graded ethanol series the cells were conventionally embedded via propylene oxide in Epon 812 and sectioned with an LKB-Ulrotome III equipped with a diamond knife. Sections were stained with a solution of 4% uranyl acetate in 50% ethanol for 20 min, immersed for another 5 min in lead citrate and rinsed with 0.1 N NaOH. In some cases fixed cells were stained in the 70% ethanol step during dehydration with a saturated solution of lanthanum hydroxide for 30 min. Sections were examined with a Philips EM300 G electron microscope at 60-80 kV.
Glycerol models

Cells grown on glass coverslips in culture dishes were immersed in a solution of 50% v/v glycerol, 10% v/v dimethylsulphoxide (DMSO) in 5 mM Sørensen’s phosphate buffer (pH 6.8), 5 mM MgCl₂ and EGTA for 2 h at —40 °C. After the extraction procedure the slides were examined with a Zeiss photomicroscope II and under visual control the glycerol medium was rapidly exchanged for a contraction medium containing 30 mM ATP (disodium salt), in 10 mM imidazole, 5 mM sodium azide (NaN₃), 17.5 mM MgCl₂, 5 mM CaCl₂ and EGTA (pH 7.0) by suction with filter paper. Controls were run with the standard salt solution either with GTP or ITP, instead of ATP.

Halothane treatments

Shaking of 10 ml culture medium with 1 ml of the anaesthetic for 10 min in a glass-stoppered bottle at room temperature gave a saturated halothane medium. One culture dish was fixed (for 30 s–1 min) immediately after halothane treatment, while in another the halothane medium was replaced after 10 min treatment by the fixation solution.

Microcinematography

An apparatus constructed by Troyer (1975a) was used with a film speed of 10–25 frames/s. The recording material was 16-mm Eastman Plus-X-negative film in a Bolex H 16 J camera.

RESULTS

The arrangement of microtubules in the inactive tentacle during the expanded and the contracted state

A detailed discussion of the ultrastructure of the tentacle of Heliophrya and especially a comparison with other suctorian species can be omitted because it corresponds, within the usual frame of interspecific variation, to other cases described already. For a general survey, the review article by Bardele (1974) may be consulted.

Compared to most species studied thus far, the long tentacles of Heliophrya (which measure up to 300 μm in the expanded state) are advantageous for an electron-microscopic analysis of the as yet insufficiently considered problems connected with the capacity for linear contraction. The latter is, indirectly, also of importance for feeding. If contractility should indeed depend upon a sliding filament mechanism, the chances of observing correlated changes at the ultrastructural level are increased in species with very long tentacles.

Transparent, unfed individuals are most suitable for the study of tentacles in the maximally expanded state. All of their 40–60 tentacles are usually fully extended and carry out only slight linear contractions when undisturbed (Fig. 4A). On the other hand, tentacles contracted to about one-third of the original length are available for study if the culture dish is kept at 5 °C for 0.5 h prior to fixation or if 10⁻⁷ M usnic acid (a dibenzofurane, typical of many lichenes such as Usnea, etc.) is added to the culture medium (Hauser, in preparation). It should be noted here that tentacle microtubules, like those of cilia, are insensitive toward low temperature.

Fig. 5A–D shows, at the same magnification, cross-sections from the mid region of the free tentacle shaft in different states of contraction. It is easily noted by comparing Fig. 5A, C with B, D that not only the arrangements but also the numbers of
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Microtubules are different. In the contracted state of Fig. 5A the microtubular ribbons projecting like septae toward the tentacular interior contain almost always six, exceptionally five microtubules, whereas the expanded state is characterized by a maximum of only five microtubules per ribbon. Furthermore, the axonemal lumen is considerably enlarged in the expanded state because the microtubule ribbons subtend a lower angle with the outer circle of microtubules. Although there are occasionally slight differences in the number of ribbons per cross-section, e.g. 20 in Fig. 5B compared to 19 in Fig. 5A, counts obtained from 20 cross-sections each (Table 1) demonstrate that the average number of microtubules is clearly higher in the contracted tentacle. Differences in the number of ribbons, on the other hand, seem to be within the limits of normal variability between tentacles. The tendency toward an increase in the average number of microtubules accompanying contraction is even enhanced in the more proximal regions of the tentacle as shown in Fig. 5C and D from cross-sections at the level of the cytoplasm. In contrast to the upper 2 pictures, the number of ribbons is the same and the difference in microtubule number is therefore all the more obvious.

The occasionally observed increased diameter of the microtubular cylinder in the expanded tentacle is not due (as might be supposed) to recruitment of microtubules from the microtubule ribbons into the cylinder but to an increase in the distances between the microtubules of the outer circle. A normal increase in the number of microtubules from the proximal toward the distal regions of the tentacle as described for many suctoria (Bardele, 1974) cannot be demonstrated in Heliophrya. As the Table shows, the differences are not significant and in some single cases the microtubule number even increases toward the tentacle base.

Concomitant with the change from the contracted to the expanded phase, the microtubular ribbons (mtr) of the tentacle shaft pass from a state of helical torsion demonstrated in Fig. 5A to a largely parallel arrangement (Fig. 5B). Simultaneously, the ribbons become more closely aligned along the inner surface of the outer circle while the number of microtubules becomes reduced. Since the distances especially between the innermost microtubules of adjacent ribbons become larger, the connecting interrow bridges (irb) are served and the arms (fa) are set free (Figs. 5B, D and 2). The extension of these ribbons toward the tentacular lumen enables them to establish contact with the peritrophic membrane when feeding occurs (see below).

Comparison of the expanded and contracted states leads to the conclusion that the linear movements of the suctorian tentacle must to some extent be accompanied by longitudinal displacements of microtubules in the axoneme. The possible involvement

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**Table 1. Microtubule (mt) counts in various phases of Heliophrya**

<table>
<thead>
<tr>
<th>Tentacle phase</th>
<th>Expanded state</th>
<th>Contracted state</th>
<th>Feeding state</th>
<th>No. of counts each state</th>
</tr>
</thead>
<tbody>
<tr>
<td>mt number, tentacle base</td>
<td>151 ± 8</td>
<td>180 ± 11</td>
<td>157 ± 3</td>
<td>20</td>
</tr>
<tr>
<td>mt number, tentacle shaft</td>
<td>155 ± 1</td>
<td>175 ± 10</td>
<td>164 ± 2</td>
<td>20</td>
</tr>
<tr>
<td>mt-ribbons</td>
<td>19 ± 2</td>
<td>20 ± 1</td>
<td>20 ± 3</td>
<td>30</td>
</tr>
<tr>
<td>mt number, total</td>
<td>147 ± 5</td>
<td>181 ± 10</td>
<td>159 ± 7</td>
<td>20</td>
</tr>
</tbody>
</table>

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of the cross-bridges which maintain the microtubule configuration within the axoneme will be discussed below. Three types of such cross-bridges are shown in Fig. 5: (in the inset of Fig. 5, cb) those which connect the microtubules of the outer circle, the inter-row bridges (irb) of Fig. 5c, and the connecting bridges (cob) which bind the

Fig. 1. Diagram of a longitudinally sectioned tentacle of *H. erhardi* in the inactive state. Besides haptocysts (ha), the knob region contains accumulations of osmiophilic granules (og) and electron-transparent vesicles with asymmetrical osmiophilic content (cv, capped vesicles). The haptocysts (ha) are held in a vertical position by the thin epiplasmic filament layer (efl). This generates local tension which causes the plasma membrane to bulge out. In the expanded state the epiplasmic filament layer originates at the anchorage site (as) of the microtubule at the transition of tentacular pellicle (pe) and knob membrane. At the anchorage site (as) the microtubules of the outer circle (ot) re-unite with those of the inner ribbons (it) from which they had separated at the level of the sleeve region (slr; compare also cross-section). In the cytoplasm, at the tentacle base, microfilaments (mf) are arranged as a ring around the microtubule skeleton (mfr) and parallel to the microtubules within and outside the microtubule cylinder.
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inner microtubule ribbons to the microtubules of the outer circle. The relation of the microtubular pattern to the phase of contraction is diagrammed in Fig. 2 where the interacting cross-bridges are designated by arabic numerals.

In longitudinal sections of tentacle axonemes (Fig. 5E) 2 types of cross-bridges are recognizable. The first type spans the larger distances, and are spaced about 20 nm apart but fail to show any pronounced periodicity (small arrowheads in the right half of the picture). The second type (parallel lines at left), not as obvious at first glance, is regularly directed at a 45° angle and has a fairly constant periodicity of 10 nm. The latter are thought to represent the short bridges between the microtubules (irb) within the ribbons. They are not noticeable in cross-sections. Because of their length and regular arrangement they are the ones most similar to the dynein arms of cilia.

**Fig. 2.** Diagrammatic representation of the 2 possible functional states of the inactive tentacle. At the left (I) the maximally expanded state, right (II) the microtubule arrangement at maximal contraction of the tentacle. There are no significant changes in microtubule number in the various tentacle regions (cytoplasmic base vs. free shaft) in either functional state. Arabic numerals (I–5) designate the various interactions of bridge- or arm-like structures within the microtubule pattern. Diagram I demonstrates how free arms of the inner rows of microtubules become available for contact with the plasma membrane during feeding as the rows of microtubules move more closely toward the outer circle. Both functional states of the inactive tentacle show significant differences in the total number of microtubules seen in cross-section. This permits the conclusion that microtubules slide past each other, either actively or passively.

**Microtubule-associated microfilaments in the inactive tentacle**

At the level of the tentacle bases (teb) filamentous layers (cmf) of about 250 nm thickness are arranged as rings around the axoneme cylinders (Fig. 6B). They consist of microfilaments of about 5–7 nm thickness (large arrowheads, Fig. 6A). The latter might represent aggregations of smaller units. With serial sections and tilting, their filamentous nature was clearly demonstrable.

It is especially striking that such ring-shaped arrangements of filaments have only been seen around axonemes of expanded tentacles (ete), i.e. those with a wide lumen, but not at the base of contracted tentacles (cte) which are recognizable by the narrow lumen (Fig. 6D). The latter are also surrounded by a halo which contains scarcely any long filaments. At best, short stretches of an apparently filamentous network are
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occasionally noticeable (Fig. 6D). One gains the impression that it might consist either of non-aggregated material or cross-sectioned filaments (mf) oriented parallel to the long axis of the axoneme because the dimensions are identical with those of circular filaments.

That microfilaments which run parallel to the microtubules do in fact exist is shown in Fig. 6c and e. In both longitudinal sections the larger arrowheads denote more or less extensive bundles of filaments of up to 700 nm length. They run either at a small distance parallel to the tentacular microtubules or, as in Fig. 6c between the 2 arrowheads, they approach the microtubules. They can also be demonstrated within the lumen of the axoneme (small arrowheads in Fig. 6e). There they may even be found in the free tentacle up to the so-called manchette region. But they have never been seen higher up in the outer tube of the tentacle shaft during the inactive state. One of the reasons for this might be sought in their possible involvement in vesicle transport into the region of the terminal knob which is known to occur in the inactive tentacle via the inner tube.

Microfilaments in the manchette region and in the inactive tentacle knob

Longitudinal sections through the upper third of the contracted tentacle show that the manchette region described already by several authors (Bardele, 1974; Hitchen & Butler, 1974; Tucker, 1974) extends in the case of Heliophrya at least over a length of 1 μm. Depending upon the degree of tentacle retraction, the microtubule ribbons are more or less contorted and turned towards the axonemal lumen (Fig. 7A). This contortion which is also found in the remaining tentacle shaft when retracted is undoubtedly most extreme in the manchette region.

The finely fibrillar material (f) which seems to hold the microtubules of the outer circle together (Fig. 7A), is in the opinion of other authors (e.g. Bardele, 1974) not identical with the cross-bridges of the proximal microtubular outer circle. From Fig. 7A, a cross-section through the proximal range of the manchette region, it is evident that the microtubules of the outer cylinder separate from those of the inner cylinder and bend in a funnel shape toward the pellicle, where they finally insert on the plasma membrane together with the microtubule ribbons at the transition of pellicle and tentacle knob (inset, as, in Fig. 7A, and Hauser, 1970). Fig. 7B shows again the extreme contortion which is indicated by the changing directions of short microtubular groups.

The most surprising observation was certainly the existence of a layer of membrane-associated filaments (3–5 nm in diameter) in the knob region. The thickness of this layer corresponds fairly well to the length of a haptocyst (approx. 100 nm). The filaments form an evenly dense coat which lines the inside of the globular knob region (in the expanded state) (Fig. 7D).

Light-microscopically one can notice a shrinking of the knob during retraction of the tentacle. In longitudinal sections of such tentacles we can now find bundled filaments (large arrows in Fig. 7B) which are preferentially oriented toward the anchorage sites (as) of the microtubules at the knob base. Normally, such a preferential direction (Fig. 7A, inset, arrow) cannot be observed. Rather, the filaments form an
unoriented network which projects in part into the space between the pellicle and the microtubule cylinder in the manchette region (compare Fig. 7A, el).

Apparently, the membrane-associated filament system (Fig. 7C) positions the haptocysts at the plasma membrane and holds the membrane under tension in such a way that the haptocyst tips project slightly above the surface. This is certainly a favourable position for establishing contact with the prey organism.

Another interesting feature of the knob is an unusual decoration of the microtubule endings in the region of the anchorage-sites (Fig. 7D, inset). These ends do not exhibit the normally smooth outlines but appear split or coated with filamentous material (Fig. 7E, bracketed area). This may be an indication of possible interaction between microtubules and microfilaments.

The action of halothane on the microtubule system of the inactive tentacle

In spite of a number of studies, the mode of action of the anaesthetic halothane is not completely clear. However, it seems to be certain that it leads to the dispersion of labile microtubules (Allison et al. 1970), while the more stable microtubule systems, as in cilia, show degenerative changes only after prolonged action (Nunn et al. 1974). The acto-myosin system also seems to be affected (Shigenaka, Watanabe & Kaneda, 1974).

Since the more stable microtubule systems are relatively insensitive toward halothane, we felt that it might be possible to gain information on the role of the microfilament system in tentacle movements by using halothane. After only one minute in a saturated solution most tentacles started to swell rapidly following a brief retraction and thereby extended to varying degrees.

Serial cross-sections of tentacles treated for various lengths of time with halothane are shown in Fig. 8B1-B3. Fig. 8B1 shows a section through a tentacle fixed immediately upon stretching. The only change it shows is an enlarged space between the outer circle of microtubules and the ribbons. After longer treatment (Fig. 8B2), the ordered microtubule pattern breaks down gradually in an orderly sequence. The rigidity of the outer circle is lost first (large arrowheads) until finally, as shown in Fig. 8B3, the connexions between the ribbons are destroyed. Only the intact microtubule ribbons themselves are left of the formerly regular pattern of microtubules. Their short interconnecting bridges are evidently more resistant than other cross-bridges. The filament layer of the tentacle knob, like the remaining filamentous material including the epiplasmic layer, is lost from the beginning.

Tentacle models and the action of ATP

All glycerol models obtained as described (p. 591) reacted to the addition of 30 mM ATP in imidazole buffer (pH 6.8) with almost complete contraction (compare Figs. 4C1 and 4C2) accompanied by folding of the membrane into a spiral (Fig. 4A, inset). However, the time before the onset of the reaction was very variable and ranged from seconds to some minutes. This is probably due to the infiltration method, the suction with filter paper permitting no reproducible speeds of exchange with the very viscous glycerol medium.
Controls with equimolar concentrations of GTP and ITP always led to negative results. At best, the tentacles bent a little after exchange of the glycerol medium and became immediately limp, while the pellicle dissolved. In the ATP medium, on the other hand, dissolution of the pellicle sets in much later.

The configuration of microtubules in the active tentacle shaft

During feeding the tentacle axonemes of *Heliophrya* exhibit a microtubule configuration (Fig. 8c) which corresponds very much to that of other species (cf. Bardele, 1974; Tucker, 1974). As Tucker (1974) has shown in *Tokophrya*, the cross-bridges of the microtubule ribbons which interact with the peritrophic membrane are not confined to the distal region but do indeed extend right down to the tentacle base within the cytoplasm (inset, Fig. 8c, small arrowheads).

Fig. 8A demonstrates that the circular arrangement of microtubules is not always maintained during food uptake. It can in some places be deformed to an elliptical shape or even become completely compressed. Even then the peritrophic membrane, though glued together in almost regular spacing by an inner electron-dense material, lines the microtubular scaffolding completely (sem, Fig. 8A). An explanation for these deformations of axonemes, which were also noted by Tucker (1974) in *Tokophrya*, might be furnished by 2 supplementary observations. In semithin and ultrathin sections where a longitudinally cut tentacle could be observed over large distances nodal points of a helical torsion with a high pitch are noted. At the nodal points the tentacles appear squashed to flatness, leading to the axoneme deformations mentioned above. Merely passive collapsing can be ruled out for static reasons alone since a labile tentacle of such length would invariably buckle as soon as the prey becomes attached.

Microcinematographic records during feeding (compare Fig. 4B1—B5) also argue against a passive event. At 10–25 frames per s almost rhythmic pulsations are observed every 0.3–0.6 s (arrowheads in Fig. 4B) which proceed as a helically rotating wave down to the tentacle base. Difficult to interpret as yet is a zone of electron-dense material in the outer tube which is always found under the most diverse conditions of fixation. It consists of osmiophilic material filling homogeneously the space between pellicular alveoli (av, Fig. 8D), limiting membrane (lm) and epiplasmic layer (el), leading to a negative contrast of microtubules and cross bridges (inset, Fig. 8D, arrowheads and lines). As a possible explanation of the nature of this material we think of solubilized osmiophilic lipid granules transported in a countercurrent to the knob region where they serve in rebuilding membrane material.

Microtubules and microfilaments in the knob region of the active tentacle

Immediately after attachment of prey the knob region undergoes a series of rapid changes. Retaining their helical arrangement, the microtubules slide into the knob where they separate in a fountain-like array and are bent backward (compare Figs. 3A and B). The problem of this bending has been discussed before (Bardele, 1974; Tucker, 1974). In relation to the microtubules it narrows down to the question whether this represents an active or a passive process.
The presence of microfilaments in the knob region of an inactive tentacle leads to the expectation that they might also be demonstrable in the active tentacle knob and that their arrangement should permit conclusions concerning their possible function in feeding.

Fig. 3 A, B. Active tentacle during invagination of the plasma membrane with adhering prey cytoplasm (pc). As the food stream flows within the inner tube into the cell interior (large arrow), a counter-current (small arrows) transports vesicles (cv) and granules (og), possibly as membrane reserve material, toward the knob region. The microtubules slide into the knob, are bent distally into a fountain-shape and insert via microfilaments in the region of coalescence with the pellicle of the prey (ppe). A tangential section at plane a(pla) shows the microtubule-rows in the region of the bend diverging helically. From their anchoring points at the plasma membrane in the distal region of the knob down into the tentacle 'gullet' the microtubules form aggregates with microfilaments (ag).
Microfilaments of 3–5 nm diameter are in fact also found during the active phase. Their distribution is no longer a uniform one. In longitudinal sections through the inner 'gullet' (Fig. 8E), the microtubules are conspicuously marked by filamentous material. In places, it appears as an interconnecting network which is dense enough to make the microtubules almost indiscernible. This is even more strikingly displayed in a cross-section of the gullet region (Fig. 9A) and at the level of the line a' in Fig. 9C. The microtubules are barely localizable at the peritrophic membrane (Im) since they are so closely coated with filamentous material. It seems therefore justified to speak of a microtubule/microfilament (mt/mf)-complex. This conspicuous association can still be demonstrated where the microtubules bend backward. In Fig. 9C the filamentous material is predominantly located in the space between the microtubules and the plasma membrane, while the area behind the microtubule bundles (right half of the figure) is practically free of it.

Special attention was devoted to the region of contact between mt/mf-aggregates and the plasma membrane because there is as yet scarcely any information about this zone which is undoubtedly of special importance in the transport of the peritrophic membrane (compare Bardele, 1974). Figs. 9B and 9c show sections of such regions of insertion which demonstrate that the microtubules remain permanently anchored at the membrane during the endocytotic phase as well as in the inactive state. In spite of the peculiar fact that the microtubules appear always in homogeneous contrast within the knob region, so that the ends of microtubules are not clearly demonstrable, it is suggested that actual attachment is mediated via filaments. Because of the constant diameter of the microtubules, however, attachment regions (mt, Fig. 9G) can be recognized as well as the bare suggestion of contortion of the filaments inserting there (Fig. 9E, G). In tangential sections close to the margin (Fig. 9D, F) it is also apparent that the mt/mf-aggregates (large arrowheads, Fig. 9D) are even cross-linked just below the membrane by microfilaments (mf, arrows) running at right angles to the former.

From this arrangement of local accumulations of membrane-associated microfilaments at microtubule bundles we conclude that a filamentous layer exists also just below the plasma membrane. It is not as homogeneous as in the inactive phase because it may perhaps contract against the microtubule bundles, which act as mechanical antagonists while the peritrophic membrane is transported into the funnel-shaped opening of the tentacle, as discussed below.

The action of halothane on the active tentacle

Although a separate publication is planned on the actions of halothane, a few pertinent results should be mentioned in this connexion.

As a first sign of the onset of halothane action the prey detaches again from the knob, no matter how long it had been attached previously. After fixation at this stage membrane-associated microfilaments are already absent in the entire knob region. The microtubules themselves are no longer 'decorated' with microfilaments and show again the usual appearance of hollow cylinders.

In the region of the tentacle shaft the peritrophic membrane detaches from the
cross-bridges of the microtubule ribbons and the microtubules themselves move more closely together, although they maintain their configuration.

Although the bridge structures of the microtubules are still retained they appear more fragile and are sometimes extended to a multiple of their original lengths. Thus, with prolonged treatment, even the very short bridges between the microtubules within a ribbon become recognizable. Adopting the viewpoint of Tucker (1974), who postulated that the bridges are extensible and resistant to pull on the basis of observed ellipsoidal form changes of microtubules during feeding, it must be assumed that halothane abolishes elasticity and, possibly, contractility of the bridges.

DISCUSSION

The suctorian tentacle can be characterized by 5 principal functional aspects: contractility, bending movements, maintenance of an asymmetrical cell appendage (rigidity), bidirectional transport, and finally irritability. For some time, these functions have been brought into connexion with its most conspicuous ultrastructural characteristic, a microtubule system which has few parallels as, for example, in axopodia of heliozoans. We know today that these are all functions or properties generally ascribed to microtubules. In the tentacle axoneme, they occur together in a unique combination.

Recent discussions of microtubule function have concentrated on the question of a mechanochemical system for the generation of motive force by microtubules. Two views held the centre of general interest in the last years, sometimes discussed in too much isolation from each other. One is based on the assumption that the almost generally demonstrable bridge structures are capable of active swinging movements which lead to a sliding of microtubules past each other (McIntosh, Hepler & Van Wie, 1969) or to the transport of adhering material along stationary microtubules (Smith, 1971). The other view is based upon the theory of a dynamic equilibrium between monomers and polymers which is subjected to precise regulation leading to assembly and disassembly of microtubules and thereby to the generation of pulling forces (Inoué & Sato, 1967). However, it should also be mentioned in this connexion that doubts have lately been raised concerning the involvement of microtubules in some cases of saltatory transport because weighty arguments have been brought forth in favour of a direct system of membrane transport (Robison & Charlton, 1973; Byers, 1974; Troyer, 1975b).

These arguments as well as the demonstration of microtubule-associated microfilaments with the dimensions of contractile proteins (compare Wohlfarth-Bottermann & Stockem, 1972) in the basal regions of inactive tentacles are certainly no encouragement for attempts to reduce the various, mostly simultaneous tentacle functions enumerated above to one basic principle. We see little hope of explaining the principal tentacle functions in their totality on the basis of microtubules alone.

While the dilation of the ribbons as well as the outer cylinder of microtubules might still be thought of as a consequence of passive stretching (Tucker, 1974), the demonstrated change of microtubule configuration in inactive tentacles in the course of various states of contraction which may, in part, be even experimentally induced,
cannot be explained with similar ease. This might involve active as well as passive sliding since the changeable microtubule configuration on the one hand and the differences in the numbers of microtubules on the other need not necessarily be due to the same cause. Thus the different numbers of microtubules in the retracted and expanded states as expressed in Table 1 are due mainly to different numbers within ribbons, and not to changes in the number of ribbons nor the number of microtubules in the outer circle.

The possibility that active sliding takes place between the microtubules of the ribbons is also supported by the halothane experiments. The latter provided indications for the existence of cross-bridges with different functions. The long ones (Fig. 2, 1-4) with more static tasks according to Tucker (1974) lose their binding properties after brief action of the anaesthetic and thus lead to a breakdown of axoneme structure, while the short interrow bridges (compare Fig. 4D with Figs. 2, 5) are considerably more resistant. Only after prolonged action is the very tight microtubule complex loosened, permitting these links to become apparent in cross-sections (Hauser, in preparation). The similarity in arrangement and length of these short arms to the dynein of A-tubules in cilia has already been pointed out.

Dislocations of complete ribbons, on the other hand, could be the result of the pull of interacting contractile microfilaments. At any rate, it is striking and scarcely by chance that the pattern of microfilaments is strictly correlated with the state of contraction. It is ring-shaped in the expanded state of the tentacle and probably parallel to the long axis of the axoneme in the retracted state.

An explanation for their concentration at the tentacle bases might be their involvement in lateral movements of expanded but otherwise completely rigid tentacles, which are not uncommon in Heliophrya.

Tucker (1974) discusses also the possibility that the epiplasmic layer which is found in practically all suctorian tentacles might be involved in tentacle contraction during feeding and in the linear movements of inactive tentacles. On the basis of the halothane experiments this possibility must be considered, since even before the typical microtubule pattern of the tentacle is noticeably affected, the epiplasmic layer has disappeared, together with the microfilaments. Light-microscopic observations on halothane-treated individuals might perhaps deserve special attention in this connexion, since they furnish proof of tension in the tentacle while it retracts concomitant with spiral folding of the pellicle. A contraction, possibly the response to stimulation, is followed by a sudden stretching of the tentacle accompanied by a balloon-like expansion of the pellicle. At the ultrastructural level, the helical arrangement of microtubules and the epiplasmic layer are now no longer demonstrable. This raises the question whether the latter is composed of the same material as are the microfilaments.

That neither active nor passive telescope-like sliding can fully explain tentacle contraction becomes evident when cases of complete retraction of tentacles are considered. However, this occurs only rarely under certain physiological conditions, e.g. in overfed animals. Because of the small diameter of the disk-shaped cell body of *Heliophrya* we are forced to assume additionally depolymerization and repolymerization.
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as does Tilney (1968) in the case of the continuously retracting and expanding axopodia of Heliozoa. The chance observation of a cross-sectioned tentacle fragment without an outer circle of microtubules could be accounted for on this basis.

The above mentioned contractility of the epiplasm, postulated by Tucker (1974) particularly because of the bending of microtubules in the knob region during feeding, deserves renewed attention, because microfilaments were found at the distal end of the manchette region where microtubules are anchored at the plasma membrane. As shown in Fig. 7A (inset) the epiplasmic layer appears dispersed to such a degree that it can scarcely be distinguished from the filamentous material associated with microtubules which are anchored in this region. This anchorage site plays a key role in the thoughts of almost all authors concerned with the transport mechanism in the suctorian tentacle. The demonstration of a filamentous layer below the plasma membrane of the knob and the proof of its close spatial relation to the microtubules at the anchorage region obviates in our view the criticisms of Rudzinska (1973) and of Hitchen & Butler (1973) against the 'grasp and swallow-model' of Bardele (1972). Bardele (1974) suspected on the basis of a casual observation of microfilaments in the knob region that the microtubules might insert flexibly at the plasma membrane. This would permit back-and-forth sliding of the microtubule ribbons, a central postulate of his model. The demonstration of microfilaments in the knob region and their largely membrane-bound arrangement in the inactive state explains also the curious anchoring of the haptocysts at the plasma membrane.

As during retraction of the knob in the contracting tentacle, where the arrangement of microfilaments points to the anchorage sites as the centres of contraction (compare Fig. 7B), the bending and sliding movements of the microtubules could be accounted for by pulling action of microfilaments in the opposite direction. This view is in accordance with Tucker (1974).

In our opinion, this idea is further supported by observations made on feeding tentacles. In this case, the microtubules form aggregates with microfilaments over wide areas. Such curiously decorated microtubules insert via microfilaments of differing lengths at the layer of membrane-associated filaments. This and the frequent cross-linking of mf-aggregates by membrane-associated filaments (compare Fig. 8E) points to an antagonistic function of the microtubules and a mechanism for transport of the peritrophic membrane by a contractile filament system. On the other hand, there is no evidence against the kind of transport mechanism proposed by Bardele (1972) if the microfilaments fulfil the double function of adhering to the membrane and effecting an oscillation in back-and-forth sliding of the microtubule system. If such sliding takes place in the form of a circular rotation in the sequence of contractions it could provide an explanation for the microcinematographic results. The latter display rapid changes in the diameter of the tentacle shaft due to a helically rotating wave which extends down to the tentacle base into the cytoplasm.

At this point, the problem of transport within the tentacle shaft has to be raised. Specifically, this includes the question as to the role played by the cross-bridges to the membrane, which were shown to exist along the whole length of the tentacle as well as in the cytopharyngeal apparatus of other ciliates (e.g. Hitchen & Butler, 1973,
There are several indications that these bridges have only static functions as mere attachment points. In cross-sections through microtubule cylinders with deep infoldings due to helical contortion, the adhering membrane reaches even into the deepest folds.

In unpublished pictures of axoneme bases which are spliced wide apart the peritrophic membrane is also still seen to adhere. The actual transport system might in this region be represented by the microtubules of the ribbons sliding past each other or by a fluidic membrane of the type discussed by Troyer (1975) in the case of the heliozoan axopodium.

The present finding of a membrane- and microtubule-associated system of microfilaments and the resulting hypothesis of generation of motive force in the system by interaction of both structural elements is supported by experiments with glycerol-extracted models capable of rapid tentacle contractions after addition of an adequate supply of ATP.

Coexistence of microtubules and microfilaments was formerly known almost exclusively from nerve cells (Smith, 1971; Ochs, 1972) and melanocytes (Tilney, 1968; Moellmann, McGuire & Lerner, 1973). Recent studies on sensory epithelia (Heywood, Van der Water, Hilding & Ruben, 1975) and the demonstration of heavy meromyosin-binding filaments in close association with the plasma membrane in Deuter's neurons (Metuzals & Mushinsky, 1974) show, however, that this is not exceptional. Reports indicating coexistence of spindle microtubules and presumably contractile microfilaments appear in increasing numbers. Recently Edds (1975) presented evidence for the coexistence of actomyosin and microtubules in the axopods of the heliozoan Echinosphaerium. The presence of actin or myosin in dividing nuclei – partly observed as filaments in electron-microscopical pictures – was deduced either from their ability to bind heavy meromyosin, their reaction to ATP, or their reaction with specific antibodies (e.g. Forer & Behnke, 1972; Jockusch, Ryser & Behnke, 1973; Hauser, 1973; Hinkley & Telser, 1974; Hauser, Beinbrech, Gröschel-Stewart & Jockusch, 1975).

An exception is possibly the especially long 10-nm filaments in melanocytes and in the micronuclei of some ciliates. Bikle, Tilney & Porter (1966) as well as Hauser & Beinbrech (1973) give reasons that the latter may consist of linearly aggregated tubulin rather than an actin-like protein. Finally it was recently shown by antibody labelling that myosin and actin can be regular components of the plasma membrane (Willingham, Oslund & Pastan, 1974; Pollack, Osborn & Weber, 1975).

The possibility of an interaction between microfilaments of an actomyosin-like nature and microtubules as postulated in the present paper gains further support from some biochemical data. Thus, Mohri & Shinomura (1973) obtained a superprecipitation-like phenomenon between microtubule protein and myosin in the presence of ATP at low ionic strength, and Puszkin & Berl (1970) succeeded in isolating a colchicin-binding protein from brain which increased the ATPase activity of myosin as does actin. On the other hand, we have at the moment no unequivocal answer with respect to possible interactions of heavy meromyosin and microtubules. Other evidence also indicates interactions between actin and microtubules, as reviewed by Forer (1974).
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REFERENCES


Microtubules and microfilaments in Suctoria


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Fig. 4. A. Overall view of *Heliophrya erhardi* with mostly expanded tentacles arranged in 4 bundles. × 200. Inset, contracting tentacle with spiral folding of the compressed pellicle.

B1-B5. From a microcinematographic run (16-mm film) of an active tentacle (*ate*) spanning a total of 1.8 s, taken at 10 frames/s, so B1-B5 represent 0, 0.4, 0.7, 1.3 and 1.8 s, respectively. Small arrows indicate changes in the diameter of the distal tentacle end as a consequence of repeated oscillations. *pr*, prey; *su*, suctorian.

C1. Suctorium which grew attached to coverslip. Treated 1 h with 50% glycerol/10% DMSO. *gcl*, glycerinated.

C4. Same preparation as C1 after replacement of solution by 30 mM ATP in imidazol buffer (pH 6.9). All previously extended tentacles react with instantaneous contraction. × 200.
Microtubules and microfilaments in Suctoria
Fig. 5. A. Cross-section through the mid region of a contracted inactive tentacle shaft. Inset, same stage with clearly discernible cross-bridges (cb) in the outer circle of microtubules. × 65,000.

B. Cross-section through the mid region of an expanded inactive tentacle shaft.

C. Base of a contracted tentacle in the cytoplasm.

D. Base of expanded tentacle in the cytoplasm.

E. Longitudinal section of the microtubule cylinder in the cytoplasm. Small arrows and parallel lines indicate cross-bridges. The oblique stripiness pointed out by parallel lines is most likely due to the type of cross-bridges which connect the microtubules within a row with a periodicity of about 10 nm. × 87,000.

cob, connective bridges; fa, free arms; irb, inter-row bridges; mtc, microtubule circle; mtr, microtubule rows; og, osmiophilic granules.
Microtubules and microfilaments in Suctoria
Fig. 6. A. Part of the base of an expanded tentacle surrounded by a ring of microfilaments. Arrows indicate longitudinally sectioned microfilaments of 5 nm thickness. × 78,000.

B. Further example of the ring-like arrangement of microfilaments (cmf) around expanded tentacles (ete). × 39,000.

c. Longitudinally sectioned microtubule cylinder in the cytoplasm with parallel orientation of microfilaments (arrows). × 39,000.

d. Base of contracted tentacle (cte) with cross-sectioned, longitudinally oriented microfilaments (mf e). × 39,000.

e. Sectioned as in c: small arrows point to microfilaments in the cylinder lumen, large arrows to microfilaments with parallel orientation. × 52,000.
Microtubules and microfilaments in Suctoria
Fig. 7. A. Cross-section of sleeve region (si). Serial sections show that further distally the microtubules can be bent even more toward the outside before they join again at the anchoring site (as) with the microtubules of the ribbons comprising the inner cylinder. The space between inner and outer cylinder enlarges with increasing contraction while the degree of torsion within the microtubule rows grows stronger. The epiplasmic layer (el) below the pellicle seems to consist of cross-sectioned filamentous material. × 52000.

Inset: Anchoring site (as) of sleeve microtubules and microtubules of the inner cylinder at the transition of tentacle shaft and knob region. The microtubules of this region are decorated with fine filamentous material. × 39000.

B. Longitudinal section of a tentacle end with retracted knob. Filament bundles (large arrows) are directed toward the anchoring sites of microtubules; ha, cross-sectioned haptocysts. × 24000.

C. Tangential section of knob region with membrane-associated filament layer. × 87000.

D. Cross-sectioned knob in the expanded state. At this stage, a uniform layer of filaments (emf) of about 3 nm thickness is found right below the plasma membrane. The thickness of this layer corresponds approximately to the length of haptocysts (ha). Outbulging of the plasma membrane around the haptocyst tips can be taken as an indication of tension in the knob membrane. × 39000.

Inset: Longitudinal section through the anchoring region with transition to the filament layer of the knob during tentacle extension. × 39000.

E. The ends of microtubules (arrows) in the anchoring region at high resolution. In contrast to more proximal regions these microtubules appear roughened due to 'decoration' with microfilaments (between brackets). × 217000.
Microtubules and microfilaments in Suctoria
Fig. 8. A. Cross-section through the basal region of an active tentacle. During feeding, the tentacle is not a solid tube throughout its length. At intervals, it takes on an elliptical or even completely flattened shape (compare Fig. 4 B₁–B₄). The peritrophic membrane in its interior appears sealed by an osmiophilic substance in such cases. *sem*, sealed membrane. × 39000.

B₁–B₃. Cross-sections through halothane-treated tentacles. B₁, after 1-min treatment with culture medium saturated with halothane, the first noticeable change is a separation of the inner microtubule ribbons from the microtubules of the outer circle; × 39000. B₂, the formerly closed outer circle becomes interrupted after 5 min in halothane and the inner microtubule ribbons are no longer held in their correct position; × 67000. B₃, after 10 min the bridges between the inner microtubule ribbons have loosened and the tentacle geometry is severely disturbed, only the integrity of the rows themselves seems to be intact. × 52000.

c. Tentacle shaft during food intake. Bridge connexion are attached to the lining membrane (*lm*) of the prey cytoplasm (small arrows, inset). This is also expressed in the course of the membrane in the survey picture. × 39000; inset, × 130000.

d. In the distal part of the tentacle, below the gullet region, material of such high electron density appears in the outer tube that the permanent tentacle structures appear here in negative contrast. This holds also for the cross-bridges (inset, lines and arrows). The dense material might represent membrane reserves (e.g. osmiophilic lipids) or it might serve to increase the rigidity of the tentacle membrane. *av*, alveoli of the pellicle; *el*, epiplasmic layer; *lm*, lining membrane; *pm*, plasma membrane. × 99000; inset × 132000.

e. Bent microtubules of the 'gullet' region during feeding which are decorated with filamentous material. × 87000.
Microtubules and microfilaments in Suctoria
Fig. 9. A. Cross-section from the region of the tentacle gullet. Microtubules and microfilaments (mt/mf) appear as closely associated aggregates; lm, lining membrane. × 39,000.

B. Anchoring of deflected microtubules in the lower knob region. Arrows point to microtubules and membrane-associated microfilaments. × 52,000.

C. Longitudinal section of microtubules in the trumpet-shaped 'gullet' region of an active tentacle. Between microtubules and the plasma membrane of the knob are fine filaments which are absent in the right half of the picture. × 39,000. a', level of sectioning in Fig. 9A; cv, capped vesicle.

D. Cross-section through the marginal zone of the knob during feeding. Large arrowheads denote microtubules decorated with microfilaments. Close to the membrane, they are cross-linked by thin strands of microfilaments (arrows, mf). × 52,000.

E. Anchoring of a microtubule (mta) at the plasma membrane. × 52,000.

F. Tangential section through the knob margin (as in D) with mt/mf-aggregation and cross-linking membrane-associated microfilaments (mf). × 52,000.

G. Region of insertion of microtubules (mi) at the plasma membrane (pm). The ends of the microtubules are closely connected with membrane-bound filamentous structures. × 130,000.