

STRUCTURES LINKING THE MYONEMES, ENDOPLASMIC RETICULUM, AND SURFACE MEMBRANES IN THE CONTRACTILE CILIATE *VORTICELLA*

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ABSTRACT

An electron microscope investigation of the interface between the myonemes of *Vorticella convallaria* and their associated endoplasmic reticulum (ER) has revealed structures of a complex morphology linking these two organelles. These structures are named "linkage complexes". Each complex contains a spindle-shaped midpiece which lies in a groove of the ER membrane. Microfilaments splay out from the tips of the midpiece and may come in contact with the inner alveolar sac membrane. Three to six raillike structures lie on each side of the midpiece and parallel it. The ER membrane appears to pass through the sides of the rails. In the lumen of the ER these rails are associated with a meshwork of filaments. A cradle of five rods lies within the groove under the midpiece. The ER membrane also passes through these rods which contact the same meshwork. In the scopular region and in the stalk the microfilaments from the midpiece form a bundle which passes into the lumen of modified basal bodies. These basal bodies are connected to the alveolar sac which, in the stalk, passes as a flattened tube along its length. The parts of the dissociated linkage complex are scattered throughout the spasmoneme of the stalk along membranes of the intraspasmonemal tubules. Thus, both stalk and body contractile bundles have linkage complexes that link their associated membrane systems to the microfibrils and, in turn, connect this membrane-microfibrillar interface to the pellicular membranes. The arrangement of the linkage complex suggests an involvement in the control of the transport of calcium ions between ER and microfibrils, and possibly the transfer of a message from the surface membranes to the sites of calcium release to trigger myonemal contraction.

INTRODUCTION

The dramatic nature of the coiling process of the stalks of certain peritrich ciliates and the simultaneous change in shape of their bodies has made these protozoans fascinating and fruitful organisms of study. In recent years the morphological structures accounting for this contractile activity have been shown to be bundles of 20–40 μm microfibrils. These bundles lie subjacent to the pellicle of the body, or zooid, of the organism and converge in the aboral region of the zooid to form one

large microfibrillar bundle which passes into and through the length of the stalk (Fauré-Fremiet et al., 1956; Sotelo and Trujillo-Cenóz, 1959).

The contractile bundle within the stalk has often been considered to be a primitive muscle most closely analogous to the smooth muscle of higher animals (Fauré-Fremiet et al., 1956; Sotelo and Trujillo-Cenóz, 1959). This idea has recently been challenged by Weis-Fogh and Amos (1972) who present arguments for a contraction process not

involving shearing forces but either involving stretching and shortening of a loose network of protein chains brought about by the formation and breaking of nonrandom, calcium-sensitive cross-links or involving the repulsion of like charges within such a network when calcium ions are removed from the system. They show that the physical properties of the microfibrils more closely resemble those of long-chained rubber than those of muscle. The configurational state of glycerinated stalk models is known to be dependent only on the calcium ion concentration available to the bundle of microfibrils; the microfibrils relax and extend when the calcium ion concentration falls below 10^{-7} g ions/liter and contract when the calcium ion concentration rises above this figure (Amos, 1971). The presence of adenosine triphosphate (ATP) is not required for this change to occur in extracted stalk models (Levine, 1956; Hoffmann-Berling, 1958; Rahat et al., 1969; Amos, 1971).

In order that the living organism be able to control this contractile process it must have some system by which calcium ions can be made available to the microfibrils as well as a system of removing the calcium ions from the microfibrils and storing these ions during periods of time between contractions. In striated muscles, which require the presence of calcium ions as well as ATP in order to contract, these functions are probably carried out by the sarcoplasmic reticulum (SR) (for reviews see Page, 1968; Podolsky, 1968; Sandow, 1970; Ashley, 1971). A system of membrane-bound saccules and tubules is also known to be associated with the microfibrils in the peritrichs (Fauré-Fremiet et al., 1956; Fauré-Fremiet and Rouiller, 1958; Sotelo and Trujillo-Cenóz, 1959; Fauré-Fremiet et al., 1962; Favard and Carasso, 1965) and has been shown by cytochemical techniques to contain accumulated calcium ions (Carasso and Favard, 1966 a, 1966 b). This prompted Carasso and Favard to propose that these saccules and tubules play the same calcium sequestering and releasing roles in ciliates as the SR plays in striated muscle of higher organisms.

These workers also suggested that the tubules within the stalk's microfibrillar bundle come into close association with the plasma membrane which lies between the microfibrillar bundle and the stalk's surrounding sheath. They postulate that these ciliates might be able to control the release of calcium in a manner analogous to that commonly thought to occur by the interaction of the trans-

verse tubular (T) and SR systems in striated muscle, if messages could pass from the plasma membrane to the membranes of these tubules (Favard and Carasso, 1965). Amos (1972) has questioned the presence of a sufficiently close connection between the plasma membrane and the tubule membranes to allow for such an electrotonic coupling.

This paper reports observations made during a study of the body and stalk myonemes of *Vorticella convallaria*. A complex structure will be described, for the first time, which lies on the surface of the membranes of the myoneme-associated saccules and tubules and links these structures to the microfibrils of the myonemes. Projections of this complex extending outward and contacting the pellicular region will also be described. Possible functions of these structures will then be considered.

MATERIALS AND METHODS

A stock culture of *Vorticella convallaria* (L.) Noland was obtained from the Culture Collection of Algae and Protozoa, Cambridge, England, strain number LB1690/1. These have been maintained in our laboratory in presterilized hay infusion medium with or without the addition of boiled wheat. The organisms feed on the natural population of bacteria which are transferred to the medium along with the ciliates.

The cells were collected from the culture surface by a Pasteur pipette and were placed in a 15 ml conical centrifuge tube. They were concentrated by means of slow speed centrifugation. Most of the culture medium was then decanted. The initial fixative consisted of a solution of 1 or 2% glutaraldehyde in cacodylate or collidine buffer. The final concentrations of collidine in the fixatives were 0.025 M or 0.05 M; both had a pH of 7.4. The collidine-buffered solutions, in addition, contained 0.02% CaCl_2 . The cacodylate-buffered fixative, pH 7.4, had a final concentration of 0.05 M sodium cacodylate and no calcium chloride. The fixation was performed by pouring fixative directly onto the concentrated cells in the centrifuge tubes, all steps including fixation were at room temperature. The fixations lasted 20 to 30 min. The cells were then washed from 30 min to 1 h in the same type of buffer used for the fixation. Postfixation was carried out in 1% OsO_4 for 45 min in the same buffer as the initial fixation and at the same concentration and pH. After this the cells were again washed in buffer. Those fixed in collidine-buffered solution were then bathed in a 0.5% aqueous uranyl acetate solution for 30 min, followed by a distilled water wash. Dehydration was in a graded series of ethanol followed by 100%

propylene oxide. The cells were "flat" embedded in Epon 812 and hardened in a 60°C oven for at least 2 days.

After the embedment the block was scanned with a light microscope and cells which appeared to be fixed well and in the proper orientation were circled. These were then cut out and glued to the tips of previously hardened epoxy-resin blocks. A diamond knife and a Sorvall MT-2 ultramicrotome were used to section the cells. The sections were picked up on unsupported 200-mesh grids or on Formvar-coated grids having one large hole. These supported grids were lightly shadowed with carbon when necessary.

Staining was for 2-5 min in uranyl acetate (Watson,

1958) followed by 5 min in lead citrate (Reynolds, 1963). The grids were examined and pictures taken in a Hitachi HU-11A electron microscope using an accelerating voltage of 75 kV and a 20 or 30 μm objective aperture.

OBSERVATIONS

Distribution of Myonemes, the Spasmoneme, and Associated Cisternae of the Endoplasmic Reticulum (ER)

Vorticella convallaria in its extended form is composed of a bell-shaped body attached to the

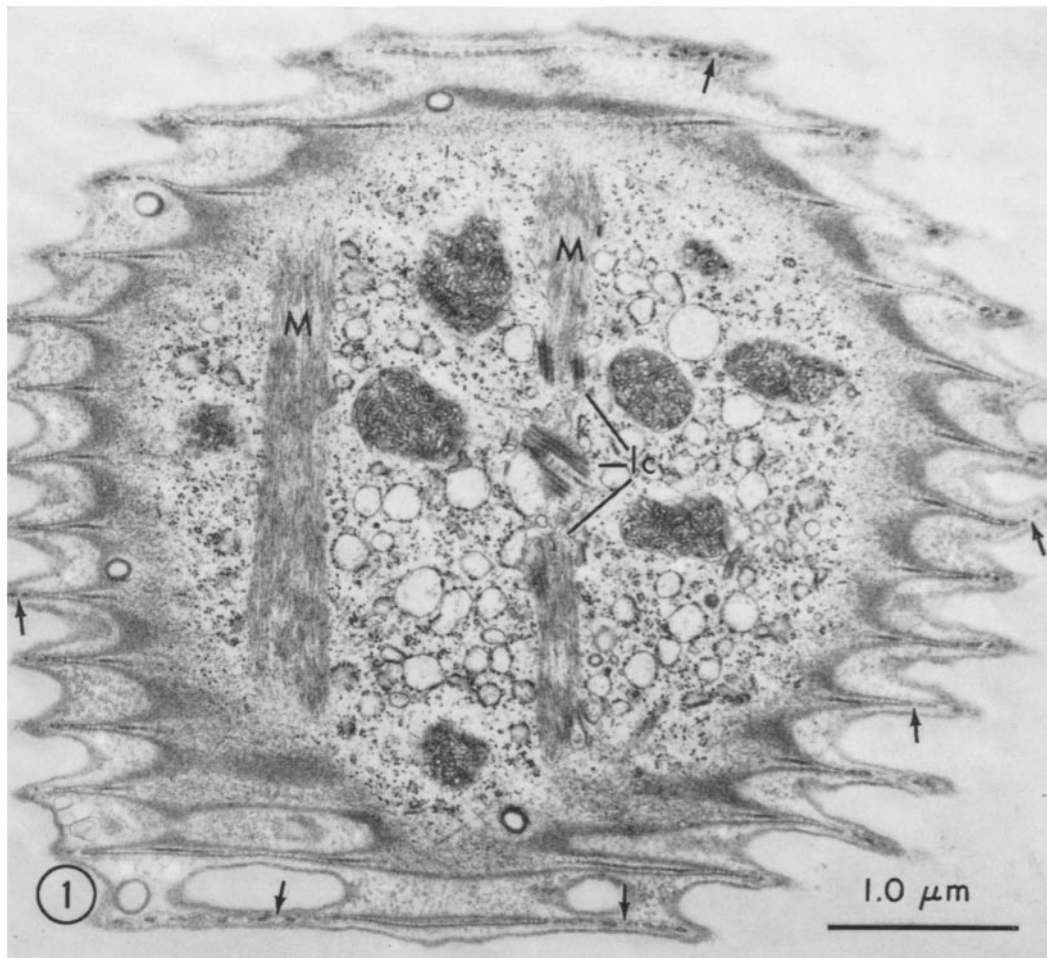


FIGURE 1 Grazing section through the surface of *Vorticella convallaria*. The annular surface rings, which are here sectioned obliquely, pass around the zooid in a fashion similar to latitudinal lines. Myonemes (*M*), of which segments of two appear here, lie subjacent to the pellicle and pass in the longitudinal direction. Portions of three linkage complexes (*lc*) are seen on the endoplasmic face of the myoneme. These linkage complexes are spaced about 1 μm apart. Around the edges of this section the septa, formed by abutting alveolar sac membranes, are perforated with large pores (arrows). $\times 25,000$.

substrate by an elongated stalk. Contractile elements are found in its body in the form of a number of longitudinal bands lying just under the pellicle and extending from the adoral zone to the scopular region where the stalk originates. These bands, known as myonemes, converge in the scopular region and merge into one large contractile fiber, the spasmoneme, which passes through the full length of the stalk.

After fixation and preparation for electron microscopy the myonemes (Figs. 1, 2, and 14) and the spasmoneme (Figs. 3, 5, and 6) are usually completely or partially contracted; the body assumes a spherical shape and the stalk becomes coiled into a helix. Thus, in the presence of fixative the cells invariably underwent complete contraction unless certain steps had been taken to reduce the amount of calcium ions which could be made available to the contractile elements. The steps can vary from placing the organisms in distilled water before fixation, placing them in oxalate before fixation, or in other chelating agents such as EDTA and EGTA before fixation. However, even after the above steps, partial contraction often occurs and the body becomes spherical; the stalk, on the other hand, may have only two or three loose turns next to the body while its distal end remains uncoiled. The pitch and diameter of the helical stalk will depend on the extent of contraction of the spasmoneme.

A special feature of the myonemes which was early recognized (Fauré-Fremiet and Rouiller, 1958; Sotelo and Trujillo-Cenóz, 1959) is their

close peripheral association and penetration by membrane-limited saccules and tubules some of which have been shown to be part of the ER. In the body the myonemes lie pressed against the epiplasm over most of their length. A flattened cisterna of the ER lies between the myoneme and the endoplasm (Fig. 2), sometimes encircling the myoneme except for the surface next to the epiplasm. Membrane-limited tubules (Figs. 2 and 18), appearing vesiculated after some fixations (Fig. 14), are frequently found within and oriented parallel to the body of these myonemes.

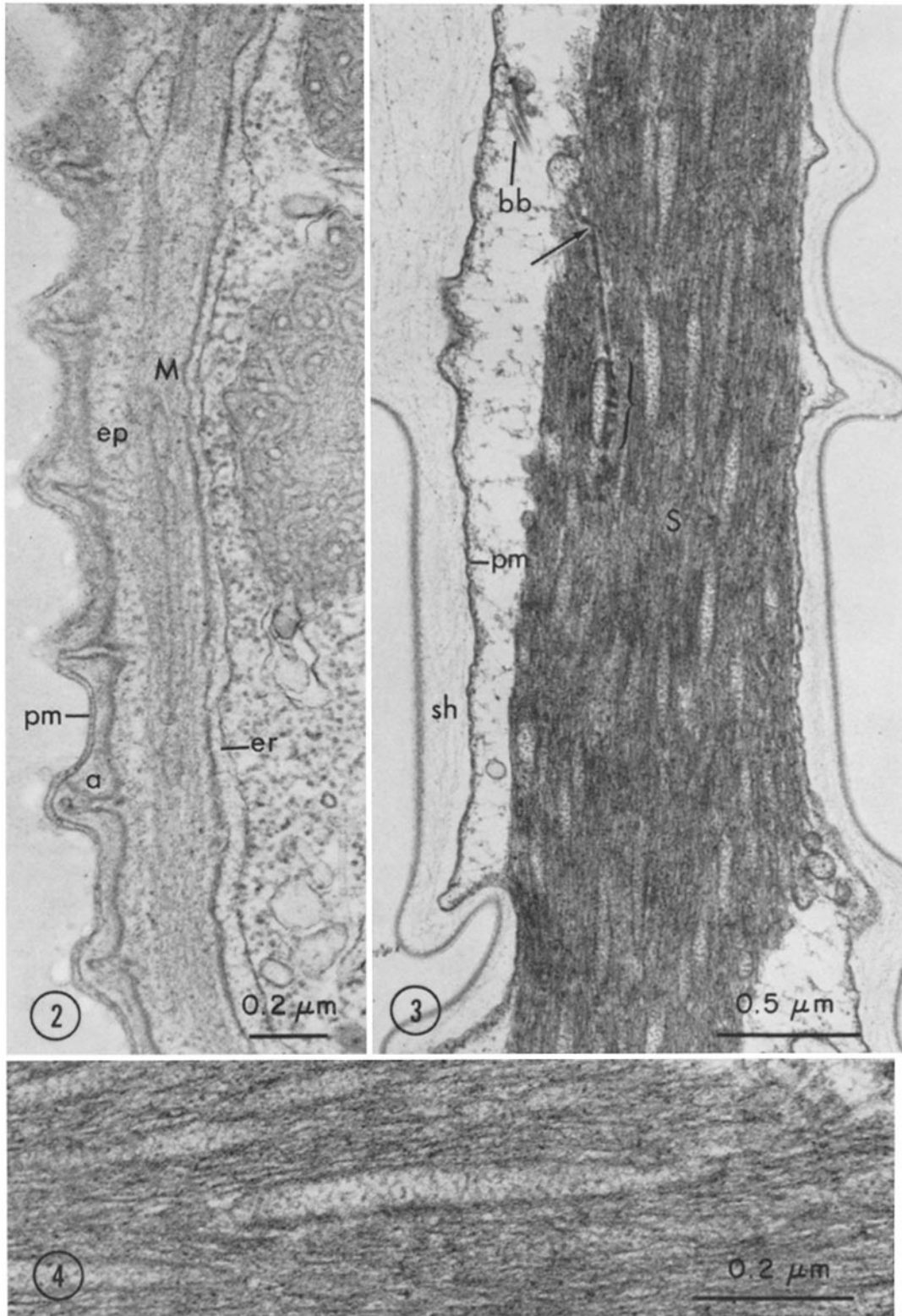
In the aboral region of the body the myonemes lie free within the endoplasm (Fig. 5). The ER at times completely encloses these portions of the myonemal bands and intramyonemal tubules are abundant.

The myonemes fuse together into one cylindrical bundle, the spasmoneme, at the proximal end of the stalk (Fig. 5). Many intramyonemal tubules continue into the body of the spasmoneme (Figs. 3 and 6) but the peripheral ER does not pass beyond this scopular region. The long axis of these tubules seems to parallel, more or less, the long axis of the spasmoneme and yet they are not seen in single sections to be continuous over very long distances even in uncontracted spasmonemes (Fig. 3). There is no consistent evidence from our pictures to suggest that the membranes of these tubules make close contact with the plasma membrane or other pellicular membranes of the stalk. The tubules (Fig. 6), of which there may be 40 or more in one cross section of the stalk, range from 30 to 100 μm

FIGURE 2 The surface in midlongitudinal sections is seen to be thrown into folds or annular rings. The pellicle includes an outer plasma membrane (*pm*) and a membrane-limited flattened alveolar sac (*a*) which is separated from the myoneme (*M*) by an epiplasmic layer (*ep*). A flattened cisterna of the ER (*er*) lies on the inside surface of the myoneme. The surface of the ER lying next to the myoneme appears to be thickened while that next to the endoplasm is studded with ribosomes. A meshwork of fine filaments lies in the lumen of the ER. $\times 60,000$.

FIGURE 3 Segment of a stalk sectioned approximately along its longitudinal axis. The sheath (*sh*), plasma membrane (*pm*), and spasmoneme (*S*) are labeled. The spasmoneme is composed of a bundle of microfibrils and segments of tubules which both appear to lie parallel to the long axis of the stalk. The tubules do not appear to come into contact with or closely adhere to the plasma membrane. The only organelle present within the cytoplasm between the spasmoneme and the plasma membrane is an obliquely sectioned basal body (*bb*). One end of a fiber (arrow) is found within the spasmoneme near this basal body. At its opposite end the fiber is continuous with a structure resembling a midpiece (bracket) of a linkage complex. This midpiece lies on the surface of a membrane-limited tubule. $\times 45,000$.

FIGURE 4 Enlargement of a tubule within the spasmoneme showing the meshwork of filaments within its lumen. $\times 100,000$.



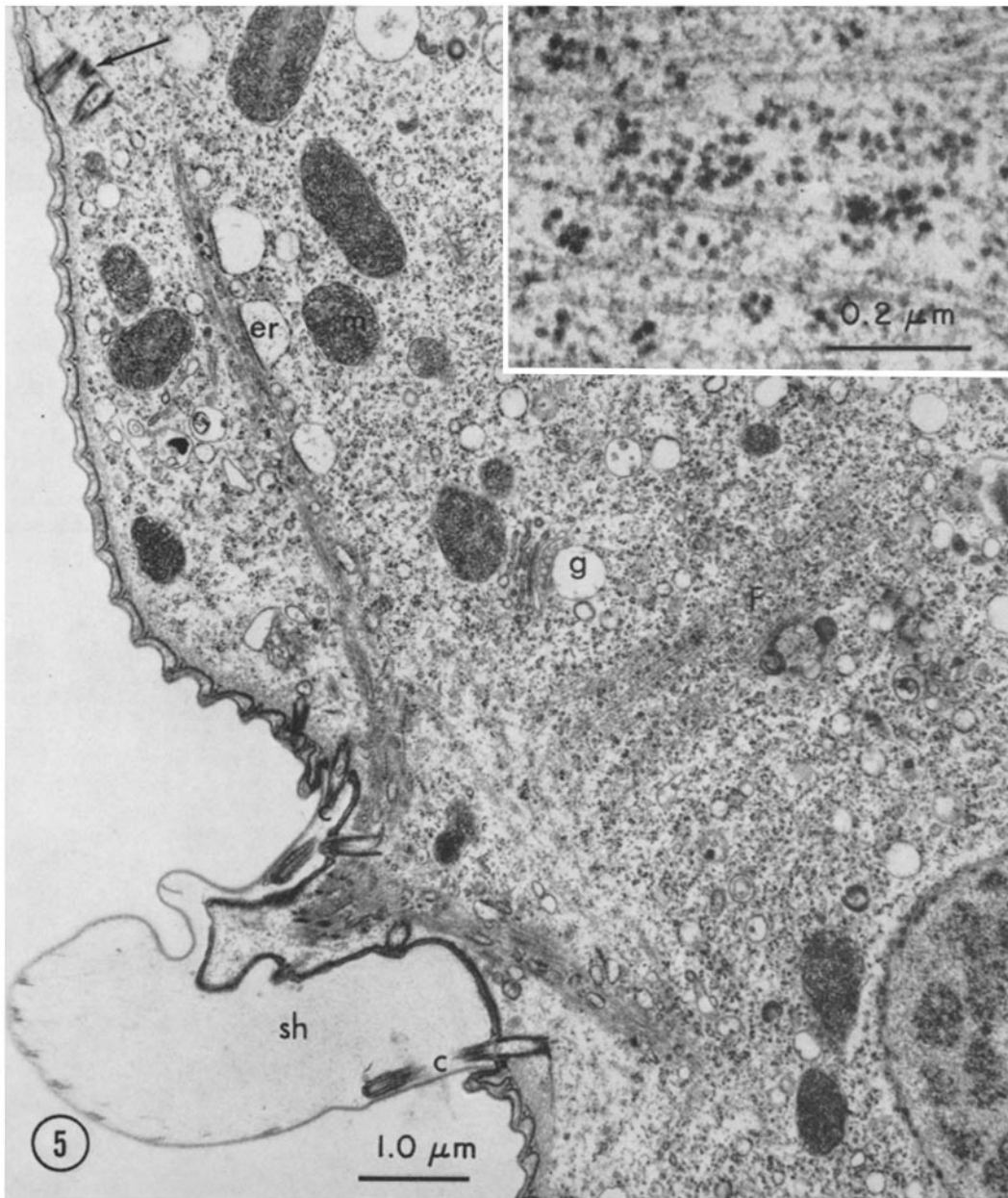


FIGURE 5 The aboral pole of the zooid. The micrograph is oriented so that the aboral-to-adoral axis passes from the lower left to the upper right of the figure. The myonemes lie free of the ectoplasm at this end of the cell. They converge in the scopular zone to form the spasmoneme of the stalk. Only the proximal end of the stalk appears in this micrograph (lower left). The location of the trochal band of basal bodies is indicated at the top left (arrow). Peripheral vesicles of ER (*er*) lie along the inner surface of the myoneme and many smaller vesicles lie within the myoneme bundles, some contain electron-opaque deposits. The intramyonemal vesicles continue into the stalk but the peripheral ER does not. Basal bodies outline the scopular region and also lie in the stalk. The scopular basal bodies are continuous with short cilia (*c*) that penetrate into the sheath for about 1 μm. The spasmoneme lies to one side of the sheath (*sh*) at this point of continuity with the body. The three membranes of the pellicle continue into the stalk but they are not sculptured into ridges as they are over the body. In this cell a column composed of thin microfilaments (*F*) passes from near the scopular region into the body. The *inset* is an enlargement of these 130–150 Å microfilaments between which ribosomes (opaque dots) are interspersed. *m*, mitochondria; *g*, Golgi complex. × 15,000. *Inset*, × 100,000.

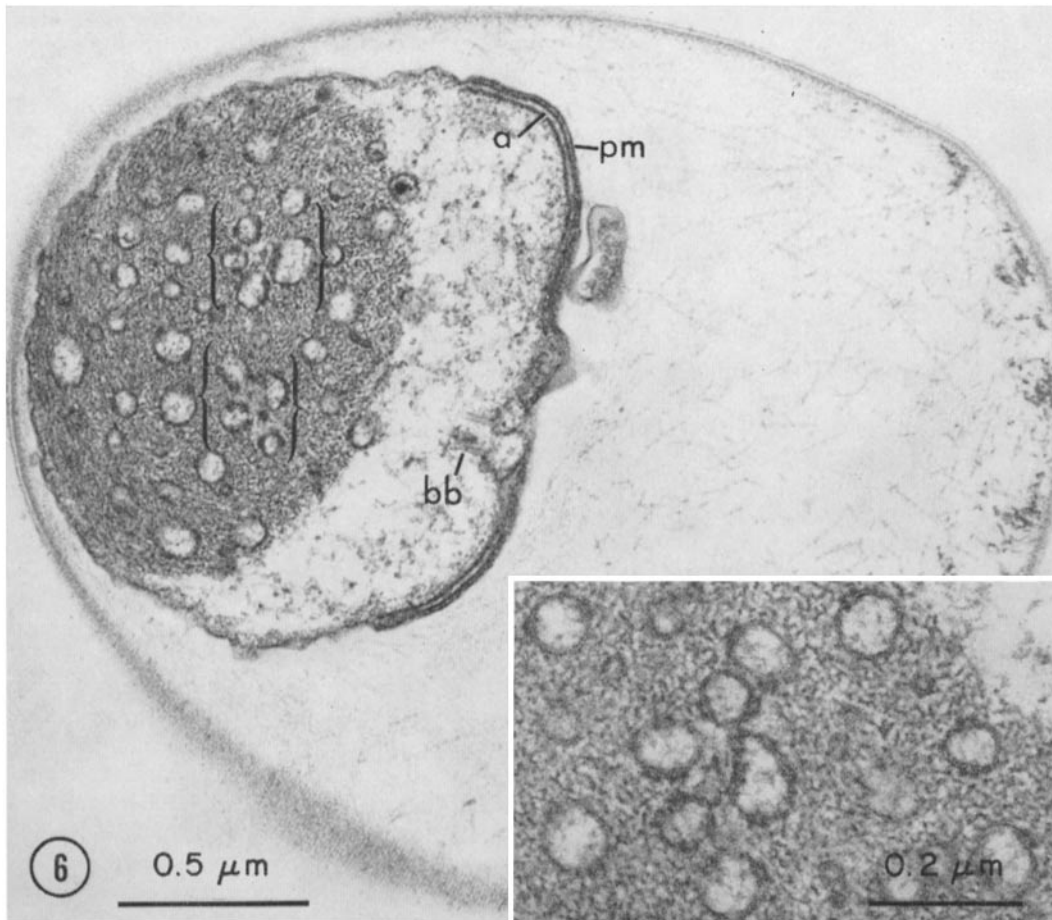


FIGURE 6 Section through a turn of a coiled stalk. The left side of the figure faces the inside of the helically-shaped stalk. The spasmome occupies most of the area enclosed by the plasma membrane (*pm*) and is placed to one side of this circular profile. This circle is placed eccentrically within the surrounding sheath. An alveolar sac (*a*) covers the internal surface of the plasma membrane opposite the spasmome. Some of the tubules lie close together within the spasmome and form clusters (brackets). These appear to surround a midpiece of the linkage complex. Most of the tubules appear to have granules on the outer surfaces of their membranes resembling cross-sectioned rails of the linkage complex. *bb*, basal body. $\times 50,000$. *Inset*: Enlarged portion of a spasmome. A cluster of four tubules surrounds a midpiece in cross section. The largest tubule bears the five-unit structure of the cradle on its limiting membrane. All tubules appear to carry rails. The midpiece, cradle, and rails are all part of the linkage complex. No microfibrils are found within the region surrounded by the cluster. Individual microfibrils of the spasmome are about 50 \AA in diameter. $\times 100,000$.

in diameter and are filled with a meshwork of fine threads (Fig. 4).

The diameters of the microfibrils of partially contracted spasmomes vary but most are $40\text{--}50 \text{ \AA}$ (*inset*, Fig. 6). This is also true for the microfibrils of contracted myonemes (Fig. 18).

Linkage Complexes at the ER-Myoneme Interface

Within the body of *Vorticella* cisternae of the ER are found next to the inside margin of the myonemes. This ER appears quite different depending

on the fixation procedure (cf. Figs. 2 and 14). In cells fixed in solutions containing 1% glutaraldehyde in 0.05 M cacodylate buffer the ER forms continuous, flattened, 40 m μ m thick sheets. However, when the cells were fixed in 2% glutaraldehyde in 0.025 M collidine buffer, the ER breaks up into vesicles, some of which remain attached to the myonemes. Those attached to the myonemes are of a rather regular size, being about 0.6 μ m in one direction by 0.4 μ m in a perpendicular direction. The endoplasmic boundary of both the flattened and vesiculated peripheral ER has electron-opaque, 20 m μ m diameter, bodies associated with it (Figs. 2 and 14). These are presumably ribosomes which, associated together, form polysomes. Ribosomes do not occur on the myonemal-facing surface of the ER. This myonemal-facing surface often appears to be thicker than the 50–100 Å of a typical "unit membrane".

On closer inspection it is seen that this thickness is due to highly complex structures lying flattened against the ER membrane and possibly even crossing through the membrane. These structures can be seen best in grazing sections of the cell through the outermost part of the cytoplasm; Fig. 1 shows such a section. In this figure one can see the arrangement of short segments of two myonemes just under the pellicle. The myoneme to the left is sectioned through the microfibrils making up its body. The myoneme to the right (center of figure) has been sectioned so that midway along the myoneme one sees the structures, in frontal view, which immediately underlie the myoneme. In this region (enlarged in Fig. 7)

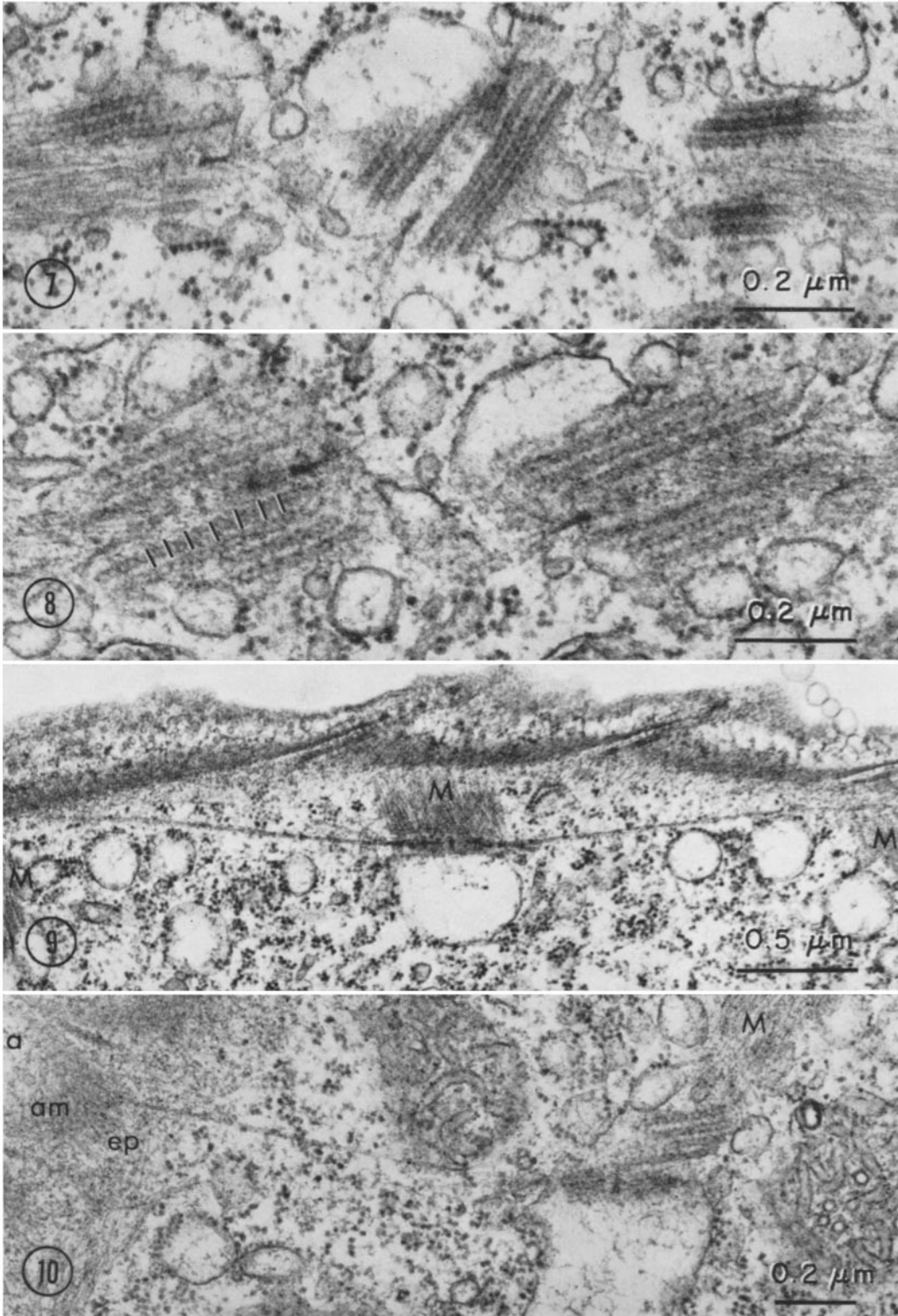
one sees portions of three coin-shaped structures having an overall diameter of 0.4–0.6 μ m which lie between the myoneme and the ER. Each coin-shaped structure will be referred to as a "linkage complex" (see Discussion for rationale). The central linkage complex of Fig. 7 and the two of Fig. 8 lie almost entirely within the plane of the sections. In this aspect the linkage complex can be seen to be composed of a midpiece and several rodlike or raillike structures lying parallel to and within the same plane as the midpiece. There are approximately the same number of rails on both sides of the midpiece, a fact which gives the structure a bilateral symmetry when viewed in this way. The most frequent number of rails per side in these micrographs is three.

The midpiece, taken as a whole, appears less electron opaque than the rails (Figs. 7, 8 and 19). It is spindle shaped, being wider in the middle and narrowing down at both ends to an electron-opaque pointed tip. It is about 500 m μ m long from tip to tip and 85 m μ m wide across its center. At this center is a prominent electron-opaque band, 65 m μ m long by 25 m μ m wide, running perpendicular to the long axis of the midpiece. Less prominent bands, three on each side of the central band, cross the spindle at periodic intervals of about 50 m μ m between the middle band and the opaque tips (above bars in Fig. 8). Fine, 10–20 m μ m, microfilaments appear to splay out from the tips of the spindle in a fashion resembling a bird's foot. In some sections (Fig. 9) these microfilaments can be seen to be as much as 1.6 μ m long and to pass in a straight line from the spindle tips. As one

FIGURES 7 and 8 Frontal views of three linkage complexes which are lying within the plane of section and portions of two others. Fig. 7 is an enlargement of the right-hand myoneme of Fig. 1. A single linkage complex is composed of a central spindle-shaped midpiece and a series of rails paralleling the midpiece, an equal number of rails is usually found on each side of the midpiece. The pointed tips of the midpiece are electron-opaque. Thin filaments splay out from these tips. Several bands, probably seven, cross the midpiece at periodic intervals (above bars), the central one tends to be prominent. The rails appear to have a periodicity reminding one of a ladder or the thread of a screw. These 0.4–0.6 μ m diameter linkage complexes lie on the interface between the myoneme (visible at edge of figures) and the underlying ER. Both figures, $\times 75,000$.

FIGURE 9 Long microfilaments pass from the tips of the midpiece of the linkage complex to the surface of the cell. In doing so they sometimes pass between adjacent myonemes (*M*) and the pellicle. The exact termination of the microfilaments is obscured by the plane of the section. $\times 35,000$.

FIGURE 10 The microfilament passing from the tip of the midpiece of this linkage complex may penetrate the epiplasm (*ep*) and make contact with the inner alveolar membrane (*am*). Although this appears to be true in this micrograph the plane of the section, oblique to the pellicle, does not permit a positive identification of such a contact. *a*, lumen of alveolar sac; *M*, myoneme. $\times 50,000$.



follows the extension of these microfilaments from the tips, it is seen that they finally impinge on the inner surface of the pellicle at an acute angle. In their passage they may cross over adjacent myonemes as in Fig. 9. Their exact point of termination is impossible to see in thin sections, which are themselves several times thicker than the microfilaments. They may, however, penetrate through the epiplasm and end next to the inner membrane of the alveolar sacs (Fig. 10).

The rails are straight, electron-opaque structures of length varying from 0.3 μm to 0.6 μm , the longest lying next to the midpiece (Figs. 7, 8, and 19). They are all of a constant width of 30 μm . The outer margins of the rails appear slightly more opaque than their centers. Opaque lines also pass from one margin across the rail to the opposite margin at periodic intervals of about 22 μm and, thus, resemble the rungs of a ladder or, possibly, the thread of a screw. Adjacent rails are separated by a minimum distance of 10 μm . There is sometimes a suggestion of periodic bridging between adjacent rails. However, since the sections are thicker than the rails, one must be careful that underlying or overlying material is not adding to the image of the true structure.

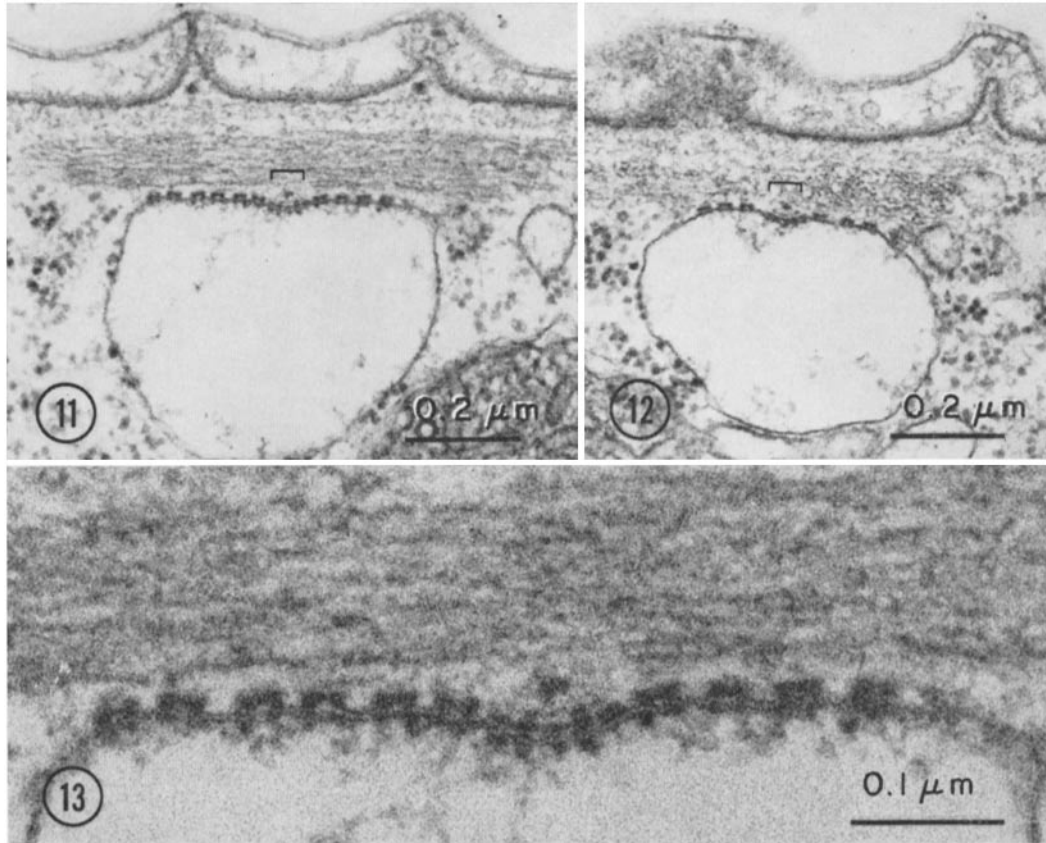
There are many linkage complexes in each organism and they are always present wherever myonemes appear in the cell. They are spaced at intervals of about 1 μm along each myoneme. These bilaterally symmetric complexes do not appear to have any preferential orientation with respect to the long axis of the myoneme but may be swiveled in any direction, i.e., the midpiece and rails may lie perpendicular, parallel or at any angle to the long axis of the myoneme but the complex always lies with its broad surface flat against the myoneme.

In transverse section (Figs. 11, 12, and 19) a linkage complex appears to be composed of separate units lined up along the ER membrane. The rails are U-shaped, in cross section, with the base of the U facing the myoneme. Only the sides of the U come into association with the ER membrane (Fig. 13). This whole structure projects 11 μm away from the membrane. Each U, or rail, is 34 μm wide with each arm being 11 μm thick. Adjacent rails have an electron-transparent space between them and, in these transverse sections, there are no apparent links between rails other than the ER membrane itself. A rail either passes through the membrane or, alternatively, there are electron-opaque granules inside the ER

membrane opposite each arm of the U. In Fig. 13 it is possible to follow the ER membrane as it appears to pass through the arms of the rails. The whole thickness of the rail from the base of the U facing the myonemes to the opposite tips of the granules within the ER is about 28 μm . The ER membrane in this region is 85 \AA thick. It is not yet clear how these rails are associated with the microfibrils of the myoneme although "wispy" material lying in the 10–15 μm space between the rails and myoneme may link these two together. The number of rails on one side of the midpiece varies from three to six in Figs. 11 and 12.

The midpiece in transverse section is much less opaque than the rails and, thus, much harder to characterize (Figs. 11 and 12). It appears to be composed of the previously described spindle which sits in a groove or cradle formed by an elaborated depression in the ER membrane. Five electron-opaque units, each similar to one arm of a U-shaped rail pass through the ER membrane in this groove. Like the rails these units have opaque granules on either side of the ER membrane. The outer tips of these five units are all interconnected by an electron-opaque line. The spindle-shaped midpiece, then, lies on this five-unit cradle. There is an opaque granule of approximately 25 μm diameter found in the middle of the transversely-sectioned spindle and several very thin, almost undetectable lines radiating out from this granule to an outer rim of dots at a distance of about 30 μm from the center of the granule. The details of the spindle next to the ER membrane can be recognized but details become indistinguishable on the side facing the myoneme.

In sagittal section the appearance of the linkage complex will depend on whether the section is through the midpiece or through the rails. In Fig. 15, an enlargement of a part of Fig. 14, the section is through the midpiece. The most prominent structure is the electron-opaque band or bar which, as noted in the description of frontal sections, crosses the middle of the spindle. The five-unit cradle under the spindle apparently does not pass under this bar but is composed of two parts, one on either side of the bar separated by a second depression of the ER membrane (55 μm wide) under the bar. This discontinuity in the cradle can be seen more clearly in Fig. 17 which is an oblique section through the cradle and through the rails on one side of the linkage complex. Each half of the five-unit cradle is about 150 μm long by 90



FIGURES 11 and 12 These two micrographs show cross sections of the linkage complex. The rails present U-shaped profiles lined up on either side of an oval profile of the midpiece (brackets). The ER membrane appears to link these units together. Under the midpiece the membrane forms a groove and has five electron-opaque lines crossing the membrane in this groove. The midpiece has a central electron-opaque dot and appears to be surrounded by an oval of fainter dots or lines. Both figures, $\times 60,000$.

FIGURE 13 An enlargement of Fig. 11. The rails, in cross section, consist of paired granules linked together on their myonemal ends. Their opposite ends appear to pass through the ER membrane. Another interpretation is that adjacent granules may be found on either side of the membrane; it is possible to follow the tripartite membrane through several of these rail arms. The ER membrane also appears to pass through the five rods forming the cradle under the midpiece. The rails are $34 \text{ m}\mu\text{m}$ wide and are separated from each other by at least $10 \text{ m}\mu\text{m}$. The details of the midpiece are difficult to interpret in transverse sections. $\times 200,000$.

$\text{m}\mu\text{m}$ wide. A periodicity of $16 \text{ m}\mu\text{m}$ can be detected along this cradle on the right side of Fig. 15. The remainder of the spindle structure is less clear in sagittal section. The $75 \text{ m}\mu\text{m}$ long opaque spindle tips (under brackets, Fig. 15) are found at the ends of the midpiece and the opaque material between the middle bar and tips must represent the other bars or bands crossing the spindle as well as the other elements already described in transverse sections. Fig. 16 is a sagittal section through

a rail. A periodicity of $22 \text{ m}\mu\text{m}$ lies along the rail. This periodicity is equal to that seen in the frontal view of the linkage complex crossing the rails.

Filamentous material can always be found in the lumen of the ER connected to the granules of the rails and the five opaque units of the cradle. In the ER which has not been disrupted by vesiculation this filamentous material seems to form a meshwork throughout the lumen (Fig. 2), passing from the outer to the inner limiting membranes of the ER.

As a concluding observation on this section dealing with the body of *Vorticella*, it should be noted that linkage complexes have not been observed anywhere else in the cytoplasm, only where the ER lies next to the contractile myonemes.

Fig. 19 is a three-dimensional drawing of a linkage complex as it appears in the body of *Vorticella*, lying between the peripheral ER and the myoneme. The ER is drawn in its nonvesiculated shape.

Linkage Complexes, Basal Bodies, and Filaments in the Aboral Region

The myonemes leave the surface of the cell just before reaching the trochal band of basal bodies. They then pass directly to the scopular region where the stalk is attached to the body. Linkage complexes are found along this free portion of the myonemes almost always on the side of the myonemes away from the nearest pellicular membranes (Fig. 5). Many tubules or rows of vesicles are found within the myonemes in this zone. In transverse sections of the extensions of the myonemes into the aboral endoplasm, it can be seen that many of these tubes or vesicles in the body of the myonemes have thickenings on their membranes corresponding to the rails of the linkage complex. This is also true of myonemes near the adoral pole of the body (Fig. 18). Some vesicles

have the midpiece with its underlying five-unit cradle as well as the rails. It is not uncommon for the lumens of some of these vesicles to contain electron-opaque inclusions.

At the scopula (Fig. 20) a very striking association of the midpiece with the scopular basal bodies occurs (the term basal body is used here instead of scopular organelle following the reasoning of Fauré-Fremiet et al., 1956). In this region the microfilaments extending out from at least one of the midpiece tips do not splay out but form a bundle, the bundle may be as much as 45 μm wide and appears to have periodic cross-striations. This bundle runs into the proximal end of a scopular basal body and continues into the lumen of this organelle at least half the distance to its distal end. In Fig. 20 the spindle can be identified by the opaque bar crossing its middle, however, the section only contains one half of the spindle. In other unpublished pictures it appears that most, if not all, of the basal bodies of the scopula have such bundles of fibers associated with them in the same manner as described here. The basal bodies of the scopula appear to be typical in most respects to those of the trochal band and to those of the peristome region of *Vorticella*, i.e., they are composed of a cylinder of nine-triplet peripheral fibers. These scopular basal bodies, however, do not contain in their lumens the electron-opaque

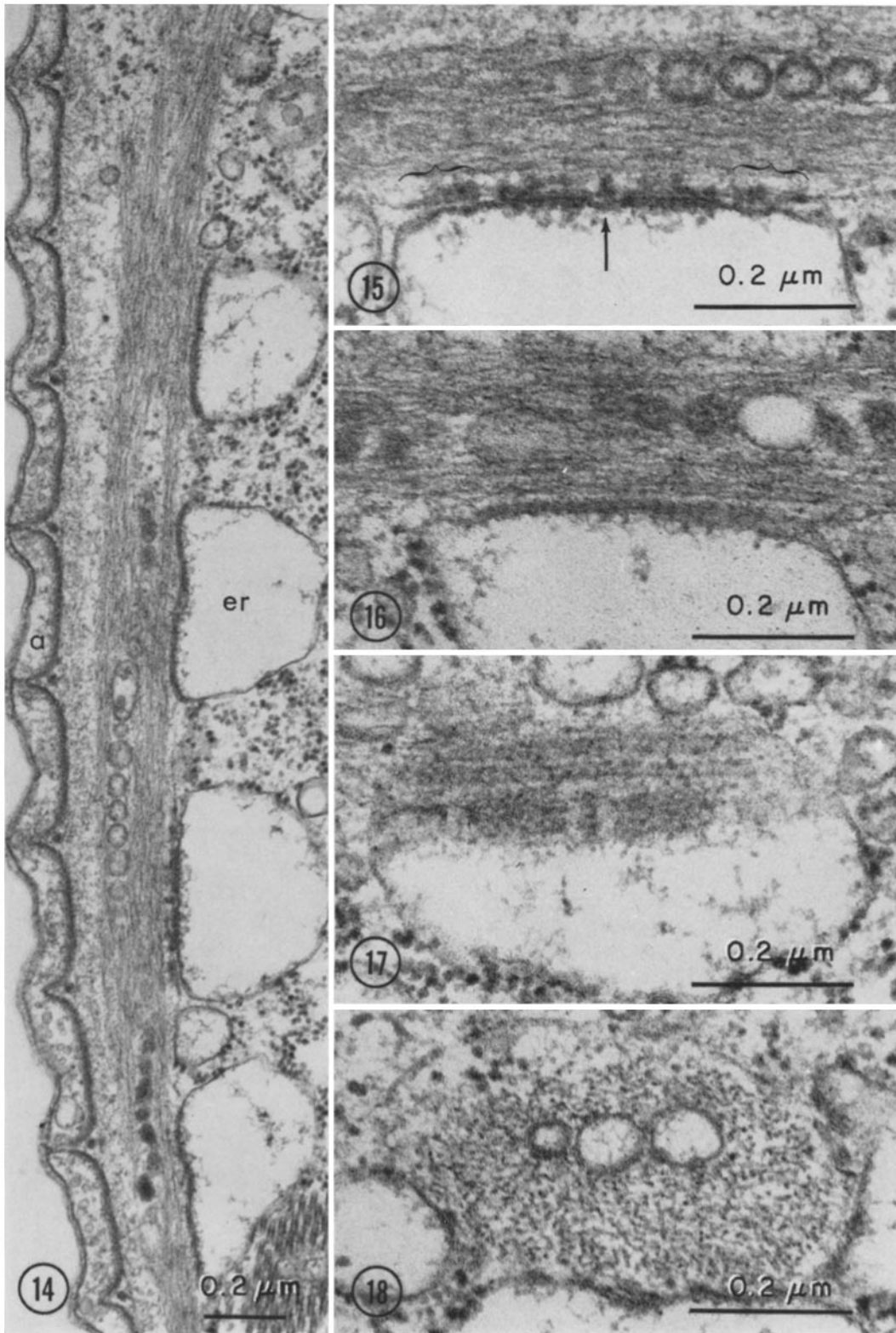
FIGURE 14 When the cells are fixed in some solutions the ER (*er*) becomes vesiculated. In this micrograph the ER has remained attached to the myoneme principally at the sites of the linkage complexes. Thus the vesicles and the linkage complexes lie at intervals of about 1 μm along the myoneme. Rows of vesicles, probably vesiculated tubules, also lie within the myoneme. The linkage complex next to the second large vesicle from the bottom shows a sagittal section of the midpiece. The alveolar sacs (*a*) are continuous over many of the ridges. $\times 50,000$.

FIGURE 15 Enlarged view of the sagittally-sectioned midpiece of Fig. 14. The tips of the midpiece lie under the brackets and the prominent central band of the midpiece is indicated by the arrow. The cradle appears to be composed of two parts, one on either side of a small depression under the central band. The ER membrane is seen passing through densities, spaced at 16 μm intervals, along the right side of the cradle. $\times 100,000$.

FIGURE 16 Sagittal section of linkage complex at the level of a rail. A periodicity of 22 μm along the rail is characteristic of these structures. A narrow relatively electron-transparent region separates the rail from the microfibrils of the myoneme. $\times 100,000$.

FIGURE 17 Oblique section of the linkage complex showing the two parts of the cradle separated by a central band. One midpiece tip appears to the left of the micrograph. $\times 100,000$.

FIGURE 18 Transverse section of a myoneme. Cross sections of microfibrils of myonemes measure about 50 \AA in diameter. Both intramyonemal tubules and peripheral ER are present. The tubules have granular thickenings on their membranes resembling the rails found on the peripheral ER. $\times 100,000$.



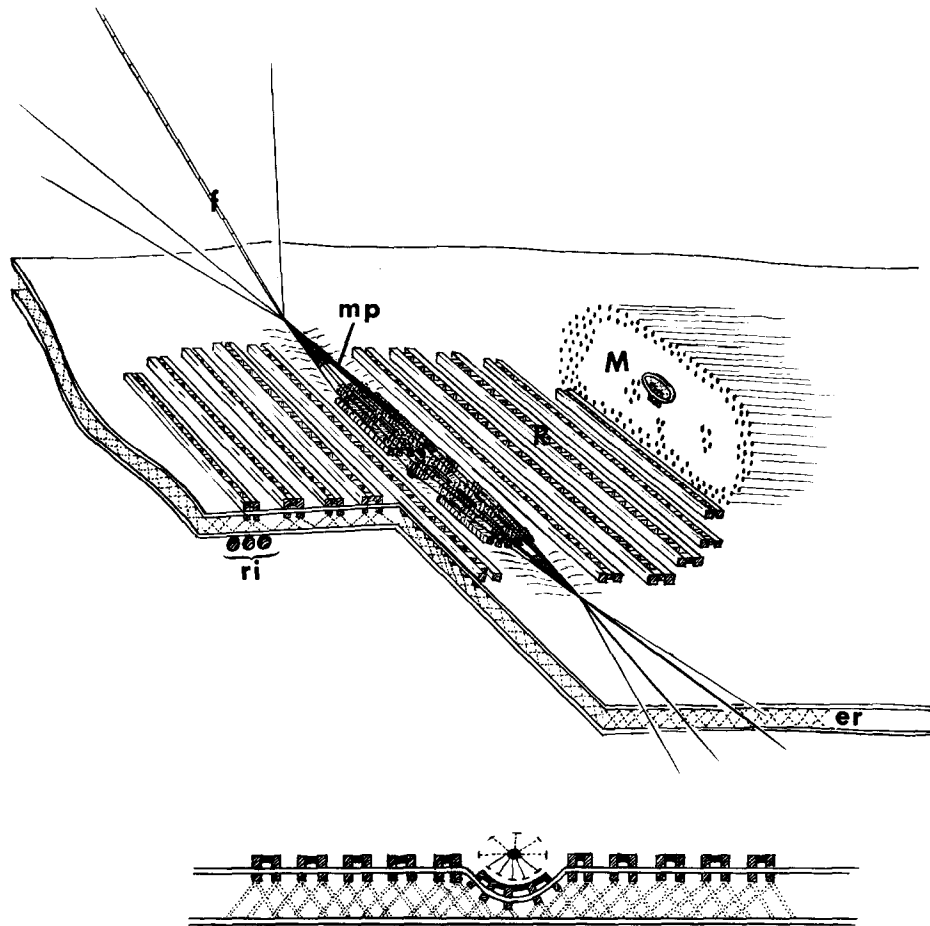


FIGURE 19 Three-dimensional drawing of the linkage complex as it appears at the interface between the peripheral ER (*er*) and a myoneme (*M*). The myoneme is shown in cross section at the edge of the complex. The ER is in its normal flattened shape. The lower drawing shows a transverse section of the linkage complex at a point somewhere between the middle and the pointed tip of the midpiece. *R*, rail; *mp*, midpiece; *f*, microfilament; *ri*, ribosomes.

amorphous clumps as do the other basal bodies of *Vorticella*. The bundle of fibrils may be tied to the internal surface of the basal body fibers by means of what appear to be fine threads. At their distal ends the basal bodies are continuous with short cilia which pass into the sheath for a distance of about 1 μm .

Above the scopular region a column of loosely associated filaments sometimes extends into the endoplasm of the zooid (*F* in Fig. 5). These filaments appear tubular in longitudinal section but no cross sections were obtained to confirm this. Their size is from 130 to 150 \AA in diameter (*inset*, Fig. 5). The width of the column is about 1 μm in diameter and the length may be as much as

5 μm . All large organelles are excluded from this column; only ribosomes are found interspersed between the filaments. No clue was obtained as to the function of these filaments.

Stalk Morphology and Linkage Complexes in the Spasmoneme

The stalk or peduncle consists of a bundle of fused myonemes, collectively called the spasmoneme, eccentrically placed within a cylinder formed by the plasma membrane (Fig. 6). This, in turn, is placed eccentrically within a secreted sheath. The eccentricity is established at the point of origin of the stalk (Fig. 5), at the scopula, where

the spasmoneme is located within and to one side of the cylindrical sheath. Basal bodies with very short, stubby cilia are found within the stalk near the scopular zone. An extension of the alveolar sacs, a system of flattened cisternae underlying the plasma membrane in the body, continues to underlie the plasma membrane at this end of the stalk (Figs. 5 and 20). The sheath is like those already described for *Vorticella* by others (Randall and Hopkins, 1962; Amos, 1972).

Farther along the stalk the alveolar sac is found coating only a fraction of the circumference of the inside surface of the plasma membrane (Figs. 6 and 23). It is always located along the outward-facing surface of the helix formed by the coiled plasma membrane-limited tube and, thus, is always separated from the spasmoneme by a layer of cytoplasm. The alveolar sac apparently passes uninterrupted along the full length of the stalk, covering the largest circumferential fraction next to the body and the smallest fraction toward the distal tip. The sac is limited by a unit membrane which surrounds a 50–150 μm wide electron-transparent space. This is much flatter than the alveolar sacs of the zooid. Similar saccules were reported in the stalk of *Carchesium* by Amos (1972) but these were not shown to be continuous with the alveolar sacs of the zooid.

The outer alveolar membrane maintains a constant distance of about 60 Å from the plasma membrane. Short bridges, 50–75 Å wide, appear to link these two membranes together (Fig. 22). Similar bridges may also cross the lumen of the alveolar sacs to link the outer with the inner alveolar sac membranes. Such bridges are now thought to be a common feature of membranes which are separated by a uniformly small distance of up to a few hundred angstroms (Franke et al., 1971; Franke, 1971).

The two membranes of the alveolar sac and the plasma membrane appear to each have a thick and a thin face when observed following the techniques used in this study. The thicker face is also more electron-opaque. The outside face of the "unit membrane" structure of the plasma membrane and the leaflets of the alveolar sac membranes facing the sac's lumen are relatively thin and more electron transparent (Fig. 22). All three membranes are 75 Å thick. The total thickness of the three membranes and two interspaces is about 35 μm in the stalk.

The stalk cytoplasm lying between the spasmoneme and the alveolar sac is unique in its almost

complete lack of ribosomes, as well as other normally occurring cytoplasmic membranous organelles. The only structures found in this cytoplasm are mitochondria, modified basal bodies, a network of background filaments, and an occasional microtubule (Figs. 3, 6, 21, 23, and 24).

At intervals along the stalk basal bodies lie next to the plasma membrane (Figs. 3, 5, 6, 20, 21, 23, and 24). These 140 μm diameter basal bodies, which are composed of a cylinder of nine single microtubules (fibers) (Fig. 23) rather than nine-triplet microtubules (near the zooid basal bodies having both doublet and singlet microtubules have been observed), seem to approach the plasma membrane only in the region where it is coated by the alveolar sacs. This basal body to alveolar sac continuity is clear only where the tripartite structure of the pellicular membranes is resolved and where the distal end of the basal body clearly meets the pellicle. The basal bodies either lie next to the edge of the alveolar sac or they pass through a pore formed in this sac (Fig. 6). These basal bodies have no sign of a cilium connected to them except for a few at the extreme proximal end of the stalk.

These basal bodies do, however, have a central striated fiber (Figs. 21, 23, and 24) (which Amos [1972] mistook to be a single microtubule) running all the way through their lumens from their distal ends to their proximal ends (Fig. 24). In Fig. 24 the fiber has a major repeat of 128 μm . From the proximal ends of the basal bodies the fibers continue into the body of the spasmoneme (Fig. 21). These fibers are apparently the same as those described in the basal bodies in the scopular region since they too can be seen in favorable sections (which are rarely obtained) to be continuous with a structure in the spasmoneme resembling the midpiece of the linkage complex found in the body (bracket, Fig. 3). The midpiece, when seen, lies next to a segment of a tubule.

The basal bodies are attached to the alveolar sac by means of short electron-opaque connectors which radiate out from close to the distal end of each tubule to the membrane-lined edge of the pore (Fig. 24). These connections are similar to those described between the alveolar sacs and the basal bodies in *Tetrahymena* (Allen, 1969) and some other ciliates. Connections of the basal bodies, or their internal fibers, with the plasma membrane are not clearly seen and it cannot be said if such do, or do not, exist.

In cross sections of the spasmoneme the different

parts of the linkage complex can be identified (Fig. 6). Most of the tubules which have a diameter greater than 50 μm show profiles of one or two pairs of membrane-associated granules which are the same shape and dimensions as the rails of the linkage complex when this is seen in transverse section. The tubules are normally regularly distributed throughout the fibrillar mass, however, occasionally four or five tubules may be seen to approach each other and form a cluster. Within such a cluster the largest tubule will be seen to contain a five-unit cradle of the linkage complex (*inset*, Fig. 6) while all the tubules will have raillike profiles. A cross section of a spindle is seen in the center of each cluster; the spindle is surrounded by the tubules. Microfibrils of the spasmoneme are excluded from the centers of these clusters.

DISCUSSION

Myoneme and Stalk Morphology

The ultrastructure of the myonemes and spasmonemes of peritrich ciliates has been described in a number of papers (Randall, 1956; Fauré-Fremiet et al., 1956; Fauré-Fremiet and Rouiller, 1958; Sotelo and Trujillo-Cenóz, 1959; Fauré-Fremiet et al., 1962; Randall and Hopkins,

1962; Favard and Carasso, 1965; Lom and Corliss, 1968; Amos, 1972). The contractile portion of these organelles is the bundle of interlaced 20–40 Å microfibrils which make up the bulk of their observable structure. Also, a unique association with membranous saccules and tubules was early recognized (Fauré-Fremiet et al., 1956; Fauré-Fremiet and Rouiller, 1958; Sotelo and Trujillo-Cenóz, 1959). These workers reported smooth-membraned tubules of 30–120 μm diameter within the myonemes and spasmonemes and flattened or dilated saccules lining the endoplasmic side of the myonemes. Later these saccules, but not the intramyonemal tubules, were seen to be in close association with ribosomes so that the continuity of the saccules with the rough ER was established (Fauré-Fremiet et al., 1962). It is not yet clear if the tubules within the myonemes are always continuous with the peripheral saccules (see Fauré-Fremiet and Rouiller, 1958; Fauré-Fremiet et al., 1962). We saw little evidence of such connections in the present study (see upper part of Fig. 2), however, such connections do exist in the peritrich *Opercularia* (Allen, 1973). The saccules and tubules are certainly similar otherwise, i.e., both contain a luminal filamentous meshwork and both have associated linkage complexes.

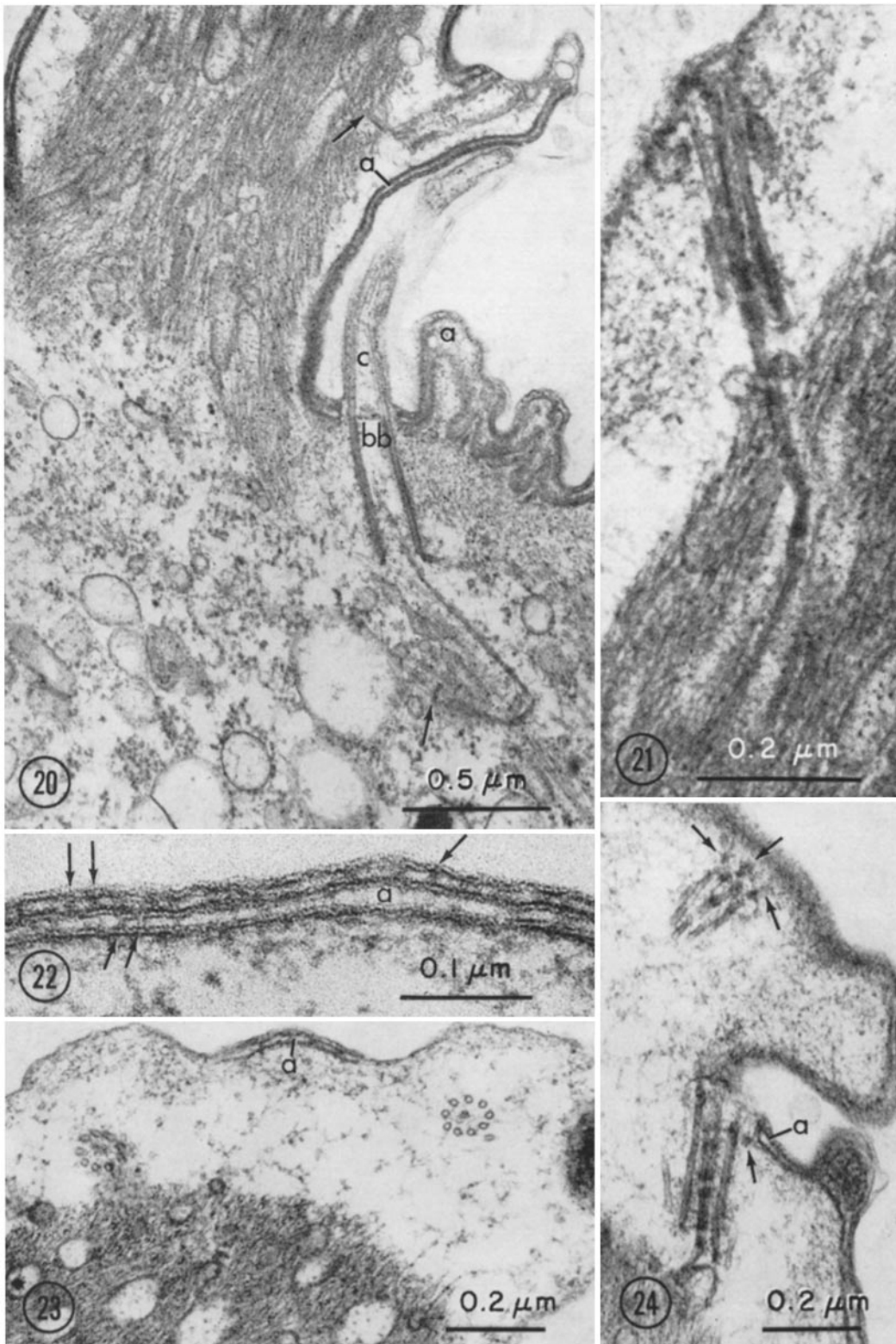
FIGURE 20 Basal body (*bb*) and short cilium (*c*) in the scopular region. A striated bundle of fibrils, continuous at one end with a linkage complex midpiece, passes into the lumen of the basal body through its proximal end. It extends at least half the length of the basal body and may be linked to the triplet tubules making up the wall of this body. The midpiece is identified by the prominent central band (lower arrow). A fibril (upper arrow) may also pass from the basal body in the stalk into the spasmoneme. The alveolar sac (*a*) continues into the stalk. $\times 45,000$.

FIGURE 21 Basal bodies in the stalk lie approximately perpendicular to the surface and contact the surface next to the alveolar sac. A striated fiber passes from the distal end of this basal body into the spasmoneme where it bends to parallel the microfibrils and tubules of the spasmoneme. $\times 100,000$.

FIGURE 22 Details of the plasma membrane and alveolar sac membranes within the stalk. Each membrane appears to have one thin and one thick leaflet. The thick margin of the plasma membrane (upper membrane) faces the alveolar sac (*a*), that of the outer membrane of the alveolar sac faces the plasma membrane. Bridges (arrows) seem to connect the outer alveolar membrane with the plasma membrane and may also cross the lumen of the alveolar sac linking the two membranes of this sac. $\times 200,000$.

FIGURE 23 Basal bodies in the stalk are composed of nine single microtubules. A fibrous bundle is located in the lumen of the basal body. A very short segment of an alveolar sac (*a*) lies next to the plasma membrane. $\times 60,000$.

FIGURE 24 Two stalk basal bodies, one in oblique section and the other longitudinally sectioned. Short electron-opaque bridges (arrows) lie between the tubules of the basal body and the alveolar sac (*a*) margin. In the longitudinally-sectioned profile the striated bundle of fibers passes through the full length of the lumen. This bundle appears to be associated with the inside surface of the microtubules which form the wall of the basal body. $\times 60,000$.



Partly on the basis of their position in intimate association with the microfibrils and partly because elements of the SR in striated muscle had been shown to play a role in controlling the contraction of muscle myofibrils through the sequestration and release of calcium ions, the ER has been implicated in a similar calcium regulating role for myonemes (Sotelo and Trujillo-Cenóz, 1959; Favard and Carasso, 1965). The cytochemical localization of calcium within these ER saccules and tubules provided additional support for this hypothesis (Carasso and Favard, 1966 a, 1966 b).

Favard and Carasso, (1965), also interpreted their electron micrographs as showing that the tubules and microfibrils of the spasmoneme were arranged obliquely across the long axis of the stalk. The tubules, which were said to be closed at their ends, were reported to narrowly adhere to the plasma membrane on one side of the stalk. Thus, they suggested that an excitation of the plasma membrane transmitted to tubule membranes across these narrow gaps might trigger the release of calcium ions from the tubules. They also suggested that the tubules might function in transporting ATP to bring about relaxation. Amos (1972) has taken exception to the idea that the tubules pass obliquely across the stalk and end near the plasma membrane. He shows convincingly that the tubules assume a helical path within the spasmoneme and do not closely adhere to the plasma membrane. This fact casts doubt on the validity of excitation transfer from the plasma membrane directly to the tubules.

Our study of *Vorticella convallaria* confirms the principal morphological observations made by previous workers. The myonemes are composed of bundles of 40–50 Å microfibrils (somewhat larger than some reports) which are in close association in the body with both peripheral saccules of ER and intramyonemal tubules; whereas, in the spasmoneme only tubules are present. We support the observation of Amos (1972) that the microfibrils and the tubules of the spasmoneme assume a helical path within the body of the spasmoneme and are not short units crossing obliquely from one side of the spasmoneme to the opposite side. This helical path can also be seen to have the same sense as that of the coiled stalk.

ER-Myoneme Association Compared with SR-Myofibrillar Association

Striated muscle myofibrils are encircled by a smooth-membraned SR which lies close to the

actin and myosin filaments of the sarcomeres and forms unique associations called triads or dyads with transverse tubular invaginations of the plasma membrane. It is this SR to which the myoneme-associated saccules and tubules have been likened (e.g., Fauré-Fremiet and Rouiller, 1958; Sotelo and Trujillo-Cenóz, 1959; Favard and Carasso, 1965).

A calcium-sequestering vesicular system has also been reported in smooth muscles of vertebrates (Carsten, 1969; Fitzpatrick et al., 1972) and in smooth muscles of invertebrates (Heumann, 1969). A similar system has recently been reported as well in the myxomycetes of the genus *Physarum* (Braatz and Komnick, 1970; Ettienne, 1972). These vesicular systems are thought to be equivalents of the SR.

The linkage complex as described here appears to have no reported counterpart in striated muscle or any other contractile system, although electron-opaque filamentous or membranous material has often been seen in striated muscle at the triad junctions and is reported to either link or form close junctions between the membranes of the SR and the transverse tubules (e.g., Revel, 1962; Franzini-Armstrong and Porter, 1964; Walker and Schrodt, 1966; Kelly, 1969). Bridges have also been reported linking SR tubules directly to the Z lines of sarcomeres in dog cardiac muscle and fetal and newborn rat skeletal muscle (Walker et al., 1968; Edge and Walker, 1970). These authors feel that these bridges crossing the 100 Å space between the SR and Z lines act as a structural tie. The linkage complexes of *Vorticella*, although differing morphologically from the above types of bridging, are similarly involved in structurally holding the ER next to the myonemes. This is clear from preparations where the fixation has resulted in the breakup of the peripheral ER into vesicles. Large ER vesicles remain flattened against the myonemes only in regions where the linkage complex is found, while only a few small spherical vesicles remain near the endoplasmic surface of the myoneme between the linkage complexes (Fig. 14). In lieu of any established physiological function for this structure we have chosen the name, "linkage complex", which suggests its morphological role.

One feature of the myoneme ER which is reminiscent of the SR is a meshwork of filaments found in the ER and tubules of myonemes and spasmonemes of *Vorticella* which is morphologically similar to a meshwork of fibrils found inside the SR of muscle in the terminal cisternae next to their junctions with the T tubules (e.g., Porter, 1956;

Revel, 1962; Peachey, 1965; Walker et al., 1971). It would be interesting to know if this material has the capacity to store calcium. MacLennan and Wong (1971) have recently isolated a strongly anionic protein from rabbit SR which may be hydrophobically bound to the inner membrane surface of the SR and may be the site of much of its calcium storage capacity. It seems possible that a similar protein might occur in the myonemal ER in *Vorticella*.

Functional Speculations Regarding the Linkage Complex

It is immediately apparent that little can be said with certainty about the function of this unique structure beyond the fact that it does tie the ER to the myoneme. However, when one compares previously reported physical and chemical properties of the myonemal contraction process along with the morphology of the linkage complex to the structure and function of the muscle-associated SR a functional hypothesis may be developed.

It is now well established that extracted contractile stalks of peritrichs require only one substance in order to contract and that is free calcium ions (Levine, 1956; Hoffmann-Berling, 1958; Rahat et al., 1969; Amos, 1971). ATP, although probably necessary for some part of the contraction-relaxation cycle in the living organism (Hoffmann-Berling, 1958) is not necessary for either contraction or relaxation of extracted stalks. A reasonable hypothesis, which has been based on extensive work on vertebrate as well as invertebrate muscles and on the studies of myonemes of peritrichs (Carasso and Favard, 1966 a, 1966 b) and of the heterotrich *Spirostomum* (Ettienne, 1970), has been to conclude that the ER is the site of calcium ion sequestration and release. It now seems reasonable to implicate these newly described linkage complexes in these physiological roles. We would like to propose (verification will need to await further testing) that the rails with their close myonemal association and their seeming continuity through the ER membrane, as well as continuity with the ER luminal filamentous meshwork, may be the routes along which the calcium moves into, and out of, the ER. If this should prove to be true, it would not be surprising to find the enzyme ATPase in intimate association with these rails.

The midpiece, with its long slender filaments either passing outward into the epiplasm of the body region, or as bundles, passing into basal

bodies of the scopula or stalk, presents a more difficult puzzle for analysis. One question for which we have not yet suggested a solution is what controls or triggers the contraction process? In looking for a morphological system in peritrichs similar to the T system of muscle, Favard and Carasso (1965) proposed that in the stalk the plasma membrane transmits excitation directly to the membranes of the closely adherent intraspasmonemal tubules. This transmitted excitation would then be able to trigger calcium release from these tubules and initiate a contraction. Amos (1972) and the present study suggest that no such close connection exists between the plasma membrane and the stalk tubules. Furthermore, no paper, including that of Favard and Carasso (1965), has suggested that the body myonemes were triggered in a manner similar to that proposed for the stalk. A relatively thick epiplasmic layer in the zooid which always separates the myoneme fibrils and their associated ER from the pellicular membranes probably accounts for this.

In *Vorticella* the only visible connections between the microfibrillar-associated saccules and tubules and the cell's surface membranes in both the zooid and stalk are the midpieces through their attached filaments. In the zooid the ends of these filaments are obscured by the epiplasm into which they pass, however, it is possible that they penetrate this epiplasm and come into contact with the inner membrane of the alveolar sac (Fig. 10). In both the scopula and stalk the filaments also appear to associate with the continuous alveolar sac via the basal bodies (Figs. 20 and 24). Fig. 24 shows that structural continuity probably exists between the filamentous bundle and the internal surface of the basal body cylinder as well as between the distal end of the basal bodies and the alveolar sac membrane. It does not seem likely that these thin filaments, especially those found connected to the zooid linkage complexes, would have a supporting function, however, it may be possible, considering their unique associations, that they are able to transmit messages from the alveolar sac membranes to the midpiece and thereby to the ER membrane. Such messages may trigger the saccules and tubules to release their load of calcium ions and, in turn, cause the microfibrils to contract.

The above hypothesis presupposes that the alveolar sac in the zooid as well as in the stalk is a continuous unbroken membrane system underlying the plasma membrane. Such continuity has been shown to be present in some other ciliates, e.g., *Paramecium* (Allen, 1971), and this may also be

true of the peritrichs. Evidence for this in *Vorticella* may be found when one observes the alveolar sac membranes at the peaks of the annular rings surrounding the body. In longitudinal sections of the body the two membranes of the alveolar sac, inner and outer, are continuous at these ridge peaks in only a fraction of the ridges (Figs. 2, 11, and 14). In oblique sections of these ridges, as in Fig. 1, the septum formed by membranes of the two adjacent alveolar sacs, along the ridge peaks, are not continuous but contain numerous pores (arrows, Fig. 1). This suggests that the pores occupy more of the ridge than does the septum and that all alveolar sacs, both their lumens and membranes, are continuous throughout the body of the peritrich and probably also into and through the length of the stalk.

Do similar linkage complexes exist in other ciliates which are known to have contractile microfibrillar bundles? In a preliminary study of *Opercularia coarctata* (Allen, 1973), a peritrich with a noncontractile stalk, structures, albeit less organized, have been observed in its body which resemble linkage complexes of *Vorticella*. However, no studies have been made of this ER-myoneme interface in those heterotrichs or gymnostomes which have contractile myonemes. Extensive membrane systems are known to lie in close contact with the myonemes in many of these cells. The author, on the other hand, saw no such complexes or any ER next to the striated bands in *Paramecium* which he concluded might be contractile (Allen, 1971) or the infraciliary lattice, another system of microfibrils. A comparative study needs to be done to see if this structure is widely scattered through the ciliates or limited only to the peritrichs.

In summary, a linkage complex has been described which may assume, in combination with the ER, some of the roles in the peritrich ciliates that the SR takes in muscle. It may help to regulate the flow of calcium ions into and away from the contractile system and it may also assume the function of the muscle cell T system by carrying the initial triggering impulse from the cell's surface to the sites of calcium storage. Neither the contractile nor the inferred calcium regulating structures of the peritrichs appear to be morphologically homologous to those of muscle but the end results of their activities may be remarkably similar.

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