# FLAGELLAR MOTION AND FINE STRUCTURE OF THE FLAGELLAR APPARATUS IN *CHLAMYDOMONAS*

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ABSTRACT

The biflagellate alga *Chlamydomonas reinhardi* was studied with the light and electron microscopes to determine the behavior of flagella in the living cell and the structure of the basal apparatus of the flagella. During normal forward swimming the flagella beat synchronously in the same plane, as in the human swimmer's breast stroke. The form of beat is like that of cilia. Occasionally cells swim backward with the flagella undulating and trailing the cell. Thus the same flagellar apparatus produces two types of motion. The central pair of fibers of both flagella appear to lie in the same plane, which coincides with the plane of beat. The two basal bodies lie in a V configuration and are joined at the top by a striated fiber and at the bottom by two smaller fibers. From the area between the basal bodies four bands of microtubules, each containing four tubules, radiate in an X-shaped pattern, diverge, and pass under the cell membrane. Details of the complex arrangement of tubules near the basal bodies are described. It seems probable that the connecting fibers and the microtubules play structural roles and thereby maintain the alignment of the flagellar apparatus. The relation of striated fibers and microtubules to cilia and flagella is reviewed, particularly in phytoflagellates and protozoa. Structures observed in the transitional region between the basal body and flagellar shaft are described and their occurrence is reviewed. Details of structure of the flagellar shaft and flagellar tip are described, and the latter is reviewed in detail.

## INTRODUCTION

Cells do work in many ways to move themselves, their internal constituents, and their environment. Contraction of muscle cells, beating of cilia and flagella, protoplasmic streaming, and ameboid motion are but a few of the known examples of this phenomenon. Cilia and flagella were first described by Leeuwenhoek in the 17th century, and his record of one such observation, of a biflagellate alga which he saw in 1702, is still relevant to the work to be presented here: "Their bodies seemed to be composed of particles that represented an oval figure; and therewithal they had [two] short thin instruments [*i.e.*, flagella] which stuck out a little way from the round contour, and wherewith they performed the motions of rolling around and going forward." [1]

In recent years the application of the electron microscope to biological materials has resulted in a wealth of information on cilia and flagella (11, 24, 27, 86, 88, 108, 112), but the mechanism of their motion remains a subject for speculation.

[1] Quoted from the translation of Dobell (21), who identifies the organism as *Haematococcus* and indicates that Leeuwenhoek probably saw the smaller-celled *Chlamydomonas* in the same sample of water from his gutter.

That the same morphological pattern of 9 + 2 units can produce a variety of motions, even in a single cell, is a simple paradox which speaks against an easy morphological solution to the question of flagellar motion, but this same paradox leads us to probe more deeply into the structure of the organelle, since such a high degree of organization demands some relation between form and function. The present study attempts to approach the problem on several levels: (*a*) the function of flagella of living cells, (*b*) the structure of the flagellum and its basal apparatus, and (*c*) correlations between structure and function, when they can be observed. A fourth level, the molecular architecture of flagellar fibers, is the subject of a separate paper (91).

The organism under study was the biflagellate alga *Chlamydomonas*, a eukaryotic cell whose small size and relative ease of culture under controlled conditions recommends it for study. The genetics and physiology of this organism have been investigated fairly extensively (see reviews by Levine and Ebersold, reference 59, and Sager, reference 101), and the basic pattern of its ultrastructure is known from the work of Sager and Palade (103, 104) and the related studies of Ris and Plaut (92) and Lang (53–55). In addition, the preliminary work of Gibbs et al. (39) has given good indication of the complexity of the flagellar apparatus in *Chlamydomonas*.

## MATERIALS AND METHODS

### *Culture Techniques*

*Chlamydomonas reinhardi* Dangeard, strain 21gr, from the laboratory of Dr. Ruth Sager, was grown in synchronous culture (10, 48) using a cycle of 15 hr light and 9 hr dark. Cells were removed for fixation during the light period, when the cells are motile and synthesis and growth occur.[2] Cultures were grown at 25°C in Medium I of Sager and Granick (102) and were bubbled with air to which approximately 1% $CO_2$ was added.

[2] Vegetative cells from nonsynchronous cultures, gametes, and zygotes soon after gamete fusion were also studied to some extent, representing all the motile stages of the life-cycle of the organism, and showed no obvious departures from the general pattern of flagellar structure to be described in synchronous vegetative cells.

### *Light Microscopy*

The mode of swimming and motion of flagella of *C. reinhardi* were studied by observing cells in viscous media. Either gelatin or agar was added to the medium to slow the frequency of flagellar beat to about one per second; cells were then observed at room temperature by phase-contrast microscopy. In other experiments, cells swimming actively in normal medium were photographed with phase-contrast or interference-contrast optics using electronic flash illumination of 0.3–0.5 msec duration.

### *Electron Microscopy*

Cells were fixed in 2% glutaraldehyde (100), washed, and then postfixed in 1% $OsO_4$, with 0.05 M collidine buffer (9), pH 7.5, as the wash solution and as the vehicle for both fixatives. Material was embedded by conventional techniques in Epon-Araldite mixture No. 1 of Mollenhauer (80) or in Luft's Epon (63). Sections were cut with a diamond knife on an LKB microtome and examined with an RCA EMU-3F electron microscope.

Information on the structure of the basal apparatus was derived to some extent from serial sections but largely from single sections of random orientation. In order to construct a three-dimensional model of the structure of the basal apparatus from random sections, it was necessary to know the orientation of each cell with respect to the section in which it appeared, so that information from many orientations could be correlated. Since the major structures of the *C. reinhardi* cell (the chloroplast, pyrenoid, nucleus, contractile vacuole, and flagella) have a fairly constant orientation with respect to one another (Fig. 1), it was possible to use these organelles as morphological markers, and, from their appearance in a micrograph, to determine at what level and angle the section had passed through the cell.

### *Terminology*

The terminology associated with motility structures is both confusing and inconsistent, since many of the terms commonly used apply to a single structure while some have more than one meaning. In general I have tried to follow the terminology of Gibbons (37, 38) and Sleigh (112) whenever possible, to introduce as few new terms as possible, and to give preference to names which are more or less self-explanatory.

For purposes of discussion, the flagellum is divided into four portions: (*a*) the tip, the region at the end of the flagellum, characterized by an alteration and disorganization of the normal 9 + 2 pattern of the flagellar fibers; (*b*) the main shaft of the flagellum, which shows a constant 9 + 2 pattern in cross-section; (*c*) the transitional region, between the shaft and the basal body, where the central fibers end and the

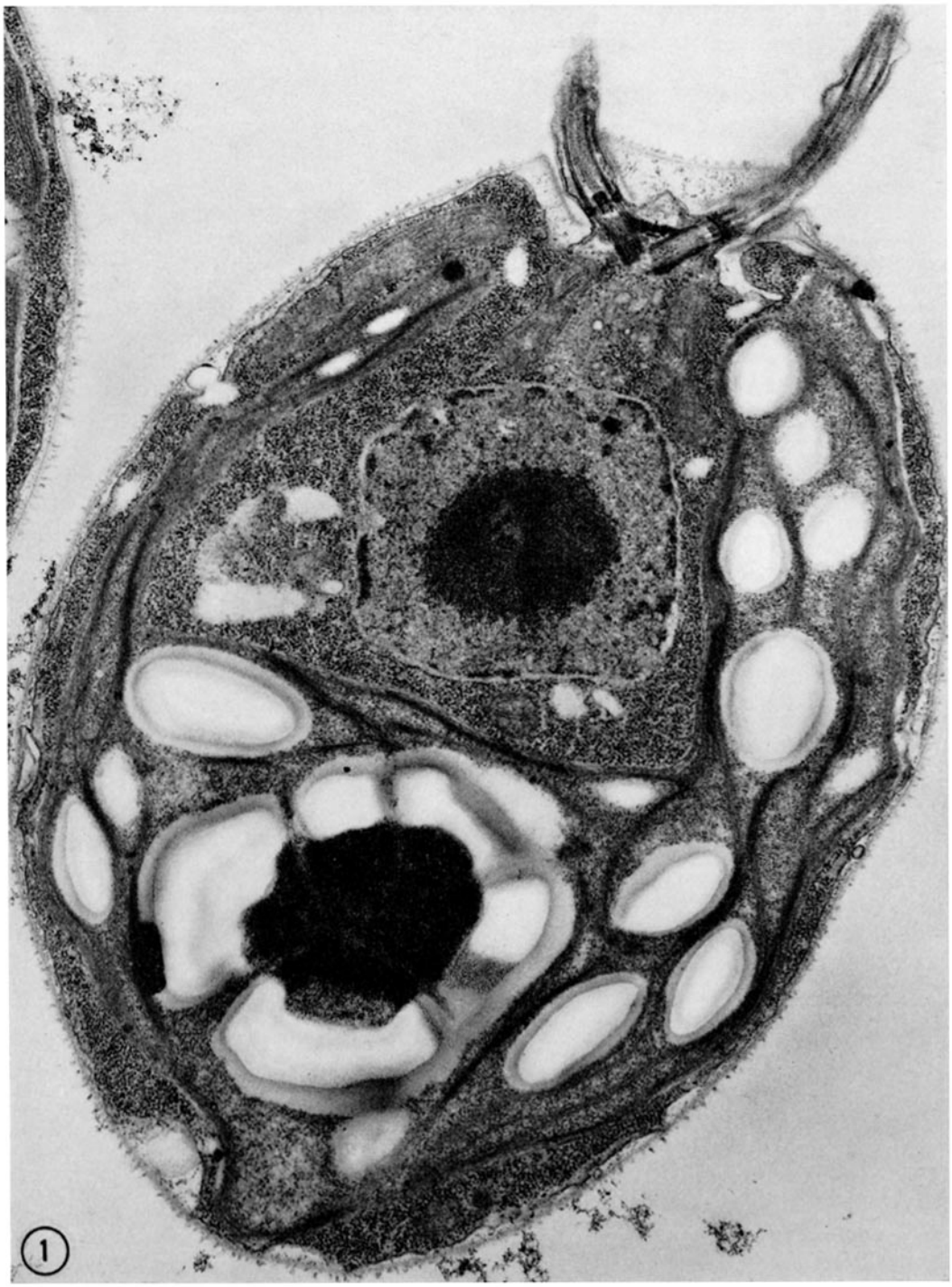



FIGURE 1 General view of a longitudinal section of the *Chlamydomonas reinhardi* cell showing the two flagella at the anterior end, with a striated fiber joining the basal bodies of the flagella. The nucleus lies near the center of the cell, surrounded by the single chloroplast which contains a pyrenoid (lower end) and many starch grains (light areas). Golgi regions lie near the nucleus but are not well preserved in this fixation. × 19,000.

flagellum shows alterations in its cross-sectional pattern; and (*d*) the basal body, the portion of the flagellum ending in the cytoplasm, where the nine peripheral fibers show a triple structure in cross-section (37).

The terms "anterior" and "posterior" are used to refer to the whole cell, the anterior end being the one which bears the flagella. "Proximal" and "distal" refer to points along the length of the flagellum; the proximal end of the flagellum corresponds to the end of the basal body lying deepest in the cytoplasm. "Below" and "above" will occasionally be used to indicate a proximal or distal direction, respectively.

The flagellar apparatus of *C. reinhardi* is sufficiently complicated that verbal description of the structures is difficult, and reference should be made to the explanatory drawings which accompany the text (Figs. 27, 28, and 30).

## RESULTS

### *Swimming*

The motion of flagella of cells in viscous media is diagrammed in Fig. 2, while Figs. 3–10 show the same patterns in high-speed flash photographs of cells swimming freely in normal medium. In normal forward swimming, cells travel with the anterior (flagella-bearing) end toward the direction of motion. At the beginning of the power stroke the flagella are straight and extended forward. The flagellum sweeps backward, remaining fairly straight and bending near the base. The return stroke begins as the power stroke is completed, with a wave of bending passing along the flagellum from base to tip; this restores the flagellum to its original position.

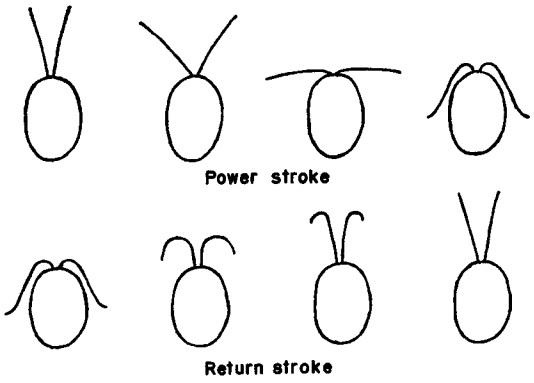



FIGURE 2 Drawings indicating the position of *C. reinhardi* flagella during forward swimming, as determined by observing cells in viscous media. Beating is synchronous and resembles that of cilia.

The positional relationship of the flagella is normally bilaterally symmetrical, the synchrony of motion being analogous to that of a human swimmer's breast stroke, but synchrony may be disturbed for brief periods. The beat of the flagellum is planar, and both flagella beat in the same plane, which also coincides with the central longitudinal axis of the cell (Fig. 10). *Chlamydomonas reinhardi* cells show little or no rotation as they swim; the appearance of rapid vibration of the forward-swimming cell is due to the alternate acceleration and deceleration of the cell during the power and return strokes.

Another type of swimming was occasionally observed in which cells reverse their direction and swim backward for short periods. In this case the flagella-bearing end of the cell faces away from the direction of motion; the flagella are extended and lie near one another (Figs. 11 and 12). Waves of bending, probably traveling from base to tip, pass along the flagella and exert a pushing force. Beating appears to be synchronous, and Fig. 11 indicates that the planar relationship of flagellar beating may be the same in backward swimming as in forward swimming. In backward swimming the cells travel smoothly, without the vibration seen in forward swimming, although the velocity is less. When cells were exposed to high intensity electronic-flash illumination (60 watt-seconds, 0.5 msec) during photography, a high percentage of the cells stopped their forward motion, swam backward for about a second, and then resumed forward swimming.

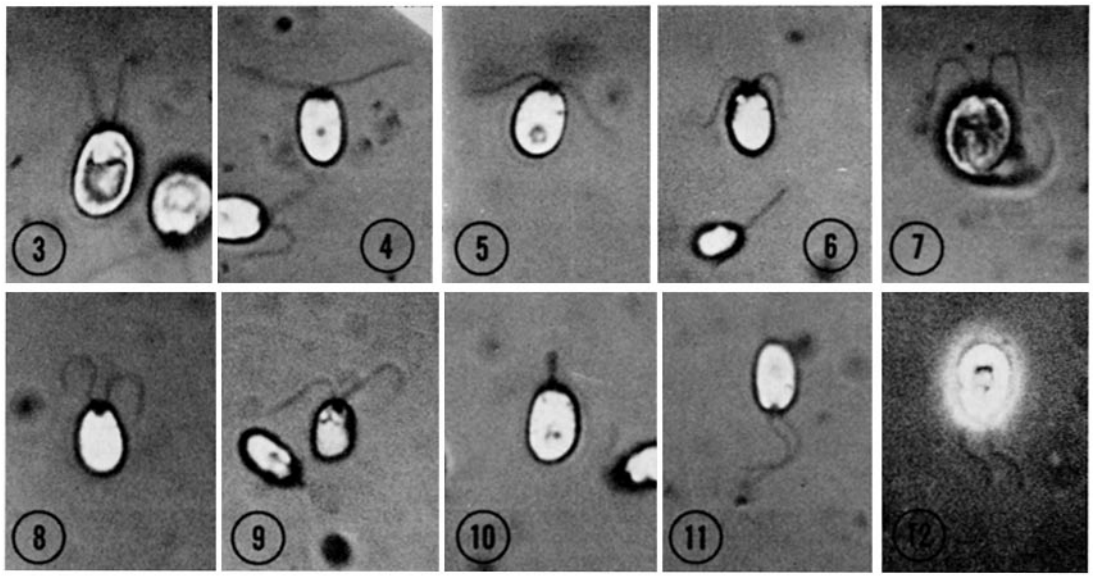
### *Structure of the Basal Apparatus*

#### General Morphology

The two flagella are inserted in the cell through specialized regions of the cell wall. Their basal bodies lie in a V configuration and are joined at the top by a striated fiber and at the bottom by two smaller fibers (Figs. 13 and 30). From the area between the basal bodies four bands of microtubules radiate in an X-shaped pattern, diverge, and pass under the cell membrane (Fig. 14). All of these structures will be described in detail below.

#### Microtubule System

The microtubule bands, seen near the basal bodies, each consist of four tubules of about 30




FIGURES 3–12 Light micrographs of cells swimming in normal medium. Figs. 3–8 show flagella in various positions during forward swimming. The cell in Fig. 9 shows slightly disturbed synchrony. Fig. 10 shows a cell whose plane of beat was perpendicular to the plane of focus of the microscope when it was photographed during the return stroke, indicating the relationship between the plane of beat of the flagella and the longitudinal axis of the cell. Figs. 11 and 12 show the pattern of flagellar motion in backward swimming. In all photographs the direction of motion of the cells is toward the top of the page. High-speed electronic flash illumination; Figs. 3–11, interference contrast; Fig. 12, phase contrast. × 1,000 (approximate).

mμ diameter in a 3-over-1 pattern (Figs. 19–21). As the bands radiate from the basal bodies, they pass near the cell membrane as a flat sheet, still with four tubular components (Figs. 18, 25, and 26). Once the tubules reach their position near the cell membrane, single tubules presumably diverge from the pattern; this leaves bands of three (Fig. 17) or two tubules (Fig. 16) which are frequently seen at the anterior end of the cell in four low ridges of cytoplasm which radiate from the basal-body region (Figs. 16–18). Individual tubules proceed for different distances just beneath the cell membrane. In a transverse section of a cell at the level of the nucleus, eight to ten tubules are often seen clearly in cross-section spaced around the periphery of the cell (Fig. 15), and all 16 tubules probably persist at least this far. The tubules are quite close to the cell membrane and often are surrounded by a region free of ribosomes. A few tubules are present in cross-section at the level of the pyrenoid (near the posterior end of the cell), so that the system of tubules encloses the whole cell to a certain extent. A diagrammatic interpretation of this pattern is given in Fig. 28.

The arrangement of the tubules near the basal bodies is quite complex. As noted above, the four bands, of four tubules each, approach the area between the basal bodies in a 3-over-1 configuration (Figs. 19–21). One of the tubules ends, probably the single one on the bottom of the 3-over-1 pattern, to leave a band of three tubules (Fig. 22). Still closer to the midpoint between the basal bodies another tubule ends. Above the remaining pair of tubules a flat plate of electron-opaque material is present (Fig. 23). In the very center of the area between the basal bodies no tubules remain; only the dense plate remains which lies just below the distal striated fiber (Figs. 1 and 13). Thus the tubule bands are not continuous with one another, and no direct connection between the tubules and any part of the basal apparatus has been demonstrated by the procedures used.

A possible interpretation of the different tubule patterns observed is given in the drawing in Fig. 27. This diagram indicates that the tubule of the triad which ends before the final pair is the one nearest to the center of the basal-body complex, and that it moves slightly upward to end just at the

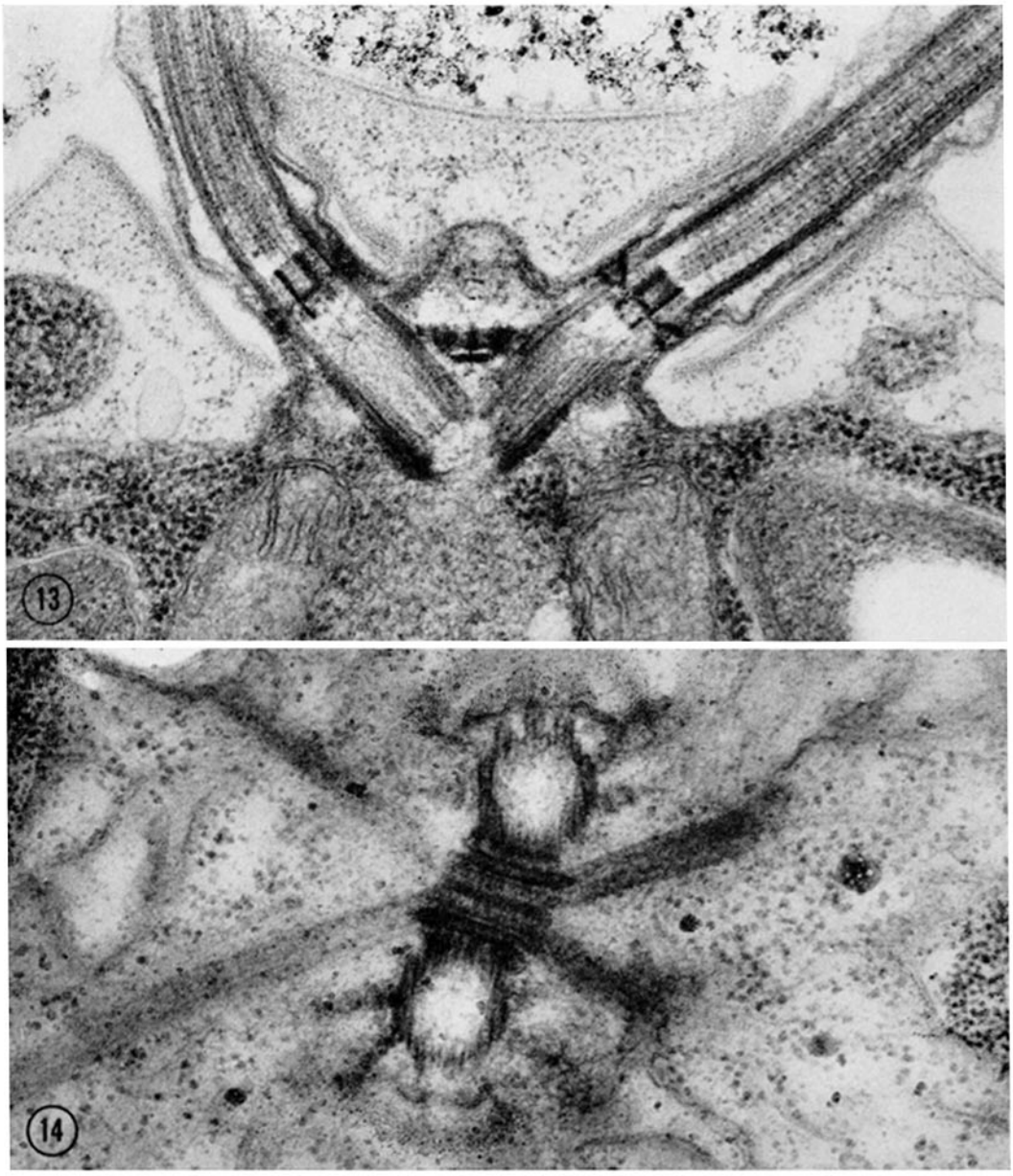



FIGURE 13 A view of both flagella in longitudinal section showing the orientation of the basal bodies. The distal striated fiber connects the basal bodies; an electron-opaque plate lies below the fiber. No microtubules are present in the region between the basal bodies. The central fibers of both flagella are visible in this section. × 63,000.

FIGURE 14 A section perpendicular to that of Fig. 13, showing the distal striated fiber in longitudinal section and the basal bodies in oblique section. Four bands of tubules approach the region between the basal bodies in an X-shaped configuration. × 63,000.

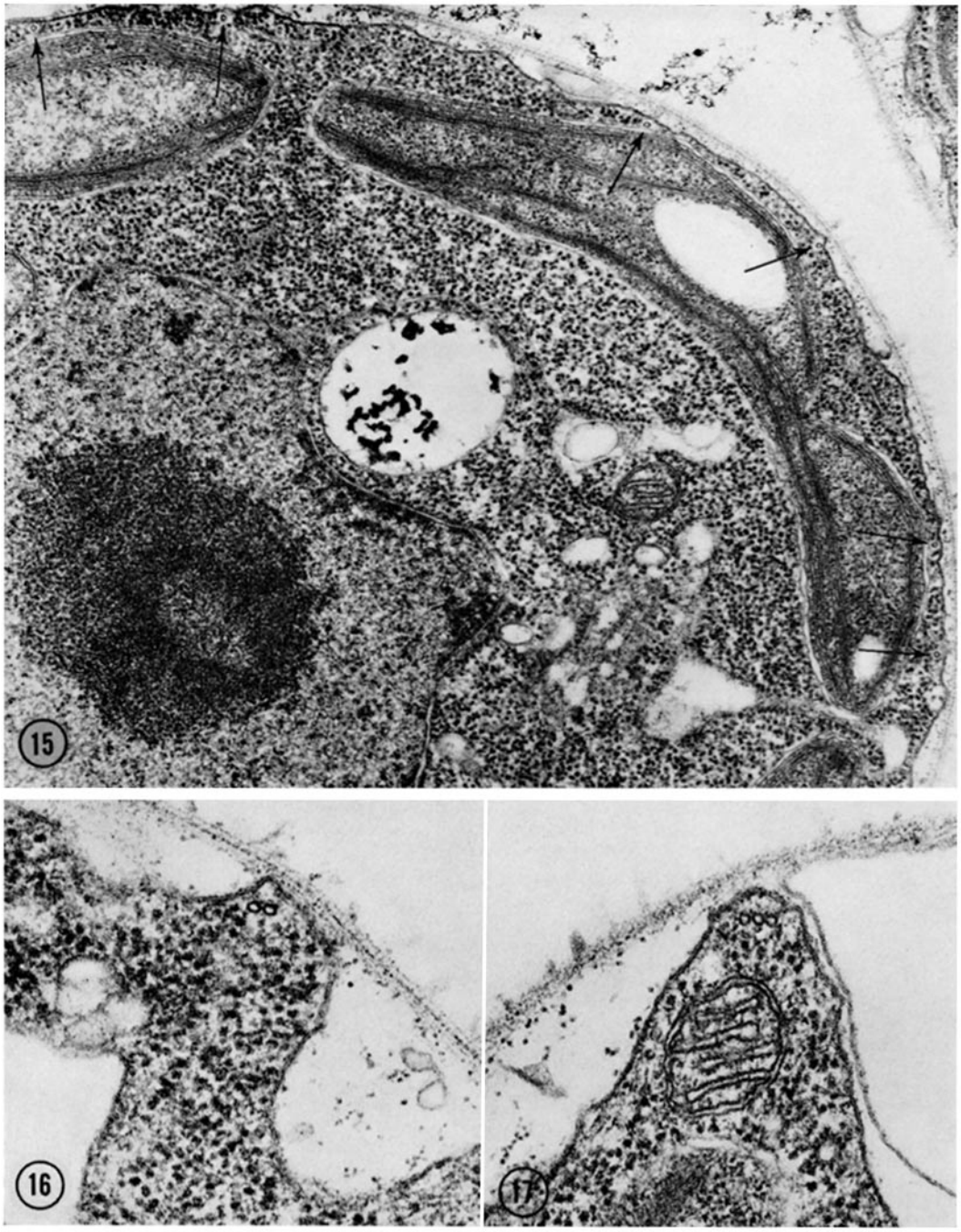



FIGURE 15 Transverse section of a cell intersecting the nucleus and nucleolus (lower left). Six microtubules are present just beneath the cell membrane (arrows). × 28,500.

FIGURES 16 and 17 Groups of two and three microtubules in cross-section near the anterior end of the cell. Contractile vacuole regions are at the lower left in Fig. 16 and the lower right in Fig. 17. × 55,500.

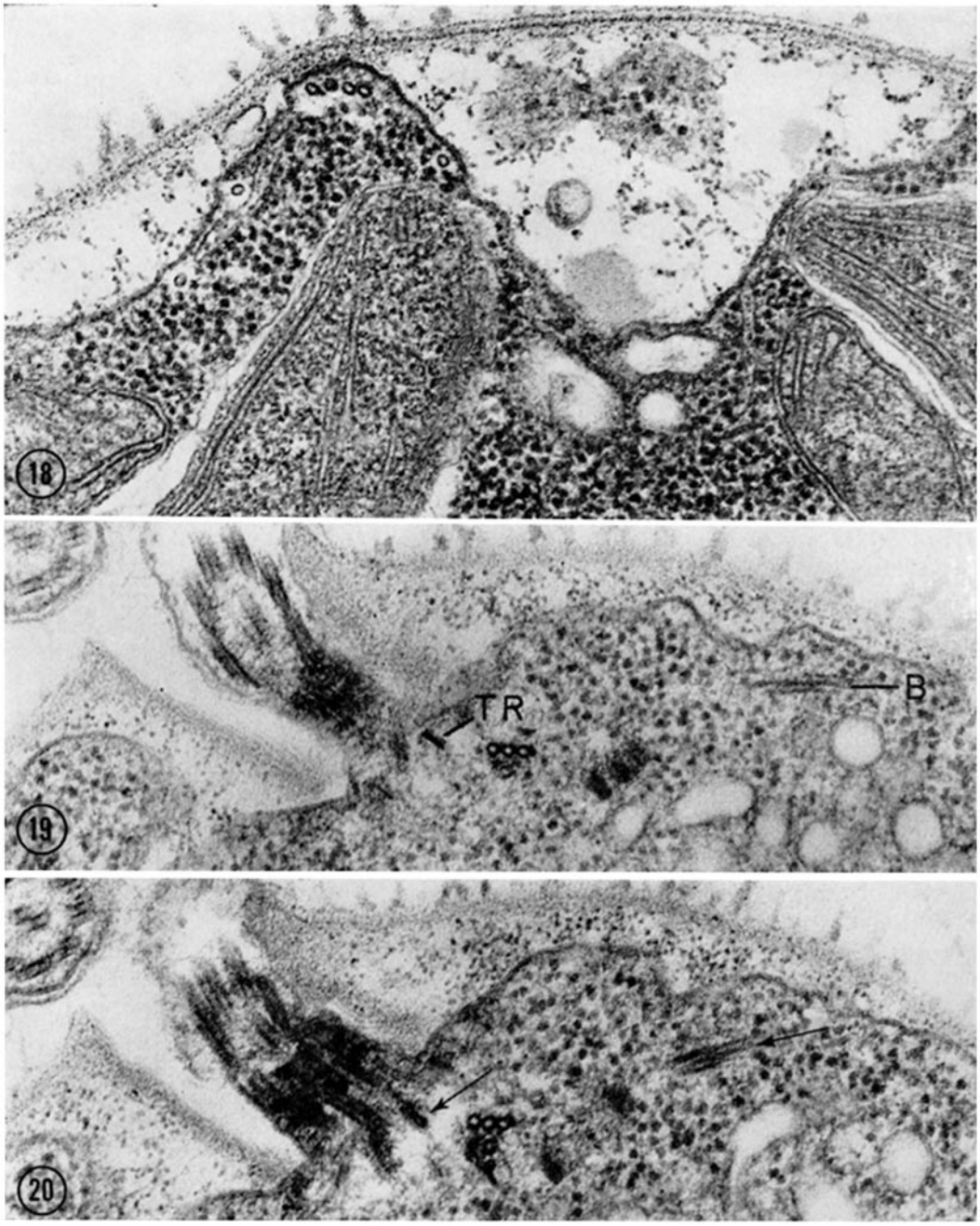
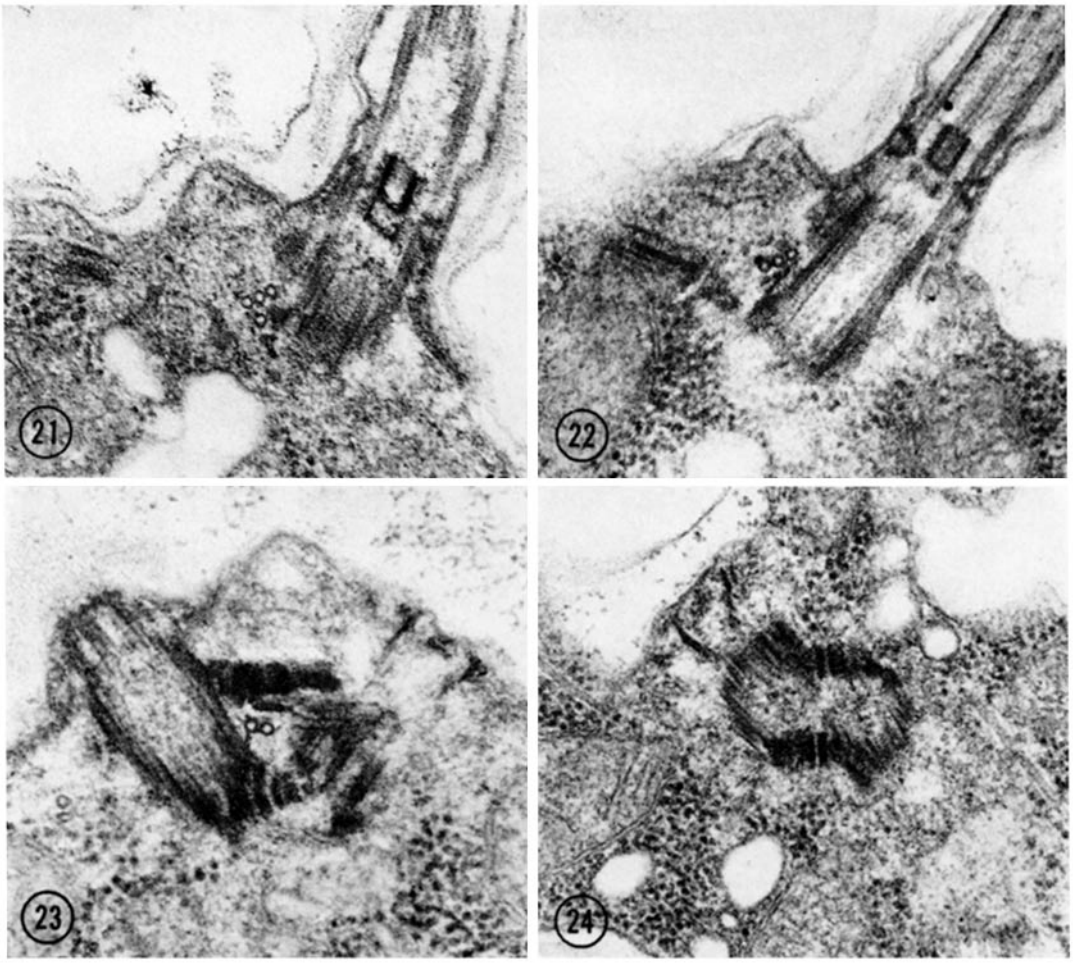



FIGURE 18 Band of four microtubules at the anterior end of a cell. Other single tubules are also present. The contractile vacuole region is near the center of the picture. × 59,000.

FIGURES 19 and 20 Successive serial sections through the anterior region of a cell, showing one microtubule band (3-over-1 configuration) in cross-section as it approaches the flagellar base (flagellum is seen at left in grazing section). The other tubule band approaching the basal bodies from that side is sectioned obliquely (*B* in Fig. 19, right-hand arrow in Fig. 20). A transitional fiber is shown in cross-section in Fig. 19 (*TR*) and connecting to the flagellar fiber in Fig. 20 (left-hand arrow). × 59,000.




FIGURES 21–23 Microtubule patterns near the flagellar bases. Fig. 21 shows the 3-over-1 configuration slightly closer to the basal bodies than in Figs. 19 and 20. The other tubule band approaches obliquely from the left. Fig. 22 shows the band of three tubules very near the basal body. The other band of tubules is at the left. Fig. 23 shows a band of two tubules in the region between the basal bodies. Below the tubules is the proximal connecting fiber; above the tubules is the electron-opaque plate associated with the tubule ends, and immediately above that is the distal connecting fiber. × 55,500.

FIGURE 24 This section has grazed the proximal ends of both basal bodies, which appear as crescent-shaped areas. The two proximal striated fibers are shown connecting the basal bodies at this level. × 45,000.

level of the dense plate (Figs. 25 and 26). This reconstruction also accounts for the fact that only one set of tubules is visible in cross-section in a given cell (Figs. 19–23). The arched path of the tubule bands, as they proceed from the basal-body region to the cell surface (Fig. 25), explains why the tubule bands only appear for relatively short distances when encountered in longitudinal section (Figs. 14, 26, and 41). It is possible that there are additional microtubules in the anterior region of the cell which do not form a part of the 16-member tubule network (see Fig. 18), but it is also possible that such views, seen infrequently, represent a stage in the replication of the 16 tubule system.

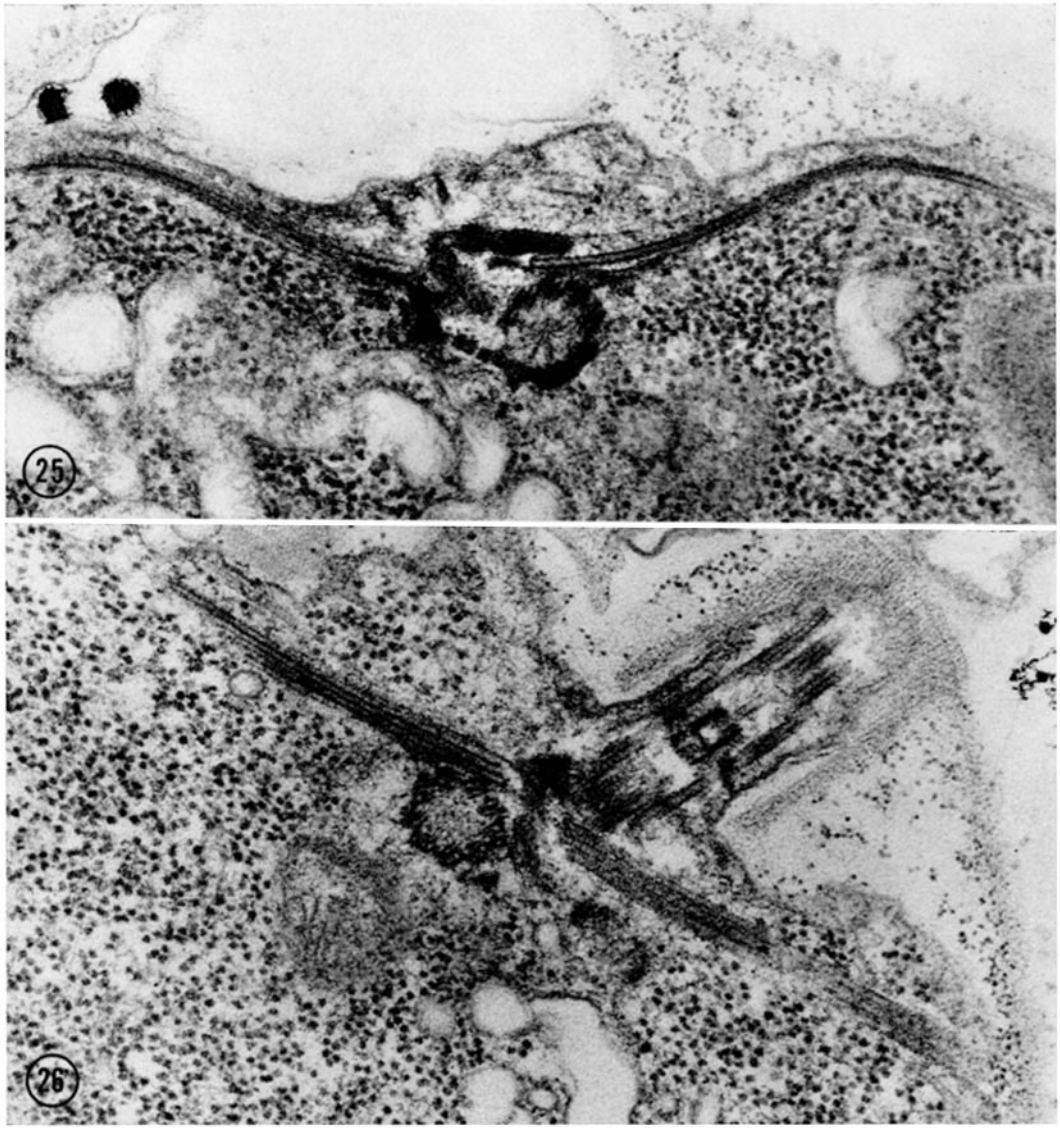



FIGURE 25 Two microtubule bands in longitudinal section, showing their form as they approach the cell membrane. The proximal end of one basal body is present in oblique section. The larger electron-opaque area is the distal connecting fiber. Immediately below it is the electron-opaque plate. One tubule ends at the level of the plate. × 48,000.

FIGURE 26 A transverse section of the anterior end of a cell at a level just below the distal striated fiber, showing two of the microtubule bands approaching the central region. × 55,000.

### STRIATED FIBERS

The distal striated fiber connects the two basal bodies at a level just below the top of the basal body (see Fig. 30). The fiber, seen in "vertical" longitudinal section in Figs. 1 and 13, in "horizontal" longitudinal section in Fig. 14, and in cross-section in Fig. 29, is about 300 mμ long, 250 mμ wide, and 75 mμ thick. The pattern of cross-banding, seen in properly oriented longitudinal sections, is complex and bilaterally symmetrical.

A pair of dark lines lies in the center, with a light line and another pair of dark lines on either side; the spacing of the central pair of striations is narrower than that of the outer pairs. Dimensions of the striation pattern are given in Fig. 31. Fine filaments running the length of the striated fiber are occasionally observed (Figs. 13 and 14), although this is not shown clearly by the present fixation.

Two smaller striated fibers, the proximal connecting fibers, link the basal bodies at their proximal ends. The long axis of these fibers is in the same direction as that of the distal striated fiber, but they are connected at the outside edge of the basal body on either side, and thus have a distance of about one basal body diameter separating them (see Figs. 28 and 30). Both of the proximal fibers are present in Fig. 24, where only the very tips of the basal body have been cut by the section. In Fig. 29 they are seen in cross-section as two ill-defined, roughly triangular pieces of material lying below the distal fiber. One of the pair of fibers is visible in Figs. 23, 25, and 40. The exact number and spacing of the cross-striations are not clear from these micrographs.

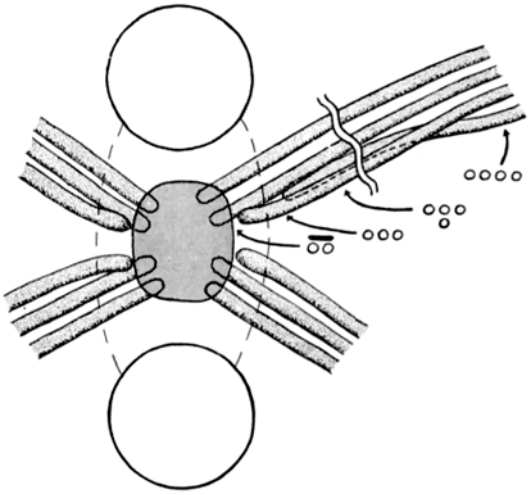


FIGURE 27 A diagram illustrating the orientation of microtubules as they approach the basal bodies. The view is from above the distal striated fiber looking in a proximal direction, with the position of the fiber indicated by dashed lines and the dense plate below it represented by the central grey area. The basal bodies are represented by two circles. In each tubule band, one tubule ends at the level of the dense plate, while two others pass below it. Only one of the bands is drawn in detail, with all four tubules shown and cross-sections indicated along its length. In the other three bands only three of the four tubules are shown.

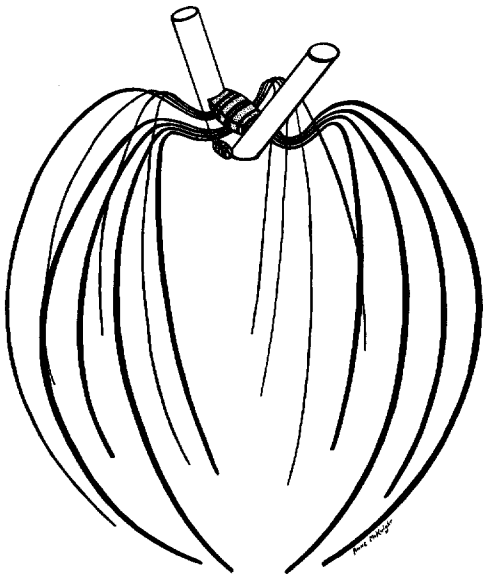


FIGURE 28 A schematic drawing of the general form of the cell's microtubule system. The emerging flagella are represented by truncated cylinders, and the relative sizes of the microtubules and basal apparatus are not to scale. Details of how the tubules end in the posterior region of the cell are not known.

Between the proximal pair of fibers and the distal connecting fiber lies the flattened plate which is associated with the ends of the microtubule bands. In addition to views in cells sectioned in the longitudinal plane of the basal bodies (Figs. 1 and 13), Fig. 29 shows the plane lying just between the basal bodies. From these and other views of known orientation (Figs. 23, 25, and 26), the approximate size and shape of the plate can be deduced. It measures 25 mμ thick and is 80–100 mμ long in both its longitudinal axes (see Fig. 27).

## Basal Body

The basal bodies are identical in structure and are otherwise unexceptional; they are composed of nine triplet fibers with connections between the A and C subfibers of adjacent triplets (Figs. 32 and 39). The basal body is 220 mμ in diameter and about 400 mμ long, with no structures apparent in the lumen. At the proximal end of the basal body a thin filament runs from the A subfiber of each triplet to a central hub, and thus form the cart-

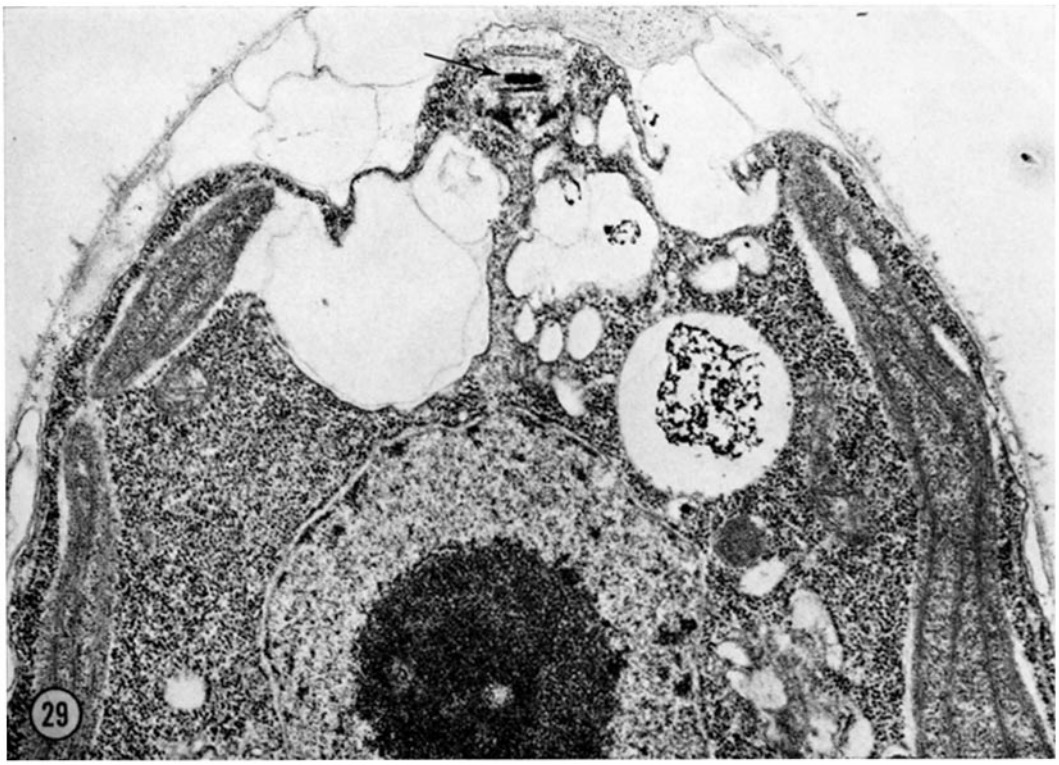



FIGURE 29 Longitudinal section of a portion of a cell made perpendicular to that in Fig. 1. The section has passed exactly between the two basal bodies, intersecting the connecting fibers in cross-section. The prominent electron-opaque area is the distal connecting fiber (arrow). Below it lies the plate associated with the microtubule system, and below that lie two irregular shapes which represent the proximal connecting fibers. × 22,000.

wheel pattern. The extent of the cartwheel structure along the length of the basal body is very slight, since it is not visible in longitudinal section and is only rarely encountered in cross-section (Fig. 40).

It is obvious from sections like that in Fig. 40 that the two basal bodies do not lie in exactly the same plane, and that while the basal bodies lie opposite one another at their tops they are skewed by a distance of somewhat less than one basal-body diameter at their bottoms. In sections where one basal body appears in exact longitudinal section, the other one is slightly oblique (Figs. 1 and 13), and other indications of the slight tilt of the basal bodies with respect to one another can be seen in Figs. 14, 26, and 41.

At the distal end of the basal body, transitional filaments radiate from the area of the B subfiber of the triplets (Fig. 38). Fig. 37 shows the point of doublet-to-triplet transition in the peripheral fibers and the appearance of the transitional filaments. One of these filaments is seen in cross-section in Figs. 19 and 20.

## TRANSITIONAL REGION

Just distal to the level of the doublet-to-triplet transition, the cell membrane approaches the peripheral flagellar fibers to form the flagellar membrane (Figs. 36 and 37). At about this level connections between the center of the doublet fibers and the flagellar membrane are often observed (Fig. 36). Only the nine peripheral doublets are visible, with no structures present in the central area of the flagellum.

At a slightly higher level, seen in cross-section, the stellate structure of Lang (54) is visible as a nine-pointed star formed by thin filaments about 50 A wide which make V-shaped connections

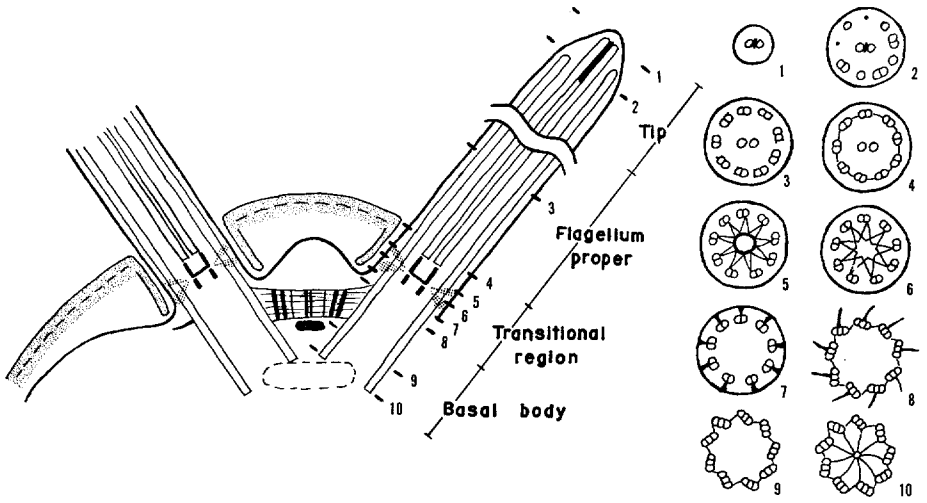



FIGURE 30 A schematic drawing of an idealized longitudinal section through both basal bodies. The tilt of the basal bodies with respect to one another is not shown, and the position of the two proximal connecting fibers, which would be out of the plane of the drawing, is indicated by a dashed line. The four regions of the flagellum are designated, and 10 typical cross-sections are shown for the numbered points marked along the length of one flagellum.

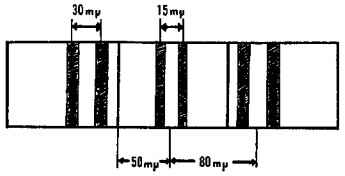



FIGURE 31 A drawing showing the approximate dimensions of the striation pattern of the distal connecting fiber.

between one of the subfibers of alternate peripheral fibers (Figs. 34 and 35). Two classes of star patterns are observed: one (Fig. 35) in which the star-forming fibers are clearly defined over their whole length and in which the apex of the V-shaped connection is visible; and another (Fig. 34) in which electron-opaque material present at the apex of the V forms a dark ring 80–90 mμ in diameter at the center of the star pattern. In longitudinal sections of flagella, the area of the star pattern appears as the profile of two cylinders about 85 mμ in diameter; this area corresponds to the ring at the center of the star. The proximal cylinder, about 50 mμ long, appears to be open at both ends, while the distal cylinder is about 100 mμ long and appears to be closed by a diaphragm at its proximal end (Fig. 32). A distance of about 25 mμ separates the two cylinders. The star pattern lacking the central ring (Fig. 35) probably represents the gap between the two cylinders. The diaphragm closing the distal cylinder has not been observed in cross-section.

The thin filaments forming the star are seen alongside the cylinders in favorable sections as lines or dots (see Fig. 32). About 10 or 12 of these are visible on either side in the best views, and, if Manton's (70) conclusion, that the star-forming filaments spiral as they connect alternate peripheral fibers and that they take two turns around the flagellum to complete the nine-pointed star, is correct, then in *C. reinhardi* the star-forming filaments would take about five or six turns along the length of the cylinders.

At certain levels of the star pattern, amorphous material is present between each peripheral doublet and the flagellar membrane. This is seen in both Figs. 34 and 35. In longitudinal sections this material appears as four wedge-shaped dense areas, two on either side of the flagellum, at levels near the space between the two cylinders (see Fig. 32 and the diagram in Fig. 30). They extend from the flagellar membrane to the peripheral fibers and overlap the latter to some extent. It is probably this material which was interpreted as a transverse diaphragm in *C. moewusii* by Gibbs et al. (39).

The distal end of the upper cylinder of the star

pattern coincides with the point of appearance of the two central fibers of the flagellum, although there does not seem to be a direct connection between the cylinder and the pair of fibers. At the level where the central fibers begin, the peripheral fibers are joined together by thin connections (Fig. 33). It is not known over what extent of the flagellum these connections occur, but they have only been observed at areas where the flagellum is within the collar of the cell wall.

The collar in the cell wall forms a cylinder surrounding the flagellum for a distance of about 0.8 $\mu$ between the point where it leaves the cytoplasm and the point where it exits from the cell wall. The collar appears to be somewhat different in organization from the rest of the wall. In general the cell wall has the appearance of an electron-opaque outer line, a denser middle line, and an amorphous inner layer (see Fig. 18). The collar does not show this layering (see Figs. 1, 13, 19, 20 and 32), and in cross-section the collar can be seen to be formed by an inner array of over a hundred 50–75 A filaments surrounded by amorphous material (Fig. 33). Lang (53) has noted a similar pattern in the flagellar channel of the colonial green alga *Volvulina*.

### Additional Basal Bodies

In addition to the normal complement of the basal apparatus of the flagella, an extra basal body is occasionally observed in cross-section lying near the normal pair (Fig. 41). The third basal body occurs to one side of the normal basal bodies in the V formed by the two tubule bands approaching the basal apparatus from that side. In addition to this fixed position, the extra basal body seems always to have its longitudinal axis in the same direction as one of the normal basal bodies, so that the tubules of the normal and extra basal bodies are both seen in exact cross-section in the same cell. The extra basal bodies observed have shown the normal triplet structure, with connections between the A and C subfibers, and show a cartwheel pattern at their lower end. Two extra basal bodies have not been observed in the same cell in the limited sample studied, and they have not been seen in longitudinal section. Therefore it is not known how their length compares with that of the normal basal body.

In other organisms with a small number of flagella, where it is possible to observe the whole complement of basal bodies in a single section, "extra" basal bodies have occasionally been seen. Manton reported one extra basal body in a small uniflagellate alga (67) and two extra ones in a biflagellate (71). In all cases the extra basal bodies seem to have some definite spatial relationship to the cell's normal complement, and probably represent newly formed basal bodies. A similar relationship has been reported in a ciliate by Bradbury and Pitelka (13).

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FIGURES 32–40 Electron micrographs of the transitional region and basal body. Figs. 33–40 represent a series of cross-sections beginning in the transitional region and progressing to the proximal end of the basal body.

FIGURE 32 A longitudinal section of a flagellum from the point where it enters the collar of the cell wall to the proximal end of the basal body. A portion of the distal connecting fiber is shown (electron-opaque area to right of basal body), and below it are three tubules of the microtubule band. The dots along the inner left side of the basal body probably represent the A–C subfiber connections of the basal body triplets seen in cross-section. × 92,000.

FIGURE 33 Cross-section of a flagellum within the collar of the cell wall. Connections between the doublets are present, and the regular structure of the collar is shown. × 120,000.

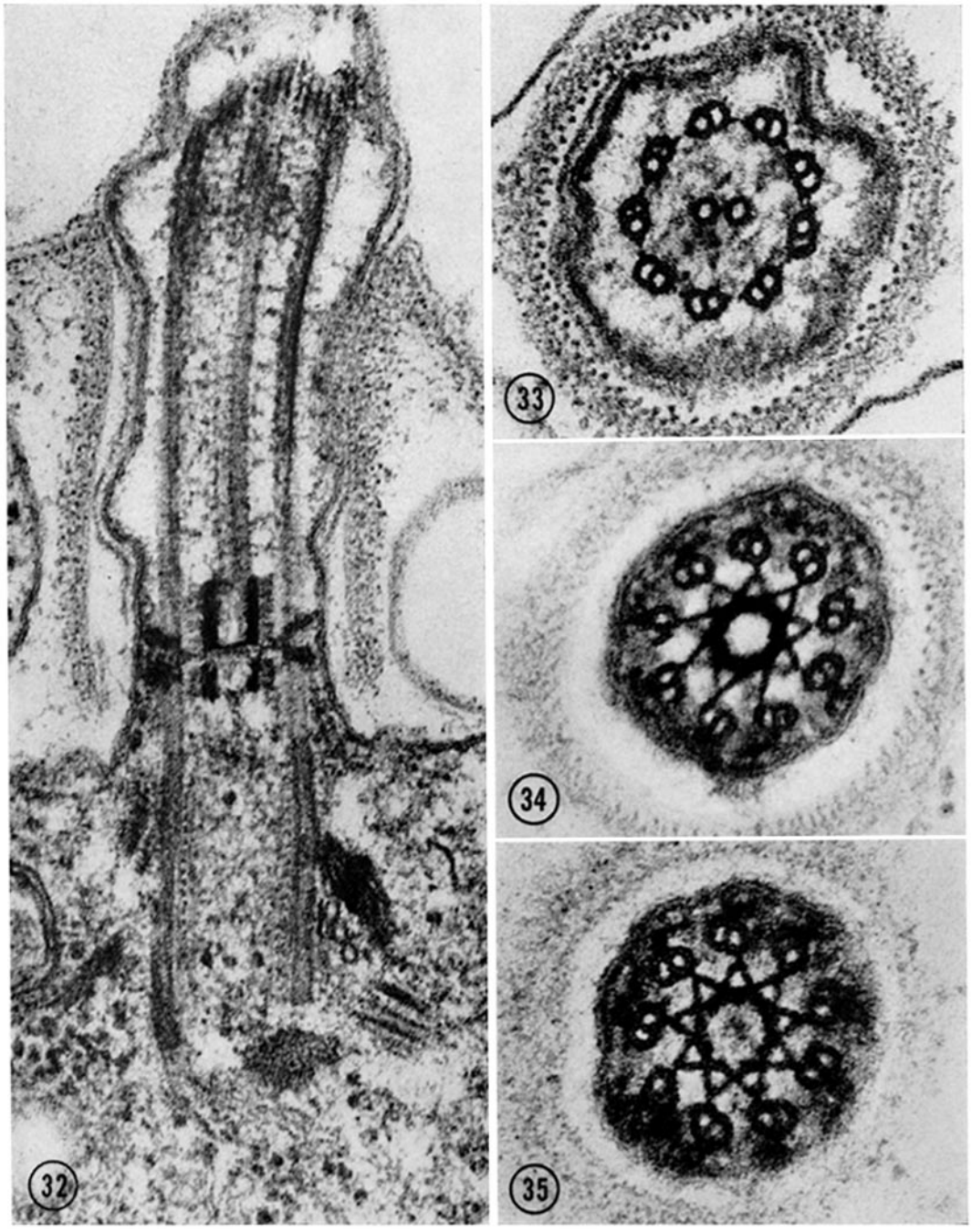
FIGURE 34 Cross-section in the transitional region showing the stellate pattern. The electron-opaque circle in the center of the pattern corresponds to the cylinder seen in longitudinal section in Fig. 32. × 120,000.

FIGURE 35 Another form of the stellate pattern, probably representing the region between the two cylinders (see text). × 120,000.

*The Flagellum*

### Flagellar Matrix

In longitudinal views of flagella, the area between the peripheral and central fibers contains material which often appears to have an orderly structure. The density of this matrix material can best be realized by comparing the normal shaft of the flagellum to the flagellar tip, which lacks the matrix material (Fig. 43). The structure



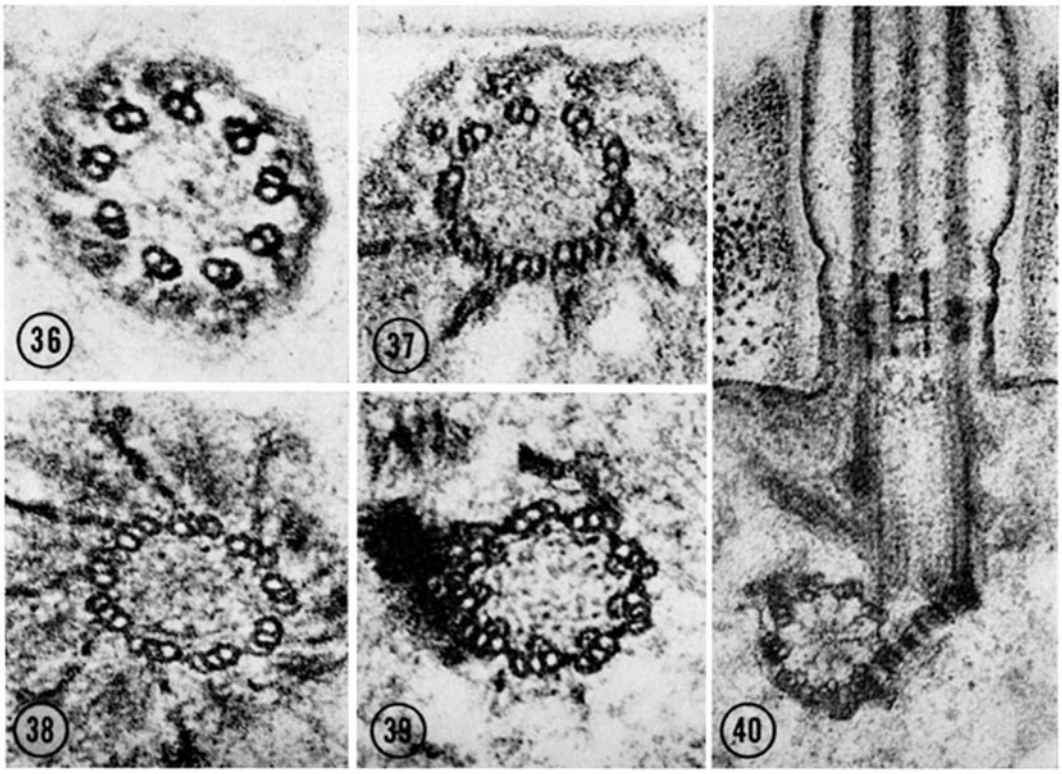



FIGURE 36 Cross-section in the transitional region below the stellate pattern, showing connections between doublets and the flagellar membrane. × 100,000.

FIGURE 37 The point of doublet-to-triplet transition between the flagellum and basal body, showing transitional fibers. × 100,000.

FIGURE 38 Cross-section at the distal end of the basal body, showing transitional fibers connecting to the B subfiber of the triplets. × 100,000.

FIGURE 39 Cross-section of a basal body, showing the normal arrangement of triplets and A–C subfiber connections. × 100,000.

FIGURE 40 Longitudinal section through one flagellum and basal body and cross-section of the very proximal end of the other basal body. One of the proximal connecting fibers is shown, along with the cartwheel pattern at the end of the basal body and the relationship of the basal bodies at their lower ends. × 80,000.

of the matrix seems to be in the form of lateral connections (probably filaments of about 50 A diameter) between the central and peripheral fibers. These connections are usually spaced at intervals of approximately 150 A along the length of the flagellum. They run perpendicular to the main axis of the flagellar fibers (Fig. 43) or are slightly inclined (Fig. 44). Unfortunately these connections are not clearly visible in cross-sections of flagella (see Fig. 48), and it has not been possible to examine enough longitudinal sections of flagella, which show the connections in sufficient detail, to draw any conclusions about their three-dimensional arrangement. These connections probably correspond to the "spokes" described by Afzelius (1) and the "radial links" of Gibbons

(36–38), which have been reported in a variety of materials (14, 56, 110) and studied most thoroughly by André (7).

### Additional Components of the Flagellum

The arms of the peripheral fibers, described by Afzelius (1), Gibbons and Grimstone (38), and others, can be seen occasionally on the fibers of flagella in *C. reinhardi*, but arms are not strikingly visible on each of the peripheral fibers (Fig. 48). In general it is at least possible to tell, in a given cross-section, in what over-all direction the arms are pointing. But the arms seem to be present more as diffuse areas on one side of the fiber rather than as distinct protrusions. Arms or arm material seem to be completely lacking in the transitional (Figs. 33–36) and the tip regions (Figs. 49–55).

Electron-opaque regions are occasionally observed against the flagellar membrane (Figs. 44, 48, 49, and 53) as are connections between the peripheral fibers and the flagellar membrane (Fig. 44). Neither the secondary fibers nor the central sheath described by Gibbons and Grimstone (38) have been observed in the present study.

### Orientation of the Central Pair

In all cases where the orientation of the central pairs of both flagella of a single cell could be determined, they were found to lie in approximately the same plane, one which coincides with the plane of beat of the flagella in forward swimming. Thus a median longitudinal section through the flagella and both basal bodies intersects all four central fibers (Fig. 13). In Fig. 1 the section misses the central pair of the right-hand flagellum, but intersects both central fibers of the left-hand one. Figs. 45–47 show the same orientation of flagella seen in cross-section over about the first 0.8 $\mu$ of their length, *i.e.*, while they are within the collar of the cell wall. In the three examples, a greater variation is seen as the flagella are sectioned in a more distal plane. Pairs of flagellar cross-sections outside the cell wall could not be positively identified as originating from the same cell without the use of elaborate serial-sectioning procedures.

### Flagellar Tip

Over about the last micron of its length, the flagellum forms a blunt or slightly pointed tip and shows a change in its internal structure (Figs. 43 and 49–57). As the flagellar fibers approach the tip, the matrix material is lost, first partially, then completely (Fig. 43). The peripheral doublet fibers become single, and these single fibers end

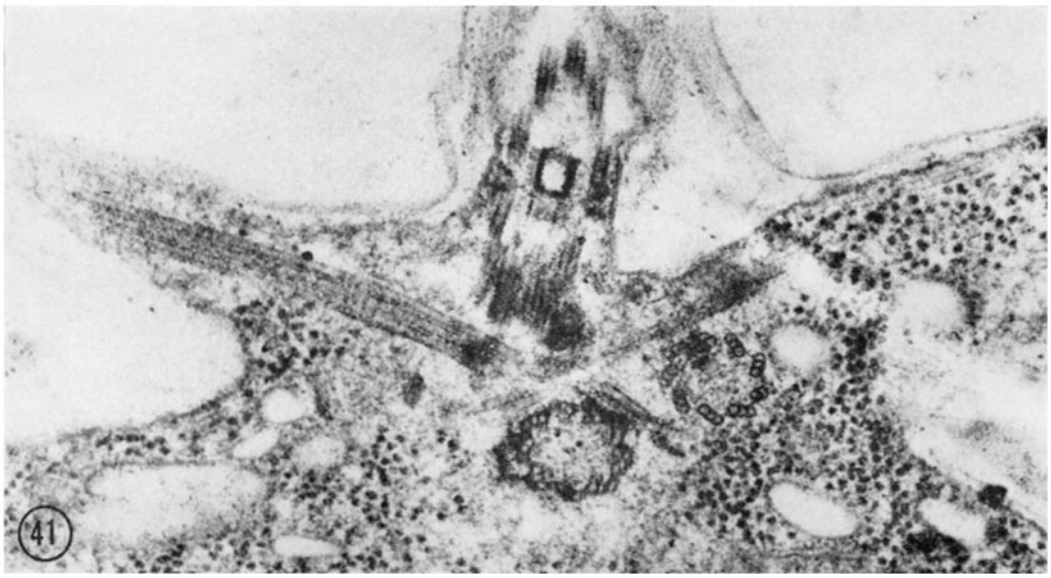



Figure 41 The normal pair of basal bodies are at the center of the picture, one in cross-section and the other above it in oblique section showing the entering flagellum. An additional basal body appears in cross-section to the right of the normal pair (see text). Microtubules can be seen approaching the central region between the basal bodies. × 58,000.

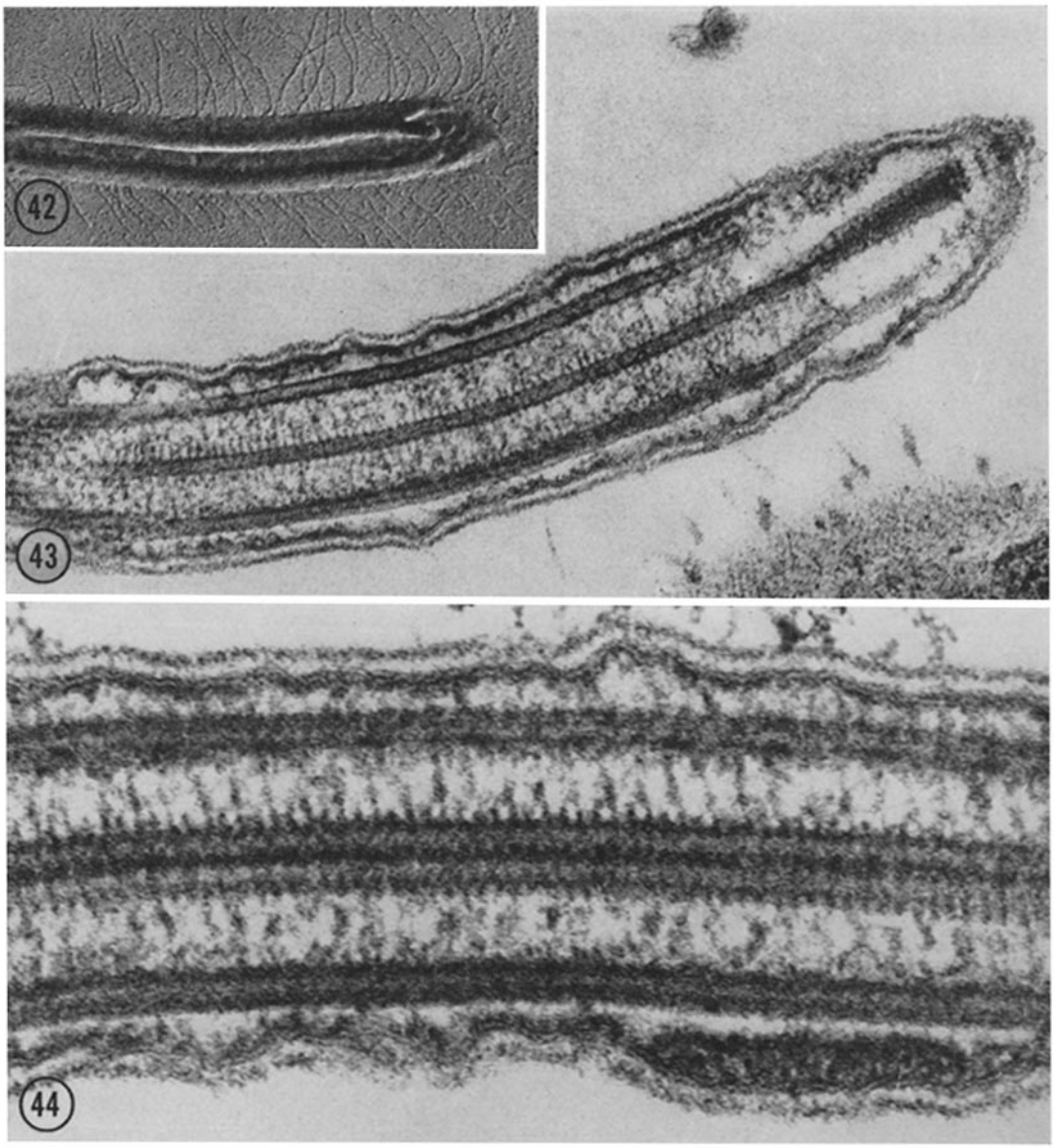
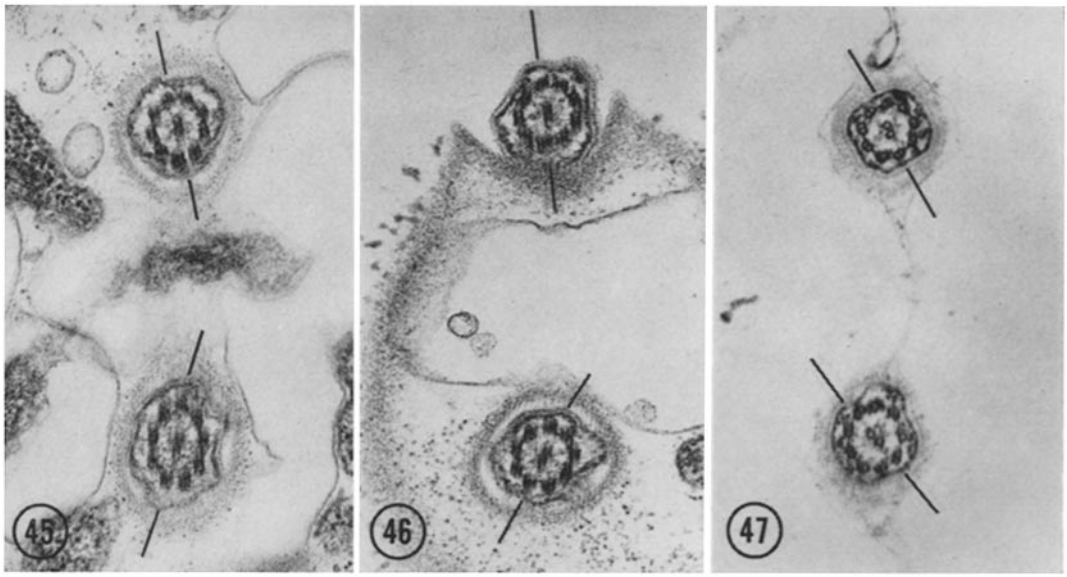



FIGURE 42 Electron micrograph of a shadowed flagellum showing its blunt end and hairlike projections. Cells were prepared by freeze-drying and shadowed with platinum-carbon. × 23,000.

FIGURE 43 Longitudinal section through the tip of a flagellum. The matrix between the central and peripheral fibers shows an ordered structure in some areas, while the matrix material is absent at the very tip. The central fibers persist to the end of the flagellum, with a sheet of material intercalated between the central pair, seen here in longitudinal section as a widened area of greater electron density. × 68,000.

FIGURE 44 Longitudinal section of a flagellum showing connections between the central and peripheral fibers. Electron-opaque material next to the flagellar membrane and connections between the peripheral fibers and the membrane are also visible in this figure. × 150,000.




FIGURES 45–47 Three sections which show the emerging flagella of single cells, with the orientation of the central pair of fibers indicated. A line, here vertical, between the two flagella would define the plane of flagellar beat in forward swimming. In Fig. 45 the flagella are sectioned fairly near the ends of the central pair, while those in Fig. 47 are almost outside the cell wall. Fig. 46 represents an intermediate position. × 40,000.

one by one, with the lumen of the tubule becoming electron-opaque just before it ends (Figs. 51–55). The level at which the fibers become single and at which they end varies among individual fibers, apparently at random. For example, in Figs. 49, 51, and 52 some of the single fibers are ending while others are still double. The single peripheral fibers usually retain their circular orientation in the tip region. The number of single fibers decreases as the end of the flagellum is reached, until only the central fibers remain. The central pair persists almost to the very end (Figs. 55–57), and its members maintain an orderly relation to one another. Near the end of the flagellum a sheet of material is intercalated between the central fibers. This tip sheet is seen in longitudinal section in Fig. 43 and in cross-section in Figs. 49, 51, and 55–57. Evidently its length varies from flagellum to flagellum, since it is present in Fig. 49 where doublets are still present, but not in Fig. 54 where the remaining seven tubules are all single. Tip sheets as much as 0.5 $\mu$ long have been measured in longitudinal sections.

### Flagellar Sheath

The flagellar sheath is a layer of material, external to the flagellar membrane, which covers the flagellum over most of its length. It is visible in longitudinal and transverse sections of flagella as an electron-opaque line spaced about 20 m$\mu$ from the flagellar membrane (Figs. 43, 44, and 48). The sheath begins at the base of the flagellar shaft at about the level of the star pattern (see Figs. 32–34) and continues to the tip of the flagellum (Figs. 43 and 48–56). The surface of the sheath shows no defined ultrastructure when seen in grazing longitudinal section, and the sheath material is evidently poorly preserved when cells are fixed in $OsO_4$ alone. Shadowed preparations of whole flagella show fine hairlike projections extending from the flagellar surface (Fig. 42). These have been seen occasionally in thin sections but have not been studied in any detail, and their relation to the sheath is not known.

## DISCUSSION

### *Motion of Flagella*

In an early study of swimming in microorganisms, Ulehla (117) examined *Chlamydomonas braunii* using dark-field illumination. More recently Lewin (60, 61) has described the swimming of *C. moewusii* using dark-field and phase-contrast microscopy and stroboscopic illumination. Both

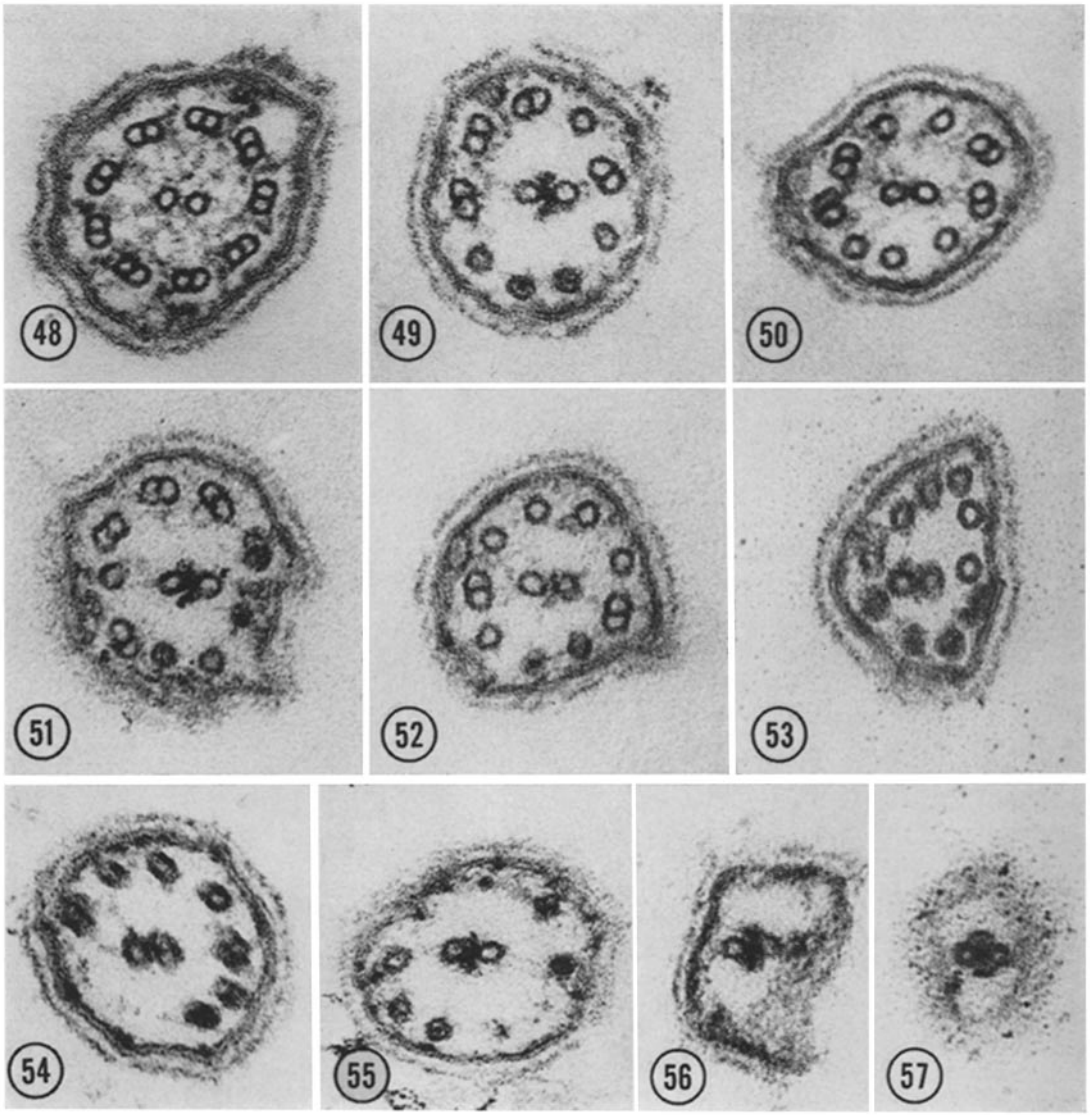



FIGURE 48–57 Sections representing the progressive changes in appearance of the flagellum in cross-section from the normal shaft through the tip region. × 100,000.

FIGURE 48 Normal cross-section of a flagellum, typical of the entire length of the flagellum between the transitional region and the tip region (see diagram in Fig. 30).

FIGURE 49–55 Cross-sections in the tip region of the flagellum showing the transition from doublets to singlets and the ending of singlets. The pattern of both changes appears to be random. Tip sheet material is visible in Figs. 49, 51, and 55.

FIGURE 56 and 57 Sections through the very ends of flagella.

these authors described forward and backward swimming, and the data presented here are in good general agreement with their accounts, except for the fact that both the organisms they studied swim in spiral paths while *C. reinhardi* does not spiral. Lowndes (62) was unable to photograph swimming cells of a large unidentified species of *Chlamydomonas* using high-speed motion picture apparatus. Instead he examined cells which he took to be "in a more or less morbid state." But from his description of them, lying on the bottom of a watch glass, having ceased to swim, with their flagella spread out and the cells vibrating, it appears that he observed normal cells whose flagella had stuck to the glass surface, as commonly occurs when cells are observed on glass slides. Lowndes' diagram of swimming in *Chlamydomonas* seems to have been based mainly on theoretical considerations and observations of other organisms, and does not agree with the data presented here or with those of the authors cited above.

Jahn and Bovee (45) have noted that the beat of *Chlamydomonas* flagella in forward swimming is similar to that typical of many cilia, with a power stroke in which bending occurs at the base and a return stroke in which a wave is propagated from base to tip (41, 112). Other examples of cilia-like beat of flagella are known, but of particular interest are the reports of various trichomonad flagellates (12, 46, 112) where, depending on the genus, the three or four anterior flagella beat synchronously in a cilia-like manner to drive the cell forward, in a fashion completely analogous to the motion of *Chlamydomonas* flagella in forward swimming.

It is interesting that both the observed types of flagellar motion in *C. reinhardi* involve a degree of synchrony. Ciliary coordination has been the subject of much study, but Pitelka and Child (88) conclude that at present neither fibrillar systems nor viscous coupling, the two most frequently invoked explanations, can completely account for the phenomena of ciliary coordination as observed in the ciliates. In the case of backward swimming in *Chlamydomonas*, where the flagella lie close together, viscous coupling probably offers a good explanation for synchrony. In forward swimming, however, where the two flagella beat in opposite directions and exert their major force on opposite sides of the cell, it is not clear whether hydrodynamic coupling could account for the synchrony observed. That synchrony may become disturbed in older cultures, as Lewin (60) reports, indicates at least some physiological effect.

There is as yet no firm evidence to implicate any of the morphological entities observed, e.g. striated fibers or microtubules, in the coordination of flagella in *Chlamydomonas*, and, indeed, any such morphological explanation faces the problem of accounting for at least two distinctive types of behavior in the same flagellar apparatus. It should be noted, however, that if the initiation of the flagellar beat in *Chlamydomonas* somehow originates in or is conveyed by the distal striated fiber, this structure is in a unique position to provide coordination between two flagella which beat in opposite directions at the same time, since it contacts the basal bodies on opposite sides, i.e. contacts each of them at the same point in relation to its direction of beat.

When isolated *Chlamydomonas* flagella are activated by ATP (15) they show an undulatory motion which is very similar to the motion of flagella in backward swimming. One might conclude from this that the property of undulatory beating is inherent in the flagella themselves (17), that the flagella are normally constrained to undergo synchronous cilia-like beating by some mechanism in the cell, and that backward swimming is the result of a temporary breakdown of this constraint. The mechanism of bending of cilia and flagella suggested by Brokaw (17), however, might account for the two types of motion by a simple difference in the way in which bending is initiated. In this connection, it is interesting to compare the flagellar motion of *Polytoma*, as studied by Brokaw (16), with that of *Chlamydomonas*. The two organisms are quite closely related, and the structure of their flagellar apparatus is probably very similar, at least in the structure of the transitional region and in the presence of a striated fiber connecting the two basal bodies (54, 55). *Polytoma* cells normally swim forward, but also show brief periods of backward swimming ("shock reaction"). In both types of swimming the flagella exhibit propagated undulatory beating normally characteristic of flagella. In forward swimming the flagella are directed backward along the sides of the cell, while in backward swimming they are directed forward as in *Chlamydomonas*. Thus forward swimming is accomplished in *Polytoma* by flagella-like beating and in *Chlamydomonas* by cilia-like beating, with the net effect being almost identical. In both organisms backward swimming is accomplished by

flagella-like beating. This comparison suggests, as does Brokaw's model (17), that the basic differences between ciliary and flagellar motion may be slight.

### *The Central Pair*

Fawcett and Porter (32) were the first to observe the uniform orientation of central fibers in fields of epithelial cilia and to indicate that the direction of ciliary beat is probably perpendicular to the plane of the central fibers. Gibbons (37), studying mussel gill cilia, and Afzelius (2), studying cilia of ctenophore swimming plates, found similar uniformity of orientation and showed clearly that the central fibers lie in a plane perpendicular to the plane of ciliary motion. Since these studies, it has been generally assumed that cilia, or flagella with a planar beat, move in a plane perpendicular to that of the central pair (27, 28). For that reason it is surprising that the orientation of the central pairs in *Chlamydomonas* flagella is opposite what would have been expected. These results remain unexplained and difficult to interpret. Some variation has been observed in other organisms, however. Satir (106) showed that nonbeating gill cilia had a uniform orientation of central fibers and gave pictures similar to those of Fawcett and Porter (32) and Gibbons (37). But Satir also found that cilia activated by potassium ion and fixed while beating showed as much as 90° difference in the orientation of the central pairs. In ciliate protozoa, Roth and Shigenaka (98) have reported that fields of cilia show a uniform orientation of the central pairs, while Pitelka (87) reported a variable orientation. Fawcett (29) has examined the orientation of the central pair of guinea pig sperm and found that it is not perpendicular to the transverse axis of the sperm head, but deviates from the perpendicular by 20–30°.

Randall and his collaborators (89, 118) have isolated several nonmotile mutants of *Chlamydomonas reinhardi* whose flagella show either loss or disruption of the central pair. However, some nonmotile strains of *Chlamydomonas* show a normal cross-section (39), and functional flagella with 9 + 0 cross-sections have been reported in other organisms (3, 78). Certainly the role of the central pair in the flagellum remains uncertain at present.

### *The Tip of Cilia and Flagella*

Information on the structure of the tip of cilia and flagella, while scarce, is now available for a wide variety of organisms: mammalian sperm (30, 31), invertebrate sperm (1, 111), gill cilia (37, 107), ciliate protozoa (25, 95, 97, 98), flagellate protozoa (38), fungal zoospores (51, 52), and unicellular algae (67). While these examples do not yet furnish a comprehensive picture, it is possible to formulate, as Satir (107) has also done, a general scheme which agrees well with the information on *C. reinhardi*.

In the area at the end of a cilium or flagellum where the fibers terminate, the arms of the peripheral fibers and the matrix and associated structures seem to be lost (14, 30, 31, 37, 38). The doublets become singlets, usually by the loss of one of the subfibers (25, 31, 37, 38, 98, 107, 111), although in guinea pig sperm each doublet forms two singlets (30). The singlets have a dense lumen near the point where they terminate (38, 107). Most reports indicate that the transition from singlet to doublet and the termination of singlets occur at different levels in different fibers, apparently at random (25, 30, 37, 38, 51, 52, 98), but Satir (107) has shown an orderly pattern of termination in gill cilia, the cross-section observed being dependent on the position of the cilium when fixed. His data support the idea that ciliary fibers do not contract, but that they slide past one another as bending occurs.

When all doublets have become single, the tip often loses its organization, and in some cases it is no longer possible to distinguish the central pair from the other singlets which originated from peripheral doublets; this description applies in the following cases: flagellate, (38), gill cilia, (37, 107); ependymal cilia, (14); guinea pig sperm, (30). In other cases, notably the ciliate protozoa studied so far, the central fibers are recognizable as such and persist to the very end (25, 95, 97, 98). Roth (97) and Roth and Shigenaka (98) report that in certain ciliates the central fibers fuse in a granule at the extreme distal point, possibly representing a structure similar to that found in *C. reinhardi* at the distal end of the central fibers. In general, the structure of the flagellar tip in *C. reinhardi* agrees closely with that reported for the ciliate protozoa. In certain fungal zoospores, which have a whiplash flagellum, the central pair persists to the tip, but it is not clear whether they continue into the whip-lash portion (51, 52); while in a small uniflagellate alga the central pair has been shown to extend past the point where the periph-

eral fibers end and form the whiplash portion of the short flagellum (67).

### *Transitional Structures*

Since Gibbons and Grimstone's classic study of termite-gut flagellates (38), several authors have studied the basal apparatus of cilia and flagella in detail (14, 26, 34, 37, 56, 87, 90, 98). Both the similarities and differences in structures observed are of interest, and attention will be given here to the range of occurrence of certain structures observed in *C. reinhardi*.

Filaments radiating from the triplets at the very distal end of the basal body were observed by Gibbons and Grimstone (38) who called them transitional fibers. These fibers have been described for the basal bodies of mussel gill cilia (37), rat trachea cilia (36), and flagellum of an amebo-flagellate (109), the primative cilium of the retinal rod (116), the kinocilium of a sensory cell in a fish (34), and the olfactory cilia of frogs (90). These fibers also appear to be present in the micrographs of ependymal cilia of Brightman and Palay (reference 14, Fig. 10) and in those of the basal structure of the eye of an arrow-worm by Eakin and Westfall (22) although these authors do not identify them as such.

Another structure which seems to occur widely is the set of links between doublet fibers and the cell membrane in the transitional region, near the point where the cell membrane becomes the flagellar membrane. These have been seen in ciliate protozoa (85, 87), termite-gut flagellates (38), sperm of an annelid (20) and of the oyster (35), cilia of oviduct (32), of mussel gill (37), and of ependymal cells (14), olfactory cilia (90), kinocilia of a lateral line organ (34) and of lobster antennae (57), and the cilia of retinal rods (116). Similar structures can be recognized in the micrographs of Lansing and Lamy (rotifer cilia, reference 56), King et al. (ciliate protozoon, reference 49), Barnes (cilia of mouse hypophysis, reference 8), Spoendlin (kinocilium, reference 114), and Roggen et al. (sensory cilia in a nematode, reference 94).

Also in the transitional region, links are sometimes observed between adjacent doublets, either at or below the level of the central pair (see Gibbons, reference 37). These structures are present in the flagellum of oyster sperm (35), in mussel gill cilia (37), rotifer cilia (56), ependymal cilia (14), olfactory cilia (90), cilia of lobster antennae (57), and kinocilia of gravity receptor cells (114); they can also be seen in the micrographs of King et al. of a ciliate protozoon (49), of Barnes of cilia in the mouse hypophysis (8), and of Roggen et al. (94) in nematode cilia.

The widespread occurrence of these three structures indicates that they are common features of cilia and flagella, and suggests that they may be normal components of such systems.

Another structure of the transitional region, the stellate structure of Lang (54) and Manton (70), is of interest because of its apparently restricted range of occurrence. So far it has been observed only in the motile cells of algae and lower plants which have been studied in detail (Table I). Studies of the form of this structure in different organisms might be expected to provide information on the phylogenetic relationships of the plant kingdom (72).

### *Striated Fibers*

The distal striated fiber joining the basal bodies of *Chlamydomonas reinhardi* was first noted by Sager and Palade (104) who described it as an array of dense parallel rods or fibers. Gibbs et al. (39), studying *C. moewusii*, saw it as an extension of the basal bodies, showing one or two transverse partitions. Lang (55) described the same structure as a broad fiber connecting the two basal bodies in *Polytoma*, a colorless phytoflagellate closely related to *Chlamydomonas*, and also noted its striated nature (54).

Striated fibers known as rootlets, generally with a period of 60–75 mμ, have often been found attached to the proximal end of basal bodies of flagella, cilia, and ciliary derivitives and have also been found associated with centrioles (105). References to fibers connecting basal bodies to one another are less common in the literature. Gibbons and Grimstone (38) described a fibrous band linking adjacent basal bodies in the termite-gut flagellate *Holomastigotoides*, with a single transverse cross-band between each basal body. The fiber appeared to be composed of finer longitudinal filaments. Another example of this sort, in the motile cells of *Oedogonium*, will be discussed below.

Among the biflagellate algae and protozoa, connections between the basal bodies have been reported in several organisms, although none have been studied in detail. In the golden-brown alga *Prymnesium* (68) the two basal bodies are joined at the distal end by a striated fiber and at the proxi-

TABLE I

*Occurrence of the Stellate Structure in Motile Cells of Plants*

| | | |
|---|---|---|
| Green algae | | |
| Volvocales: | *Chlamydomonas reinhardi** | |
| | *Polytoma obtusum* | (54) |
| | *Polytoma uvella* | (54) |
| | *Chlorogonium rosae* | (70) |
| | *Eudorina elegans* | (54) |
| Ulotrichales: | *Stigeoclonium* zoospore | (69, 70) |
| | *Draparnaldia* zoospore | (70) |
| Charales: | *Nitella* sperm‡‡ | |
| Siphonales: | *Dicotomosyphon* sperm‡‡ | |
| Classification uncertain: | *Heteromastix rotunda* | (70) |
| | *Pedinomonas minor* | (23) |
| Golden-brown algae | | |
| | *Prymnesium parvum* | (70, 76) |
| Lower plants | | |
| | *Equisetum* sperm‡‡ | |
| | *Marsilea* (fern ally) sperm‡‡ | (79) |
| | Fern sperm‡‡ | |
| | *Reboulia* (liverwort) sperm‡‡ | |
| | *Polytrichum* (moss) sperm | (82) |

* Palade, G. E. Personal communication. Also present paper.

‡‡ Turner, F. R. Personal communication.

mal end by two, or perhaps more, smaller striated fibers, a situation very similar to that in *Chlamydomonas*. In the green biflagellate *Mesostigma*, the micrographs of Manton and Ettl (75) clearly indicate a fibrous material joining the basal bodies at the proximal and distal ends. In the quadriflagellate green alga *Prasinocladus*, the four flagellar bases seem to be linked as pairs by two striated fibers which join the basal bodies near their distal ends (83). Anderson has reported electron-opaque material joining the two basal bodies of the protozoon *Chilomonas* near their distal ends, but no details of structure are available (4); and in *Stephanosphaera*, a green biflagellate related to *Chlamydomonas*, Joyon (47) has diagrammed an arched connection between the two basal bodies and a general arrangement of basal bodies similar to that in *Chlamydomonas*, but again no details are given.

Although the zoospore and spermatozoid of *Oedogonium*, studied by Hoffman and Manton (43, 44), are multiflagellate cells (the main difference in the two cells, besides their position in the life cycle of the plant, is their size and number of flagella), there is a great similarity between the arrangement of the basal apparatus of their flagella and that of *Chlamydomonas*. In these cells, adjacent basal bodies are linked by fibers at the proximal and distal ends of the basal body, forming a ring of flagellar bases (see below and Fig. 58). In the zoospore, the proximal linking material does not have a fibrous appearance, while in the spermatozoid a fiber is suggested. The striation pattern of the distal connecting fiber is most clearly exhibited in the zoospore, with a complex pattern very similar to, though not identical with, that of *Chlamydomonas*. The pattern in the spermatozoid is also similar, though less clearly defined. In both cases the distal fibers appear to be made up of finer longitudinal elements.

Although little detailed information is available, there seems to be at least some general similarity between the flagellar apparatus of the phytoflagellates studied so far. The pattern found in biflagellates may represent the basic organization, with quadriflagellates showing a doubling of the same basic structure, while the multiflagellate cells, like those of *Oedogonium*, may represent a "polymer" of a basic biflagellate unit. This line of reasoning is given some credibility by the fact that the zoospore and spermatozoid of *Oedogonium* have the same general flagellar organization, differing mainly in the number of flagella, about 30 in the spermatozoid and about 120 in the zoospore (42, 43). In the spermatozoid, Hoffman and Manton found

that the number of flagella born by individual cells varied from 24 to 34 in the sample studied (44), suggesting that the flagellar band is, in some sense, a polymer with a variable number of units.

### *Microtubules*

Although microtubules have been reported in a variety of cells, their function is, to a great extent, still uncertain in specific cases. Roth originally suggested that microtubule systems were responsible for the coordination of cilia and flagella (96). In some instances their presence has been implicated in the production of motion, as in the axostyle of flagellates (42) and in certain nonflagellate sperm (19, 93). Microtubules may be responsible for the elongation of palisading epithelial cells (18) and for the form of scale cells in insects (80). They are probably the supporting structural elements in the haptonema of algae (84), the axopods of heliozoans (50, 115), and the tentacles of suctorians (99), and may also be related to the motion or streaming of these appendages. Microtubules also play a structural role in maintaining the shape of amphibian erythrocytes (33) and of certain sperm cells (64, 65, 111).

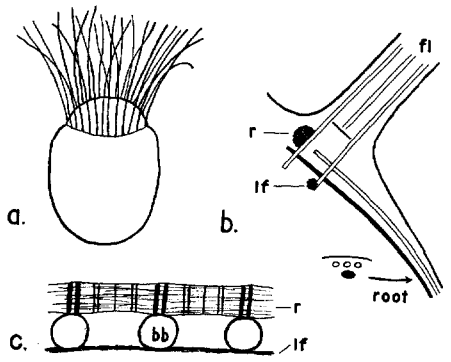



FIGURE 58 Diagrams showing the arrangement of flagellar apparatus in the zoospore and spermatozoid of *Oedogonium*, based on the work of Hoffman and Manton (43, 44), to indicate points of similarity to the flagellar apparatus of *Chlamydomonas*. (a) General form of the motile cells of *Oedogonium*, showing the ring of flagella at the anterior end. The zoospore has about 120 flagella, the spermatozoid about 30. (b) One flagellum and its basal body, indicating the levels of attachment to the basal body by the proximal (*lf*) and distal (*r*) connecting fibers, and the relation of the microtubule band (*root*) to the basal body. The root lies midway between adjacent basal bodies; the position of the dense component associated with the microtubules is indicated in cross-section. (c) Fibers connecting the basal bodies to form the ring of flagella. The striation pattern shown is that of the zoospore. *fl*, flagellum; *bb*, basal body; *r*, fibrous ring; *lf*, lower fibers.

Microtubules are commonly found associated with the basal apparatus of cilia and flagella, but apparently only, or at least most extensively, in single, free-swimming cells. In ciliate protozoa several distinct systems of bands or sheets of tubules may be present in the same cell (85, 86), commonly with one group of tubules running between groups of cilia and another group of tubules lying just beneath the cell membrane (97, 119). In both ciliates and flagellates orderly groups of tubules, often bands of less than 10, are frequently seen arising near the ciliary or flagellar basal bodies and passing away from the basal-body region just under the cell surface.

In the ciliates *Paramecium* (87) and *Colpidium* (85), rows of microtubules (four or five in the case of *Paramecium*) originate beside each basal body and extend toward the cell surface. In *Euglena* tubules extend from the basal-body region around the reservoir membrane, and tubules lie under the ridges of the pellicle (58, 113). The trypanosomid flagellates show regularly spaced microtubules below the cell surface (40), and orderly groups of tubules have been seen to extend from the basal bodies to the surface membrane (6, 88). The axostyle of the flagellate *Tritrichomonas* is a layer of tubules which arise near the basal bodies and run beneath the cell membrane (5).

Of special interest in relation to the flagellar apparatus of *Chlamydomonas* is the extensive work of Manton and her collaborators on the motile cells of algae. She has described groups, which she calls "roots," of varying numbers of microtubules associated with the basal bodies of several uni-, bi-, and quadriflagellate organisms. In Manton's terminology, *Chlamydomonas reinhardi* would have four four-member roots. Manton has found four roots in several other organisms: the biflagellate zoospore of *Draparnaldia* (74) and the quadriflagellate zoospore of *Stigeoclonium* (69) both have two two-member roots and two five-member roots; the biflagellate zoospore of *Chaetomorpha* (74) and the uniflagellate *Pedinomonas* (23) have two two-member roots and two three-member roots. These roots are evenly spaced sheets or bands of microtubules which originate near the basal bodies and radiate toward the cell membrane in a symmetrical manner and are in general very similar to the microtubule bands observed in *Chlamydomonas*.

One obvious difference is that some of the bands observed by Manton have electron-opaque material on one or both sides of the band of tubules; this material appears amorphous in cross-section and striated in longitudinal section (69).

Manton also observed variations in the arrangement of tubules in a band, similar to the changes seen in *Chlamydomonas* as the group of tubules approaches the basal bodies. The five-member root of *Stigeoclonium* is arrayed as five tubules side by side near the basal bodies, but further away may assume a 4-over-1 or 2-over-3 configuration (69). In the biflagellate *Heteromastix*, which has a two and a seven member root which extend from the basal body to the cell membrane, the seven member root is reduced to six or five members as it approaches the cell surface (77). Other algal cells reported to have tubule systems are the uniflagellate sperm of the brown alga *Dictyota* in which about eight tubules arise near the basal body and pass to the cell membrane (66), and the biflagellate *Mesostigma* in which at least 40 tubules begin on either side of the flagellar bases and extend under the cell membrane (75).

Two unusual appendages of algal cells, the haptonema of *Prymnesium parvum* and the "proboscis" of *Fucus* sperm, arise in the same manner as flagellar roots and are probably homologous with them. The haptonema of *Prymnesium* (68) is a short flagella-like appendage which consists of a compact sheath of three membranes, all continuous with the cell membrane, enclosing seven single tubules. These tubules originate near the two basal bodies in the region where the basal bodies are connected together by fibers. At the lowest level there seem to be nine tubules. The number decreases to eight, and then to seven which continue out into the membrane sheath to form the haptonema. The main difference between this and the other groups of tubules described above is that it extends outside the normal bounds of the cell. Another group of tubules, a sheet of about 20, originates near the same basal bodies and passes under the cell surface in normal fashion (76). The proboscis of the biflagellate sperm of *Fucus* (73) begins as a group of about 13 microtubules near one of the flagellar bases. These extend outward, arching to form a flat sheet which protrudes from the front of the cell and is covered by the cell membrane. The tubules then arch back into the body of the cell and pass just below the cell membrane, in a fashion typical of a flagellar root.

As mentioned above, the motile cells of *Oedogonium* present a situation similar to that of *Chlamydomonas* in that the basal bodies are linked at their proximal and distal ends (43, 44). Between each pair of basal bodies a band of three microtubules originates, passing between the proximal and distal connecting fibers in much the same way as the tubule bands of *Chlamydomonas* (see Fig. 58). Proximal to the tubules is a dense fiber which is striated in longitudinal section but amorphous in cross-section. This fiber extends on for a short distance in the space between the basal bodies after the tubules end and offers a slight suggestion that the dense plate in *Chlamydomonas*, which lies at the point between the basal bodies where the tubules end, may represent the same sort of material in very reduced form.

In none of the above cases where microtubules were found closely associated with basal bodies has a distinct connection between the two structures been noted. Yet when the *Oedogonium* zoospore or spermatozoid was dried and shadowed, the tubular roots remained with the ring of basal bodies when the other parts of the cell, including some flagella, were lost (43, 44). This suggests that a certain amount of mechanical cohesion exists between the tubules and basal apparatus, possibly in the form of an apparently structureless matrix material which is not made visible by present techniques.

### *Function of Microtubules and Fibers*

Some evidence for the function of microtubules as structural or "cytoskeletal" elements was noted above, as was the common association of microtubule bands with ciliary and flagellar bases in free-swimming cells. In *Chlamydomonas*, as the flagella beat, stresses must exist between the active and passive portions of the cell, particularly in the region of the flagellar bases. The radiating array of microtubules, provided it has some mechanical cohesion with the joined basal bodies, is ideally located to distribute the stresses of acceleration and deceleration over the surface of the cell. Its symmetrical arrangement would also allow it to maintain the alignment of the flagella with respect to the longitudinal axis of the cell.

The striated fibers which link the basal bodies lie in the plane of flagellar beating, and together with the basal bodies which they join they form a triangular unit which might be expected to have

dimensional stability. This would maintain the orientation of the flagellar bases with respect to one another and resist the opposing forces which the two flagella exert at their bases as they beat in opposite directions.

Evidence for these functions of the fiber and microtubule systems is largely circumstantial, since the physical properties of the structures are not known directly, but they may provide a justification for the existence in *Chlamydomonas* and similar cells of such an elaborate array of structures associated with the flagella.

## CONCLUSION

The present study has demonstrated that it is possible, in the defined and relatively simple system of a small flagellate, to obtain comprehensive knowledge of the structure of the flagella and their associated structures. Thus for the first time outside of the well-studied case of mammalian sperm (30) a detailed picture of the motility apparatus of a single cell is available, along with a description of what may be the entire microtubule system of a cell. The feasibility of correlating function and ultrastructure in a small organism has also been shown, although much remains to be done in this area. The intricate and regular arrangement of the microtubules observed raises questions about their function and also about their morphogenesis and replication. The description of flagellar apparatus given here should provide the basis for further studies using *Chlamydomonas* and other phytoflagellates as model systems, particularly in such areas as the beating and coordination of flagella, the replication of flagellar apparatus and microtubule systems, and the chemical and genetic (89, 118) modification of flagellar apparatus.

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