THE STRUCTURE AND FORMATION OF CILIA AND FILAMENTS IN RUMEN PROTOZOA

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ABSTRACT

The large oligotrich rumen protozoa *Diplodinium ecaudatum* and *Ophryoscolex caudatus* have been studied by electron microscopy during interphase and division. The structure of mature cilia is contrasted with that seen during their formation particularly in a tuft where development lags and is arrested. Here the shaft is only a few micra long and is composed of filaments that have circular cross-sections not in the typical circular arrangement. In their diameter and appearance the filaments are similar to filaments associated with the nuclei during division. The macronucleus has within it randomly directed filaments, while the micronucleus contains well aligned filaments and other arrangements typical of an intranuclear mitotic process. An extranuclear filament system is also present and is elaborated during division. The infraciliary filament system is particularly elaborate in these organisms. Filaments ranging from 14 to 22 mμ have been observed with some tendency for a bimodal distribution in diameters of 15 and 21 mμ. Formation of such filaments has been observed and consists of an initial orientation of very fine elements followed by filament formation. The observations are discussed in relation to filament involvements in cell movements. The concepts are discussed that filaments are metastable structures and that the transitions from one state to another are functionally significant.

INTRODUCTION

Filaments are associated with many systems for cell movements. An understanding of the system of filaments in striated muscle has progressed rapidly by the correlation of careful structural and biochemical studies. The contraction of striated muscle is effected by the interaction of actin, myosin, and adenosine triphosphate. Actin is localized in the smaller secondary filaments, while myosin is localized in the larger, primary filaments. In both cases the filaments extend only part of the sarcomere length (15).

In the more primitive motile systems, the filaments tend to extend continuously through the functional unit. Examples are cilia, where the individual filaments extend essentially continuously from base to tip, and the mitotic apparatus, where filaments probably extend continuously either from pole to pole or pole to chromosome. Further generalizations suggest that these systems do not necessarily have two morphologically different filament types and may not have regular geometric arrangements.

This study deals with such continuous filament systems. Each organism studied in division has been found to contain forming and formed infraciliary filaments, cilia, and nucleus-associated

filaments. Information relating to structure of these systems at known states of function and formation is presented and then related to questions and hypotheses of filament formation and function and cell movements.

A comment on terminology is necessary. The large amount of literature encompassed by this area is characterized by almost random uses of the terms "fiber," "fibril" and "filament." One of the first steps requisite to comparative discussions of the several systems is the adoption of coherent terminology. We have chosen to use "filament" uniformly in reference to the tubular-appearing elements with a diameter of 12 to 27 mμ. Such filaments are arranged into groups called "fibrils" or "bundles."

MATERIALS AND METHODS

Collections of rumen protozoa were obtained from fistulated cattle maintained by Dr. W. R. Woods of the Department of Animal Science at Iowa State University. Organisms were withdrawn, 4 to 6 hours after feeding, from approximately the center of the rumen. Since the rumen fauna vary from one cow to another, a particular animal was chosen for its supply of the large-sized species *Diplodinium* and *Ophryoscolex*; this cow is maintained largely on dry rations.

Protozoa were fixed for 15 to 35 minutes at 23°C in 1 per cent osmium tetroxide buffered with Veronal acetate (final concentration of 0.047 M sodium barbital and 0.047 M sodium acetate) to pH 7.4. Frequently, divalent cations were added to the fixation solution to enhance filament preservation (33) either as 0.002 M calcium chloride or as similar concentrations of both calcium and magnesium chlorides. Dehydration consisted of two 10-minute changes of 50 per cent, one 15-minute change of 75 and 95 per cent and two 10-minute changes of absolute ethanols. Methacrylate infiltration was performed in three steps (usually 20, 30, and 20 minutes) after which the organisms were pipetted into gelatin capsules. The methacrylate was a mixture of 2 parts ethyl and 3 parts *n*-butyl monomers with 1 per cent benzoyl peroxide (w/v) added; this mixture was dried by filtration through anhydrous, powdered Na_2SO_4.

Individual organisms were selected for division configurations before fixation, in 75 per cent ethanol, or after embedding. Both capsules and flat embeddings were used and trimmed for a selected organism excluding all others. Such procedures do not allow the most precise classification, so that species identification is difficult to determine; genera could always be ascertained. We are probably dealing with *Diplodinium ecaudatum* in all cases; however, we have only designated *Diplodinium* sp. In some cases we sectioned organisms appearing to be *Ophryoscolex caudatus* and will report supplementary findings; similarly, this genus is ascertained, but the species is open to some doubt.

Sections were cut with an LKB microtome at settings of 10 to 15 mμ and were mounted on thin Parlodion or methacrylate membranes, stained with potassium permanganate (21), and overlaid with a methacrylate membrane to reduce sublimation (31). An RCA EMU 3F operated at 50 kv or 100 kv with 25- or 50-μ objective apertures were used.

The study utilized many organisms in the interphase and about fifteen dividing organisms.

OBSERVATIONS

Studies of these and closely related organisms have been reported by Noirot-Timothée (24, 25) and Bretschneider (5, 7). A more detailed description of the organisms is now possible and is necessary to allow comparisons of mature cilia with newly forming cilia and with the filamentous structures of the cytoplasm and dividing nuclei. Non-filamentous components are largely excluded and may be the subject of later reports.

THE STRUCTURE OF MATURE CILIA: All cilia in *Diplodinium* and most in *Ophryoscolex* are located anteriorly and are customarily arranged in four groups; dorsal, oral, adoral, and esophageal (Fig. 2); helpful descriptions of ciliature and infraciliature are given by Rees (27) and Bretschneider (5, 6). A diagrammatic summary of ciliary structure in these organisms is presented in Fig. 1; no differences in ciliary structure have been noted in these species. The ciliary shaft is composed of the classical filamentous pattern, nine peripheral doublets and two central simple filaments (Figs. 3 to 5). A fine material is observed between these filaments (Figs. 3 and 5, *FM*) usually irregularly oriented, although some longitudinal sections show a regular shaft spacing particularly around and between the central filaments as diagrammed in Fig. 1. Linear arrangements of this material (1) extending from central to peripheral filaments have been observed in several cases. "Arms" protruding from the peripheral filaments as described by Afzelius (1) and Gibbons and Grimstone (11) have been seen but their occurrence on only one side of the filaments has not been established.

The basal portions of the cilia are seen, in cross-section, to lack central filaments which have terminated proximally in contact with a granule

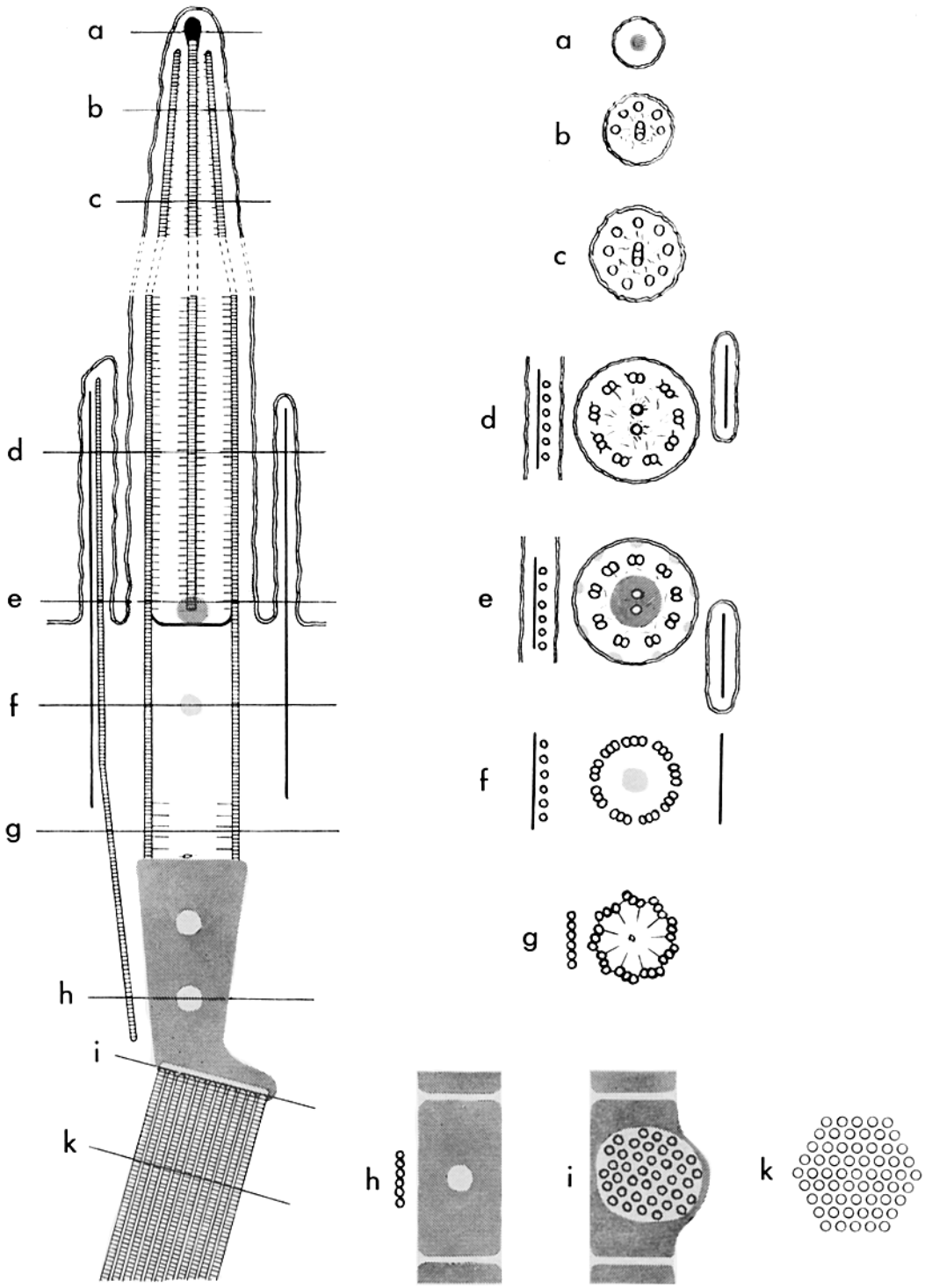


FIGURE 1 Diagrammatic presentation of ciliary structure in *Diplodinium ecaudatum*. The plane of the drawing is perpendicular to the planes of Figs. 3 and 4. Variations exist in the placement of the structures auxiliary to the cilium, such as the microvilli included in levels *d* and *e*.

(Figs. 4 and 9, *G*) near the level of the plasma membrane (Figs. 3 and 4, *P*). The peripheral filaments have triplet cross-sections and are arranged as described in flagella (11) and in centrioles (10, 39), so that a line drawn through the centers of the three components is nearly a tangent at the distal end but is approximately 30° to a tangent at the proximal end (Figs. 6 and 13, *PF*).

In longitudinal section, the basal portion of each peripheral filament appears as four parallel lines while the shaft portion appears as three lines. This transition in structure takes place rather abruptly, the deleted subfilament appearing to be closed (Fig. 3 inset, *PF*).

The tapered tips of cilia are recognized in cross-section not only by their reduced diameter but by peripheral filaments with simple circular cross-sections (Figs. 7 and 8). In certain sections of individual cilia, some of the peripheral filaments have doublet, while others have circular, cross-sections; the level of transition is not uniform (Fig. 8, *PF*). In such sections and those more distal, the central filaments have material surrounding them causing them to be less well defined; the central filaments extend to the extreme distal point where they fuse into a homogeneous granule (Fig. 8, *C*) that is included within the membrane. Between these two levels, the peripheral filaments terminate successively so that cross-sections reveal nine (Figs. 7 and 8, *PF*) or fewer (Fig. 8, *PFR*) peripheral elements but always readily recognized central filaments (Figs. 7 and 8, *B*). Termination of peripheral ciliary filaments before central ones has been demonstrated in *Euplotes* (29) and *Isotricha* (32) but contrary evidence has been presented from hypermastigote protozoa (11) and in ciliated epithelium (28); variations undoubtedly exist.

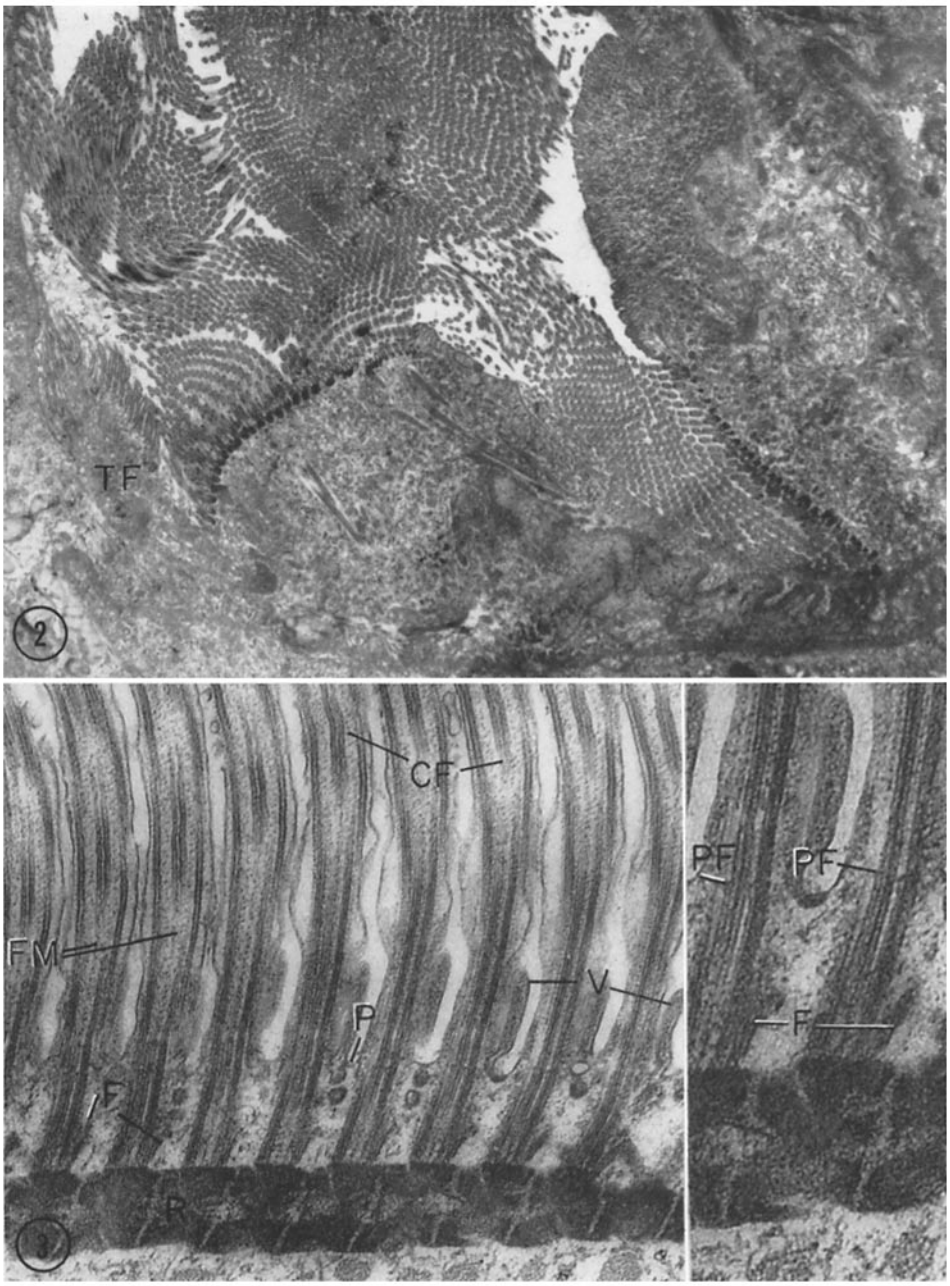
Cilia of two different sizes exist in these organisms. This phenomenon was first demonstrated by Bretschneider (6) and has also been observed in this study: comparisons can be made between the larger bases (Fig. 17, *LC*) and others at the same magnification (Fig. 19). Cilia with the smaller diameter greatly outnumber those with the larger; Bretschneider suggests that only one row of large cilia is present. Measurements show the diameter comparison to be about 130 mμ to 190 mμ, with the basal bodies appearing to be shorter in the cilia of larger diameter.

STRUCTURES ASSOCIATED WITH CILIARY BASES: A large dense rod lies at the extreme proximal end of the basal portion and interconnects the cilia in one row (24). This rod is cross-striated with one unit per cilium, has central, somewhat irregular regions of lower density (Figs. 3 and 4, *R*), and has a maximum diameter of 350 mμ.

Deeper in the cytoplasm and connecting these rods is a complex filamentous system. Bundles of filaments hexagonally packed extend from the endoplasm to these rods (Fig. 9) where their junction has a characteristic arrangement (Fig. 10). In the endoplasm, filaments are frequently seen lying in small regularly spaced groups about 1 μ from the macronuclear envelope. They may also be found in ectoplasmic regions in groupings of bundles (Fig. 11) that form a volume of several cubic micra containing little but filaments; such a region suggests the "motorium" originally de-

FIGURE 2 The anterior tip of *Diplodinium* in survey showing the regions of ciliation among which are non-ciliated areas that are locations for ingestion of particulate food. A tuft of cilia (*TF*) is found in organisms at most stages and includes a large number of cilia in arrested development. × 4,100.

FIGURE 3 Longitudinal section through mature cilia in *Ophryoscolex caudatus*; the structure differs very little from that of *Diplodinium*. The plane of section parallels the large dense rod (*R*) that connects basal bodies and shows central ciliary filaments (*CF*). Fine material (*FM*) is present between the ciliary filaments. Villus-like processes (*V*) are present between the cilia and have filaments (*F*) extending into them from the basal tip of the cilium. The plasma membrane (*P*) has small vesicles associated with it regularly. Inset: Higher magnification of the portion of Fig. 3. The basal portion of each peripheral filament (*PF*) terminates at a well defined point below which a longitudinal section shows the filament as four parallel lines and above which it is three lines. × 36,000; inset, × 69,000.



scribed by Sharp (37) in *Diplodinium*. Filaments may also be arranged in sheet-like arrays immediately inside of the plasma membrane (Figs. 4 and 12, *F*). They are also present in a small sheet of about four short filaments lying nearly parallel to each ciliary base (Figs. 3 and 6, *F*) and extending into villus-like membrane protrusions (Fig. 3, *V*).

These filaments are circular in cross-section with a low-density center. Their diameters are variable as indicated on the included nomogram (Fig. 14). The largest number are in the 13- to 16-mμ range with a marked skewing toward higher values, the extent of which gives a slight indication of a bimodal distribution; the second peak is in the 19- to 22-mμ range.

Adjacent cilia are interconnected by two structures at the extreme proximal end of their bases (Fig. 13, arrows). The structural arrangement is presented schematically (Fig. 15) to emphasize the salient features of the interrelationships. These interconnecting structures do not appear to contact the peripheral filaments; rather, a small separation of about 5 mμ exists. Granules, as diagrammed, are regularly present at some positions. Since some of these structures are subjacent to others, the proximal one-fifth of the base is estimated to be involved.

The orientation of the central filaments in the shaft with respect to the dense rod at the base is quite constant. A line drawn through the center of the central filaments parallels the length of the rod (see Fig. 3 which is in such a plane), suggesting that the plane of beat is perpendicular to the dense rod, and, therefore, that the interconnecting structures in this organism join ciliary rows perpendicular to the plane of beat, an arrangement contrary to that typical of many other cilia. Unfortunately these organisms do not lend themselves to analysis of ciliary beat or synchrony.

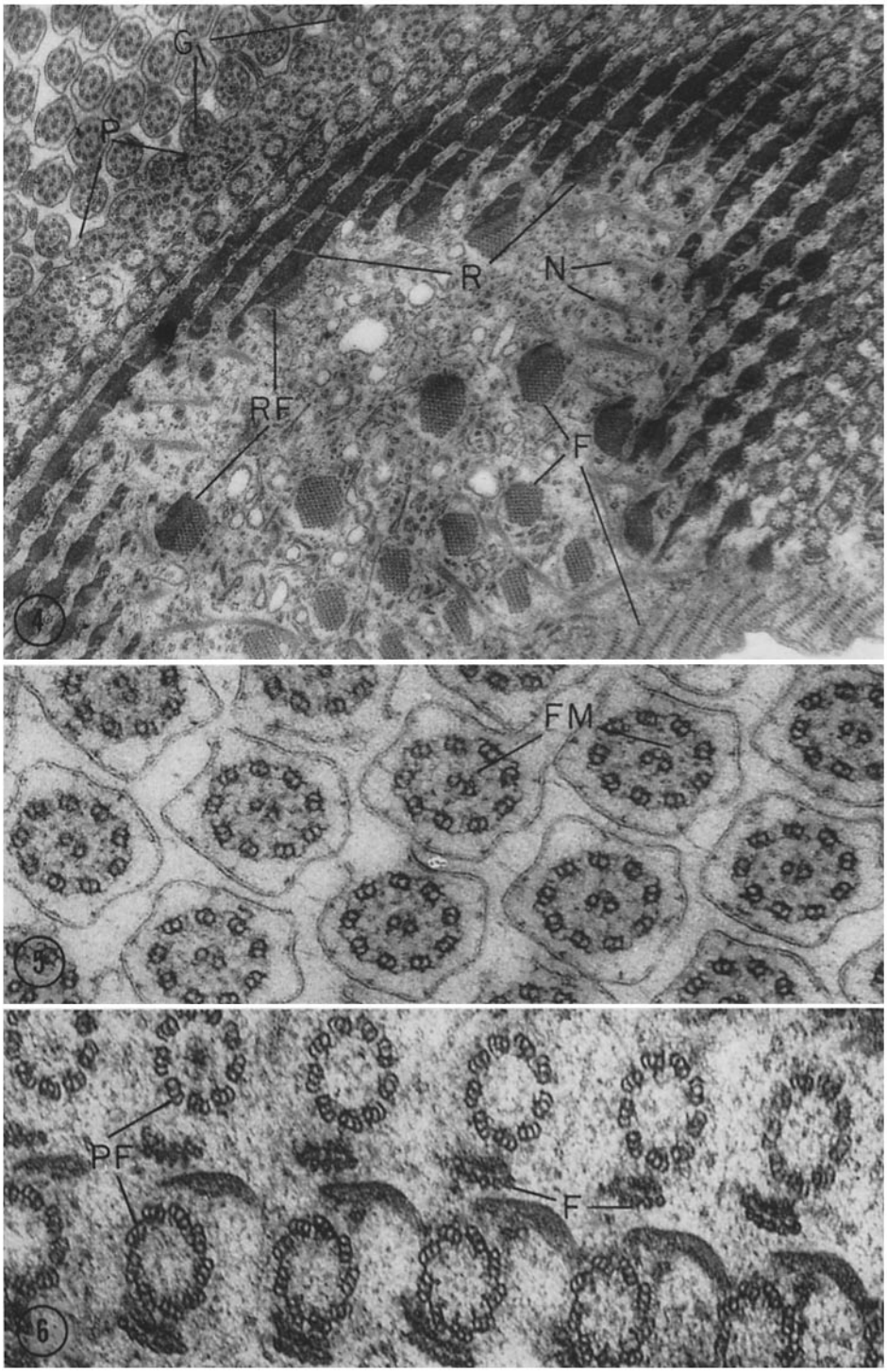
In the regions where mitochondria are usually concentrated in aerobic ciliates, membrane-limited vesicles with an internal granularity of variable degree but characteristic appearance are found in high numbers (Figs. 19 and 24, *M*). Because of their localization, they are referred to as mitochondrion-like bodies though no knowledge of their function or biochemical evidence for such function has yet been obtained in these anaerobic organisms. Studies to determine whether these vesicles are sites of phosphorylation will perhaps be made in the future.

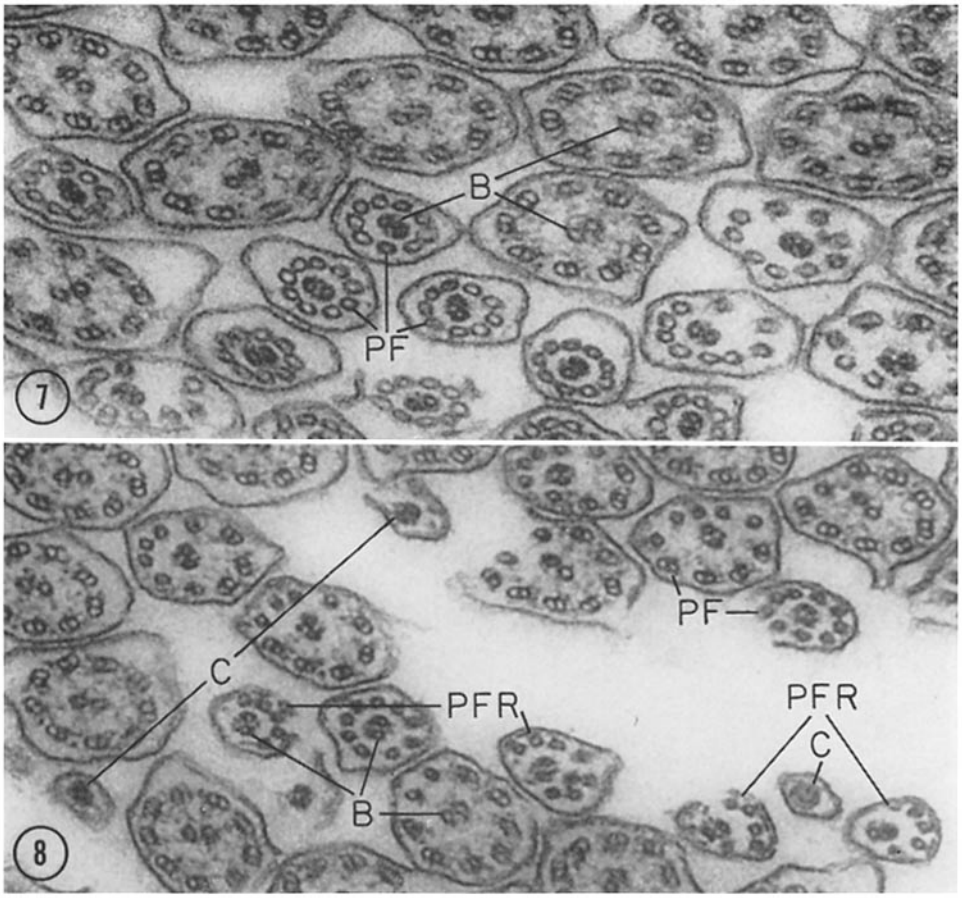
FORMING CILIA: Organisms with early constriction furrows have largely completed the formation of new cilia. Resorption of cilia has been observed (and will be the subject of a subsequent publication), indicating that an entirely new ciliature has probably been formed in both sisters of the pair; however, in the posterior sister, it is certain that a new ciliature is just being completed. If the ciliature of the posterior sister is studied at the time of furrowing, a single tuft of cilia is found in which basal bodies and very short cilia are seen. A similar tuft can be found in the anterior sister (Fig. 2, *TF*) and in organisms not known to be in division, indicating that the tuft probably persists in essentially this condition throughout the interphase. Bretschneider (7) first described these tufts in other species of rumen ciliates, gave them a name "paralabialorgan," and assigned them a possible sensory function. Their persistence throughout the interphase is interpreted by us to be developmental arrest of shaft formation; whether the arrest is temporary

FIGURE 4 Survey micrograph of *Ophryoscolex* perpendicular to both Figs. 1 and 3. The large rods (*R*) have one unit for each cilium and have filament bundles (*F*) that join to them (*RF*). Bundles of non-filamentous material (*N*) are frequent in these organisms during interphase and division. Cross-sections of the cilia show a granule (*G*) just above the basal body and above the deepest penetration of the plasma membrane (*P*). × 23,000.

FIGURE 5 The shaft of the cilium contains the typical filamentous array with fine material interspersed in irregular manner. From *Ophryoscolex*. × 80,000.

FIGURE 6 Cross-sections of basal bodies in *Diplodinium* show peripheral filaments (*PF*) that are triplets. In the distal portion (upper row), each filament is arranged so that a line through the centers of each part of the triplet almost coincides with a tangent, while more proximally (lower row) the line is perhaps 30° to a tangent. Auxiliary structures including filaments (*F*) are present between ciliary rows. × 95,000.





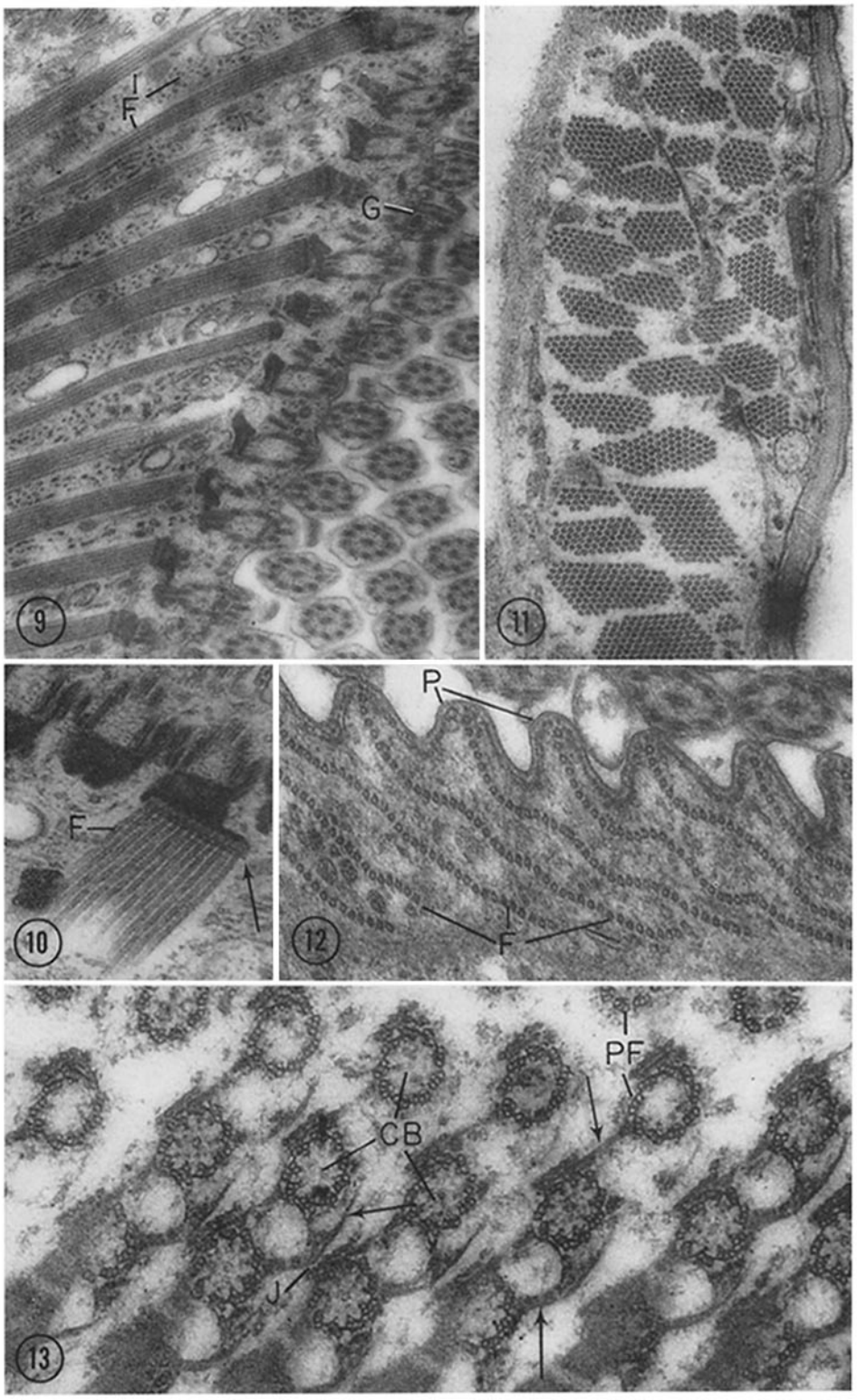
FIGURES 7 and 8 Cross-sections of the tips of cilia in *Diplodinium*. Tips are recognized by reduced diameters and by the presence of peripheral filaments with circular cross-sections (*PF*). The number of peripheral filaments is progressively reduced (*PFR*) while the central filaments (*B*) extend to the tip and end in a granule (*C*). Fig. 7, × 137,000; Fig. 8, × 118,000.

FIGURES 9 and 10 In *Ophryoscolex*, filaments in tightly packed bundles (*F*) extend from deep in the cytoplasm to the ciliary dense rod. This junction (arrow) has a typical appearance. G, granule above basal body and above deepest penetration of plasma membrane. Fig. 9, × 27,000; Fig. 10, 47,000.

FIGURE 11 In certain regions near the pellicle in *Ophryoscolex*, high concentrations of filament bundles are present in parallel array. Filaments in a given bundle are constant in diameter and hexagonally packed. × 30,000.

FIGURE 12 Sheet-like filament arrays (*F*) are found just beneath the plasma membrane (*P*) in locations such as the region of ingestion of particulates at the anterior end of the esophageal tube. This tube is an intracellular channel leading to an enlarged digestion area, both of which are separated from the remainder of the cytoplasm by a wall composed of filaments (32). *Diplodinium*. × 65,000.

FIGURE 13 Cross-sections, oriented almost exactly perpendicular to the basal bodies (*CB*) and passing through their proximal end, demonstrate structures (arrows) connecting adjacent cilia at particular filaments (*PF*) and adjacent rows of cilia (*J*). *Diplodinium*. × 69,000.



or permanent is not yet known, since it is impossible to follow the structure through a division cycle.

Our observations of this tuft are largely limited to the posterior sister where an early constriction exists, so that tuft formation is still in progress or has just been arrested. Longitudinal sections show some basal bodies that have not yet formed shafts (Figs. 16 and 17, *BB*). The basal bodies are similar in size to those of the smaller cilia described earlier, but differ by frequently having several granules included within them (Fig. 16, *BG*). The spacing of these new basal bodies is quite close, and few accessory structures are peripheral to them. At their proximal ends, however, all basal bodies

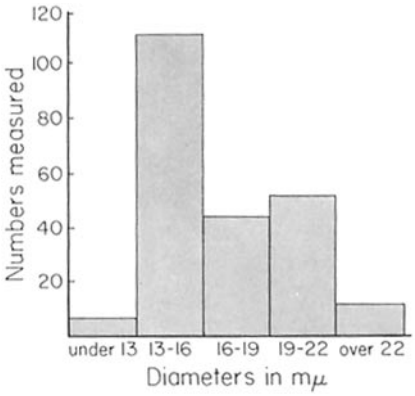


FIGURE 14 Nomogram showing the distribution of diameter measurements of infraciliary filaments in *Diplodinium*.

observed already have infraciliary filaments that extend into the cytoplasm (Figs. 16 and 17, *F*).

The early shaft elongation of cilia in these organisms takes place into closed vesicles that form at a few micra below the pellicle. Noirot-Timothée (25) has presented a detailed light microscopic account and some electron micrographs of this stage. By contrast with the tufts, the spacing between basal bodies is greater, the separation from the surface membranes is noticeable, and no membrane folds are present; infraciliary filaments have already formed, as observed in the tufts (Fig. 18).

The dense rod at the proximal end of the mature ciliary bases is not present in the forming cilia (Figs. 16 and 17). Rather, this structure, which will be interposed between ciliary base and infraciliary filaments, arises later. In some dividing organisms, cilia may be seen when partially formed dense rods (Fig. 19, *R*) are penetrated by infraciliary fibrils (Fig. 19, *F*).

Folds of the plasma membrane are regularly arranged, one between each row of basal bodies, on the surface of these tufts. The basal bodies are directed toward the spaces between each fold, not into the cytoplasm contained in each fold (Fig. 17). Each fold contains a sheet of filaments measuring 15 mμ in diameter (Figs. 17 and 20, *FT*) contoured beneath the plasma membrane (Fig. 20, *P*). Another layer is located between the plasma membrane and the filament sheet and is not layered as a membrane (Fig. 20, *LM*).

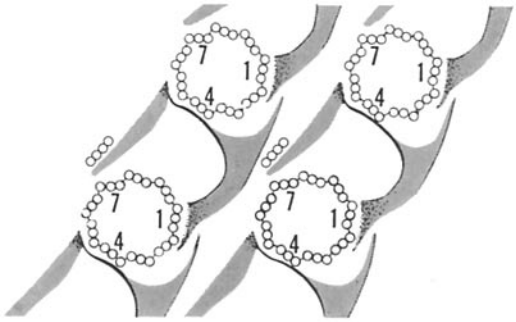


FIGURE 15. Diagram of structures connecting basal bodies of cilia in a row and adjacent rows.

The large central volume of these tufts is filled with infraciliary filaments, smooth-surfaced membrane-bounded vesicles, and some finely granular material (Fig. 21). Rough-surfaced endoplasmic reticulum is conspicuously absent though found in quantity elsewhere in the cell.

When dividing organisms are cross-sectioned, the tuft is seen to be long (about 20 μ with a 3 μ width), the folds are oriented lengthwise, and the cilia are observed to be forming in straight rows numbering about sixteen with an equal number of folds (Fig. 22). Filaments and forming cilia are now cross-sectioned. The infraciliary filaments are 15 mμ in diameter and number from four to six (Fig. 23, *F*); later the number of filaments per cilium will be much higher. The basal bodies are composed of the typical nine triplets, indistinguishable from those in mature cilia, and already have most of their accessory filaments (Fig. 23, *BB*). In these aspects the morphological

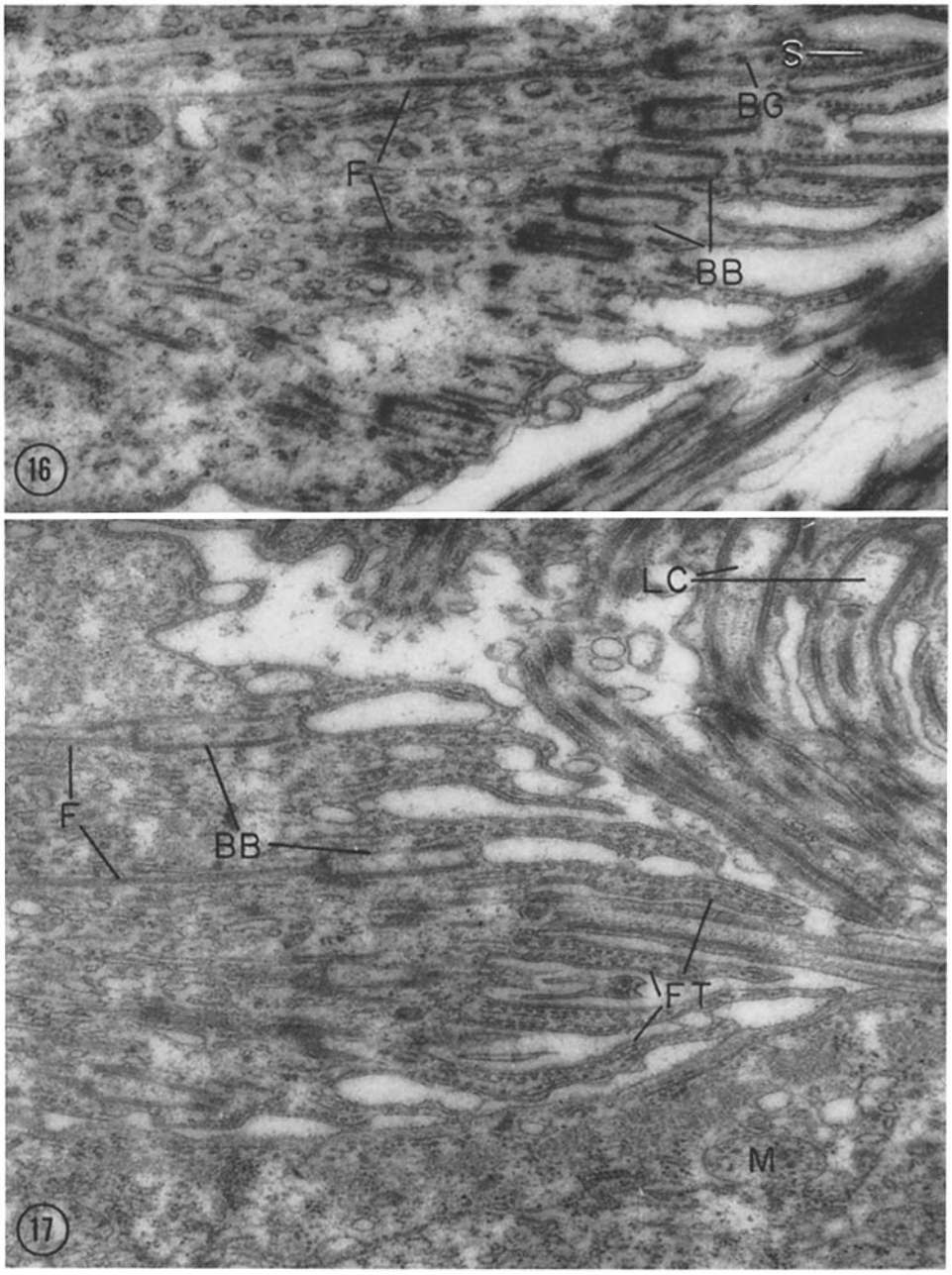


FIGURE 16 The tuft of forming cilia as seen in organisms sectioned longitudinally. Basal bodies (*BB*) are closely packed, may contain granules (*BG*), and already have infraciliary filaments (*F*). A short ciliary shaft has been formed (*S*). *Diplodinium*. × 36,000.

FIGURE 17 Tuft of cilia from another organism. The basal bodies (*BB*) with their infraciliary filaments are directed toward the spaces between the membrane folds. The filaments contained in the folds are cross-sectioned (*FT*) since they parallel the long dimension of the folds. Basal bodies of the larger cilia that occur in low frequency in these organisms (*LC*) and a mitochondrion-like body (*M*) are included. *Diplodinium*. × 46,000.

appearance of forming cilia is not greatly different from that of mature cilia.

However, the shaft structure is unusual. Immediately distal to the base, nine doublet filaments are present and arranged in a typical cylinder; however, slightly beyond that, the filaments are randomly arranged, and the ciliary membrane is usually flattened to a non-circular cross-section (Fig. 23, *S*). Moreover, each filament does not exhibit the typical bipartite cross-section but is circular with a lower density material arranged around it. The number of such filaments is usually nine except in a few cases where a count of ten is possible (Fig. 23, *S*, upper right). A longitudinal section of such a forming cilium shows a short, tapering shaft (Fig. 16, *S*).

Longitudinal sections of the membrane folds between these cilia show three layers on each side: the plasma membrane (Fig. 23, *P*), the layer below it (Fig. 23, *LM*), and the filaments each of which now appears as two lines (Fig. 23, *FT*).

Infraciliary filaments must also be forming in dividing organisms, and such regions have been located. Rather large masses of fine material are present, at the periphery of which filament bundles are typically seen (Fig. 24). Single filaments are occasionally present within the mass and are not parallel to each other (Fig. 24, *F*). In other cases in such organisms, formed infraciliary filaments are seen at the basal body, but the filament bundle fades into a partially oriented non-filamentous mass (32). Included in the vicinity are many vesicles with single membranes (Fig. 24, *V*) and many mitochondrion-like bodies (Fig. 24, *M*).

DIVIDING NUCLEI: Organisms with constriction furrows contain macronuclei undergoing division and micronuclei in metaphase or anaphase. The division of the macronucleus is amitotic while the division of the micronucleus is mitotic with an intranuclear, acentric, and non-convergent apparatus. In these stages both nuclei contain filaments not found in the interphase nuclei, and both are surrounded by continuous nuclear envelopes throughout division.

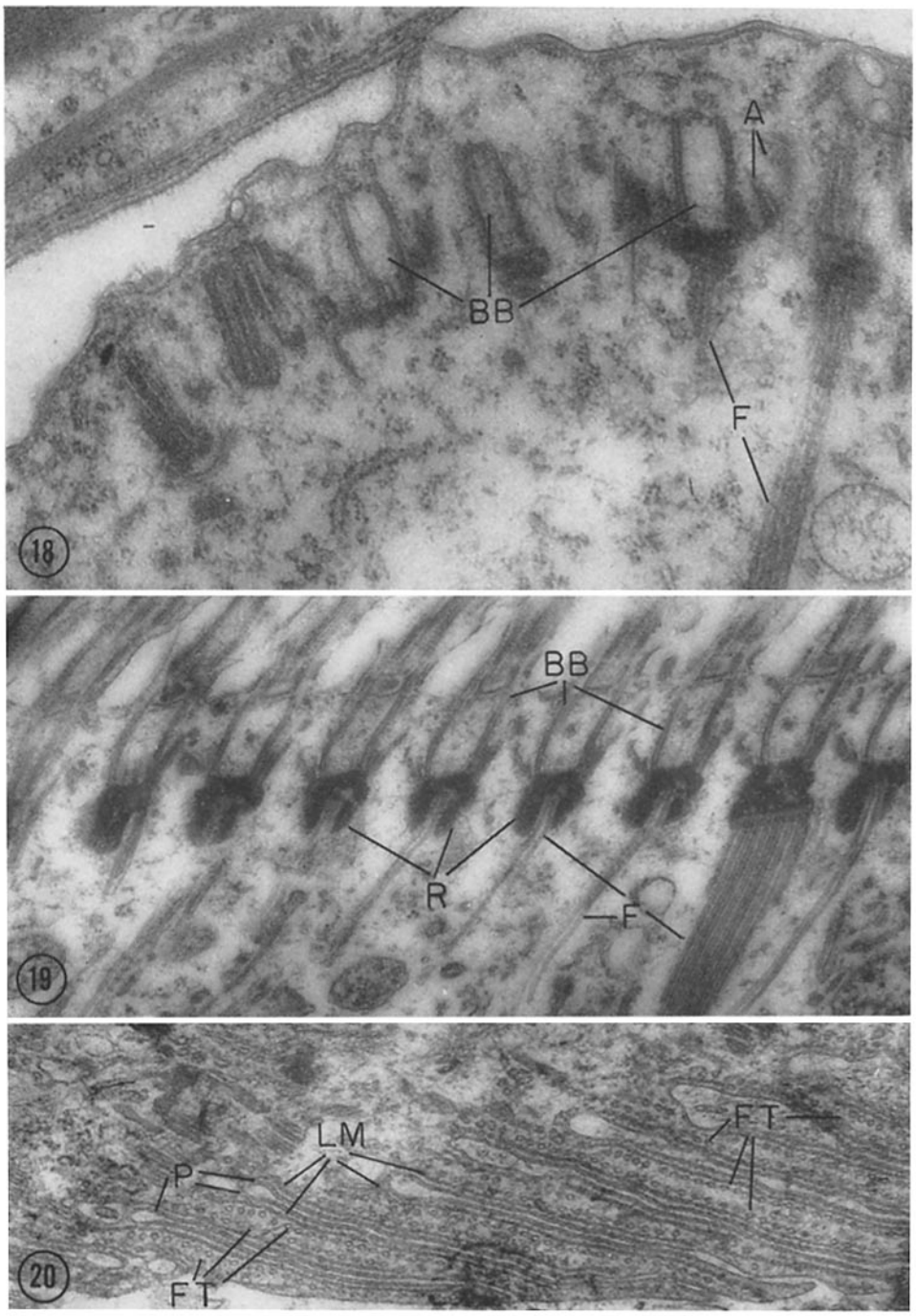
The appearance of the macronucleus at division is not greatly altered from its interphase appearance. Irregular masses of chromatin (Fig. 25, *MB*) fill the largest part of the nuclear volume. A fine network is found between these masses, and all is surrounded by an envelope not readily shown to be composed of membranes and in which no annuli have been seen at any stage. Two additional structures are present in division. First, smaller, denser granules (Fig. 25, *MG*) are seen between or on the chromatin masses. Second, a filamentous component arranged in small bundles can be seen between the masses; these filaments are circular in cross-section, have a diameter of 15 mμ (Fig. 25, *MAF*), and may be many microns long. Granules and filaments of these types have been observed in other dividing ciliate macronuclei (34) and are undoubtedly structures of general occurrence in such nuclei.

The micronucleus contains filaments identical in size and appearance (Figs. 25, 26, and 27, *MIF*). They have a dense cortex, measure 15 mμ in diameter, and have wisps of material of varying size adhering to their surfaces at irregular intervals (Fig. 26, *O*). In anaphase these filaments are predominately located between the chromosome plates and lie almost exactly parallel with each other (Fig. 26), although some are found between the chromosomes and a few others are on the "poleward" side of the chromosomes. In earlier stages, probably metaphase, the micronucleus is largely filled with a tight chromosome grouping with only enough space between chromosomes for a few filaments. In all mitotic stages

FIGURE 18 Basal bodies (*BB*) before formation of shafts in a small unidentified rumen ciliate. Even though the basal bodies are not yet in contact with the membranes, infraciliary filaments (*F*) and accessory structures (*A*) are present. × 40,000.

FIGURE 19 Cilia in a dividing *Diplodinium*. Forming cilia initially lack the dense rod in this species; in this case, the rod (*R*) is partially formed at the junction of the basal body (*BB*) and filaments (*F*). × 46,000.

FIGURE 20 High magnification of structures in the tuft folds in *Diplodinium*. The plasma membrane (*P*) has another layer of material (*LM*) under it and a still deeper sheet-like arrangement of filaments (*FT*) on each side of each fold. × 55,000.



observed, a layer of material with a density similar to that of the filaments lines the inner surface of the envelope in regions near the chromosomes (Fig. 27, *X*). In the interzonal region of the anaphase nucleus, this material is absent (Fig. 25). The envelope of the micronucleus, as that of the macronucleus, seldom shows well defined membranes (Figs. 25, and 27, *E*).

Ribosome-like bodies, characteristically found in large numbers in the mitotic apparatus of other cells (12, 14, 33), are not present in the mitotic apparatus of the micronucleus.

Filaments are found closely surrounding the nuclei in the cytoplasm during division. Filaments of small diameter are typically found between the dividing macro- and micronucleus (Fig. 25, *CF*).

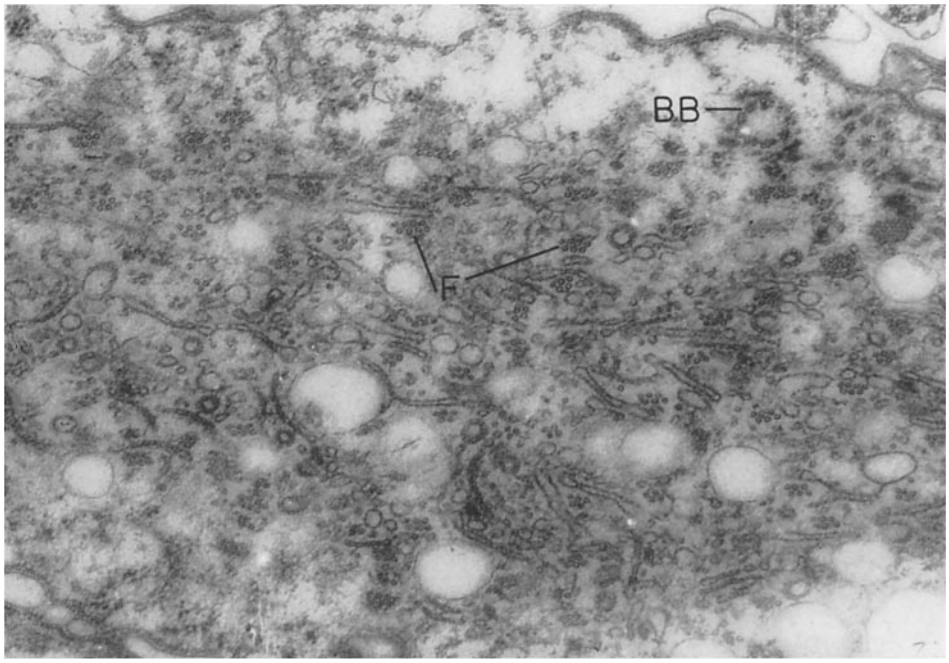


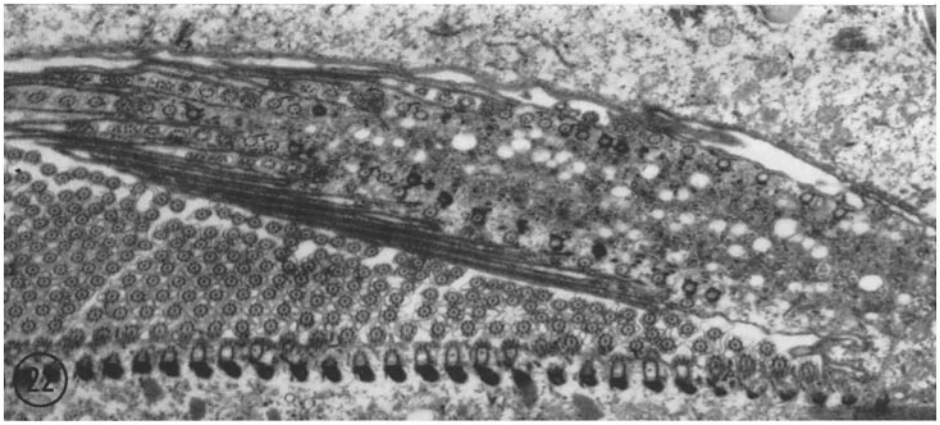
FIGURE 21 The deeper part of each tuft is filled with small flattened vesicles and small bundles of filaments (*F*). The surface of this tuft is indicated by the basal bodies (*BB*). *Diplodinium*. × 54,000.

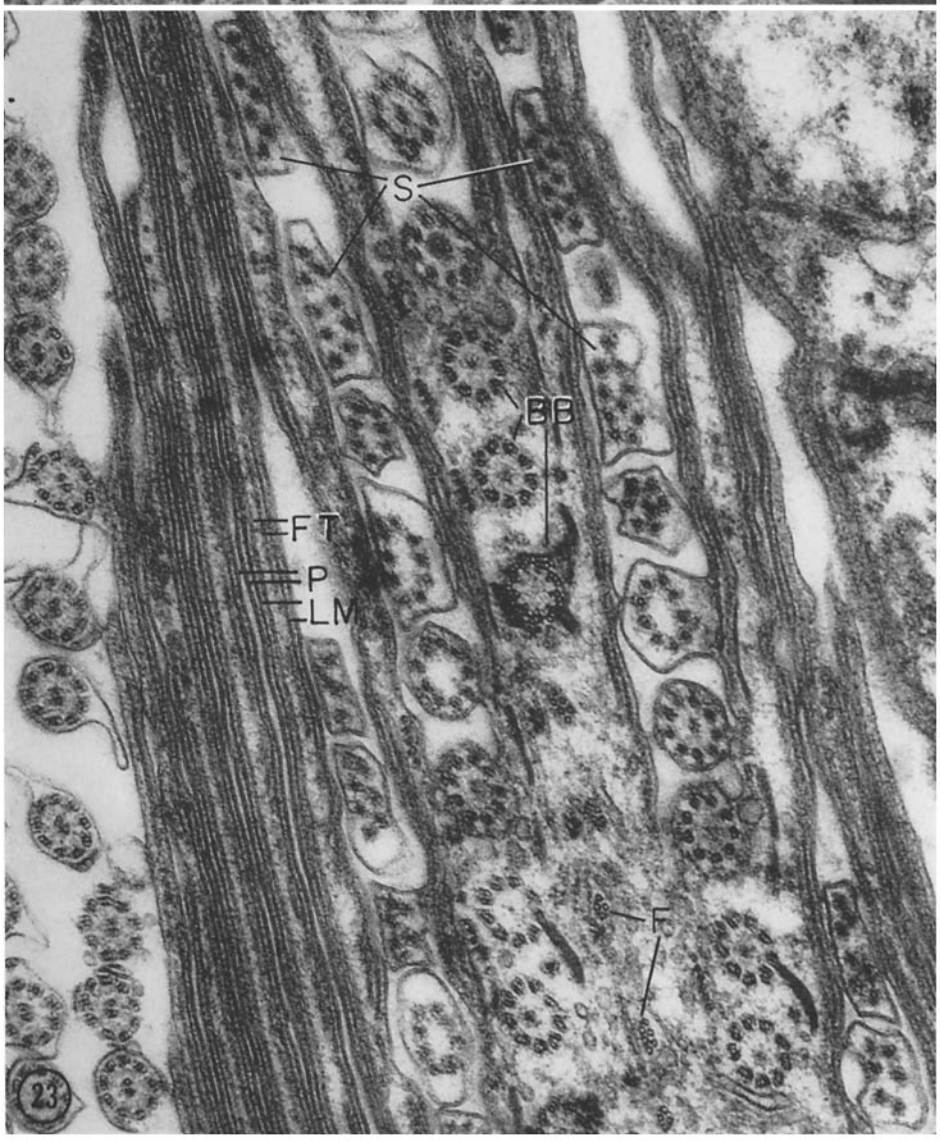
Anaphase chromosomes appear as dense, elongated rods that usually show no internal structures (Fig. 27, *CH*). However, in cases where tangential sections of chromosomes appear to have been produced, an indication of banding can occasionally be seen (Fig. 27, *CHB*).

However, a filament system of greater magnitude encloses the nuclei in all stages and is elaborated to greater complexity during division (Figs. 28 and 29, *NF*). The latter two micrographs are from a section through a slightly curved macronucleus; serial sections show that the nucleus is

FIGURE 22 The tuft as seen in *Diplodinium* when cross-sectioned. The tuft is quite long and contains several rows of basal bodies. × 7,000.

FIGURE 23 Higher magnification of such a section through the tuft. The basal bodies (*BB*) are typical and have accessory structures present. The short shafts that are now cross-sectioned are flattened and contain filaments with circular cross-sections and without their typical arrangement (*S*). The filaments in the short shafts are similar in appearance and diameter to those in the infraciliature (*F*). The folds are again shown to be composed of the plasma membrane (*P*), inner layer (*LM*), and filaments (*FT*). × 68,000.





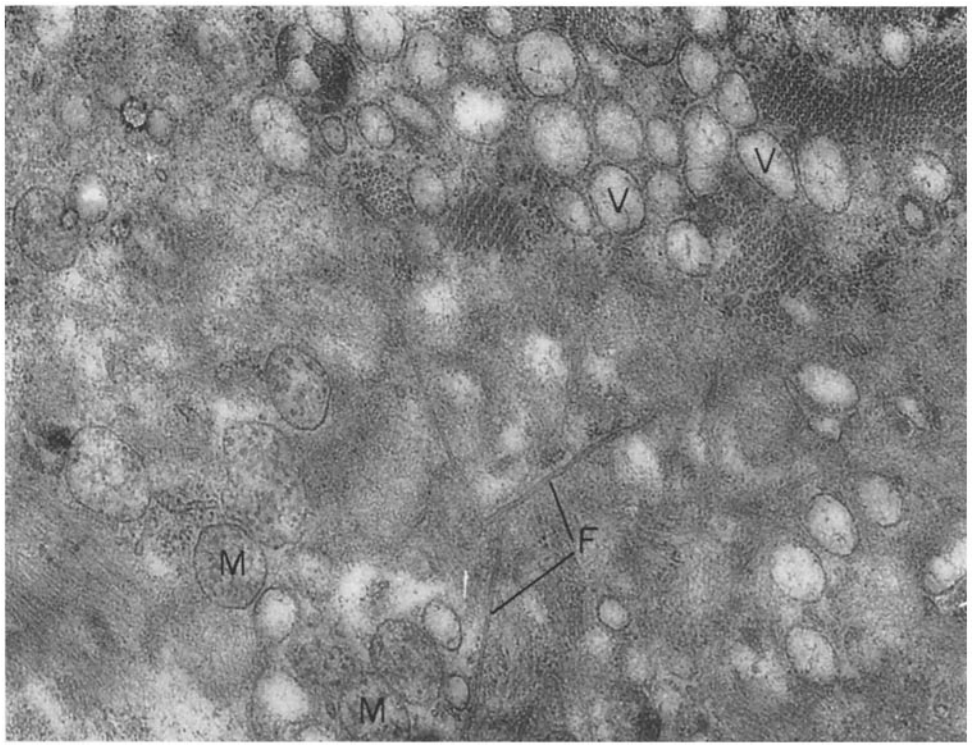


FIGURE 24 Section from a dividing *Diplodinium* a few micra below the plasma membrane. Many infraciliary filaments must be formed at division; regions such as this appear in dividing organisms and suggest that filaments are formed by arrangement of material present in relatively disorganized masses. Filaments (*F*) that are randomly oriented and others that are arranged into bundles are present either in or at the edges of such masses of fine material. In such regions, small vesicles (*V*) and mitochondrion-like bodies (*M*) abound. × 37,000.

not yet divided but is curved out of the plane of section in the central part of the illustration. Thus the section shows a tangential cut through the nucleus at top and bottom and a cut above the nucleus centrally. This extranuclear system is composed of multiple layers of filaments arranged at varying angles with respect to each other (Fig. 29, arrows indicate directions).

Such filaments are also present in interphase less than a micron from the nuclei. At that time, however, they are arranged in small bundles each of about six, regularly spaced filaments parallel to each other.

DISCUSSION

Filaments are ubiquitous in the cytoplasm of these organisms. In interphase they are abundant in the pellicle, throughout the cytoplasm, and in close association with the surface of the nuclei, while in division they are also present within the nuclei. In every case, the filaments have a dense cortex and a light center.

THE FORMATION OF FILAMENTS: That filaments of this type are formed under the influence of particular structures in the cell is now established. For example, both kinetochores and centrioles have a considerable influence on the formation of filaments in the mitotic apparatus (18). The basal bodies of cilia, in addition to influencing the formation of the ciliary shafts, also influence the formation of infraciliary filaments extending into the cytoplasm of the cell. The process of filament formation, therefore, can be considered to be composed of two steps: first, the synthesis of molecules; and secondly, their aggregation into filaments. The influence of structures such as kinetochores and centrioles needs to be involved only in the step of aggregation or alignment of

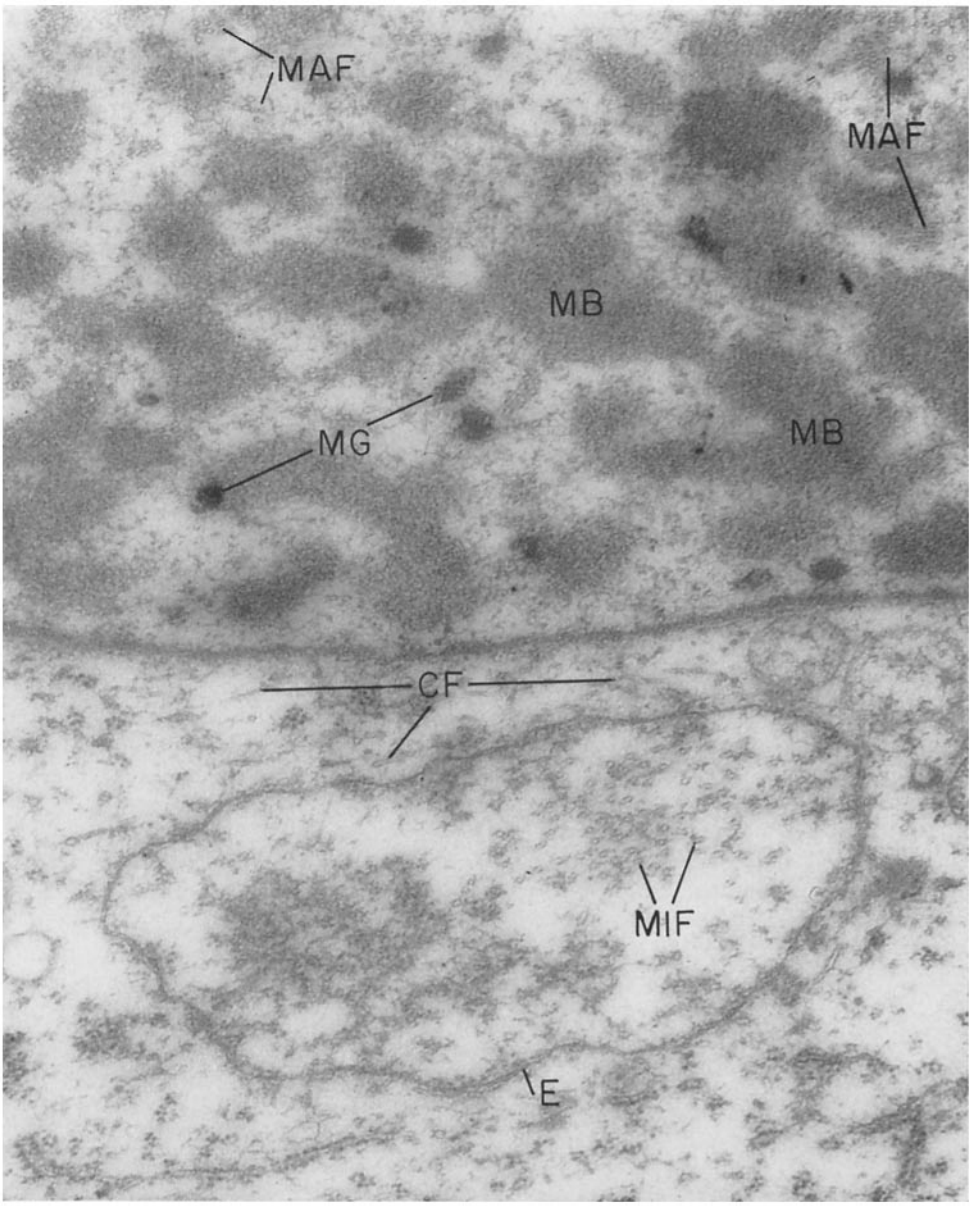


FIGURE 25 Portion of the macronucleus and micronucleus from a dividing *Diplodinium*. The macronucleus contains large masses (*MB*) that are probably chromatin in both interphase and division but during division also has small denser masses (*MG*) and filaments in small bundles (*MAF*). The micronucleus here is probably in anaphase and sectioned in the interzone, so that it does not contain the typical thick layer on the inner surface of the envelope (*E*); it does contain filaments (*MIF*). Between the nuclei, a few filaments of smaller diameter are found (*CF*). × 57,000.

previously synthesized molecules. In this regard, however, their involvement must be quite intimate.

The intense basophilia (*e.g.* 4, 36) and presence of ribosome-like particles (12, 33) in the mitotic apparatus has caused speculation that synthesis of protein and filament formation take place concurrently (12). In regard to such a hypothesis,

Went (41) has presented contrary evidence and our study of the micronucleus shows that no ribosomes are present when the micronuclear filaments aggregate and function. Thus ribosomes are absent during the division of a nucleus that had no nucleoli and into which no cytoplasmic ribosomes could move during prometaphase. Thus, the synthesis of molecules and their aggregation into filaments are probably separate events in both time and location.

The intranuclear mitotic apparatus of ciliate micronuclei presents unanswered questions concerning the origin of filament protein. Cytoplasmic contributions of protein for the mitotic apparatus, thought to be necessary for its formation in several cells (23), must cross a continuous nuclear envelope. A partial solution to this problem may be indicated by the layer of material located just inside the envelope. If this material is a pool of protein for the formation of filaments, then the micronucleus maintains a ready intranuclear store. The origin of this material is unknown, since the micronucleus is thought to be metabolically inert and shows no indication of ribonucleic acid. Thus the postulation of cytoplasmic origin seems appropriate but assumes a mechanism for transport across and accumulation inside the nuclear envelope.

THE FORMATION OF CILIA: At first the shafts of forming cilia have randomly arranged filaments of circular cross-section. Proceeding from that observation, this study suggests that the formation of ciliary shafts should be considered in two stages that are perhaps separate in time and space.

The first event is the formation of the filaments in the shaft. The circular cross-sections of filaments in the early forming cilium are the same as those found in the tips of mature cilia. This structural equality suggests that the tips of the peripheral filaments are formed first in association with the basal part of the cilium and that, as development proceeds, the doublet nature of the filament begins to form. Growth and elongation could take place by accretion at the tips of the shaft filaments or by the addition of molecules at the basal body.

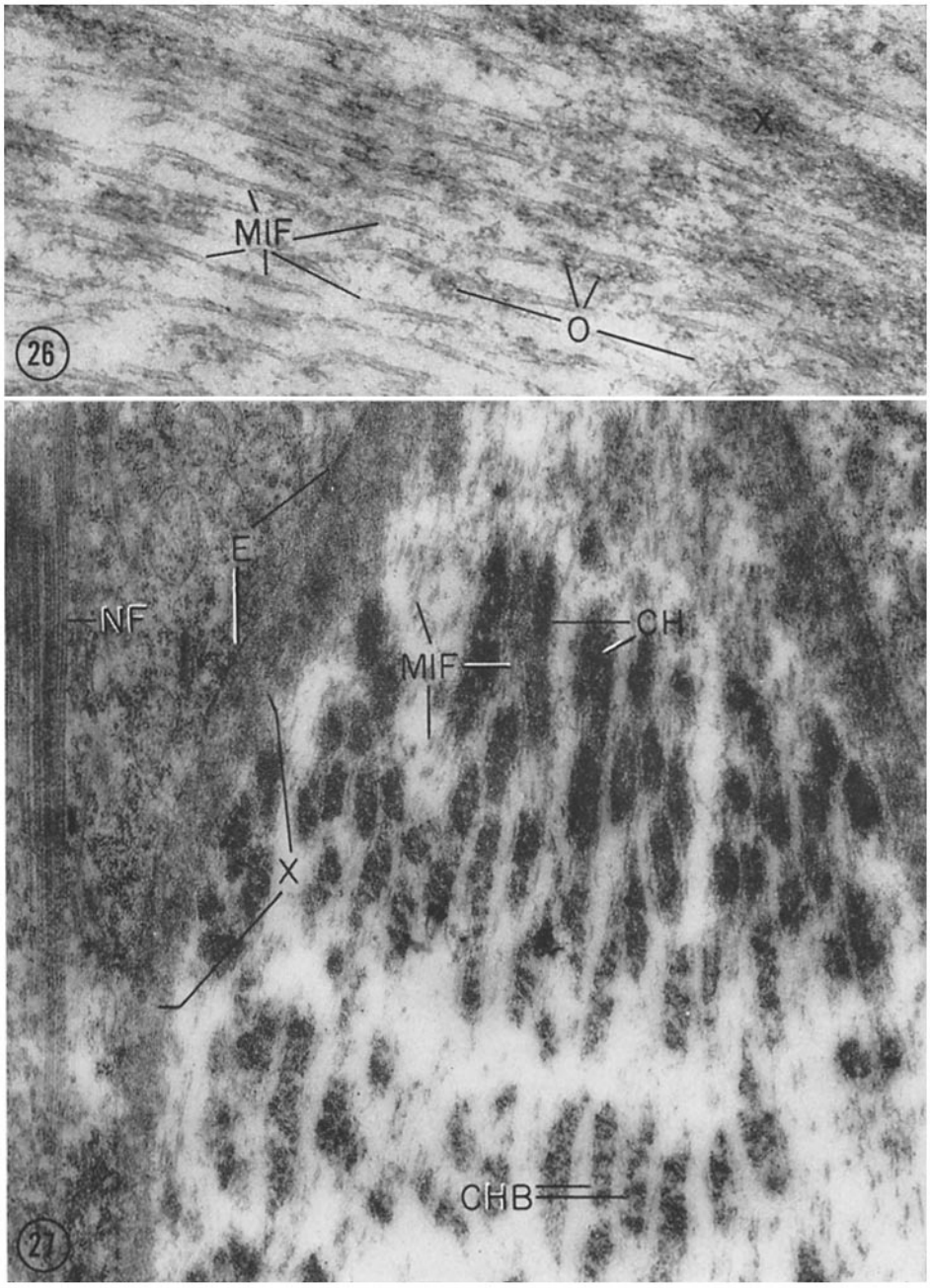
After the nine subunits are formed, they must become arranged into a circular complex. Evidently the circular arrangement of filaments in the basal body is not sufficient in itself to confer a stable circular configuration upon the newly formed filaments in the shaft. Organization of the peripheral filaments into a circular configuration probably takes place after the formation of doublet filaments. Gibbons and Grimstone (11) hypothesized that the "arms" of the peripheral filaments perform the role of maintaining the spacing and orientation of central and peripheral filaments due to their structural arrangement in the shaft compared to their reported disarrangement in the tip. In our studies, loss of spatial arrangement has not been observed in ciliary tips where central filaments persist, so that evidence for this specific role of the "arms" is uncertain.

To summarize, the formation of the ciliary filament system may be considered to consist of the following steps: (*a*) synthesis of protein, (*b*) aggregation of molecules into filaments with circular cross-sections, (*c*) a further aggregation process to form doublet filaments, and (*d*) arrangement of filaments to form the typical filament grouping.

THE FUNCTIONING OF FILAMENTS: Rapidly repeated movements requiring a relatively slight shortening as in cilia can perhaps best be explained as configurational changes in the component molecules of filaments. However, in the mitotic apparatus, the great degree of spindle elongation found in many cells precludes the possibility that increased length can be provided

FIGURE 26 Higher magnification of the filaments within the dividing micronucleus in *Diplodinium*. The filaments (*MIF*) have other fine material (*O*) surrounding them, while other material with similar appearance (*X*) forms a layer just inside the envelope. × 74,000.

FIGURE 27 Survey of a portion of the micronucleus and cytoplasm in anaphase in *Diplodinium*. Chromosomes (*CH*) usually appear as dense rods except in regions where tangential sections show banding (*CHB*). Filaments (*MIF*) are present between chromosomes. The dark layer of material (*X*) is clearly shown under the envelope (*E*) which is continuous throughout mitosis. In the surrounding cytoplasm, filaments are also present (*NF*). × 31,000.



in this way. For example, the description of spindle elongation by Short (38) and Berkeley (3) in the giant amebae indicate that the continuous filaments increased from 10 μ in metaphase to over 60 μ at the end of anaphase; similar elongation takes place in the intranuclear mitosis of the micronucleus in *Diplodinium*. Major additions of substance to each filament must account for such elongation in these cells. The reader is referred to more detailed treatments of these phenomena by Inoué (17), Mazia (22), and Roth (31).

Filaments participate in movements or contribute structural rigidity. In all of the systems studied, the filaments have a dense cortex and diameters ranging from 12 to 27 mμ (2, 8, 9, 13, 14, 19, 20, 25, 26, 30, 32, 33, 35, 39). Attempts to formulate unifying concepts on the basis of these morphological and functional similarities have been made (*e.g.* 39), but the data do not yet allow definitive formulations.

However, one comparison can perhaps be profitably cited. Numerous cells, especially protozoa and spermatozoa, have been shown to contain filaments of circular cross-sections and of 21 mμ diameter; a survey paper (29), and a later review on protozoan fine structure by Pitelka (26), deal with this subject. Other filaments also of circular cross-section and of 15 mμ diameter have been described in the mitotic apparatus (13, 14, 33). In this study, preliminary evidence is given suggesting that filaments of both these sizes may exist in the infraciliature, and further that the smaller may be formed during division stages while the larger predominates during interphase. This evidence is strengthened by two other studies of different material. Harris has described the mitotic apparatus of sea urchin blastomeres (13, 14) which contain filaments measuring 15 mμ in diameter. Kane (19) has described filaments measuring 21 mμ in diameter, using very similar material and studying it by similar electron microscope sectioning methods, but having first isolated the mitotic apparatus from the blastomeres. Thus, both filament types are reported from very similar cells when different procedures were used.

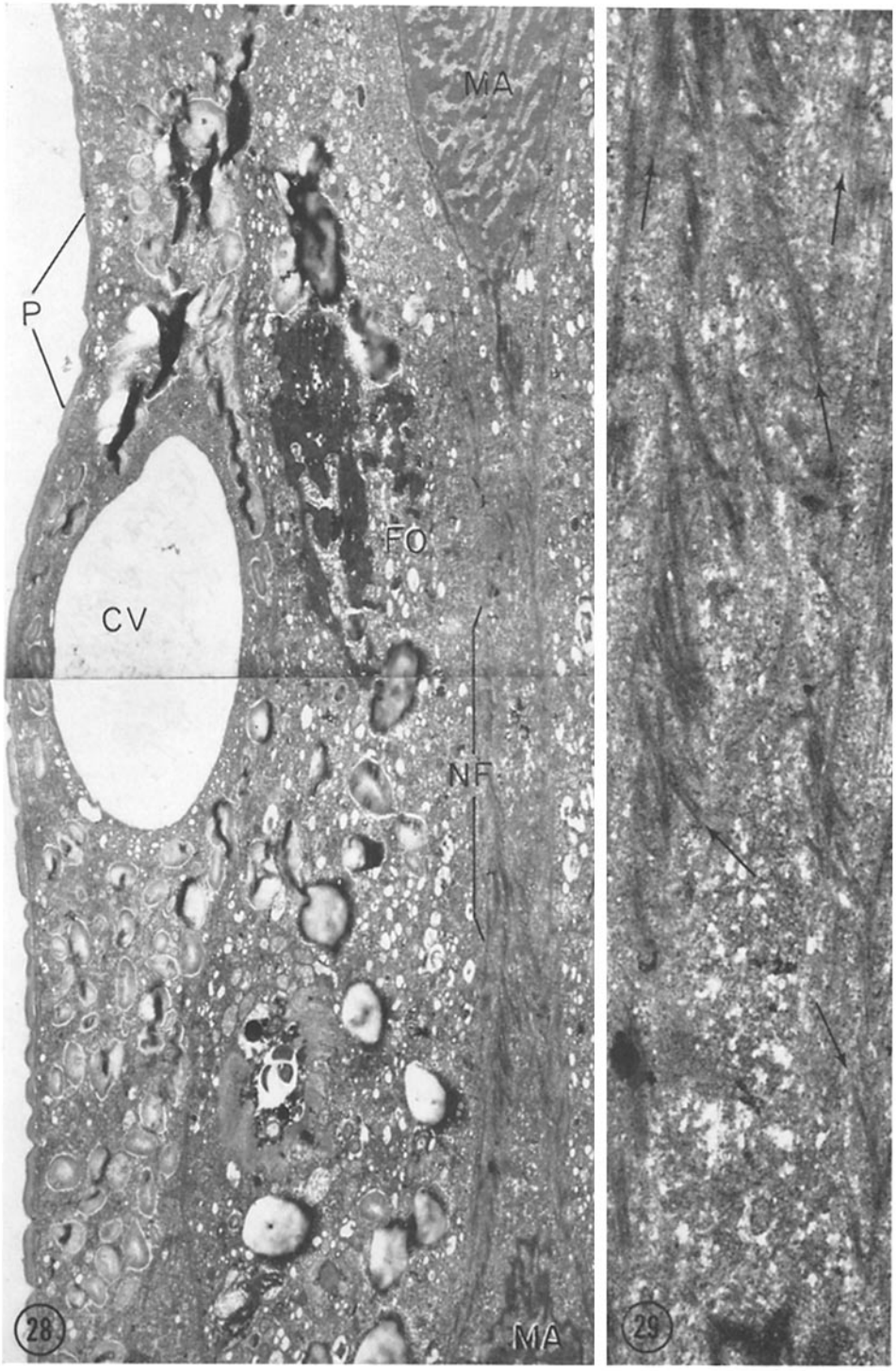
The possibility of a 15- to 21-mμ transition is one of several transformations. Inoué has demonstrated a generalized loss of birefringence in the mitotic apparatus when the temperature is reduced (17) and a localized loss with ultraviolet microbeam irradiation (18). The effects of pH and divalent cations also alter filamentous nature of the mitotic apparatus (22, 33). Only the highly labile mitotic apparatus has been amenable to such observations thus far, although the more stable filament systems such as cilia and the protozoan infraciliature should be similarly studied.

On the basis of these studies, several hypotheses can be formulated. First, a cell can transform the molecules *in vivo* from one to the other of several conditions. Second, such transformations and metastability are functionally important. Third, the several filament systems (cilia, mitotic apparatus, infraciliature, centrioles, and bacterial flagella) are chemically very similar or perhaps even identical in molecular composition. When such hypotheses have been tested, much more will be known about cell movements.

In electron microscope as well as all other biological studies, the conditions of preservation and observation need to be prescribed carefully. Ion concentrations, pH, temperature, and length of fixation are examples of the parameters that need to be controlled. Studies of the mitotic apparatus best illustrate this principle, since they

FIGURE 28 Survey micrograph of the region near the constriction furrow and including the elongated dividing macronucleus of *Diplodinium*. Surrounding the nuclei is a filament system (*NF*) that is elaborated in division and encases both nuclei. The macronucleus (*MA*) has not yet divided and is out of the plane of section in the central part of the montage. The pellicle (*P*), contractile vacuole (*CV*), and food vacuoles (*FO*) are included, as well as many cytoplasmic food-reserve granules. × 5,000.

FIGURE 29 Higher magnification of a portion of Fig. 28. The filament system is composed of filaments oriented in two general ways (arrows). The arrangement suggests opposed spiraling filament sets. × 17,500.



have shown that, in both protozoa and metazoa, conditions different from those used routinely are necessary to observe filaments (33). Biochemical studies of ribosome variations in different magnesium-ion concentrations provide another cogent illustration (16, 40). In electron microscope studies, careful definition of fixation conditions may also be useful in suggesting chemical similarities.

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