ASPECTS OF CILIARY FINE STRUCTURE IN EUPLOTES PATELLA*

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PLATES 79 TO 81

The current renewal of interest in cilia has been motivated by the possibilities of studying their fine structure at much improved levels of resolution with the electron microscope. The arrangements of cilia in the hypotrichous ciliates aroused our interest, and the organism *Euplotes* was chosen for such work since much is known from several light microscope studies of the cytology in resting organisms by Yocom (12), Taylor (9), and Turner (11). Hammond (4) and Turner (10) have published light microscope observations during conjugation and division.

Materials and Methods

Euplotes patella was isolated from local soil and grown on a lettuce infusion inoculated with *Aerobacter aerogenes*. Pierson's taxonomic study (6) was used in classification.

The organisms were concentrated with a sintered glass filter of medium porosity and changes of fluid were made by aspiration after the organisms had settled out of suspension. No centrifugation was used. The fixation fluid was 1 per cent osmium tetroxide in 0.9 per cent sodium chloride with MacIlvaine's buffer at pH 7.4, 0.05 molar. After a fixation of 1 hour at 22°C., the organisms were washed for 15 minutes in a solution containing the same concentrations of sodium chloride and buffer as the fixation fluid. Dehydration was by one 15 minute change in 50 per cent, 75 per cent, and absolute ethyl alcohols, followed by two 30 minute changes of methacrylate. After transferring to gelatin capsules, the organisms were embedded in 40 per cent ethyl methacrylate and 60 per cent *n*-butyl methacrylate catalyzed by 1 per cent luperco CDB (0.5 per cent 2,4-dichlorobenzoyl peroxide) using ultraviolet light, with the organisms at a temperature of 60°C.

Equipment used included a Porter-Blum microtome and an RCA EMU-3A microscope (100 kv. beam).

OBSERVATIONS

The Cirri.—The cilia in *Euplotes* are arranged in localized groupings called cirri, in which five to eight rows of cilia are hexagonally packed so that three row directions are possible. If the row direction giving the best symmetry is chosen, a typical cirrus (Fig. 3) would have three cilia in the first row, four in the second, five in the third, five in the fourth, four in the fifth, and three

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in the sixth row (a 345543 arrangement). Other typical arrangements are 56776 (Fig. 1), 34554, and 34555432. The cirri are usually found in slight depressions of the pellicle with no sheath or membrane covering the cirrus as a whole. Likewise there has been no extraciliary matrix seen and the cilia appear to be in free contact with the surrounding fluid. Membranelles consisting of two or three longer rows of cilia and showing no matrix, sheath, or extraciliary membrane are also present.

The Pellicle.—This term is used here to denote an outer zone composed of two separate membranes. The outermost is a double-layered membrane having a thickness of 90 A (each layer 30 A thick with a separation of 30 A). The membrane (here called the "pellicular membrane") is characterized by frequent, small protuberances (Figs. 3 and 6), which may appear as slight elevations or marked outward extensions of greater length; their frequency is as high as one hundred for each square micron of surface.

A second double-layered membrane (called here the "cytoplasmic membrane"), lies directly below the pellicular membrane at a distance of about 40 A at the points of closest approximation. It presents a much straighter appearance than that described for the pellicular membrane. Its two layers are each 40 A thick with a separation of 40 A (Fig. 6).

The Ciliary Rootlets.—The structures called by the light microscopists "the neuromotor fibrils," which connect cirri and membranelles, are composed of a number of subfibrils or filaments, each of which is 120 A in diameter, with a less dense central portion. No periodic structure has been observed. Similar filaments also appear to interconnect the cilia of neighboring membranelles and cirri directly. In addition, short, direct connections are frequently demonstrable between the adjacent cilia in each membranelle and cirrus, linking the interior cilia of a cirrus to the peripheral cilia. Thus the rootlet filaments need not contact directly all the cilia in a cirrus and numerous cilia are seen without direct rootlet contact.

The Cilia.—The structure of the ciliary shaft demonstrated by Fawcett and Porter (3) is seen here including the nine double peripheral fibrils and the two single central fibrils. However, the only indication of a ciliary matrix is a slight accumulation of material immediately around the central fibrils. Since it has been possible to observe the tips of the cilia and to examine the basal regions more thoroughly, certain additions to our idea of ciliary structure are presented.

In oblique sections at the distal ends of membranelles are seen numerous ciliary sections, each one slightly nearer to the tip (Fig. 4). The cilium tapers at the tip; the eleven fibrils terminate shortly beyond the point at which the diameter has been reduced from the typical 300 mμ to about 150 mμ, the peripheral fibrils terminating before the central ones. At diameters of 100 mμ, it is still possible to see as many as five peripheral fibrils, in addition to the

two central ones (Fig. 4). The ciliary membrane probably remains intact to the end for it has been possible to measure tips as small as 50 mμ in diameter in which the membrane is clearly present.

The ciliary membrane, which is double-layered (each layer 30 A thick with a separation of 30 A) (Fig. 5), is in direct continuity with the pellicular membrane (Fig. 6). Both membranes have the same dimensions, and both exhibit extensions or protrusions (Figs. 3 and 6 *p*). All the ciliary fibrils continue below the level of the pellicle into the cytoplasm for a distance of 330 mμ. Each of the double peripheral fibrils is 350 A in diameter in cross-section, with the subfibrils measuring 180 A by 350 A, both in the shaft and in the basal region. At the point of junction of the peripheral fibrils and the rootlet filaments a dense granule appears in most instances. There is also a second layer of granule-like structures 170 mμ distal to the first which seem to be short, direct interconnections between the peripheral fibrils of adjacent cilia (Fig. 2). The plate-like appearance at these two levels as seen in the light microscope studies of Taylor (9) may be attributed to the combination of rootlet filaments, granule-like regions, and ciliary interconnections.

Although it has not been generally accepted that central fibrils are present in the basal region, they can be clearly seen here (Figs. 2 and 6), and certain characteristic features can be distinguished. No rootlet filaments have been seen in contact with the central fibrils. In addition cross-sections of the basal region may show two, three, or four sections of the central fibrils; longitudinal sections show that each central fibril penetrates to a depth equal to that of the peripheral fibrils, at which level the central fibrils both turn back toward the pellicle and join together just below the level of the cytoplasmic membrane. Only parts of this structural relationship can be seen in any one ciliary section (Figs. 2 and 6). It can also be observed that the basal portions of the central fibrils are much less straight in appearance than the shaft portions. Also seen are small dense regions regularly arranged in two or more rows on the central fibrils (inset, Fig. 6); these granules measure 40 A in diameter and are separated by 80 A.

Immediately distal to the cytoplasmic membrane is a small disc-shaped granule associated with the central fibrils (arrow, Fig. 2), measuring 100 mμ in diameter by 50 mμ thick; cross-sectional views show that it is penetrated by the central fibrils (Fig. 6).

DISCUSSION

For many years, it has been assumed that in the Hypotricha, cirri were groupings of fused cilia. It has been reported by Taylor (9) that cirri may be split into loose bundles of numerous cilia, but that even with rough handling, cilia cannot be completely separated; he then suggests that the cilia are embedded in a gelatinous matrix and the whole is surrounded by a membrane.

Similar suppositions as to the method of unification of cilia were made by others (12), but it is apparent now that neither an extraciliary matrix nor membrane is present. Rather it appears on the basis of the present observations that the only ciliary structures which contribute to functional unity in the cirrus are the protrusions of the ciliary membrane. These protrusions are rather long extensions (about 1 μ) which intertwine among the cilia; since cross-sections rarely show continuity of these with the ciliary membrane, it is unlikely that they are short connections between adjacent cilia. Thus, adjacent cilia probably have no structural continuity beyond the superficial entangling of membrane extensions and the coordination of beat now takes on greater importance in achieving functional unity in cirri than previously recognized.

The foregoing functional concept does not preclude the possibility that these structures also serve the purpose of increasing absorptive or secretory surface. The mastigonemes of protistan flagella, which Pitelka and Schooley (7) describe as increasing mechanical effectiveness in motion, also bear strong resemblances to these structures.

Some features of the central fibrils are also significant. The central fibrils are not oriented in the same plane in sections of adjacent cilia and probably spiral around each other. The basal portions in *Euplotes* differ in appearance from the shaft portions, and from cilia in other organisms in which the central fibrils are missing entirely. Further, the cilia composing cirri and membranelles possess the ability to move in more than one plane, while the cilia reported by Fawcett and Porter (3) as having the central fibrils uniformly oriented may move in only one plane. These relationships point toward a possible association between the plane of ciliary beat and the orientation of central fibrils.

In making comparisons between the central fibrils and the peripheral fibrils, several differences have been reported. Hodge (5) has reported a greater resistance to pepsin digestion by the central fibrils. Afzelius (1) has reported differences in size and shape in the fibrils of three species of sea urchin sperm tails, the central ones being round with a diameter range of 252 to 278 A and the peripheral pair ranging from 202 to 224 A in radial width and from 340 to 380 A in tangential width. These differences are also seen in *Euplotes*, where the central fibrils are round with a diameter of 240 A and the individual fibrils of the peripheral pairs are 180 by 350 A. In addition, the granular structure demonstrated here in the basal regions of the central fibrils (Fig. 6, inset) has not been seen in the peripheral fibrils. Such evidence indicates a difference in the composition of the central and peripheral fibrils.

The complexity of interconnections of most cilia is exceedingly great in this organism, and in all cases observed, the interconnections seem to be directed only to the peripheral fibrils of the cilia; that is, cilia in a cirrus or

membranelle are joined by several direct connections with the peripheral fibrils of adjacent cilia, and in addition, short rootlet filaments from the peripheral fibrils are often seen to extend directly from cirrus to cirrus or from membranelle to membranelle. Bradfield (2) speaks of a similar system in discussing bull and ram sperm in which the peripheral fibrils are gathered into three bundles in the neck region "as if there were three primary contractile units, each subdivided to facilitate finer gradation of structure. Three such units would presumably be the minimum apparatus necessary to produce three dimensional waves in a sperm tail consisting of straight contractile fibrils."

The central fibrils, on the other hand, do not appear to be involved in these interconnections; there are no connections with the rootlet filaments here. It may be pointed out also that in some other organisms, central fibrils have not been observed in the basal region (3, 8).

These observations on *Euplotes*, together with the other evidence cited, are consistent with the hypothesis that ciliary motion is produced by the contraction of the peripheral fibrils, while the central fibrils perhaps determine the plane in which the cilia can bend.

SUMMARY

1. The functional unity of cirri and membranelles can result structurally only from extensions of the ciliary membrane.

2. The pellicle is composed of an outer pellicular membrane and an inner cytoplasmic membrane.

3. The ciliary rootlets are composed of numerous filaments 120 A in diameter with central areas of low density. They have no periodic structure.

4. The ciliary membrane is a double-layered structure continuous with the pellicular membrane. The cilia show the typical arrangement of nine double, peripheral and two single, central fibrils. All fibrils pass into the basal region, the peripheral ones joining with the rootlet filaments, while the central fibrils from the extreme proximal position of the basal region turn back toward the pellicle and appear to unite just beneath the cytoplasmic membrane.

5. The cilia (300 mμ diameter) taper at their tips to a diameter at least as small as 50 mμ. At a diameter of about 150 mμ, the fibrils begin to show a reduction in number.

6. The central ciliary fibrils may determine the possible directions of ciliary beat. These fibrils show an intrafibrillar structure in their basal portion, which involves regularly spaced 40 A granules.

7. These observations on *Euplotes*, together with the other evidence cited, are consistent with the hypothesis that ciliary motion is produced by the contraction of the peripheral fibrils, while the central fibrils perhaps determine the plane in which the cilia can bend.

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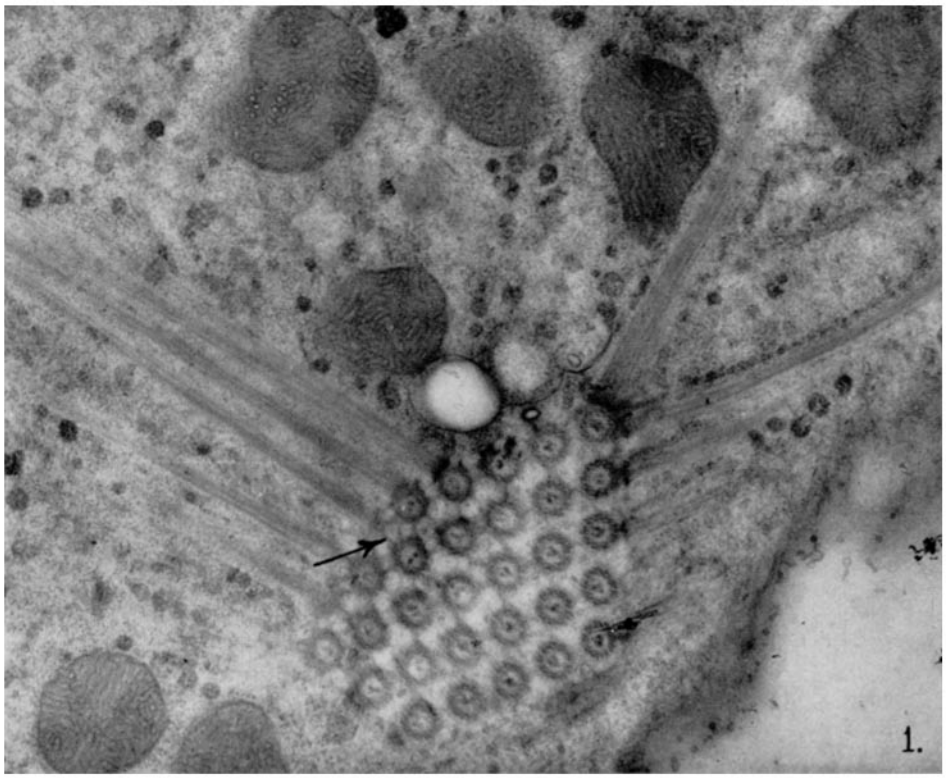
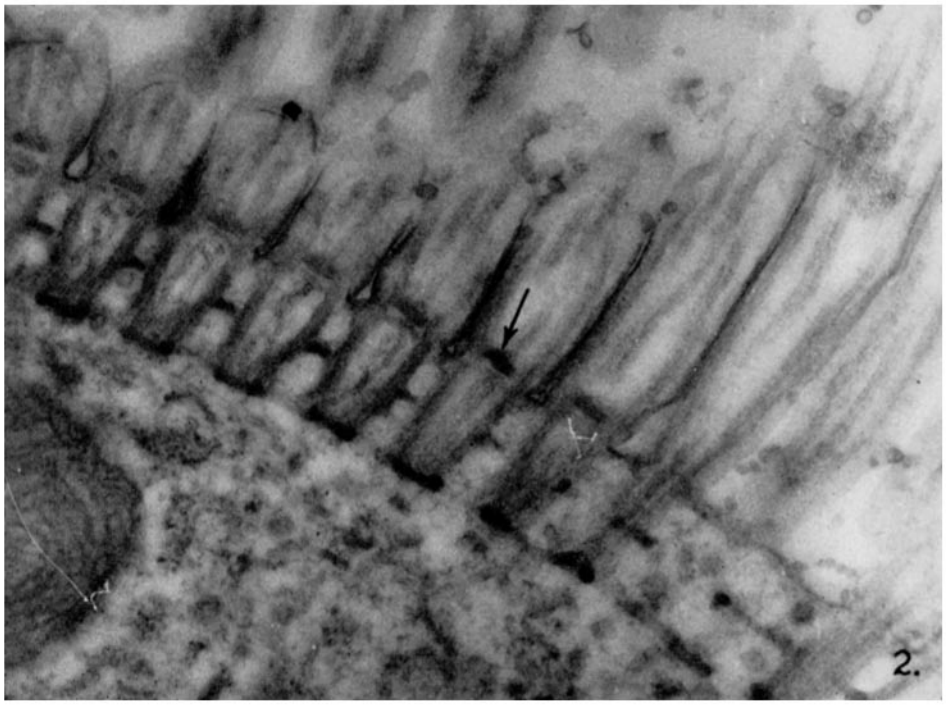
EXPLANATION OF PLATES

PLATE 79

FIG. 1. A typical cirrus sectioned just under the pellicle. Rootlet filaments are shown approaching the cirrus from several directions and direct connections of the peripheral fibrils of adjacent cilia are indicated (arrow). Numerous mitochondria can be seen. × 25,000.

FIG. 2. A membranelle in nearly longitudinal section. Granules similar to those appearing at the bases of the peripheral fibrils are seen at a second more distal plane. This second group of granules serves to interconnect the peripheral fibrils of adjacent cilia. The disc-shaped granule associated with the central fibrils in each cilium is just distal from the cytoplasmic membrane in seven of these cilia (arrow). × 41,000.





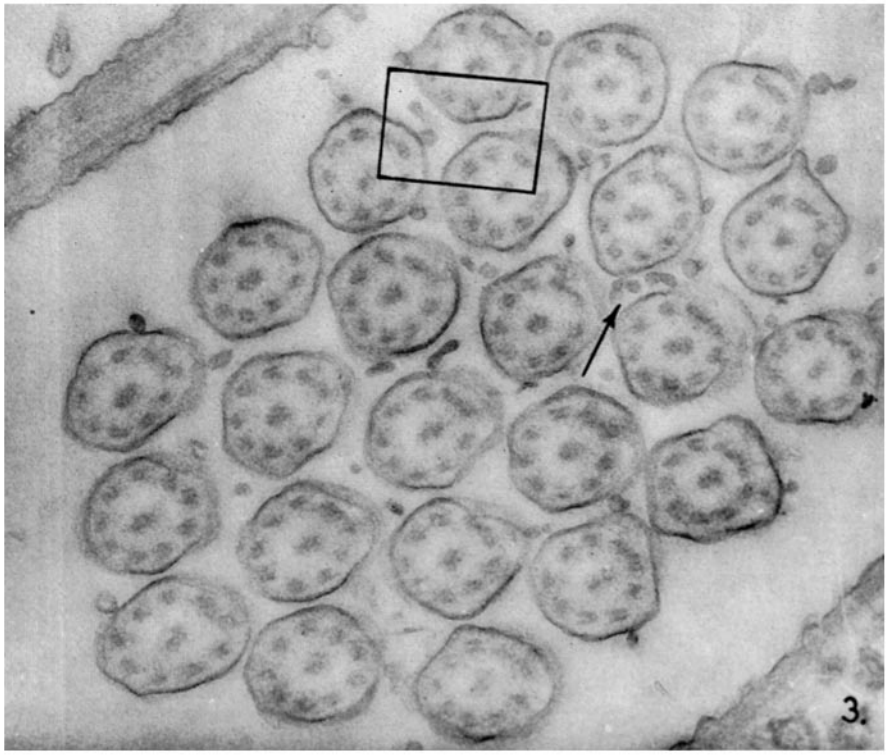
(Roth: Ciliary fine structure in *Euplotes*)

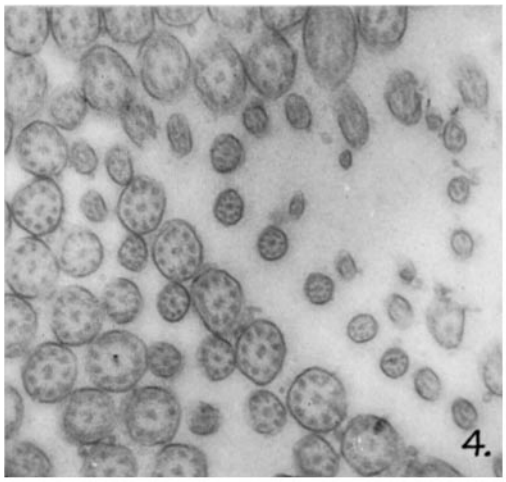
PLATE 80

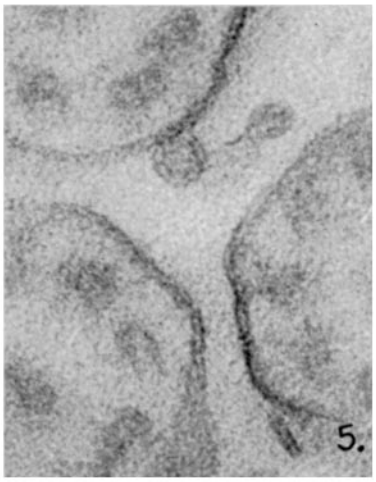
FIG. 3. A typical cirrus sectioned just distal to the pellicle showing symmetry of the ciliary arrangement. The interciliary extensions of the ciliary membrane are seen in numerous examples (arrow). No sheath or matrix is seen, so that the interciliary tubules are the only structures present which could give the structural unity demonstrated by Taylor (9) to the cilia composing this cirrus. × 52,000.

FIG. 4. Rows of cilia composing membranelles. Such ciliary configurations allow optimum study of tip structure of cilia. The ciliary fibrils are still present even at the level of much reduced diameter in the tapering tips. × 27,000.

FIG. 5. A greater enlargement of a portion of Fig. 3 (see marked area) showing the double-layered structure of the membranes bounding the cilia and the interciliary extensions. × 163,000.



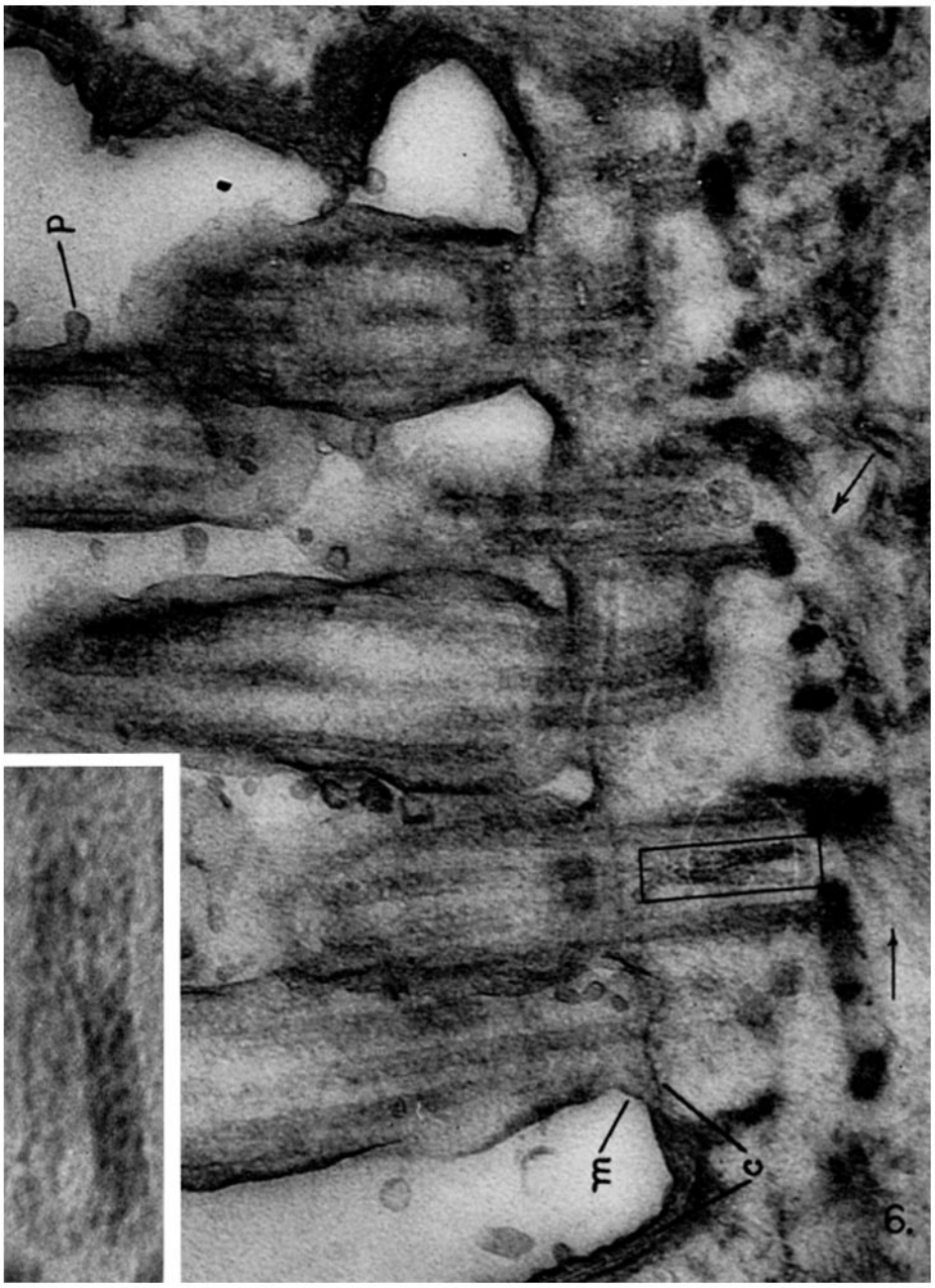




(Roth: Ciliary fine structure in *Euplotes*)

PLATE 81

FIG. 6. A cirrus sectioned in the basal region of some of the cilia. The continuity of the ciliary fibrils into the basal region and the close association of the rootlet filaments (arrows) with the peripheral fibrils are shown. Two examples of the central fibrils turning back toward the pellicle are seen as well as the intrafibrillar structure of the basal portion of the central fibrils, a marked area of which is further enlarged in the inset. The granular appearance of this region shows possible molecular relationships in the fibril. The continuity of the pellicular membrane and the ciliary membrane is shown (*m*) and the cytoplasmic membrane is also shown (*c*). An interciliary protrusion (*p*) of the ciliary membrane is seen at the point of origin. × 82,000. inset × 240,000.



(Roth: Ciliary fine structure in *Euplotes*)