FINE STRUCTURE OF THE CILIA OF ROTIFERS

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ABSTRACT

The fine structure of the coronal cilia of the rotifer *Philodina citrina* has been studied in detail. Specimens were fixed with OsO_4 and embedded in butyl–methyl methacrylate, Epon 812, or Vestopal and sectioned with a Porter-Blum microtome. The details of structure of the rootlets, basal bodies, basal plates, and free cilia are described. The general structure of the rotifer ciliary apparatus conforms well to that established for other species. One of the main observations is the difference in structure of the peripheral filaments in the opposing halves of a cross-section of the free cilium. Also, in longitudinal sections evidence is offered for the existence of a helical structure in the peripheral filaments.

This is the first of a series of studies on the electron microscopical structures of rotifers. The rotifer, which has not been systemically characterized from a cytological viewpoint, is unusually well suited for study of senescence in that its life span is measured in days, it manifests determinate development so that all body cells are of the same age, it is easily reared in homozygous stocks by virtue of its parthenogenetic reproduction, and it lends itself well to standardized breeding in the laboratory (11). Previous studies (12, 13) which established the existence of a transmissible, cumulative, and reversible accelerator of senescence in rotifers have stimulated interest in the fine cytology of the rotifer.

In an attempt to extend these early studies to characterization of the fine structural and cytochemical changes that may be associated with senescence in the rotifer, a beginning is being made with analysis of the coronal cilia. Stroboscopic measurement of the rate of ciliary beat has confirmed an old impression (12) that the cilia beat more slowly in the senile rotifer. During most of the life span the coronal cilia beat at 1200 complete strokes per minute, but in the senile rotifer pilot studies indicate that they slow down to 900 to 1000 beats per minute.

This report summarizes the current status of our study of the normal fine structure of the cilia of the rotifer. It is our intention to establish a base line for analysis of possible age changes which may be correlated with the decrease in beat rate. Some of our observations have encouraged us to develop a model of ciliary structure which may further our understanding of the nature of ciliary beat.

MATERIAL AND METHODS

The bulk of these observations have been made on *Philodina citrina* with occasional reference to a giant rotifer of the genus *Rotifer* which as yet has not been fully identified. The animals were raised in mass and isolation culture essentially as described previously (11). Instead of artificial pond water, deep well water from the Pittsburgh environs buffered at pH 8.0 was used. The animals were fed a uniform diet of *Chlorella vulgaris* raised under artificial light on non-nutrient agar slants as before.

For electron microscopy the excess pond water in the pyrex depression slides used to raise rotifers was carefully drawn off with fine pipettes and 1 or 2 per

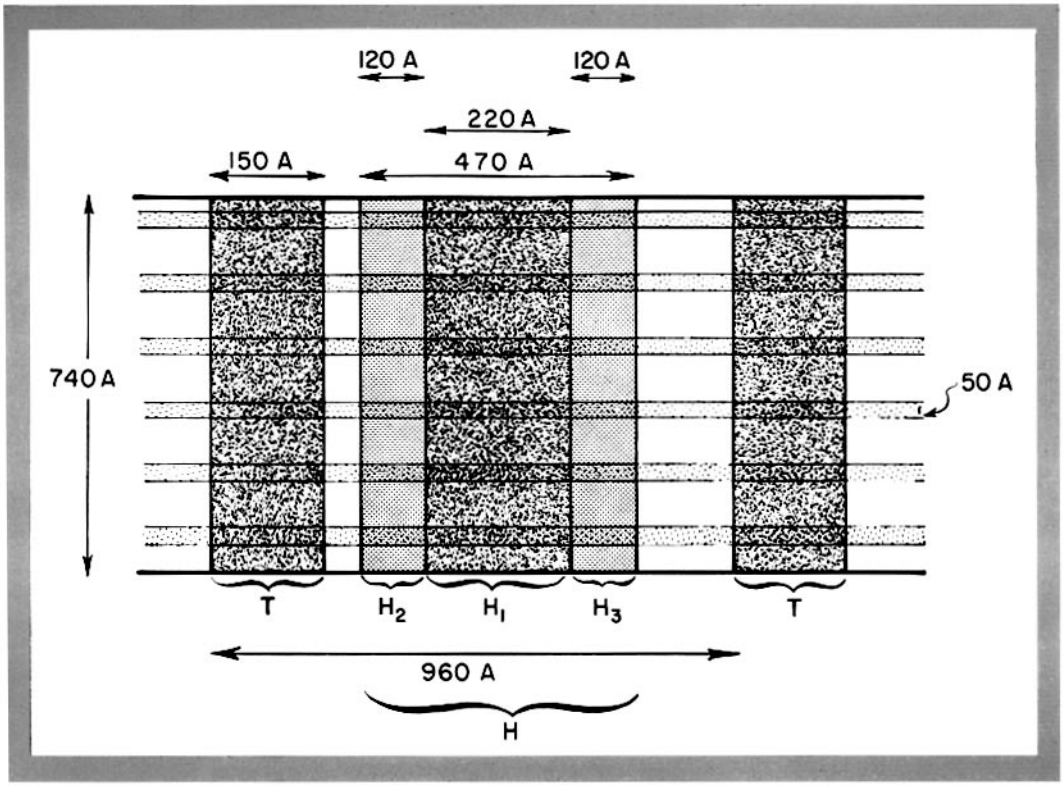


FIGURE 1

Diagram illustrating the longitudinal fibrils and pattern of transverse periodicity in the rootlets of the cilium. The light bands flanking the dense H band (consisting of H_{1-3}) are of variable and unequal width.

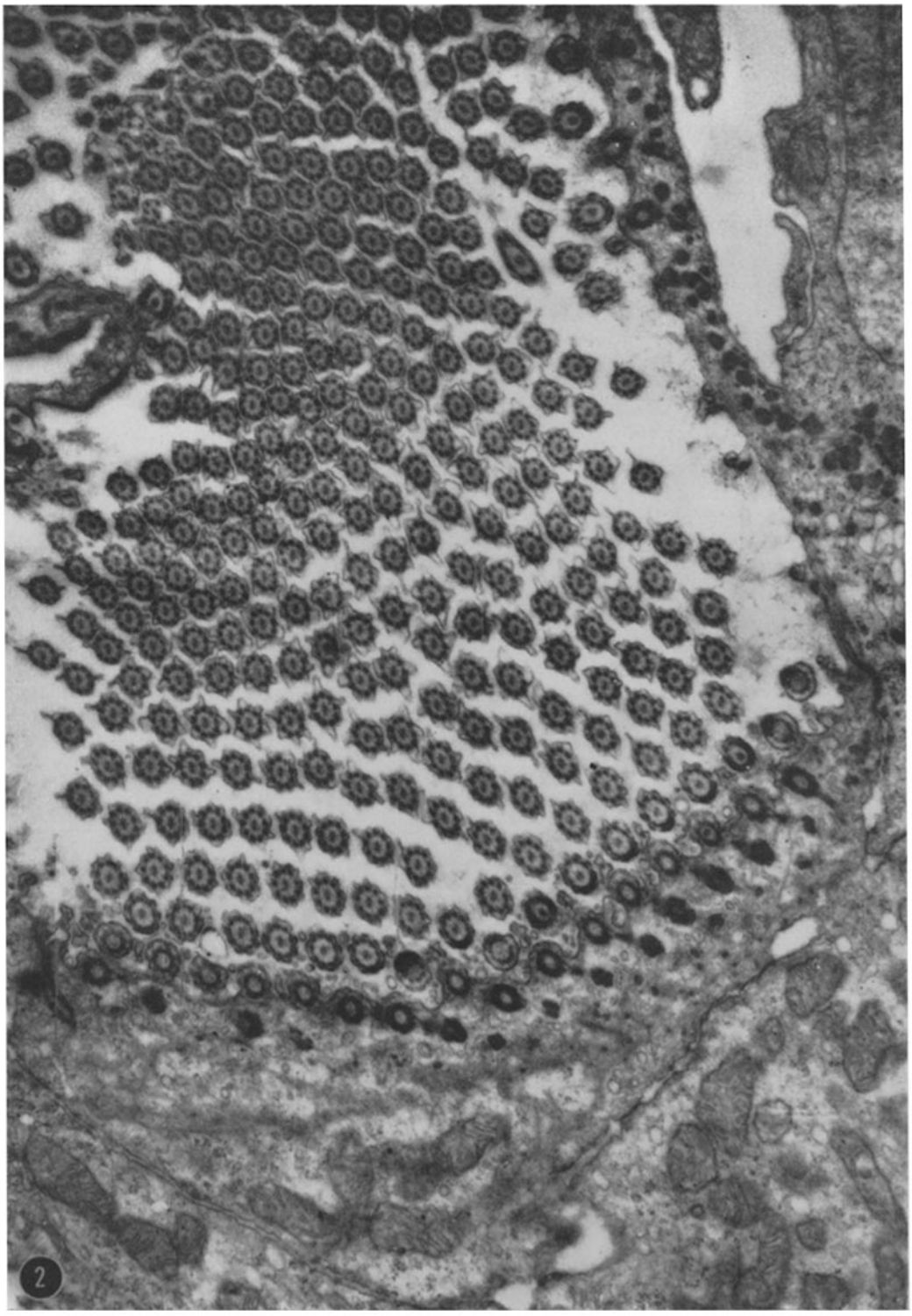
cent unbuffered osmium tetroxide in distilled water was rapidly added. No significant difference was recognized between these two concentrations of osmium tetroxide. Time of fixation varied from 15 to 30 minutes, again with no apparent differences. After thorough washing with distilled water the rotifers were dehydrated in ethanol and embedded in butyl–methyl methacrylate (9:1) in the usual manner, in Vestopal (10), or in Epon 812 (7). It should be noted that although the cilia were reasonably preserved in methacrylate, the bulk of the rotifer body suffered from severe explosion damage. On the other hand, Epon 812 or Vestopal eliminated explosion damage to the cells of the body and also preserved the limiting membrane of the cilia, which frequently was damaged in methacrylate.

Sectioning was done with a diamond knife mounted in the Porter-Blum microtome, and the electron micrographs were taken with a Philips EM-100B using Eastman Kodak spectroscopic 35 mm. film (No. 649-0) or Ilford "photomechanical" lantern slide plates. One electron micrograph was taken with the Philips EM-200 prototype (Fig. 7). The electron micrographs were taken at screen magnifications between 12,000 and 46,000 and photographically enlarged as necessary.

All illustrations refer to *Philodina citrina* and Vestopal embedding except as specifically noted.

FIGURE 2

Electron micrograph of a transverse section through the corona of the rotifer illustrating the relations between the intracellular components of the cilium and the free part of the cilium, as well as the appearance of cross-sections of cilia at different levels. × 26,000.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OBSERVATIONS

Rootlets

The coronal cilia of rotifers possess very well developed single rootlets whose transverse periods fade indistinctly in the region of junction with the basal body. These rootlets appear to have a mean maximum diameter of 740 A with a range of 722 to 758 A. The repeating transverse period, T to T (see Fig. 1), has a mean spacing of 960 A with a range of 922 to 1022 A. Between these narrow T bands of a mean width of 150 A (range 103 to 173 A) there is the prominent H band whose mean width is 470 A (range 427 to 530 A). In suitably thin sections it is possible to resolve three distinct bands in the H band: a thick central band with a mean width of 220 A, designated H_1, is flanked by two bands designated H_2 and H_3 each of which has a mean thickness of 120 A. The H band is not equidistant between the T bands but rather is displaced toward one of these bands so that one band of low electron density between H and T is twice the width of the other.

In several preparations, one of which is illustrated in Fig. 7, we have been able to resolve fine longitudinal fibrils, apparently six in number across the diameter of the rootlet, which are equally spaced. At the point of intersection of these longitudinal fibers, which measure 50 A, and H_2 or H_3 there are slight thickenings which may be due to superposition of longitudinally and transversely disposed material or may constitute actual nodules. Thus far we have not been able to clarify this point.

Basal Body

Every longitudinal section including both rootlet and basal body that we have examined is characterized by a narrow region, at the point of junction of these two structures, in which the transverse periods of the rootlet becomes very indistinct and blend rapidly into a dense fibrous mat characteristic of the wall of the basal body (Figs. 10 and 14). In both methacrylate-embedded and Epon 812-embedded material the medulla of the basal body is characterized by a very low electron density. The basal body characteristically is located in a protuberance of cytoplasm like a hillock.

In suitably thin sections it is apparent that the basal body is a complex structure. The dense fibrous wall is confined to the proximal two-thirds of the basal body, while the distal third is of relatively low density and is composed of longitudinally oriented filaments, nine in number. These filaments are of the same approximate diameter as the filaments of the free part of the cilium and are interconnected by a dense material (Figs. 3 and 6). The nine filaments within the substance of the basal body pass through the basal plate of the cilium and are continuous with the peripheral filaments of the free cilium.

Fig. 4 illustrates the anatomical continuity between the cross-striated rootlet, the basal body, the basal plate, and the peripheral filaments of the free cilium.

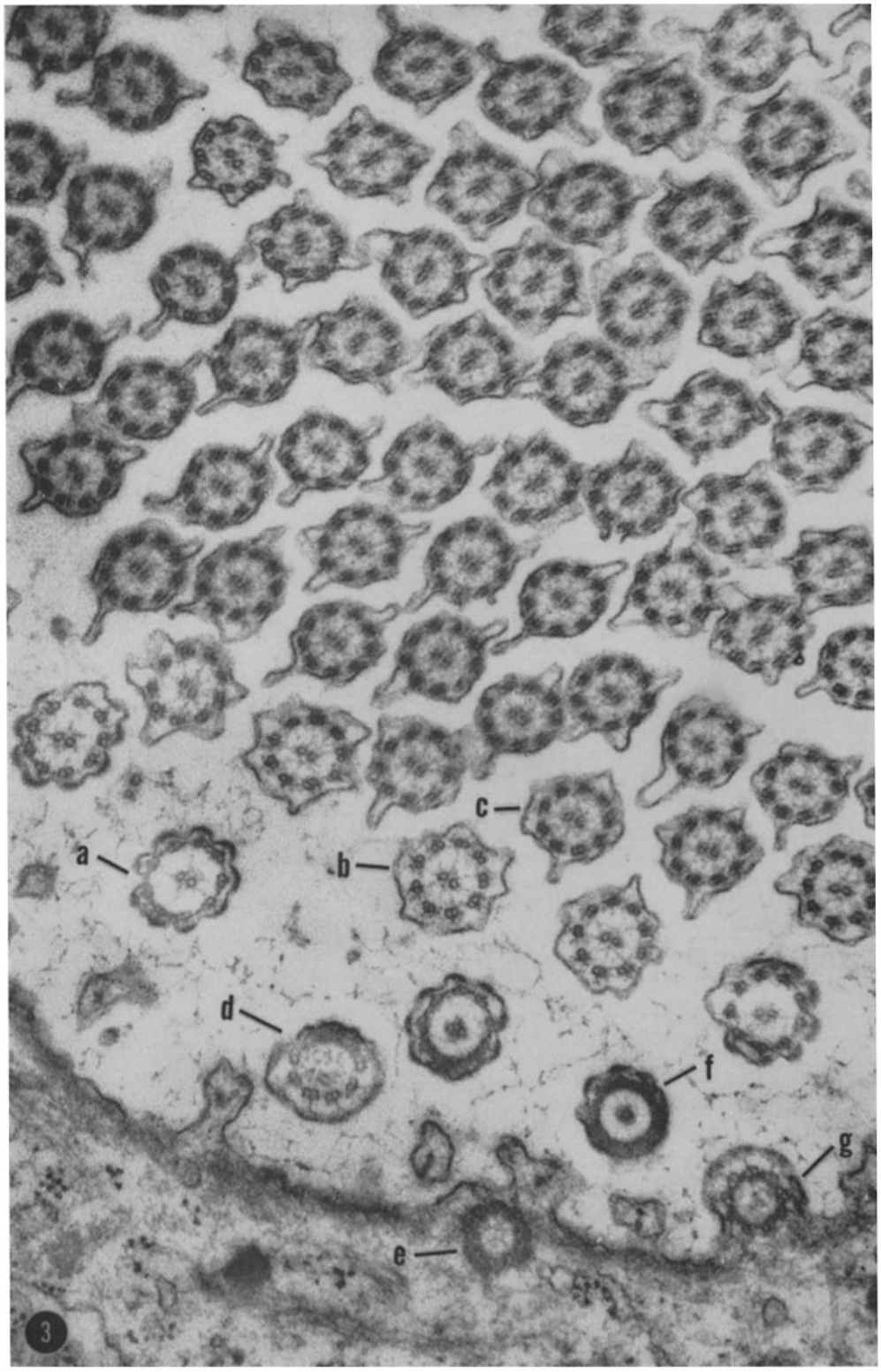
So far as the medullary part of the basal body is concerned, our sections have not enabled us adequately to characterize this region. Specimens fixed in 5 per cent $KMnO_4$ showed a very low electron opacity for the medulla of the basal body. With 1 or 2 per cent OsO_4 the medulla showed indistinct, sparsely distributed granules, while similarly fixed material stained with lead acetate as recommended by Dalton (5) revealed the presence of some strands and granules not unlike material found in the adjacent cytoplasmic substance.

Basal Plate

The basal plate of the cilium is strikingly conspicuous because of its intense density. It is best described as a shallow cup or pan whose bottom is only one-third as thick as the lateral walls. The material which makes up the walls and base of the basal plate appears to be homogeneous.

FIGURE 3

Electron micrograph of transverse sections of cilia illustrating the variations that occur at different levels: *a*, through distal edge of lateral wall of the basal plate; *b*, through bulb at the very base of the free part of the cilium; *c*, through the free part of the cilium; *d*, same as *b* but oblique section; *e*, through wall of basal body, illustrating filaments in cross-section; *f*, through lateral wall of the basal plate; *g*, same as *e* but more distally located (see Fig. 20). × 58,600.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3

As already noted, the peripheral filaments of the free part of the cilium pass through the lateral wall of the basal plate and are continuous with filaments in the basal body. The paired central filaments of the free cilium extend down to, and appear to fuse with, the floor of the basal plate. At the region of junction of the central filaments with the floor of the basal plate there is a slight swelling and increased density. In no case have we observed central filaments extending below the basal plate into the basal body.

Free Cilium

In the main, the structure of the free part of the cilium of the rotifer conforms to that described for other species (Fig. 2), the literature for which has recently been summarized (8). In addition to the established pattern of nine pairs of peripheral filaments and two separated central filaments, we have, in suitable cross-sections, observed the existence of the "arms" and "spokes" described by Afzelius (1) and substantiated by Gibbons and Grimstone (8). The radial distribution of the "spokes" is illustrated in Fig. 8.

Fig. 15 summarizes observations made on methacrylate-, Vestopal-, and Epon-embedded material regarding the relatively fixed radial distribution of the peripheral filaments. Based upon analysis of 18 sections of cilia cut in normal section (as judged by sharp, circular outlining of the pair of central filaments), it seems that the distribution of peripheral filaments conforms well to the pattern suggested by Cleland and Rothschild (4). Projection through the long axis of the central filaments will pass through one pair of peripheral filaments (Fig. 15). Similarly, projection of an axis perpendicular to the long axis through the central filaments will also pass through a pair of peripheral filaments. In keeping with the numbering system of Bradfield (2) and others, the peripheral pair in the perpendicular axis is numbered 1 and the other filament pairs are numbered consecutively 2 to 9 in the direction of the filament pair in the longitudinal axis. Inspection of Fig. 15 further indicates that the positions of filament pairs 2 through 7 are quite fixed, while the positions of pairs 4, 5, and 6 show considerable variability. Also, while the interval between filament pairs 1 and 2, 2 and 3, 5 and 6, 6 and 7, and 7 and 8 is slightly over 40°, the spacing between 3 and 4, 4 and 5, 8 and 9, and 9 and 1 is roughly 10° less. In selecting the 18 sections for measurement, care was exercised not only to use normal sections, but also to avoid compression-distorted specimens which would not exhibit a circular distribution of the peripheral filaments. This observation is consistent with that of Cleland and Rothschild (4), who noted that the peripheral filaments of the bandicoot spermatozoon are not distributed in a "equiangular radial way."

Of further interest in cross-sections of cilia was the observation that the members of the peripheral pairs of filaments are not identical (Fig. 13). One member is slightly larger than the other and truly circular, whereas the smaller member appears to be an arc of a circle with one wall in common with its mate. In addition, as illustrated in Fig. 17, one member of a pair of peripheral filaments exhibits a greater electron opacity than its mate. Counting counterclockwise it appears that the first member of a pair is the one that exhibits the greater electron opacity.

Figs. 8 and 9 illustrate a phenomenon of consistent occurrence in all sections cut perpendicular to the long axis of the cilium except those at the very base of the cilium (Fig. 16). In sections of the

FIGURE 4

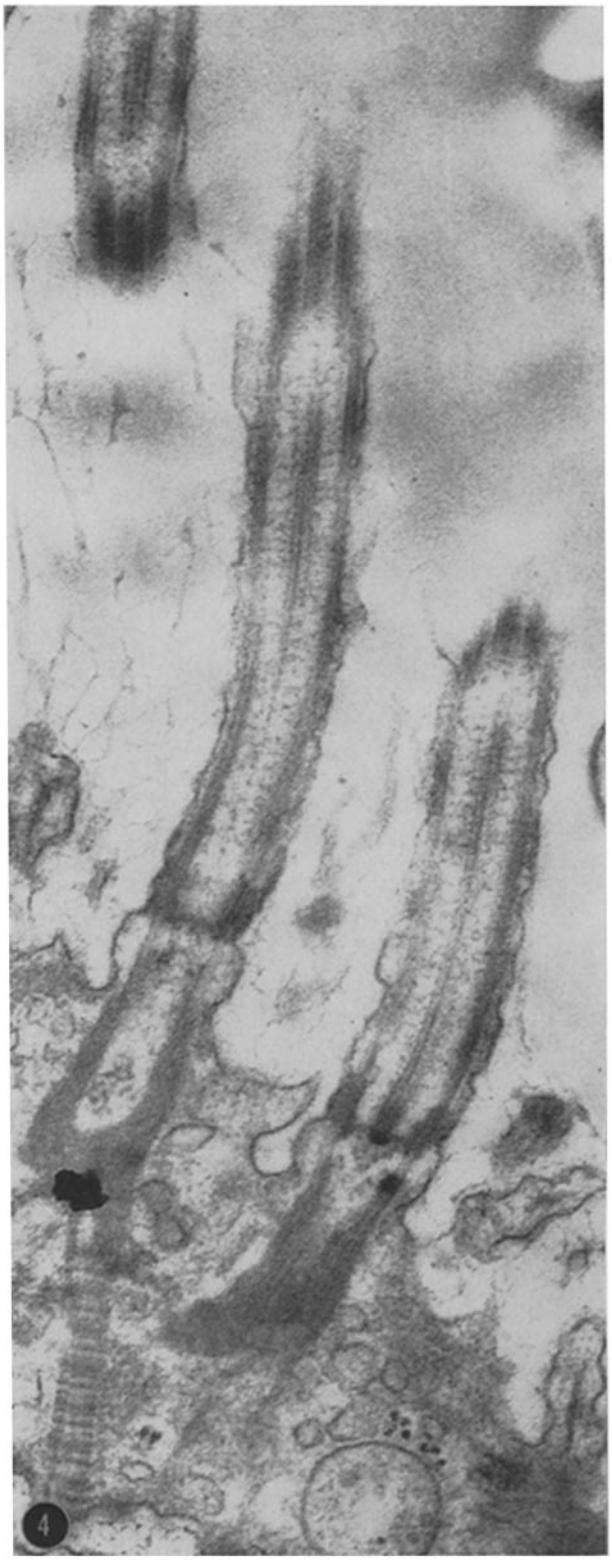
Longitudinal section through two adjacent cilia showing the anatomical continuity between rootlet, basal body, and free cilium. × 54,900.

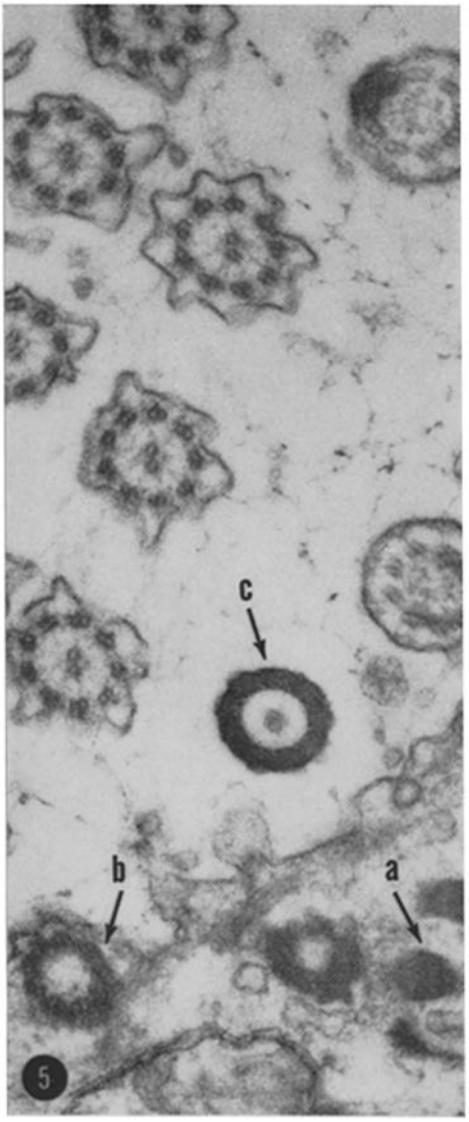
FIGURE 5

Transverse section illustrating the rootlet (*a*), wall of the basal body (*b*), and lateral wall of the basal body (*c*). × 54,900.

FIGURE 6

Transverse section showing the connections between peripheral filaments in the lateral wall of the basal body (*d*) and a section just proximal to the basal plate (*e*). × 40,400.

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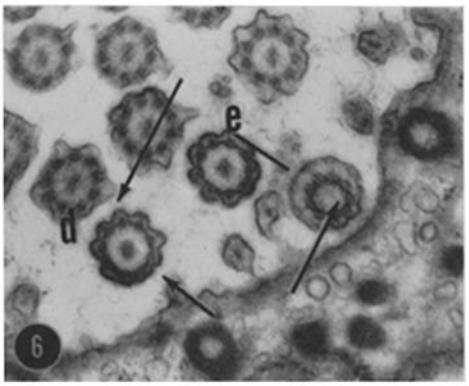
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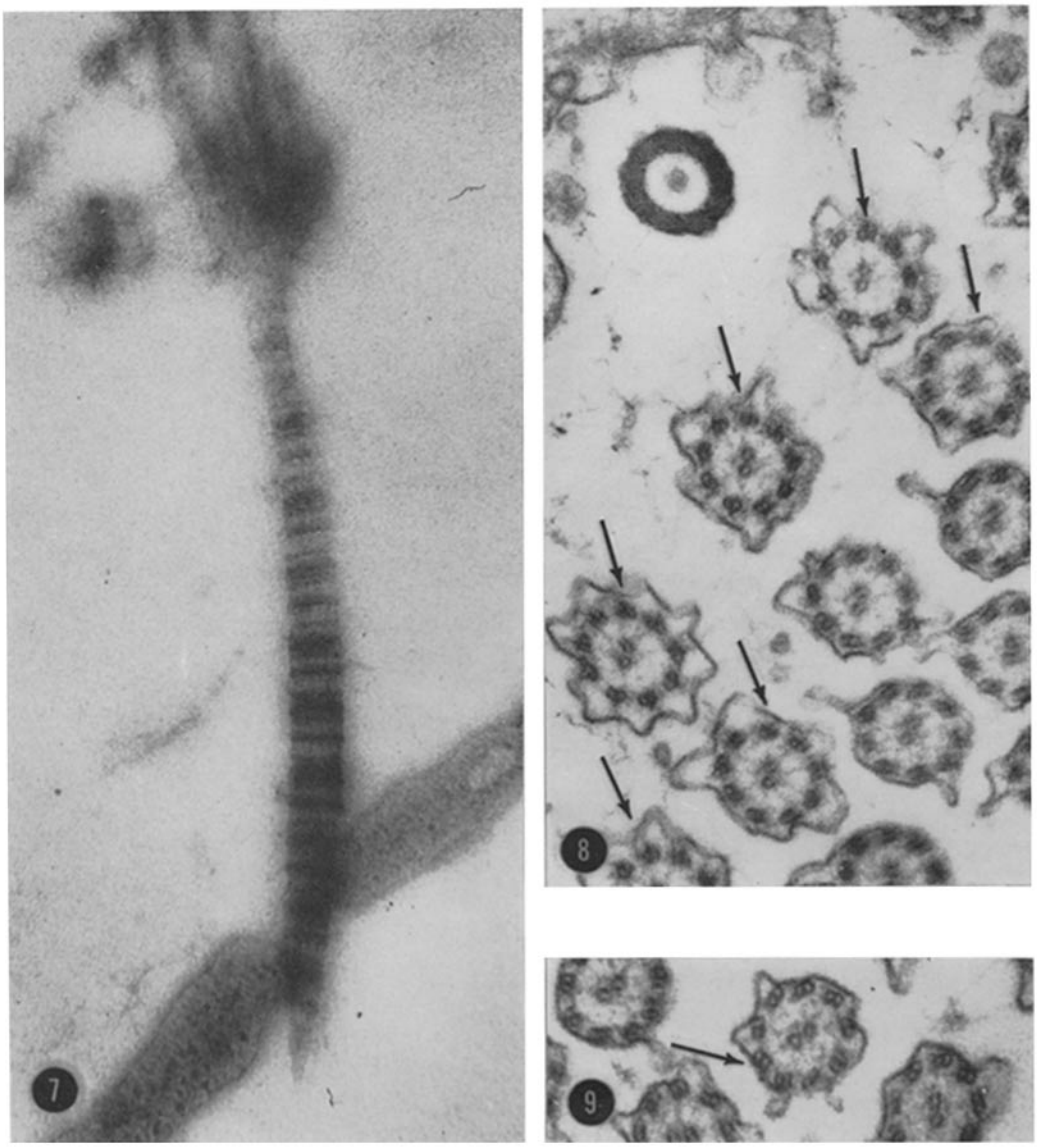


FIGURE 7

Longitudinal section through a rootlet illustrating the longitudinal fibrils and transverse periodic structure. Epon 812-embedded, Philips EM-200, Eastman Kodak medium contrast plate. × 93,600.

FIGURES 8 and 9

Transverse sections through free parts of cilia illustrating the almost semicircular differences in appearance of peripheral filaments (arrows). Peripheral filaments in one semicircle are sharply outlined while the filaments in the opposite semicircle are indistinct. × 56,000.

latter, the central pair of filaments and all nine pairs of peripheral filaments are sharply outlined as would be expected in a normal section. In all other sections it seems clear that peripheral filaments 9, 1, 2, 3, and 4 differ in appearance from filaments 5, 6, 7, and 8. When filaments 9 to 4 inclusive appear sharply outlined along with the pair of central filaments, filaments 5 to 8

inclusive are indistinct and disoriented. Conversely, if filaments 9 to 4 are indistinct and poorly outlined, filaments 5 to 8 are well defined. This phenomenon is observed only in truly normal sections.

In longitudinal sections, the central filaments are readily recognized by the presence of four distinct walls (Fig. 11) and the peripheral filaments are distinguished by the existence of three walls (Fig. 12), indicating that one wall is common to the two members of the pair.

Favorable sections have consistently shown a suggestion of periodic structure in one member of a pair of peripheral filaments which is difficult to resolve. As illustrated in Fig. 10, the pitch or interval between the centers of the dark bands of the period is approximately 140 A, while the dark band itself is of the order of 40 A. Fig. 18 further illustrates the appearance of this transverse periodicity, which seems, at magnifications of 60,000 to 100,000, to have a definite pitch associated with a helical structure. Photographic enlargement to 500,000 of a selected field illustrated in Fig. 18 results in the pattern of structure shown in Fig. 19. The apparent angle of the helix shown in Fig. 19 is about 60° with respect to the long axis of the filament.

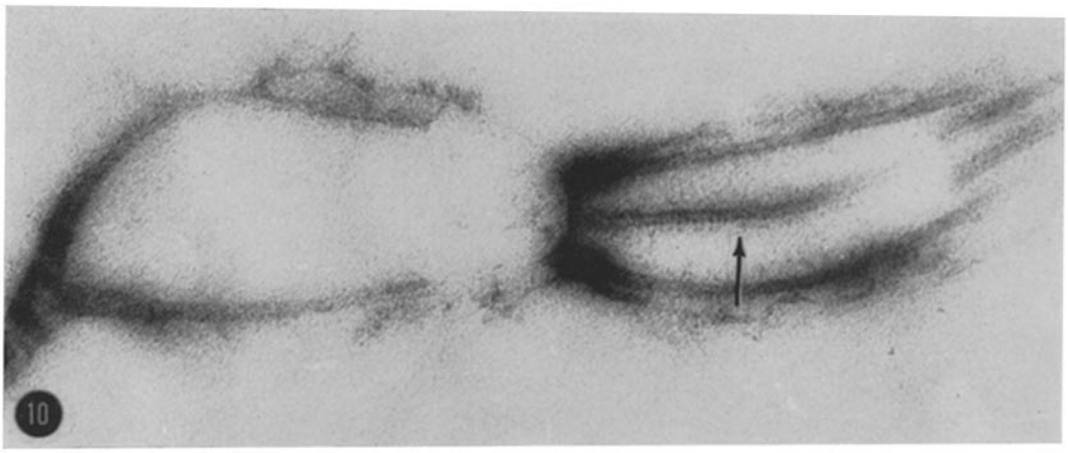
DISCUSSION

As might be expected from the observations of Fawcett and Porter (6), the architecture of the cilium in rotifers conforms in the main to the generalized pattern noted in a number of other species. The fine structural differences from the now classical structure of the cilium are differences of detail rather than principle, but in addition we have noted some structural features which may contribute to an understanding of the factors involved in ciliary beat. Fig. 20 summarizes the general architecture of the cilium of the rotifer.

The cilium of the rotifer possesses a well developed rootlet which is single in *Philodina citrina*, the species principally studied, and forked in other species which were examined casually. The dimensions of the rootlets are somewhat greater than those described in detail by Fawcett and Porter (6). The major repeating period is about 960 A with alternating thick and thin bands measuring 470 A and 150 A respectively. We have also found clear evidence of the presence of fine longitudinal filaments in the rootlet which measure about 50 A. Although not illustrated in this paper, we have found, in several electron micrographs, a network of fine fibers which appear to interconnect the rootlets of adjacent cilia. We have not yet been able to recognize structural detail in these fibers nor have we been able to determine absolute continuity between the fibers and rootlets.

The basal body continues to be an intriguing structure. As noted by Burgos and Fawcett (3) and Sedar and Porter (14), there are anatomical similarities between centrioles and cross-sections of peripheral filaments of the sperm flagellum and cilium, and there is nothing in our observations to gainsay this. Also, there are convincing indications that ciliary basal bodies and centrioles are intimately associated (Inoué, 9). Yet the absolute anatomical continuity between the rootlet, basal body, and free part of the cilium coupled with the clear evidence of continuity between the peripheral filaments of the free cilium, peripheral filaments in the wall of the basal body, and longitudinal filaments in the rootlet make it difficult to visualize the role of the centriole in this system.

Gibbons and Grimstone (8) and Fawcett and Porter (6) have noted the variable structure of the region of junction of the basal body and free cilium. In the rotifer this region of the cilium, which we have designated the corona, is exceedingly dense. In both longitudinal and cross-sections the paired peripheral filaments of the free cilium continue through the coronal wall and extend into the wall of the basal body with diminishing diameters. The pair of central filaments invariably terminate abruptly in the basal plate of the corona. As illustrated diagrammatically in Fig. 20, the peripheral filaments in the region immediately distal to the lateral walls of the basal plate are interconnected by dense material. It is also in this region that cross-sections of the cilia show a very low electron density in all the peripheral filaments. In contrast to this, the remainder of the free parts of the cilium, in cross-section, show marked electron density in one of the paired peripheral filaments. Thus far, we have consistently found that the dense member of the pair is the first one encountered in counterclockwise order. Obviously, if the section were inverted, symmetry would result in a clockwise rather than counterclockwise order. Since we have not observed this, and since there is no randomness in the technique of mounting sections on a grid, it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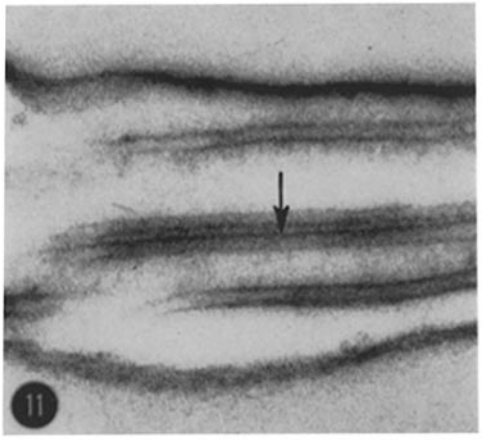
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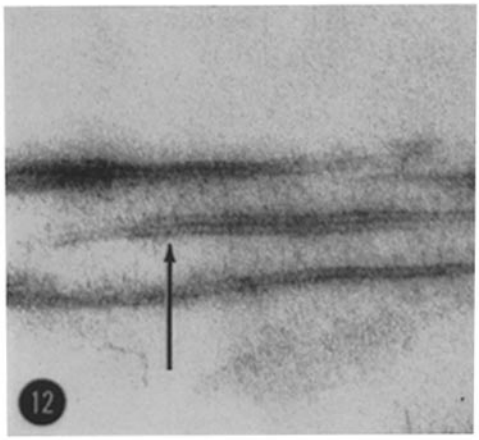
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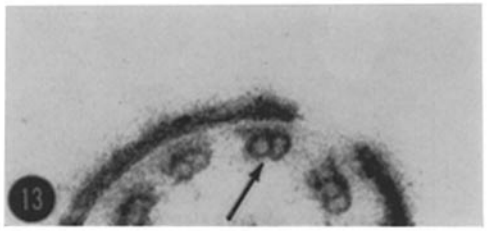
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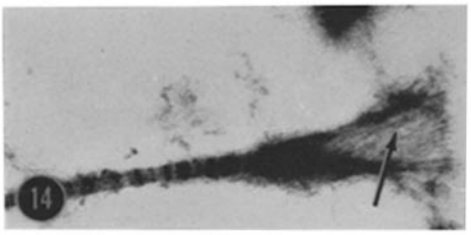
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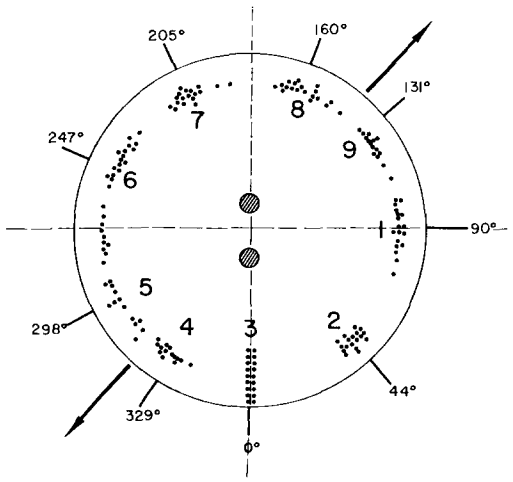


FIGURE 15

Graphic illustration of the radial distribution of peripheral filaments of the cilium. Note that one pair of peripheral filaments is in the same plane as the longitudinal axis through the central filaments and another is in the perpendicular axis (filament 1). Arbitrarily we chose to measure all angles from filament 3 because the latter is readily located. Arrows indicate axis of semicircular differentiation.

would seem that there is a preferential orientation of rotifers in the block. This might well occur during sedimentation of the animals in the unpolymerized plastic.

The proximal part of the free cilium differs from the remainder of the cilium in another respect. In normal sections, sections cut perpendicular to the long axis of the proximal part of the cilium (the swollen base of the cilium) show all central and peripheral pairs of filaments in sharp outline (Fig. 16). This does not occur in the principal length of the free cilium (Figs. 3, 5, 8, 9, and 17). Here we find a consistent, almost semicircular difference in appearance of the peripheral filaments which is not a function of angle of section, astigmatism, or embedding matrix. Angle of section is not a factor, since the cross-sectional shape of the cilium is essentially round, not elliptical; astigmatism is not a factor, since individual peripheral filaments are sharply outlined; and embedding matrix is not a factor, since the same phenomenon has been observed with three different embedding matrices. As described earlier, peripheral filaments 9 to 4 differ significantly in appearance from filaments 5 to 8 in normal sections. In some sections filaments 9 to 4 are sharply outlined like the central filaments, while 5 to 8 are markedly disoriented and indistinct; in other sections the converse is true. One formal interpretation of this phenomenon, which we have also noted in electron micrographs published by others, is that the peripheral filaments of one half of the cilium are in extension

FIGURE 10

Figures 10 to 14 illustrate butyl–methyl methacrylate–embedded specimens.
Longitudinal section through basal body and free part of cilium showing transverse "banding" in peripheral filament (arrow). × 60,700.

FIGURE 11

Longitudinal section through central filaments of free cilium illustrating separation of the filaments (arrow). × 79,200.

FIGURE 12

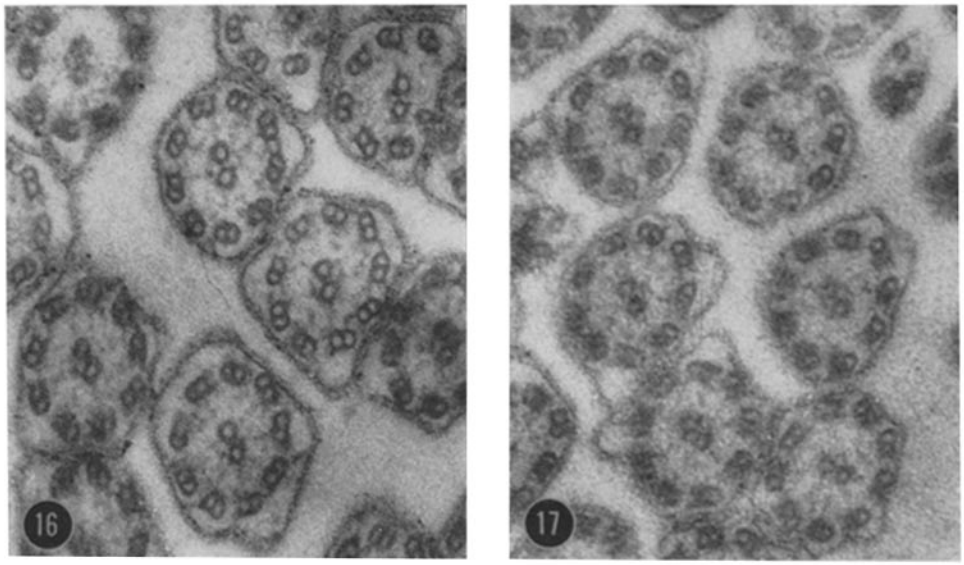
Longitudinal section through peripheral filaments. Arrow points to area in which the common wall between the pair of filaments can be clearly distinguished. × 50,600.

FIGURE 13

Transverse section through peripheral filaments illustrating the common wall between a pair of filaments (arrow). × 110,500.

FIGURE 14

Longitudinal section through a rootlet and basal body illustrating the fibrous nature of the wall of the basal body (arrow). × 37,500.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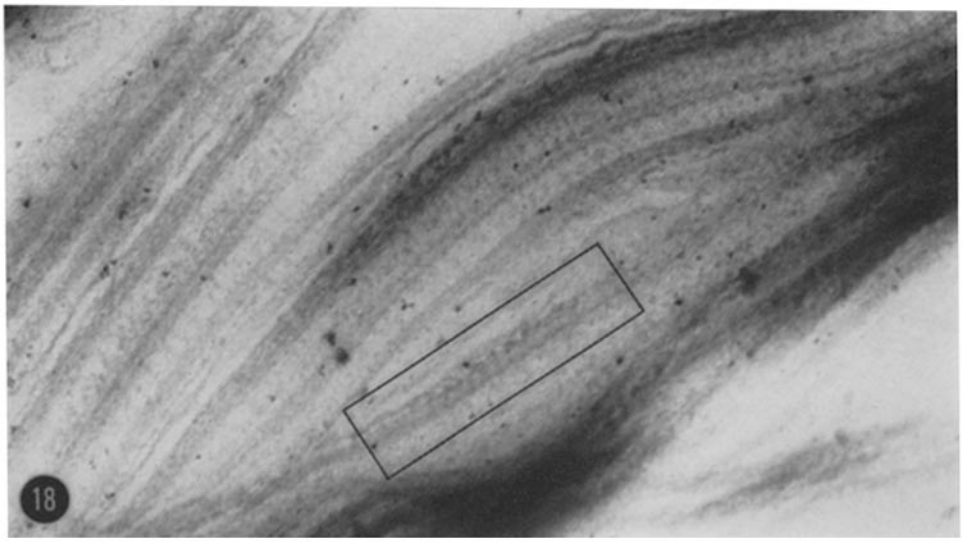
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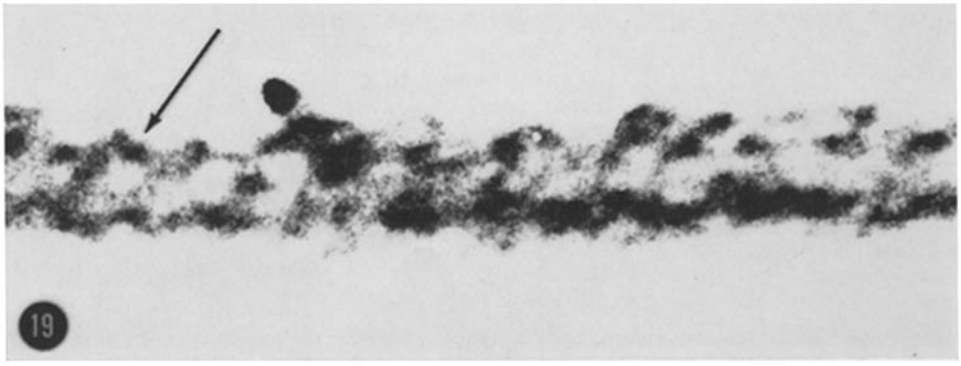
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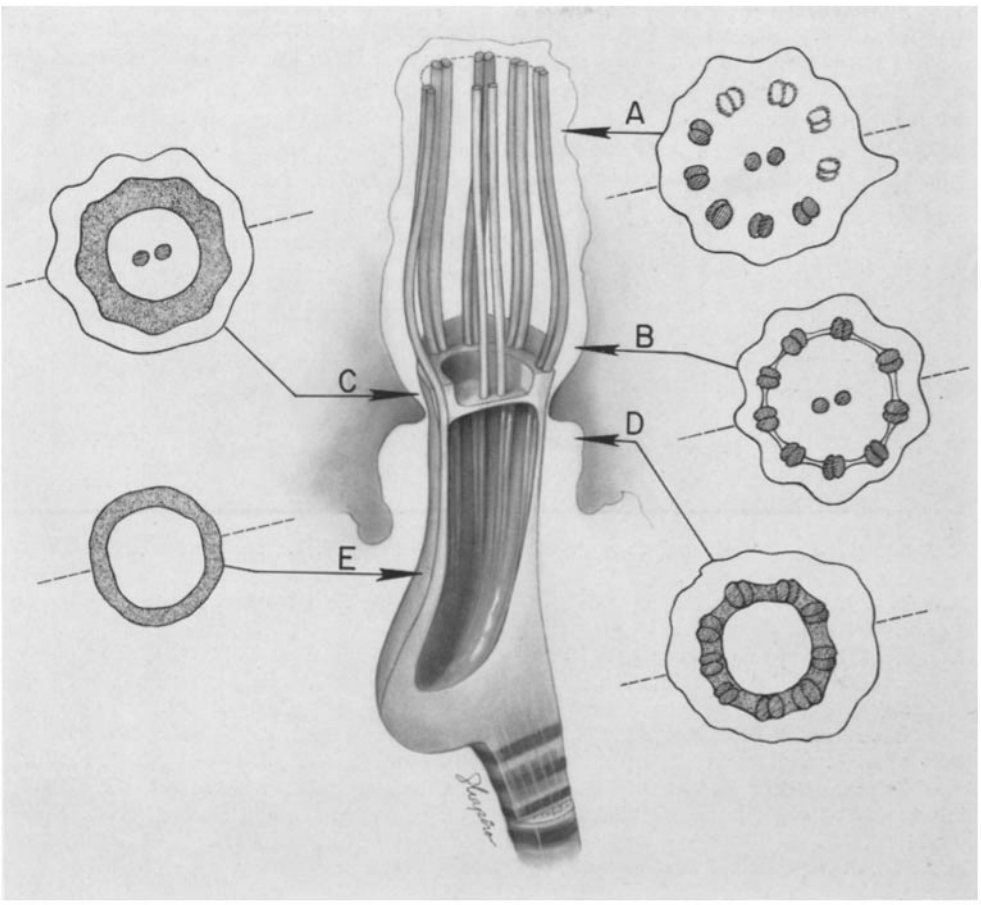

FIGURE 20

Semidiagrammatic reconstruction of the rotifer cilium in longitudinal and transverse axes. Note that the central filaments, unlike the peripheral filaments, do not extend into the wall of the basal body.

FIGURE 16

Transverse section through the bulbar base of the free part of the cilium. In this region all peripheral filaments are sharply outlined in the normal section and are of equally low electron opacity. × 104,000.

FIGURE 17

Transverse section through the main body of the free cilium illustrating the difference in electron opacity between members of a pair of peripheral filaments. × 104,000.

FIGURE 18

Longitudinal section through free cilium. Area in enclosure is enlarged in Fig. 19. × 107,000.

FIGURE 19

Enlargement of area shown in Fig. 18 illustrating the existence of a helical structure (arrow) within the peripheral filament. × approximately 500,000.

while those of the other half are in contraction. This would result in loss of parallel relationship between filaments as well as possible differences in crystallinity between filaments which are extended or contracted. In the light of this observation it is clear that the plane of beat of the cilium is not perpendicular to the plane of the two central filaments, but rather makes an angle of approximately 40° with the accepted plane of beat as described by Fawcett and Porter (6) and Bradfield (2).

It is not difficult to visualize the possibility that a pendular beat by the cilium could be derived from a contraction of peripheral filaments in one semicircle of the cilium with a corresponding extension of peripheral filaments in the opposing semicircle. The observation of a helical structure in longitudinal sections of the peripheral filaments is consistent with the possibility that a contractile and expansile system does exist in the peripheral filaments.

Current studies are being conducted to explore further the nature of the helical structure in the peripheral filaments and to determine the possible influence of this system on ciliary beat.

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