

ULTRASTRUCTURE OF CILIARY MICROTUBULES AFTER CRITICAL POINT DRYING

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INTRODUCTION

Many studies have been conducted on the fine structure of the "9 + 2" microtubules from cilia and flagella. The techniques used include both thin sectioning of whole cilia or flagella and negative staining of isolated microtubules. In this study, cilia from four diverse species of ciliate protozoa: *Euplotes eurystomus*, *Spirostomum ambiguum*, *Tetrahymena pyriformes*, and *Colpoda*; and sperm from the insect *Oncopeltus fasciatus*, were prepared by spreading on a Langmuir trough (12) followed by drying from liquid CO₂ (2). The combined procedures (5) will hereafter be referred to as LTCD. In each case, small spherical periodic masses occur attached to one of the doublet peripheral microtubules (11).

MATERIALS AND METHODS

Cells from which the cilia or flagella were studied were placed between two clean glass slides and ground by circular rotation. Both slides were then dipped into the clean water surface of a Langmuir trough on which a film of cellular contents including cilia or flagella readily spreads. The surface film was picked up on stainless steel, Formvar-coated, carbon-stabilized grids by touching them to the water surface. They were stained in 0.2% uranyl acetate and dried from liquid CO₂ by the critical point method. Cilia from all organisms were studied without fixation prior to the spreading procedure mentioned above, while *E. eurystomus* was also studied after fixation in osmium tetroxide or glutaraldehyde. In some cases, the grids were shadowed at an angle of 1:2 with platinum. All figures except Figs. 7 and 8 were calibrated against a

carbon grating of 54,800 lines/inch. Grids were examined with either a Hitachi HU 11-A or J.E.M. 7A electron microscope.

RESULTS

In each organism in which there was no fixation, ciliary microtubules tend to separate from each other but show no clear substructure such as the protofibrils (16, 3). The microtubules consistently possess nodules, possibly the arms which occur on the "A" microtubule (1). Nodes do not appear to be doublet structures in these preparations. Typical micrographs for each organism are those of Fig. 1 (*E. eurystomus*), Fig. 2 (*S. ambiguum*), Fig. 3 (*Colpoda* sp.), Fig. 4 (*T. pyriformis*), and Fig. 5 (*O. fasciatus*—sperm). A high magnification view of an unusually well-dried preparation from *T. pyriformis* (Fig. 6) shows the doublet microtubule and nodes. Nodes on microtubules which have remained in close association after LTCD appear

oriented in a straight line (Fig. 4, *inset*; Fig. 5). In each case, the line is at an angle of about 30° from a perpendicular to the long microtubule axes. Approximate dimensions of microtubules, their nodes, and spacing of nodes are presented in Table I.

An additional feature is the occasional presence of fine strands (Figs. 2, 3; arrow) interconnecting fibrils. These often, though not always, are located at points adjacent to the nodes, and may represent the fibrous connection shown by many workers, i.e., Gibbons (6), and recently Williams and Luft (17) in cross-sections of *Tetrahymena* cilia.

When *E. eurystomus* is fixed by fuming in osmium tetroxide prior to spreading, the resulting ciliary microtubules (Fig. 7) do not possess nodes. They also have a more rigid appearance than for unfixed material. Fixation of the same organism in glutaraldehyde followed by LTCD routinely reveals

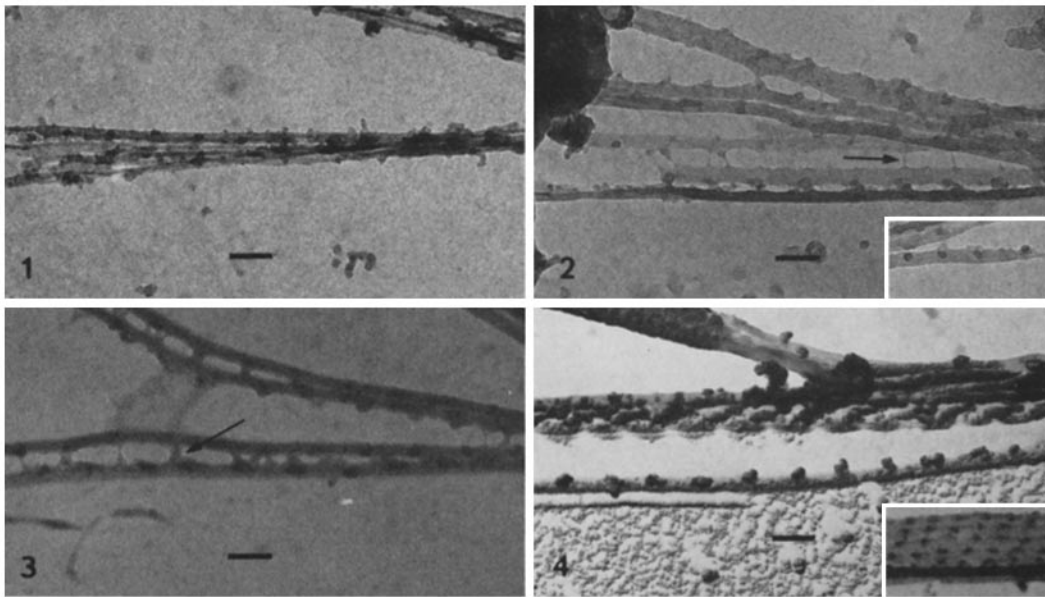


FIGURE 1 Peripheral ciliary microtubules from *E. eurystomus*. $\times 60,000$. Bar length equals 0.1μ in all photographs.

FIGURE 2 Ciliary microtubules from *S. ambiguum*, showing fine strands (arrow) interconnecting microtubules of cilia. *Inset*, clear view of nodes on a microtubule. $\times 60,000$.

FIGURE 3 Ciliary microtubules from *Colpoda*. Strands are seen interconnecting microtubules (arrow). Some beam contamination is apparent, and nodes are not so clear as in other preparations. $\times 60,000$.

FIGURE 4 Shadowed, reversal-printed ciliary microtubules from *T. pyriformis*. *Inset*, low-magnification view showing linear orientation of nodes. $\times 60,000$.

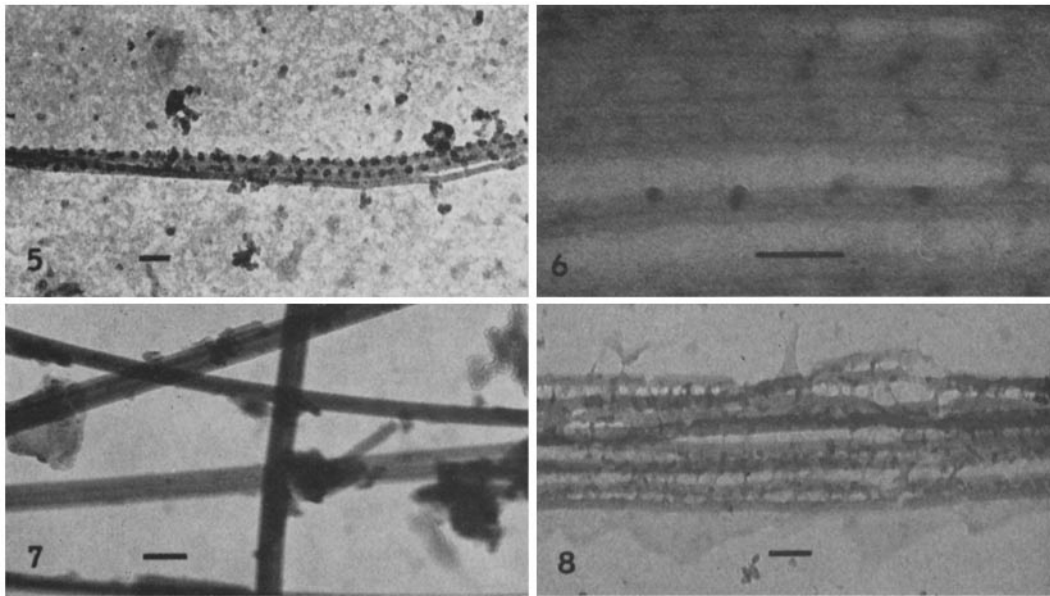


FIGURE 5 Nodes and microtubules from sperm of *O. fasciatus*. $\times 41,000$.

FIGURE 6 High magnification view of ciliary microtubules from *Tetrahymena* showing doublet tubules and several nodes. $\times 125,000$.

FIGURE 7 Smooth microtubules from cilia of *E. eurystomus*. Fixation by OsO_4 fuming. Several microtubules are in close, side-by-side association. $\times 60,000$.

FIGURE 8 Glutaraldehyde-fixed ciliary microtubules from *E. eurystomus*. $\times 60,000$.

TABLE I
Microtubule and Node Measurements

Organism	Microtubule diameter	Nodes		
		Length	Width	Center-to-center
	m μ	m μ	m μ	m μ
<i>Euolotes</i>	22	22	19	92
<i>Spirostomum</i>	28	20	20	75-100
<i>Colpoda</i>	20	46	27	110
<i>Tetrahymena</i>	31	33	30	100
<i>Oncopeltus</i> (sperm)	24	25	25	44

ciliary microtubules (Fig. 8) with poorly defined nodal regions, and many interconnecting strands.

DISCUSSION

It seems reasonable that the nodular periodicities on microtubules of cilia and flagella are the "arms" first incompletely described by Manton and Clarke

(15). Kraneveld (13) air-dried flagella from *Trypanosoma evansi* and made note of cross-striations with a 50 m μ period on the subfibrils; possibly these, too, were arms. Afzelius (1) first designated them as arms and indicated that they were attached to subfibril A of the doublet. The subfibrils (microtubules) with nodes were first obtained after LTCD preparation by S. L. Wolfe (who kindly contributed Fig. 5) while studying sperm from *O. fasciatus*.

Arms were studied in sectioned material of *Pseudotriconympha* by Gibbons and Grimstone (9) and described as double structures which were 12-15 m μ long by 5 m μ wide with a center-to-center spacing of 13 m μ . Recently, a number of investigations have shown a variety of armlike fibrils radiating from cytoplasmic or nuclear microtubules. These often form a complex interconnecting network (see Wilson, 1969). Gibbons and Rowe (10) isolated the arms from *Tetrahymena*, and found 14S and 30S fractions after sedimentation. The 14S fraction consisted of spherical entities measuring

7–10 μ high and 18 μ wide. They postulated that these polymerize to form the 30S fraction which was between 40 and 50 μ long with nodes of 13 μ center-to-center spacing. It is of particular interest that this period corresponds well with that mentioned above for the flagellate protozoan *Pseudotrichonympha*. Crane fly spermatids isolated and negatively stained by Behnke and Forer (4) had “regularly spaced projections . . . 600 Å apart” on the microtubules and are somewhat similar to the LTCD nodes. In their Fig. 24, however, adjacent nodes appear to be almost touching. Microtubules of the flagellar shaft from developing chicken sperm recently have been reported to have projections with a period of 24 μ (14). Whether this was sectioned or isolated-dried material was not mentioned. In the LTCD preparations shown here, the microtubules possess nodes of variable dimensions: 20–46 μ length (dimension parallel to long microtubule axis), 19–30 μ width, and a center-to-center distance of 44–110 μ . These nodes are considerably wider than the arms described by Gibbons and Grimstone, yet they are strikingly similar to the nodular appearance of the isolated 14S fraction. The internode distance is $3\frac{1}{2}$ –8 times greater than that for the arms described by earlier workers.

Among the possible explanations for the apparent discrepancies in center-to-center spacing of nodes are: (a) Nodes in LTCD preparations are not arms but some other structure. (b) Some arms, occurring at periodic distances, are of a somewhat different nature than the others or are bound to the microtubule differently, and they alone are retained during the preparation. In this regard, Gibbons (8) reported that arms are readily detached during isolation and negative staining of cilia from *Tetrahymena pyriformis*. This thesis may also be partially supported by the lack of arms or nodes in LTCD preparations of rat sperm tail (19). (c) Different cilia have arms with different periods. This is clearly the case for the nodes seen after LTCD preparation. A similar variation in center-to-center spacing has not been clearly shown for thin-sectioned material and may be resolved after careful sectioning of cilia from each organism. Comparison of the work of Gibbons and Rowe (10) with the present study suggests a real discrepancy in arm-to-arm distance for *Tetrahymena* prepared by different methods—13 μ in the isolated fraction and 110 μ after LTCD. (d) The peripheral

fibrils are stretched during LTCD preparation—though the degree of stretching required for the observed periodicity is very great. The first and fourth explanations are unlikely, but which of these, if any, is the answer awaits subsequent investigation.

The second notable discrepancy between this and earlier work is that arms isolated in this way do not appear as double structures. Perhaps, during LTCD the double components collapse upon one another and combine with other cellular proteins to form the rather uniform-looking nodes.

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