

## SUBSTRUCTURE OF FLAGELLAR TUBULES

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The 9 + 2 array of ciliary and flagellar tubules was described in the early days of electron microscopy when osmium tetroxide was the standard fixative. Cytoplasmic microtubules, which are not generally in evidence after osmium tetroxide fixation, were rarely seen until after the advent of aldehyde fixatives. In some glutaraldehyde-OsO<sub>4</sub>-fixed material, flagellar and ciliary tubules are

more electron-dense than microtubules (3). From their differing fixation characteristics, one must infer that microtubules differ chemically from ciliary and flagellar tubules; nevertheless, one is struck by the morphological and functional similarities between them. The tubules of the 9 + 2 complex of cilia and flagella have approximately the same diameter as cytoplasmic microtubules. Microtubules, like flagellar tubules, may be concerned with cellular movement; in some cases, sperm apparently are propelled by a microtubular rather than or in addition to a flagellar system (1, 2). In early spermatids of the fungus gnat *Sciara*, tubules forming the axial complex are initially indistinguishable in their fixation characteristics from microtubules in the adjacent cytoplasm, but during spermiogenesis they gradually acquire the fixation characteristics of flagellar tubules (3). This suggests that the two types of tubule may be initially similar.

Subunits have been discerned in both microtubules and flagellar tubules, and the number of units per tubule has been reported to be different in the two types. Ledbetter and Porter found, by examining thin sections of meristematic cells of *Juniperus* and *Euphorbia*, that 13 circular subunits could be seen in cross-sections of cytoplasmic microtubules (4). Gall (5) found that negatively stained microtubules of salamander red blood cells are composed of 12, 13, or 14 subunits, but he was unable to verify whether the number is exactly 13. In flagella, on the other hand, Pease found that each tubule of the central pair of negatively stained rat sperm axial complexes consists of 10 filaments (6), and André and Thiéry (7), using similar techniques, determined that each tubule of the central pair of tubules in human

sperm is composed of 10 or 11 subunits which they termed "protofibrilles".

We have examined thin sections of testes of a number of species of Diptera, Coleoptera, Hemiptera, and Homoptera. Testes were fixed in either 1% OsO<sub>4</sub> (pH 7.6) or 2.5% glutaraldehyde (pH 7.0) followed by 1% OsO<sub>4</sub> (pH 7.6). Fixing solutions were buffered in Sorenson's phosphate buffer. The testes were dissected in cold (0 to 4°C) fixative, and subsequent fixation was carried out in the cold. Tissues were dehydrated in cold ethanol and embedded in Epon 812 according to Luft (10). Sections were cut on a Porter-Blum MT-1 ultramicrotome, stained in 3% aqueous uranyl acetate for 12 hours, and examined with a Siemens Elmiskop I.

Many insect sperm have, in addition to the conventional 9 + 2 array of tubules, 9 singlet tubules situated peripheral to the doublets. In some species, these 9 peripheral singlet tubules, as well as the central pair of singlets, contain a central tubule approximately 85 Å in diameter (Fig. 1). Also, in all the insect species examined, two small arms similar to those described by Gibbons and Grimstone (8) occur on subfiber A, but the position and shape of the "spokes" and the arms on subfiber B and on the 9 peripheral singlets vary somewhat from one species to another.

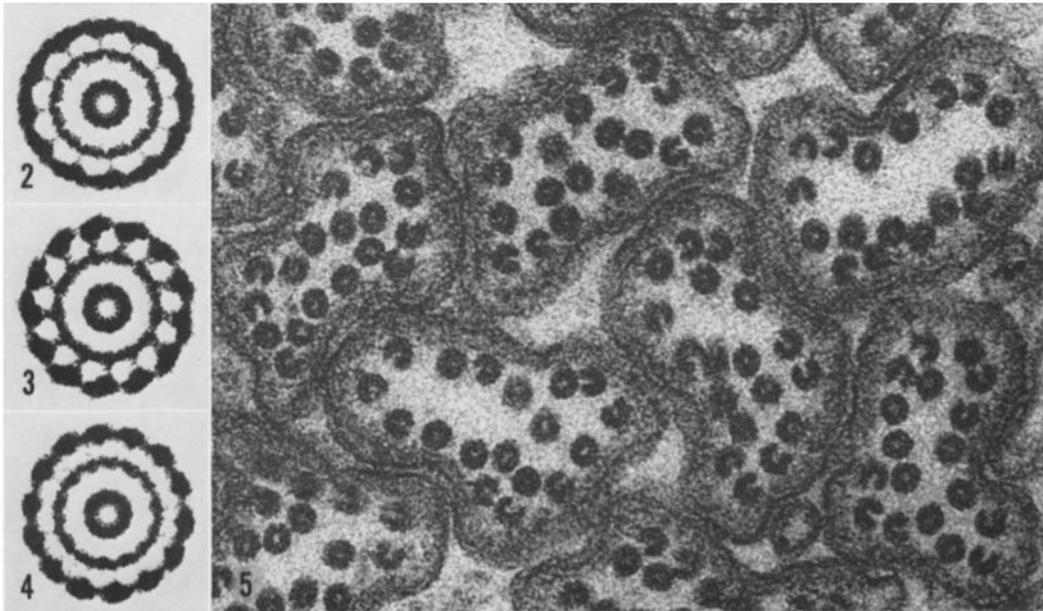
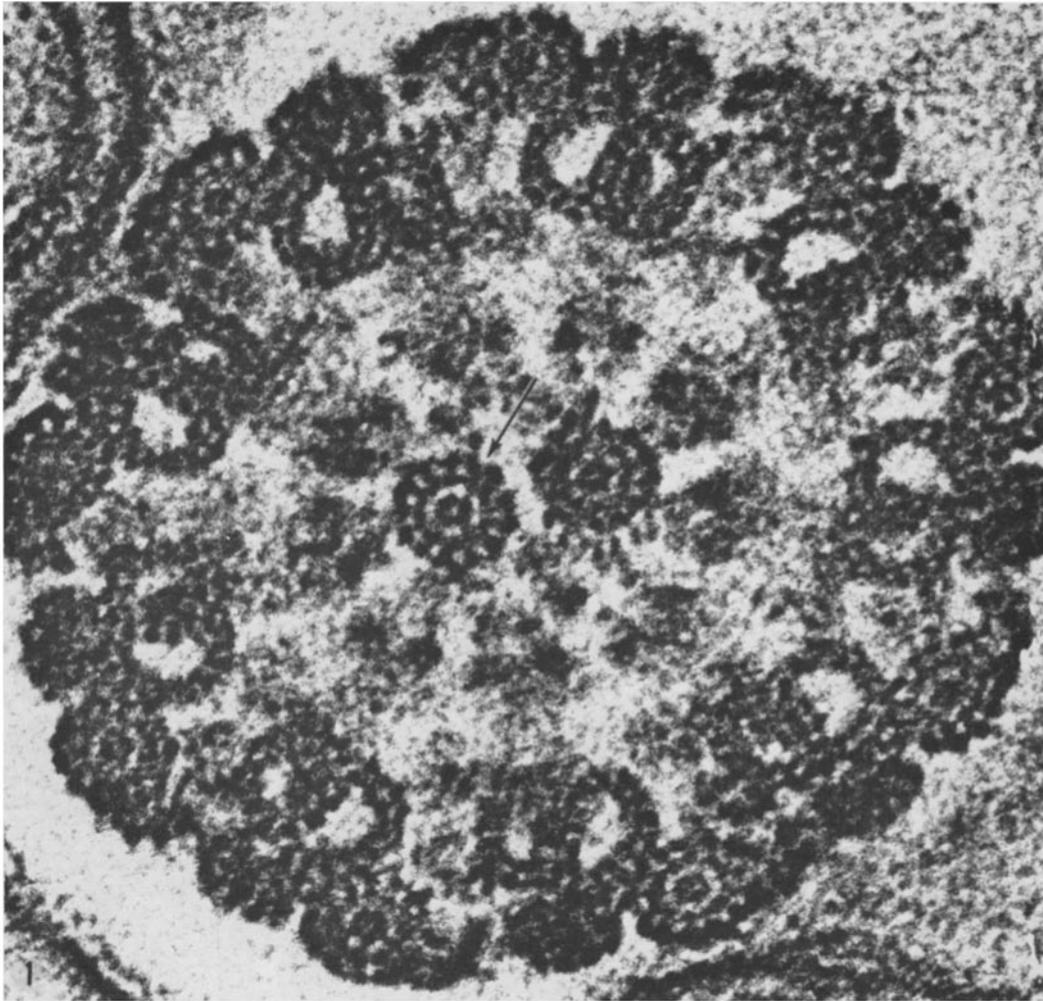
In cross-section, the flagellar tubules appear to be made up of protofibrils with circular profiles similar to those described by Ledbetter and Porter. The protofibrils appear circular in both overfocus and underfocus as well as focused micrographs, which indicates that the circular appearance is not a focus artifact. They are in evidence after either OsO<sub>4</sub> or glutaraldehyde-OsO<sub>4</sub> fixation. Although protofibrils appear circular in transverse

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FIGURE 1 Transverse section of a spermatozoon from the black scavenger fly, *Sepsis*. The flagellar tubules are composed of subunits with circular profiles. The arrow indicates the tubule analyzed by Markham's rotation method. Glutaraldehyde-OsO<sub>4</sub>, uranyl acetate.  $\times 570,000$ .

FIGURES 2 to 4 Markham rotation technique employed on the tubule indicated by the arrow in Fig. 1. (Fig. 2)  $n = 12$ ; (Fig. 3)  $n = 13$ ; (Fig. 4)  $n = 14$ . The greatest reenforcement of the image occurs when  $n = 13$ .  $\times 900,000$ .

FIGURE 5 Transverse section of the posterior portion of several spermatozoa from the leafhopper *Draeculacephala*, where subfiber A and subfiber B of the doublet have separated from each other. One of the separated subfibrils appears in cross-section as a circle whereas the other is "C"-shaped. This suggests that the flagellar doublet is composed of one complete tubule and one incomplete, C-shaped tubule. Glutaraldehyde-osmium, uranyl acetate.  $\times 156,000$ .



section, they may not actually be tubular. It is possible, for instance, that the uranyl ions are bound to the periphery of a subunit which is fibrillar rather than tubular. Each protofibril is approximately 70 Å in diameter and possesses an electron-lucid center 30 to 40 Å in diameter (Fig. 1). In favorably aligned central or peripheral singlets, 13 protofibrils are distinguishable. The image of the 13 protofibrils may be reinforced by employing the Markham rotation technique (9) (Figs. 2 to 4). This is further evidence of a similarity between cytoplasmic microtubules and flagellar tubules. However, the findings of Pease and of André and Thiéry suggest that generic differences in number of protofibrils exist.

Even in favorably oriented doublet tubules of the axial complex, it has not been possible to count the number of protofibrils. This is partially due to the difficulty of deciding which of the protofibrils in the area where the two tubules of the doublet are contiguous should be ascribed to which tubule of the doublet. This area where the two tubules are in close association consistently appears thickened, often giving the appearance that the two tubules overlap. This suggests that the doublet is composed of two complete tubules, rather than one complete and one incomplete tubule.

In spermatozoa of the ladybird beetle, *Hippodamia*, and the leafhopper, *Draeculacephala*, subfiber A of the doublet is separated from subfiber B in regions posterior to the point where the peripheral 9 singlets and the central pair have terminated. In these posterior regions of the sperm, the 9 doublets appear in cross-section as 9 complete circles and 9 "C's" (Fig. 5). In ladybird beetle sperm, it is possible to ascertain that the "C"-shaped member is derived from subfiber B of the doublet, whereas the complete tubule is subfiber A. This suggests that, in spite of their appearance, doublets are composed of one complete tubule and one incomplete tubule.

The flagella of insect sperm appear to possess two classes of tubules. One class includes the central pair and the peripheral singlets. These

tubules are composed of 13 protofibrils, and in some species contain a small central tubule. The other tubule type is found in the doublets which are usually closely associated but in some cases disassociated into one complete and one partial tubule. The difference in morphology between the two classes of tubule suggests a difference in the role they play in flagellar movement.

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