

## MODIFIED MICROTUBULES IN THE TESTIS OF THE WATER STRIDER, *GERRIS REMIGIS* (SAY)

F. RUDOLF TURNER. From the Cell Research Institute, The University of Texas at Austin, Austin, Texas 78712. Dr. Turner's present address is the Department of Zoology, Indiana University, Bloomington, Indiana 47401.

### INTRODUCTION

Microtubules are generally considered to be unbranched, tubular structures consisting of a dense wall surrounding a lumen of lower electron opacity. Exceptions to this simple tubular morphology include the doublet microtubules of cilia

and flagella (12), and the triplet microtubules of centrioles (8, 29) and basal bodies (8). Diameters given for most cytoplasmic microtubules fall in the range of 200–250 Å. However, reports of both larger and smaller diameters are not uncommon (5). They have been the subject of a number of

recent reviews to which the reader is referred for additional information on microtubule structure and function (4, 5, 19, 23, 31).

Occasional examples of partial microtubules (C microtubules) are seen in the literature (17, 21). In most instances these appear to be transitory structures associated with microtubule formation or degradation (21, 27). Arms are present on ciliary doublet microtubules (1, 12) but they are chemically distinct from microtubular protein (10, 11).

The present study describes a population of microtubules which exhibit structural modifications of conventional microtubule morphology.

#### MATERIALS AND METHODS

Adult specimens of *Gerris remigis* (Say) were collected locally in the Austin, Texas area. The testes were dissected out and fixed in a mixture of 4% glutaraldehyde and 0.7% picric acid buffered with 0.1 M sodium cacodylate (pH 7.2). After fixation at room temperature for 0.5–8 hr the material was rinsed with buffer and postfixed for 0.5–2 hr in the vapor of a 2% OsO<sub>4</sub> solution. After a brief water wash the testes were stained in 0.5% uranyl acetate (6 hr, 4° C), dehydrated in an ethanol series, and embedded in a mixture of Epon and Araldite (mixture No. 1 of Mollenhauer [14]). Thin sections cut on a diamond knife were stained with uranyl acetate and lead citrate and examined with an RCA-EMU 3G or Hitachi HU-11C electron microscope.

To study the effects of colchicine on the modified microtubules, the testes were dissected out into the glutamic acid–glycine (GG) medium of Shaw (24). The epithelial sheaths of the testes were broken open, and the testes were placed into fresh GG medium containing 0.1 (2.5 × 10<sup>-3</sup> M), 0.2, or 0.4% colchicine. After 4–6 hr the testes were removed from the colchicine and fixed immediately or were allowed to recover by placing into fresh GG medium for 1 hr before fixation.

#### RESULTS

In the testis of an adult *Gerris* each bundle of approximately 128 late spermatids or mature spermatozoa is enveloped by a cellular sheath derived from a cyst cell (18) (Fig. 1). At the ultrastructural level this sheath is most conspicuous in the anterior region of the sperm bundle where it surrounds the developing acrosomes (Fig. 2). During earlier spermatid stages the individual acrosomes within a cyst are separate, and each is surrounded by a portion of the cyst cell. As spermiogenesis progresses the anterior portions of the

acrosomes within each cyst become embedded as a group in an electron-opaque matrix (Fig. 2).

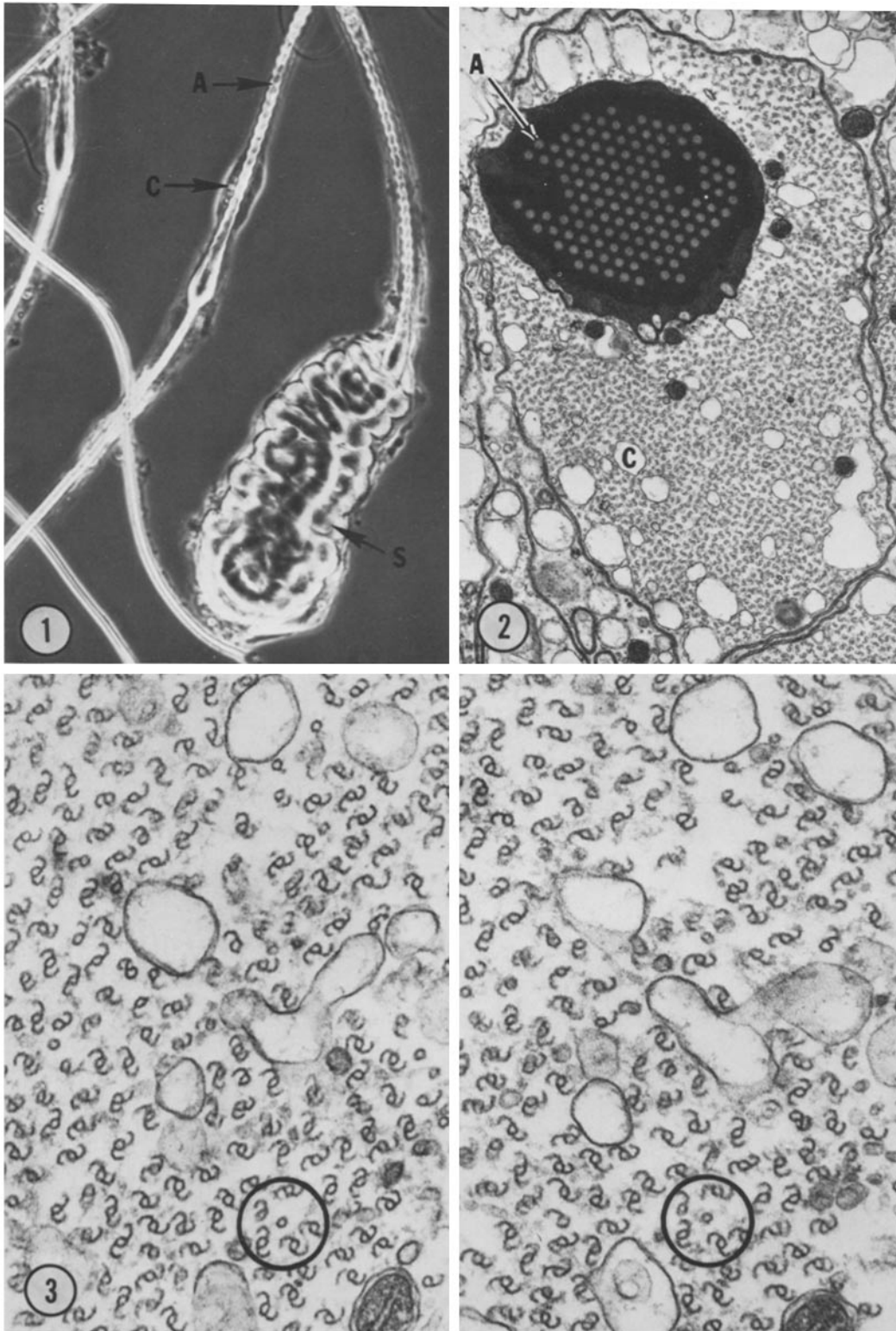
The cytoplasm of the cyst cell in the region of the differentiating acrosomes is filled with a large number of modified microtubular structures oriented parallel to the long axis of the sperm bundle (Figs. 3, 4, 5). In transverse section a few of these structures show the simple circular profile characteristic of conventional microtubules (Fig. 4). These circular profiles have a diameter of approximately 270 Å. A majority of the profiles possess one to three long projections extending out from various regions of the microtubule wall (Figs. 3, 4). The most prevalent form consists of a microtubule with two long, curved projections extending out from opposite sides of the microtubule wall (Fig. 4), but close examination of Figs. 3 and 4 will reveal various other profiles as well. The lengths of the projections appear to vary. They are never straight but usually have a radius of curvature somewhat greater than that of the wall of the microtubule (Figs. 3, 4).

Both longitudinal (Fig. 5) and serial cross-sections (Fig. 3) of these modified microtubules indicate that the projections are continuous along relatively long lengths of the microtubules. In areas where the cyst cells apparently extend beyond the tip of the sperm bundle, two or more of the modified microtubules may become linked together, presenting an even more complex profile (Fig. 6).

In transverse sections through a single cyst the projections on the microtubules, with very few exceptions, all curve in the same direction (Fig. 4). In a single section the projections of the microtubules in adjacent cysts may curve in the same or in opposite directions. It has not yet been determined if the direction of curvature of the projections is the same in every cyst cell when viewed in the same direction, i.e., towards the anterior end of the sperm bundle.

After colchicine treatment the profiles of the microtubules in transverse section are mostly circular, lacking the projections (Fig. 7). They appear to be surrounded by a considerable amount of flocculent material. Longitudinal sections of the treated material confirm the observation that the projections are no longer present (Fig. 8).

The results were similar for all concentrations of colchicine tested. In control testes incubated only in GG medium the projections on the microtubules were not affected.



**FIGURE 1** Phase-contrast micrograph of a portion of a sperm bundle fixed in glutaraldehyde. The sperm tails (*S*) are wound in a series of tight gyres while the region containing the acrosomes (*A*) is fairly straight. Remnants of the cyst cell (*C*) can be seen surrounding the sperm bundle.  $\times 250$ .

**FIGURE 2** Survey electron micrograph through the acrosomal region of a sperm bundle showing the enveloping cyst cell (*C*). At this level the acrosomes (*A*) are embedded in an electron-opaque matrix.  $\times 5500$ .

**FIGURE 3** Two serial sections through a number of modified microtubules cut in transverse section. Note continuity of the projections (circled) in adjacent sections.  $\times 44,400$ .

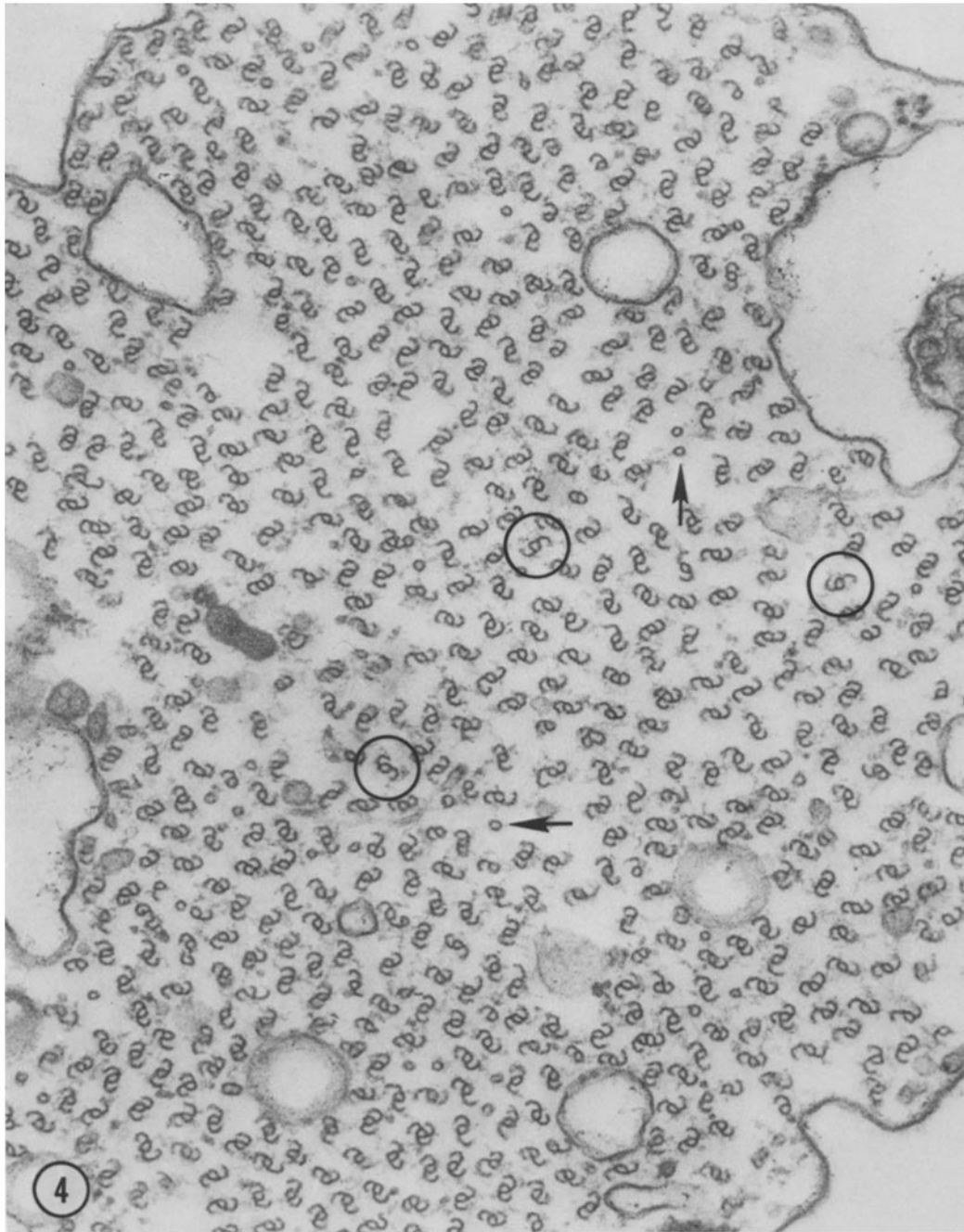


FIGURE 4 Section through a portion of a cyst cell showing a number of modified microtubules in transverse section. Several different profiles may be seen including those of conventional microtubules (arrows). The circled microtubules possess a polarity opposite that of a majority of the profiles.  $\times 52,400$ .

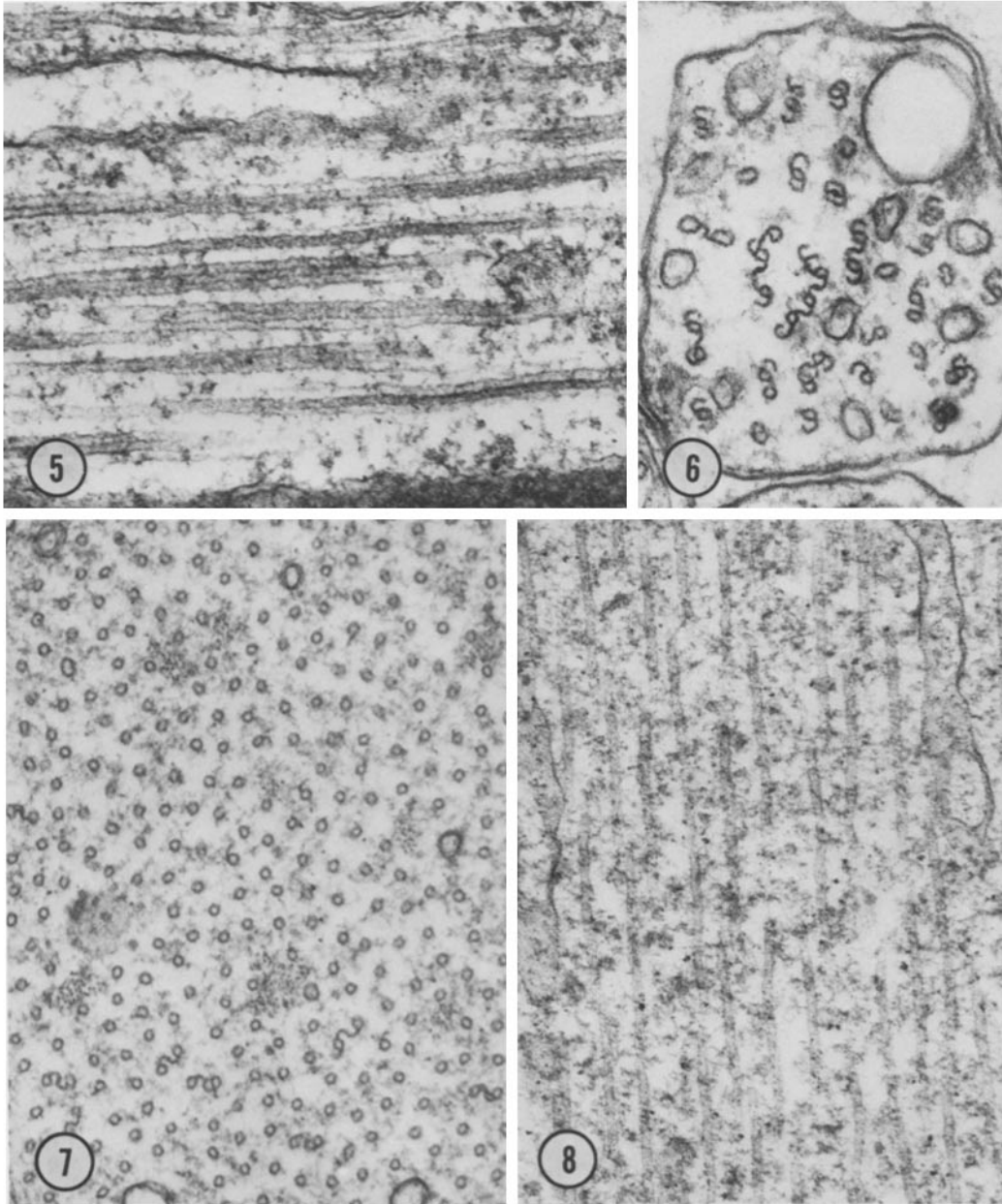


FIGURE 5 Several modified microtubules sectioned longitudinally. The projections are continuous along the lengths of the profiles.  $\times 62,400$ .

FIGURE 6 Apical portion of a cyst cell showing several modified microtubules which are apparently connected together.  $\times 88,600$ .

FIGURE 7 Portion of a cyst cell fixed after colchicine treatment (0.2%, 6 hr). Most of the projections have been lost from the microtubules.  $\times 63,600$ .

FIGURE 8 Similar to Fig. 7 except that the microtubules are sectioned longitudinally. The projections are no longer evident. Compare with the untreated microtubules in Fig. 5.  $\times 59,600$ .

## DISCUSSION

The simplest of the microtubules in the cyst cells are morphologically similar to conventional cytoplasmic microtubules. These microtubules are also the basic structure common to all of the more complex forms. The slightly larger diameter of 270 Å is not considered significantly different from the 200–250 Å usually quoted.

On the basis of morphological criteria the projections described in the present study appear to bear no similarity to the arms present on the A subfiber of ciliary microtubules (1, 12). Nor do they resemble the arms and bridges associated with various types of cytoplasmic microtubules (7).

The fixation and staining characteristics of the projections suggest that they are similar if not identical in composition to the wall of the microtubule. Microtubules are believed to be comprised of a number of protofibrils or protofilaments (2, 9, 16), commonly 10–14 (3, 13, 20), each of which is composed of globular protein subunits (25, 26). Stephens (28) has shown that under certain experimental conditions solubilized microtubular protein can be made to reassociate in linear arrays to form long ribbons which "closely resemble the wall of a spread microtubule." The projections on the modified microtubules may represent a similar type of ribbon, one edge of which is bound to a protofilament in the wall of the microtubule. The different lengths of the projections could be ascribed to different numbers of protofilaments comprising the ribbons.

The results of the colchicine treatment may be interpreted in several ways. It is known that not all types of microtubules are equally sensitive to colchicine (4, 6, 32, 33). One explanation for the selective disappearance of the projections is a chemical difference between projections and microtubules. Biochemical analysis of isolated modified microtubules will probably be necessary to clarify this point.

Alternatively, the lability of the projections in colchicine could be attributed to differences in binding of the protofilaments. The protofilament along the leading edge of a projection may be more susceptible to colchicine action than the other protofilaments, of either the projection or the microtubule, which are bound on two sides to neighboring protofilaments. If this assumption is correct we could expect a sequential removal of the protofilaments in the projections when ex-

posed to colchicine. Further research is necessary to determine if the projections are removed one protofilament at a time and if the action of colchicine is reversible. The projections did not reappear after a recovery period of 1 hr under the specified experimental conditions.

The acrosome of *Gerris* is approximately 2.5 mm long, about half the length of the mature sperm (30). Tandler and Moriber (30) have described two types of microtubules associated with acrosome differentiation in this organism. During its transformation from a spherical structure to a long tapering rod, the acrosome is filled with a mass of tubules about 130 Å in diameter. These tubules, oriented parallel to the long axis of the acrosome, are believed responsible for its rigidity. The second class of microtubules (diameter approximately 220 Å) was found in the cytoplasm of the spermatid immediately surrounding the elongating acrosome. It was suggested that these cytoplasmic microtubules might be involved in the transport of acrosome precursors in addition to providing rigidity for this organelle. The modified microtubules of the cyst cells were not described by these authors.

The function of the modified microtubules must remain speculative, because the amount of information available is limited. These microtubules could be expected to possess greater structural rigidity than similar microtubules lacking projections. This increase in rigidity would be asymmetric with respect to a plane passing through the long axis of the microtubule. The close association of the cyst cell with the elongating bundle of spermatids and the parallel orientation of the microtubules are both circumstantial evidence for a cytoskeletal function. The relatively straight character of the acrosomes in the isolated sperm bundle, compared with the tightly coiled sperm tails, is further evidence for a supportive function.

The curvature of the projections imparts a polarity to the cyst cell microtubules. The fact that almost all of the microtubules within a cyst cell possess the same polarity strongly suggests a directional function. This could indicate a unidirectional transport mechanism similar to that described for different regions in the tentacle of *Tokophrya* (22). Elaboration of these points awaits further clarification of the function of the cyst cell.

Of considerable interest in the future will be the elucidation of the chemical nature of the projections and a comparison of the modified

microtubules with conventional cytoplasmic microtubules present in other cells of this organism. Possible differences in the environment of the modified microtubules (cyst cell cytoplasm) and in the nucleating sites for these microtubules must also be considered as possible factors responsible for their unusual morphology.

The authors wish to thank Dr. W. G. Whaley and Dr. Harriett E. Smith for advice and encouragement during the preparation of this manuscript. In addition, Dr. J. C. Schaeffner's help in identifying the species of *Gerris* used is gratefully acknowledged.

This study was supported in part by the Cell Research Institute, The University of Texas, Austin, and by a grant from the Faith Foundation to Dr. W. G. Whaley, Director of the Institute. Additional support was obtained from National Aeronautics and Space Administration grant NAS 9-9211 to Dr. Paul G. Mahlberg.

Received for publication 4 November 1971.

#### REFERENCES

1. AFZELIUS, B. 1959. Electron microscopy of the sperm tail: results obtained with a new fixative. *J. Biophys. Biochem. Cytol.* **5**:269.
2. ANDRÉ, J., and J. P. THIÉRY. 1963. Mise en évidence d'une sous-structure fibrillaire dans les filaments axonématiques des flagelles. *J. Microsc.* **2**:71.
3. ARNOTT, H. J., and H. E. SMITH. 1969. Analysis of microtubule structure in *Euglena granulata*. *J. Phycol.* **5**:68.
4. BEHNKE, O., and A. FORER. 1967. Evidence for four classes of microtubules in individual cells. *J. Cell Sci.* **2**:169.
5. BERTOLINI, B., G. MONACO, and A. ROSSI. 1970. Ultrastructure of a regular arrangement of microtubules and neurofilaments. *J. Ultrastruct. Res.* **33**:173.
6. BURTON, P. R. 1968. Effects of various treatments on microtubules and axial units of lungfluke spermatozoa. *Z. Zellforsch. Mikrosk. Anat.* **87**:222.
7. FRIEDMAN, M. H. 1971. Arm-bearing microtubules associated with an unusual desmosome-like junction. *J. Cell Biol.* **49**:916.
8. FULTON, C. 1971. Centrioles. In *Origin and Continuity of Cell Organelles*. J. Reinert and H. Ursprung, editors. Springer-Verlag New York Inc., New York. 170-221.
9. GALL, J. G. 1966. Microtubule fine structure. *J. Cell Biol.* **31**:639.
10. GIBBONS, I. R. 1963. Studies on the protein components of cilia from *Tetrahymena pyriformis*. *Proc. Nat. Acad. Sci. U. S. A.* **50**:1002.
11. GIBBONS, I. R. 1967. The organization of cilia and flagella. In *Molecular Organization and Biological Function*. J. M. Allen, editor. Harper and Row, Publishers, New York. 211-237.
12. GIBBONS, I. R., and A. V. GRIMSTONE. 1960. On flagellar structure in certain flagellates. *J. Biophys. Biochem. Cytol.* **7**:697.
13. LEDBETTER, M. C., and K. R. PORTER. 1964. Morphology of microtubules in plant cells. *Science (Washington)*. **144**:872.
14. MOLLENHAUER, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. *Stain Technol.* **39**:111.
15. NEWCOMB, E. H. 1969. Plant microtubules. *Annu. Rev. Plant Physiol.* **20**:253.
16. PEASE, D. C. 1963. The ultrastructure of flagellar fibers. *J. Cell Biol.* **18**:313.
17. PHILLIPS, D. M. 1966. Substructure of flagellar tubules. *J. Cell Biol.* **31**:635.
18. POLLISTER, A. W. 1930. Cytoplasmic phenomena in the spermatogenesis of *Gerris*. *J. Morphol.* **49**:455.
19. PORTER, K. R. 1966. Cytoplasmic microtubules and their function. In *Principles of Biomolecular Organization*. G. E. W. Wolstenholme and M. O'Connor, editors. Little, Brown and Company, Boston. 308.
20. RINGO, D. L. 1967. The arrangement of subunits in flagellar fibers. *J. Ultrastruct. Res.* **17**:266.
21. ROTH, L. E., and Y. SHIGENAKA. 1970. Microtubules in the heliozoan axopodium. II. Rapid degradation by cupric and nickelous ions. *J. Ultrastruct. Res.* **31**:356.
22. RUDZINSKA, M. A. 1965. The fine structure and function of the tentacle in *Tokophrya infusionum*. *J. Cell Biol.* **25**:459.
23. SCHMITT, F. O., and F. E. SAMSON, JR. 1969. Neuronal fibrous proteins. *Neurosci. Res. Symp. Summ.* **3**:301.
24. SHAW, E. I. 1956. A glutamic acid-glycine medium for prolonged maintenance of high mitotic activity in grasshopper neuroblasts. *Exp. Cell Res.* **11**:580.
25. SHELANSKI, M. L., and E. W. TAYLOR. 1967. Isolation of a protein subunit from microtubules. *J. Cell Biol.* **34**:549.
26. SHELANSKI, M. L., and E. W. TAYLOR. 1968. Properties of the protein subunit of central-pair and outer-doublet microtubules of sea urchin flagella. *J. Cell Biol.* **38**:304.
27. SHIGENAKA, Y., L. E. ROTH, and D. J. PIKLAJA. 1971. Microtubules in the heliozoan axopodium. III. Degradation and reformation after dilute urea treatment. *J. Cell Sci.* **8**:127.

28. STEPHENS, R. E. 1968. Reassociation of microtubule protein. *J. Mol. Biol.* **33**:517.
29. STUBBLEFIELD, E., and B. R. BRINKLEY. 1967. Architecture and function of the mammalian centriole. *Symp. Int. Soc. Cell Biol.* **6**:175.
30. TANDLER, B. and L. G. MORIBER. 1966. Microtubular structures associated with the acrosome during spermiogenesis in the water-strider, *Gerris remigis* (Say). *J. Ultrastruct. Res.* **14**:391.
31. TILNEY, L. G. 1971. Origin and continuity of Microtubules. *In Origin and Continuity of Cell Organelles.* J. Reinert and H. Ursprung, editors. Springer-Verlag New York Inc., New York. 222-260.
32. TILNEY, L. G., and J. R. GIBBINS. 1968. Differential effects of antimitotic agents on the stability and behavior of cytoplasmic and ciliary microtubules. *Protoplasma.* **65**:167.
33. TURNER, F. R. 1970. The effects of colchicine on spermatogenesis in *Nitella*. *J. Cell Biol.* **46**:220.