THE CELL

FAWCETT

Chapter 14: Sperm Flagellum

Second Edition

THE CELL

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PREFACE

The history of morphological science is in large measure a chronicle of the discovery of new preparative techniques and the development of more powerful optical instruments. In the middle of the 19th century, improvements in the correction of lenses for the light microscope and the introduction of aniline dyes for selective staining of tissue components ushered in a period of rapid discovery that laid the foundations of modern histology and histopathology. The decade around the turn of this century was a golden period in the history of microscopic anatomy, with the leading laboratories using a great variety of fixatives and combinations of dyes to produce histological preparations of exceptional quality. The literature of that period abounds in classical descriptions of tissue structure illustrated by exquisite lithographs. In the decades that followed, the tempo of discovery with the light microscope slackened; interest in innovation in microtechnique declined, and specimen preparation narrowed to a monotonous routine of paraffin sections stained with hematoxylin and eosin.

In the middle of the 20th century, the introduction of the electron microscope suddenly provided access to a vast area of biological structure that had previously been beyond the reach of the compound microscope. Entirely new methods of specimen preparation were required to exploit the resolving power of this new instrument. Once again improvement of fixation, staining, and microtomy commanded the attention of the leading laboratories. Study of the substructure of cells was eagerly pursued with the same excitement and anticipation that attend the geographical exploration of a new continent. Every organ examined yielded a rich reward of new structural information. Unfamiliar cell organelles and inclusions and new macromolecular components of protoplasm were rapidly described and their function almost as quickly established. This bountiful harvest of new structural information brought about an unprecedented convergence of the interests of morphologists, physiologists, and biochemists; this convergence has culminated in the unified new field of science called cell biology.

The first edition of this book (1966) appeared in a period of generous support of science, when scores of laboratories were acquiring electron microscopes and hundreds of investigators were eagerly turning to this instrument to extend their research to the subcellular level. At that time, an extensive text in this rapidly advancing field would have been premature, but there did seem to be a need for an atlas of the ultrastructure of cells to establish acceptable technical standards of electron microscopy and to define and illustrate the cell organelles in a manner that would help novices in the field to interpret their own micrographs. There is reason to believe that the first edition of *The Cell: An Atlas of Fine Structure* fulfilled this limited objective.

In the 14 years since its publication, dramatic progress has been made in both the morphological and functional aspects of cell biology. The scanning electron microscope and the freeze-fracturing technique have been added to the armamentarium of the miscroscopist, and it seems timely to update the book to incorporate examples of the application of these newer methods, and to correct earlier interpretations that have not withstood the test of time. The text has been completely rewritten and considerably expanded. Drawings and diagrams have been added as text figures. A few of the original transmission electron micrographs to which I have a sentimental attachment have been retained, but the great majority of the micrographs in this edition are new. These changes have inevitably added considerably to the length of the book and therefore to its price, but I hope these will be offset to some extent by its greater informational content.

Twenty years ago, the electron microscope was a solo instrument played by a few virtuosos. Now it is but one among many valuable research tools, and it is most profitably used in combination with biochemical, biophysical, and immunocytochemical techniques. Its use has become routine and one begins to detect a decline in the number and quality of published micrographs as other analytical methods increasingly capture the interest of investigators. Although purely descriptive electron microscopic studies now yield diminishing returns, a detailed knowledge of the structural organization of cells continues to be an indispensable foundation for research on cell biology. In undertaking this second edition I have been motivated by a desire to assemble and make easily accessible to students and teachers some of the best of the many informative and aesthetically pleasing transmission and scanning electron micrographs that form the basis of our present understanding of cell structure.

The historical approach employed in the text may not be welcomed by all. In the competitive arena of biological research today investigators tend to be interested only in the current state of knowledge and care little about the steps by which we have arrived at our present position. But to those of us who for the past 25 years have been privileged to participate in one of the most exciting and fruitful periods in the long history of morphology, the young seem to be entering the theater in the middle of an absorbing motion picture without knowing what has gone before. Therefore, in the introduction to each organelle, I have tried to identify, in temporal sequence, a few of the major contributors to our present understanding of its structure and function. In venturing to do this I am cognizant of the hazards inherent in making judgments of priority and significance while many of the dramatis personae are still living. My apologies to any who may feel that their work has not received appropriate recognition.

It is my hope that for students and young investigators entering the field, this book will provide a useful introduction to the architecture of cells and for teachers of cell biology a guide to the literature and a convenient source of illustrative material. The sectional bibliographies include references to many reviews and research papers that are not cited in the text. It is believed that these will prove useful to those readers who wish to go into the subject more deeply.

The omission of magnifications for each of the micrographs will no doubt draw some criticism. Their inclusion was impractical since the original negatives often remained in the hands of the contributing microscopists and micrographs submitted were cropped or copies enlarged to achieve pleasing composition and to focus the reader's attention upon the particular organelle under discussion. Absence was considered preferable to inaccuracy in stated magnification. The majority of readers, I believe, will be interested in form rather than measurement and will not miss this datum.

Assembling these micrographs illustrating the remarkable order and functional design in the structure of cells has been a satisfying experience. I am indebted to more than a hundred cell biologists in this country and abroad who have generously responded to my requests for exceptional micrographs. It is a source of pride that nearly half of the contributors were students, fellows or colleagues in the Department of Anatomy at Harvard Medical School at some time in the past 20 years. I am grateful for their stimulation and for their generosity in sharing prints and negatives. It is a pleasure to express my appreciation for the forbearance of my wife who has had to communicate with me through the door of the darkroom for much of the year while I printed the several hundred micrographs; and for the patience of Helen Deacon who has typed and retyped the manuscript; for the skill of Peter Ley, who has made many copy negatives to gain contrast with minimal loss of detail; and for the artistry of Sylvia Collard Keene whose drawings embellish the text. Special thanks go to Elio and Giuseppina Raviola who read the manuscript and offered many constructive suggestions; and to Albert Meier and the editorial and production staff of the W. B. Saunders Company, the publishers.

And finally I express my gratitude to the Simon Guggenheim Foundation whose commendable policy of encouraging the creativity of the young was relaxed to support my efforts during the later stages of preparation of this work.

DON W. FAWCETT
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CONTENTS

SPERM FLAGELLUM

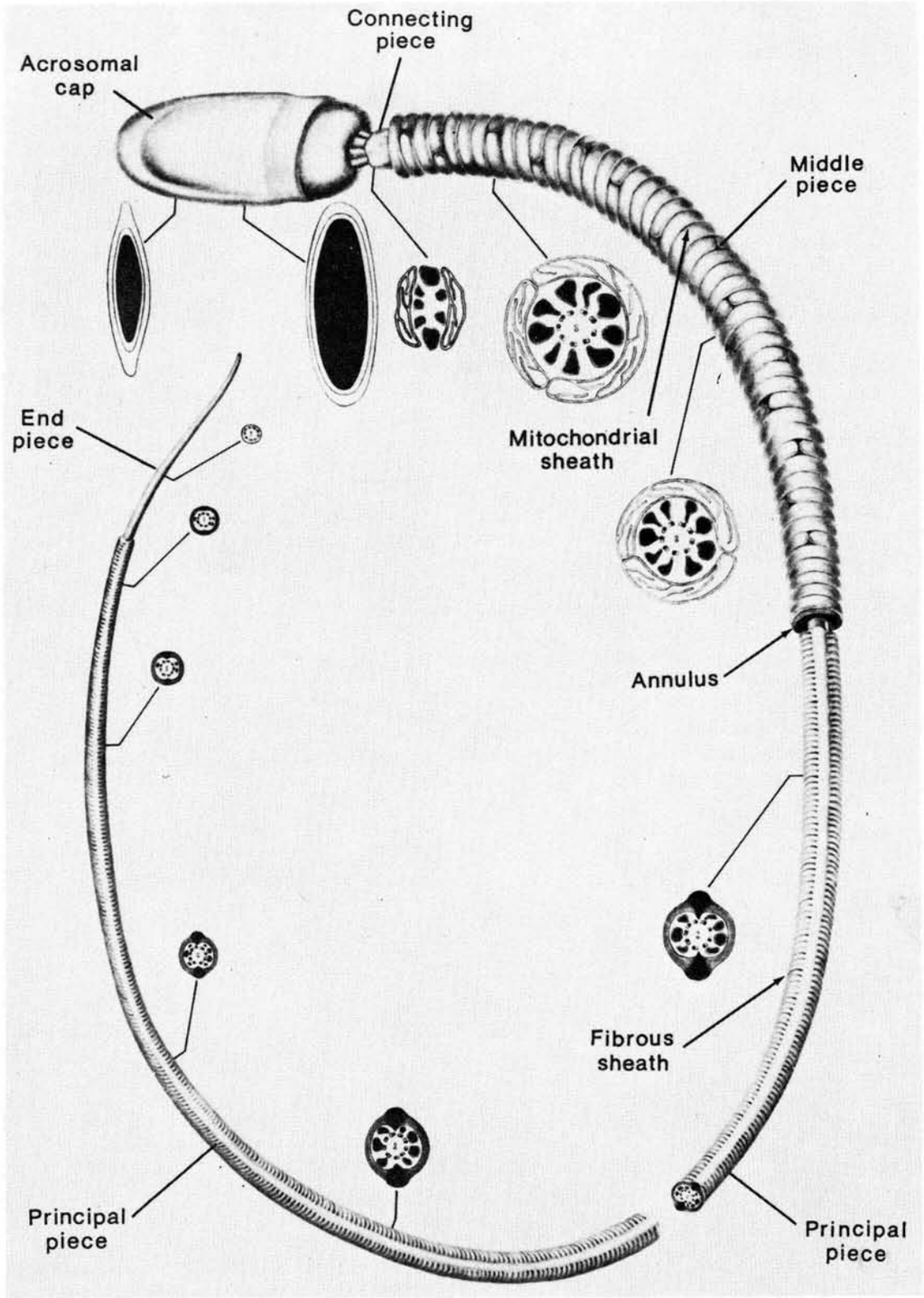
MAMMALIAN SPERM FLAGELLUM

The spermatozoon was first observed in 1677 by Leeuwenhoek, who described it as progressing by undulating movements of its long tail, like eels swimming in water. Flagella were seen by him and other early microscopists on protozoa. However, no internal structure was resolved in these slender processes until the latter part of the 19th century, when Jensen (1887) and Ballowitz (1890) observed that the tip of sperm flagella occasionally frayed during preparation into a tuft of exceedingly fine fibrils. Little significance was attached to this observation until it was verified in the earliest electron micrographs of dissociated and desiccated mammalian sperm tails (Seymour and Benmosche, 1941; Baylor et al., 1943). Estimates of the number of fibrils ranged from 8 to 24 due to the different degrees of fragmentation and artifacts of drying.

When satisfactory methods of fixation and thin sectioning were developed, there was general agreement on the nine peripheral and two central fibrils of the axoneme (Watson, 1952; Fawcett, 1954) but the mammalian sperm flagellum was found to have an additional set of nine *outer dense fibers* around the axoneme (Challice, 1953; Bradfield, 1953). In transverse section these vary in size and shape, with three of the nine usually being larger than the other six. With the conventional system of numbering, the larger fibrils are numbers 1, 5, and 6. The presence of nine outer fibers in addition to the axoneme (9+9+2) was found to be characteristic of the majority of animal species with internal fertilization, while lower forms that release their sperm directly into sea water retain the more primitive 9+2 pattern of flagellar microtubules. It was speculated that the outer fibers were accessory motor elements that evolved to overcome the greater resistance encountered in the secretions of the female reproductive tract. This interpretation persisted until the outer fibers were isolated and analyzed (Price, 1973; Baccetti et al., 1973). They were found to consist of four polypeptides ranging from 11,000 to 55,000 daltons. Their amino acid profile was unlike that of any known contractile protein, and the high content of cysteine was more suggestive of a scleroprotein like keratin. Therefore the outer dense fibers are now interpreted as passive stiffening elements, but it is not obvious what advantage is conferred by adding resilient elastic components around the axoneme of the sperm flagellum.

A coiled strand wrapped around the proximal part of the sperm tail was described by Jensen (1887), who was among the first to recognize that this was composed of mitochondria. Subsequent investigators were divided among those who regarded it as a continuous sheath formed by end-to-end fusion of mitochondria (Retzius, 1909; Schnall, 1952) and those who insisted that the mitochondria are coiled helically around the axoneme but retain their individuality (Gresson and Zlotnik, 1945; Challice, 1953; Yasuzumi, 1956). Electron microscopy established the latter interpretation as the correct one for mammals, but fusion of the mitochondria is common in reptiles, birds, and invertebrates (Fawcett, 1958). The segment of the tail enclosed by the *mitochondrial sheath* is called the *middle piece*, or *midpiece*. Its length varies greatly from species to species, ranging from about a dozen mitochondrial turns in humans to over 200 in some rodents.

At the distal end of the midpiece is the *annulus (Jensen's ring, terminal ring)*. This dense circular structure is firmly attached to the flagellar membrane, where the latter is reflected from the midpiece onto the next segment, called the *principal piece*. The



Schematic representation of a generalized mammalian spermatozoon as it would appear with cell membrane removed, and in cross sections at various levels. (From D. W. Fawcett, The Mammalian Spermatozoon, Dev. Biol. 44:394–436, 1975.)

shape of the annulus and its firm attachment to the cell membrane suggest that it serves to stabilize the mitochondrial sheath and prevent caudad displacement of mitochondria during vigorous movements of the tail.

In the long principal piece of the flagellum, the axoneme is invested by a *fibrous sheath (tail helix, cortical helix).* Light microscopists described it as consisting of one or more continuous fibers wound around the axoneme in a tight helix. With the electron microscope, it proved to consist of a series of circumferentially oriented *ribs* that extend half way around the tail and terminate in two *longitudinal columns* which run along the dorsal and ventral aspects of the sheath for its entire length. The closely spaced ribs are usually of uniform thickness but may occasionally branch and anastomose with neighboring ribs (Bradfield, 1953; Fawcett, 1954, 1958). The outer dense fibers 3 and 8 terminate near the end of the midpiece. In the principal piece, their place is occupied by inward extensions from the longitudinal columns of the fibrous sheath that attach to appendages on doublets 3 and 8. The position of the longitudinal columns would seem to impose some restraint to flexion in a dorsoventral plane, but the principal plane of tail bending is perpendicular to the line joining the central pair of microtubules, and movement in this plane can be accommodated by alternate widening and narrowing of the interspaces between ribs on the two sides of the sheath. The function of the fibrous sheath is unclear, but it has been suggested (Challice, 1953) that if lateral flexion of the tail is to be produced by a propagated wave of contraction passing along the axoneme, there should be parallel resistances to provide the necessary couple. If the fibrous sheath is endowed with elastic properties, it may provide the needed resistance. This explanation is less than convincing, however, since flagella of primitive sperm function effectively without a fibrous sheath. Although the longitudinal columns and ribs of the fibrous sheath appear to have a different substructure, fibrous sheaths have now been isolated and found to consist of a single polypeptide (Olsen, 1977).

The fibrous sheath ends about 10 μm from the tip of the tail and the terminal segment of the axoneme enclosed only by the flagellar membrane constitutes the *end-piece* of the sperm tail.

In a scanning electron micrograph of spermatozoa on the surface of the uterine endometrium, the smooth contour of the long tapering tails gives no hint of their regional differentiation or of their complex internal organization.

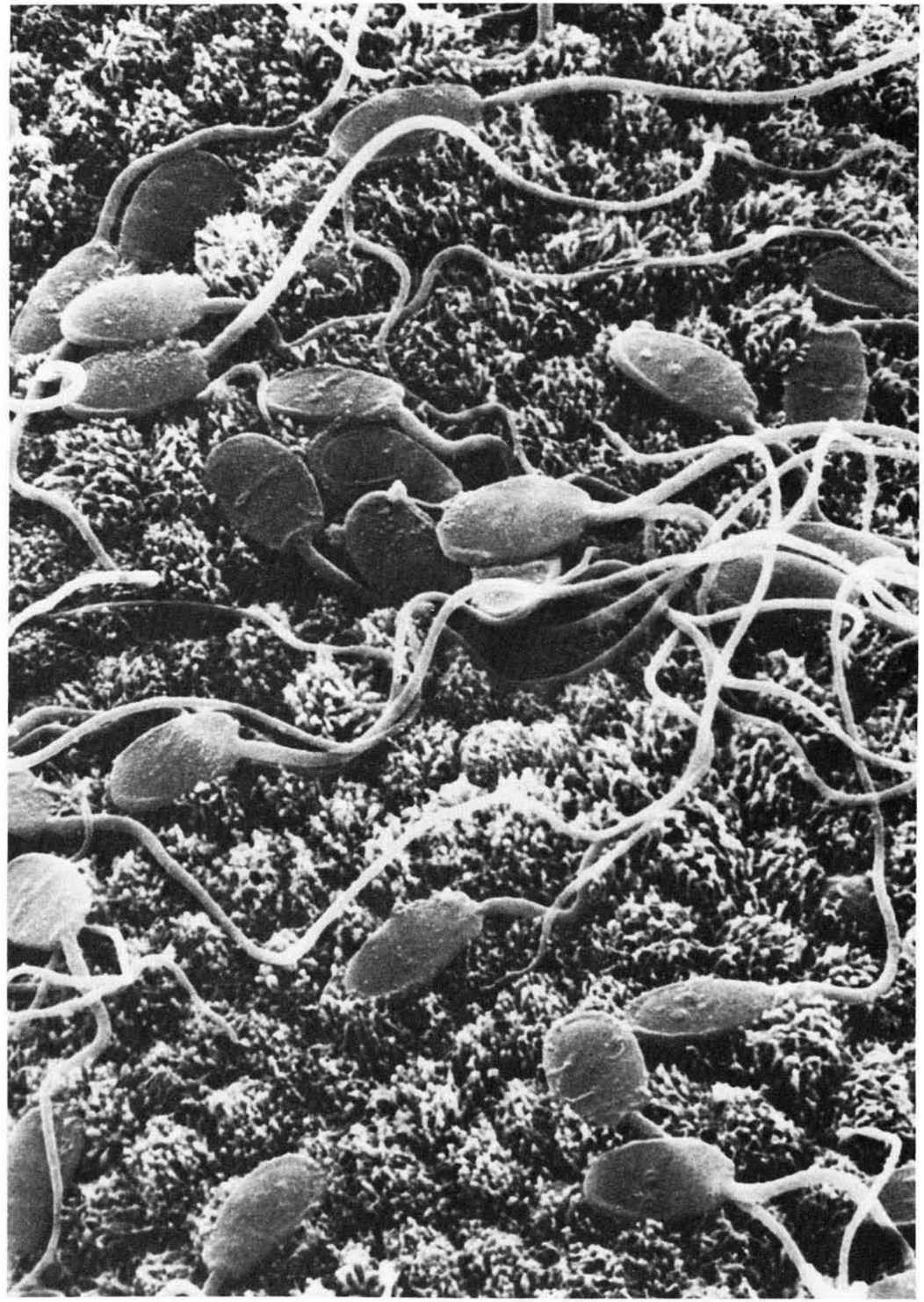
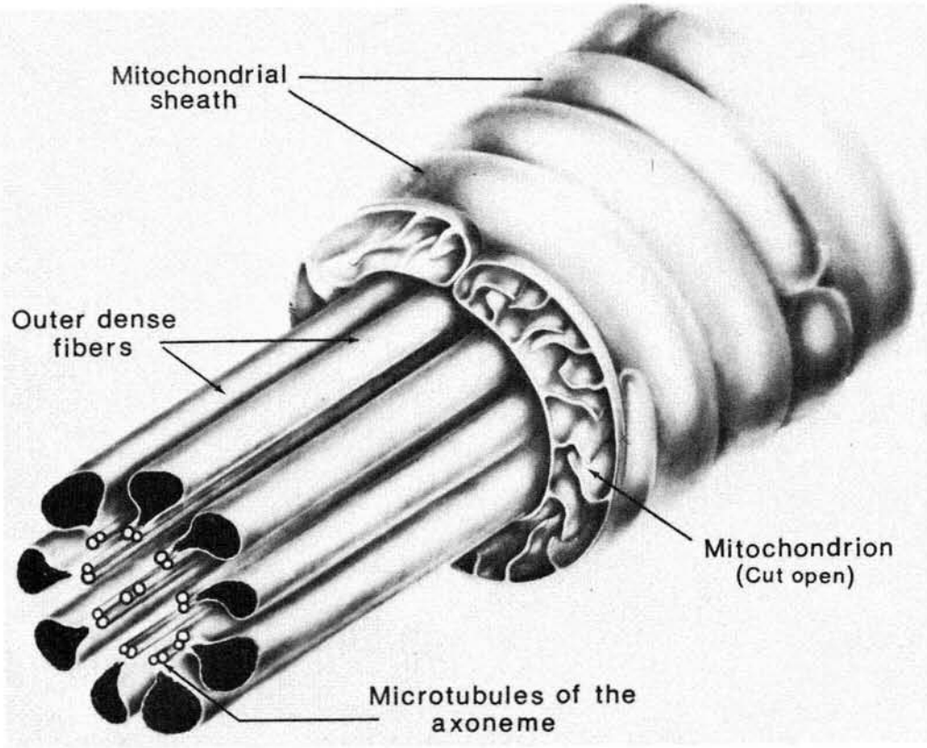


Figure 328. Scanning electron micrograph of rabbit spermatozoa on the endometrium of the uterus. (Micrograph courtesy of David Phillips.)

Figure 328



An extended diagram of the components in the midpiece of a mammalian spermatozoon tail. (From D. W. Fawcett, Dev. Biol. 44:394–436, 1975.)

In the extended diagram (above) showing the arrangement of components in the midpiece of the mammalian sperm tail, the helical mitochondrial sheath is seen to be closely applied to the longitudinal dense fibers. This relationship is illustrated in the transverse sections on the facing page. The dense fibers display distinctive differences in size and shape. In most species, numbers 1, 5, and 6 are somewhat larger than the others. A variable number of small *satellite fibrils* of unknown provenance and function are found in the interstices between the outer dense fibers.

Figure 329. Transverse sections through the midpiece of Chinese hamster spermatozoa. (Micrograph courtesy of David Phillips.)

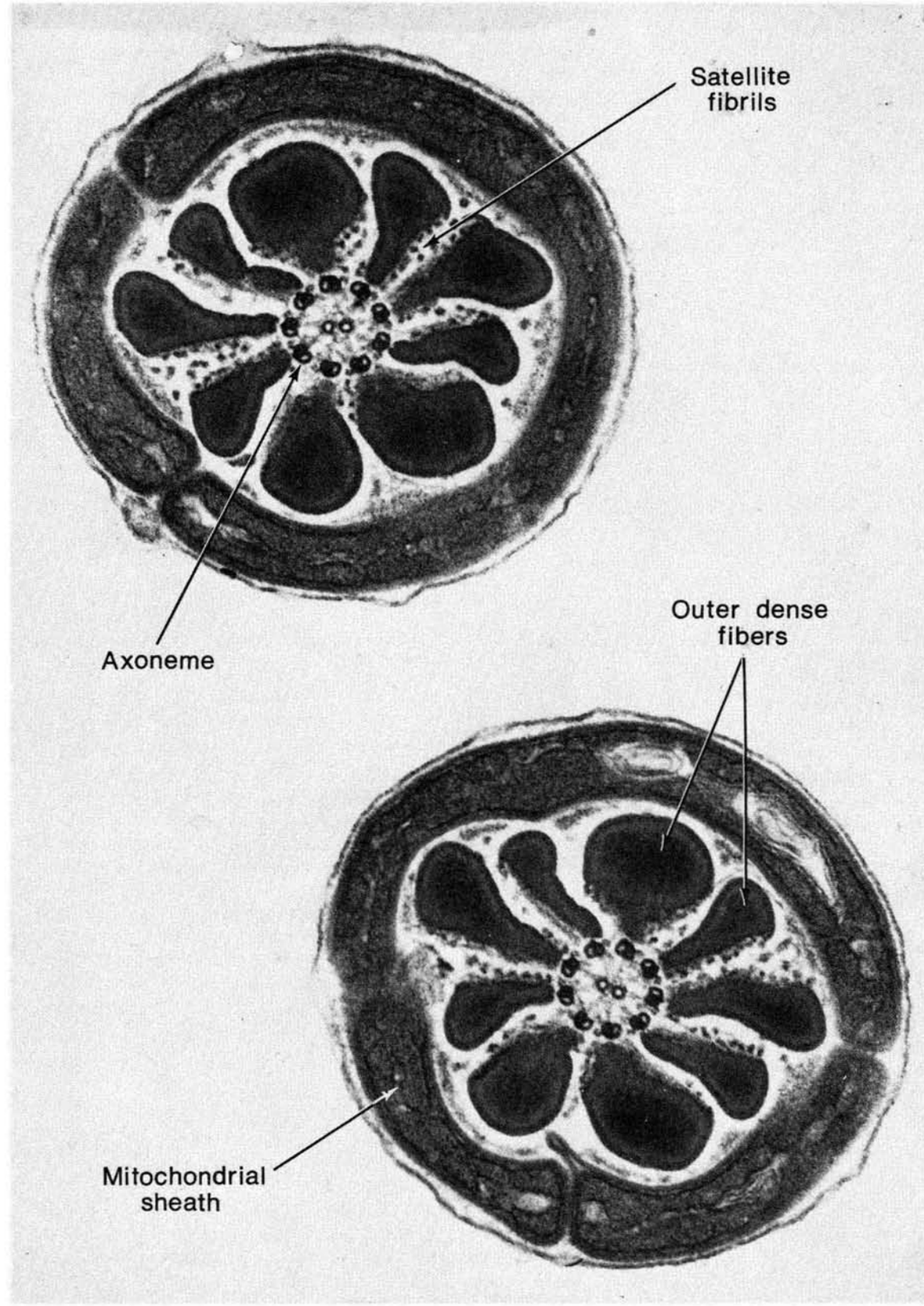
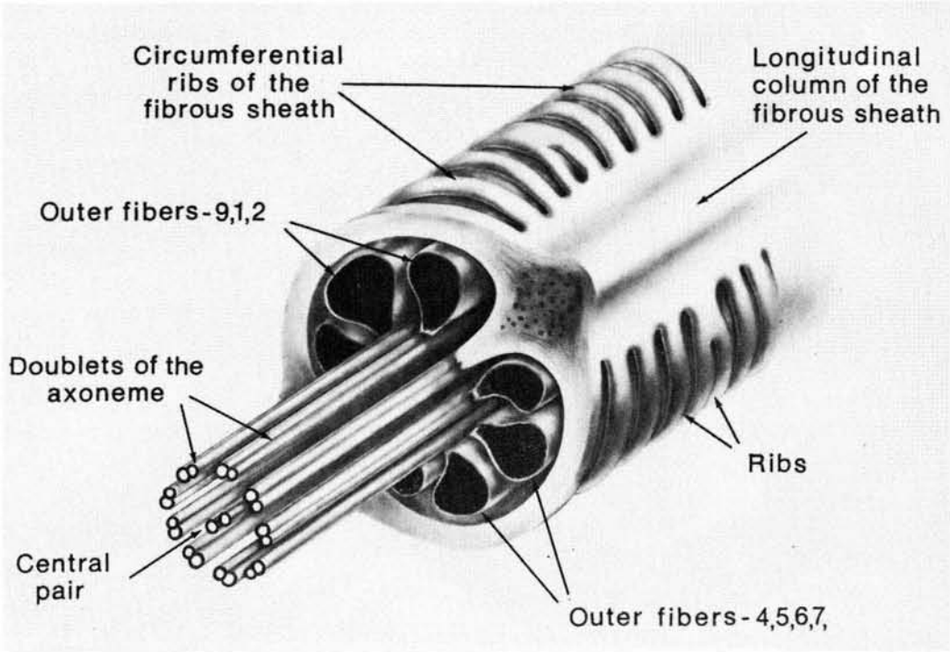


Figure 329



An extended diagram of components of the principal piece of a mammalian spermatozoon. (From D. W. Fawcett, Dev. Biol. 44:394–396, 1975.)

The topographical relationship of components in the principal piece of the mammalian spermatozoan is shown above. The fibrous sheath consists of continuous dorsal and ventral longitudinal columns joined by circumferential ribs. The columns are coplanar with the central pair of microtubules and hence offer little resistance to bending in the plane at right angles to the central pair. Bending in this plane is accommodated by widening or narrowing of the spaces between the ribs of the fibrous sheath.

The micrograph on the opposite page presents cross sections at various levels in the tapering principal piece of several spermatozoa. Note that outer dense fibers 3 and 8 have terminated and their positions are occupied by inward projections from the longitudinal columns, which attach to the corresponding doublets of the axoneme. This attachment probably prevents these doublets from participating in the microtubule sliding responsible for wave generation.

Figure 330. Transverse sections through the principal piece of testicular sperm in Chinese hamster. (Micrograph courtesy of David Phillips.)

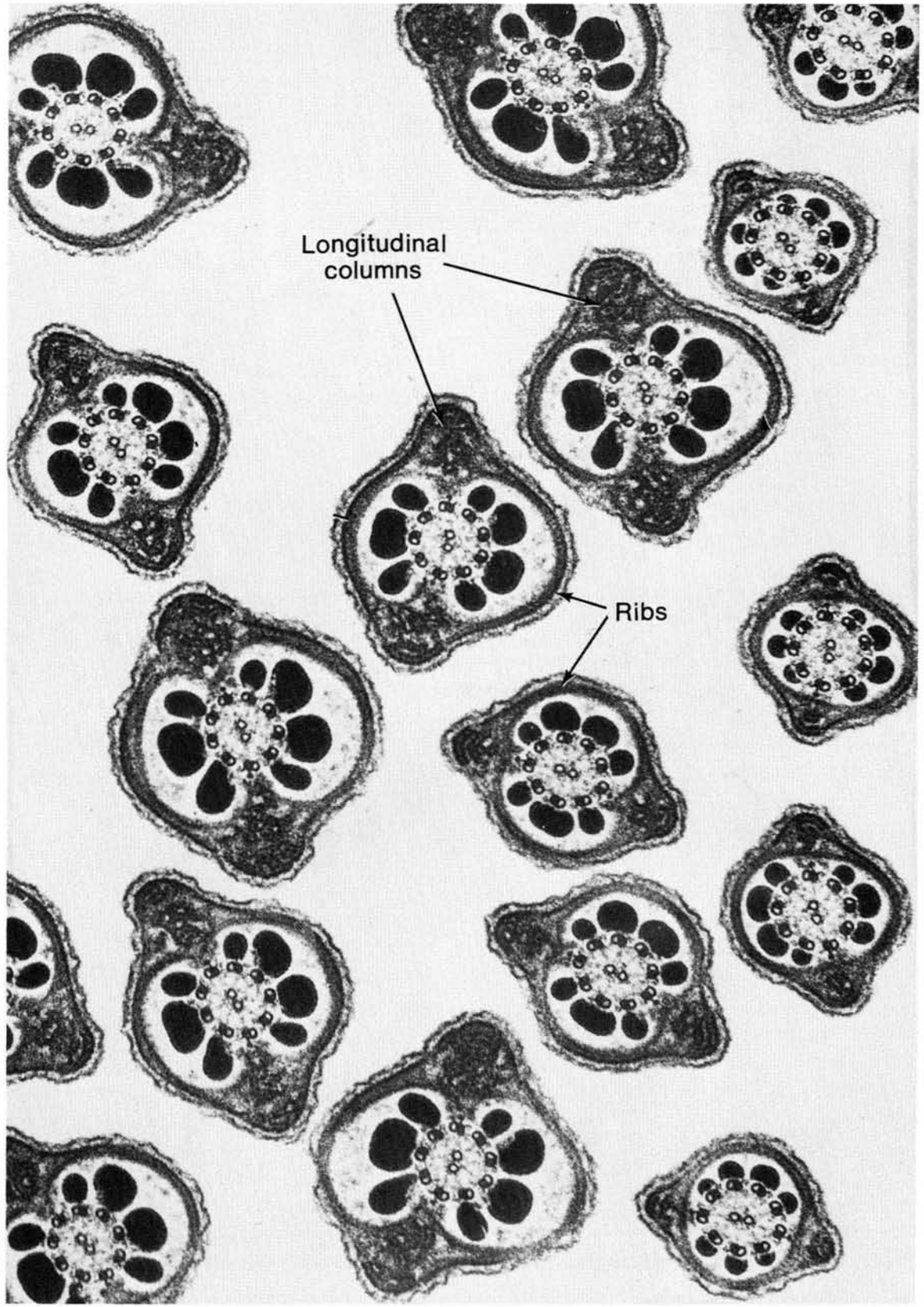


Figure 330

The accompanying micrographs permit a comparison of longitudinal sections of the midpiece (left) and principal piece (right). The principal piece shown here is not entirely typical, for in this species there is considerable fusion of neighboring ribs of the fibrous sheath near their insertion into the longitudinal columns. The individual ribs are seen more clearly in the micrographs that follow.

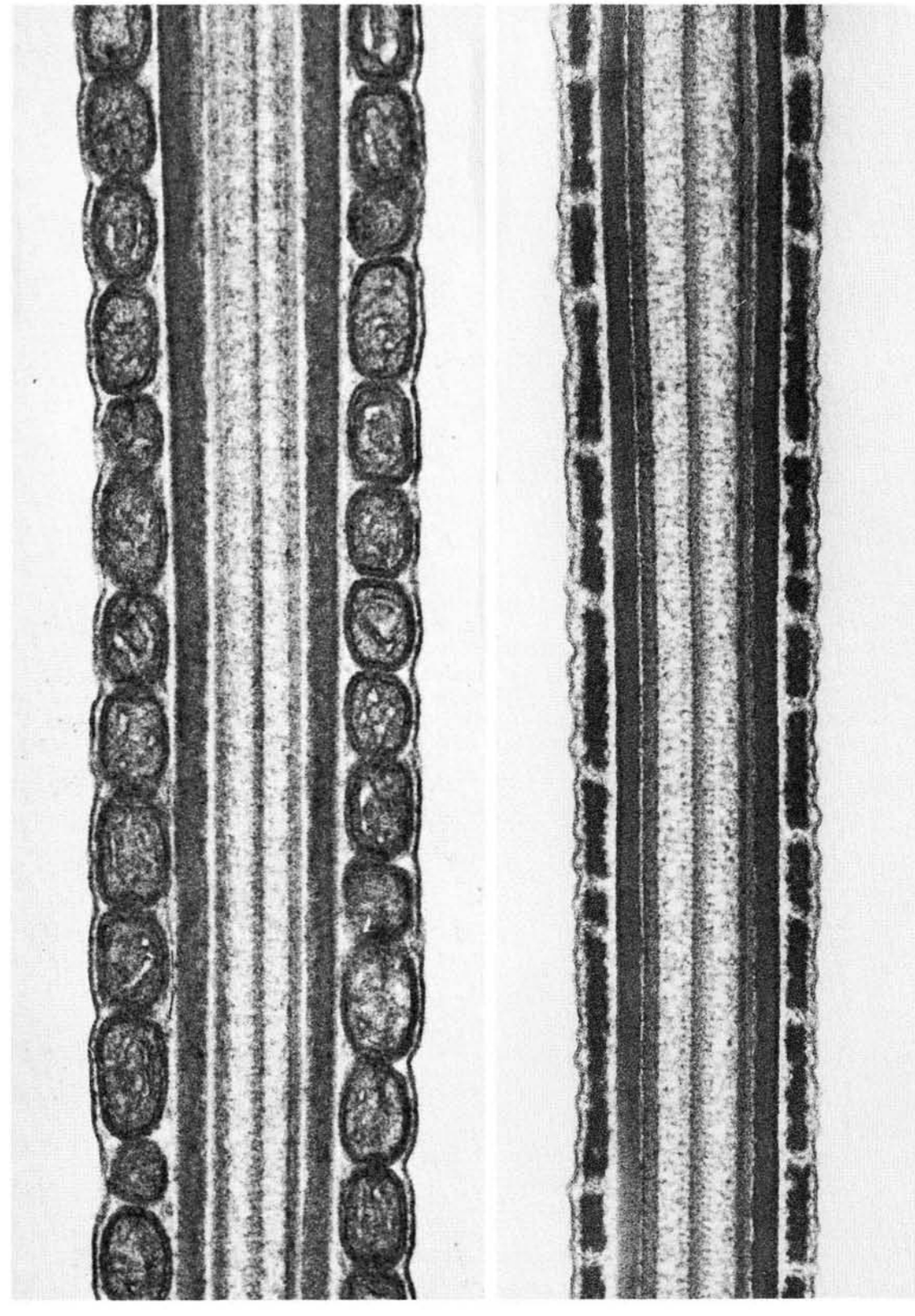
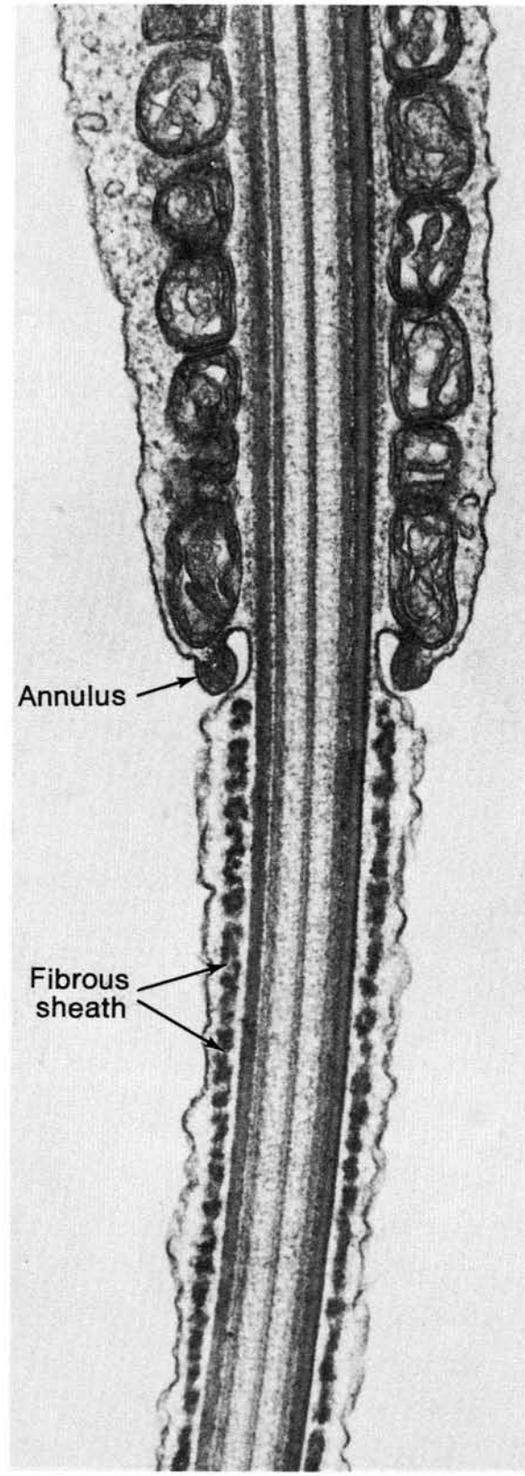


Figure 331. Longitudinal section of midpiece from sperm of the suni *Nesotragus moschatus*.

Figure 332. Longitudinal section, principal piece, mouse sperm.

Figure 331, *left* **Figure 332,** *right*

At the junction of the midpiece and principal piece of mammalian sperm is the annulus (Jensen's ring), a dense structure firmly attached to the overlying flagellar membrane. The configuration of this region shows considerable species variation. In some there is a groove or recess between the annulus and the motor apparatus. In others, the surface contour is flat and the annulus is triangular in section, with the base at the plasma membrane and the apex projecting beneath the last gyre of the mitochondrial sheath. It is speculated that the annulus is a stabilizing structure preventing caudal displacement of mitochondria in actively motile sperm tails.



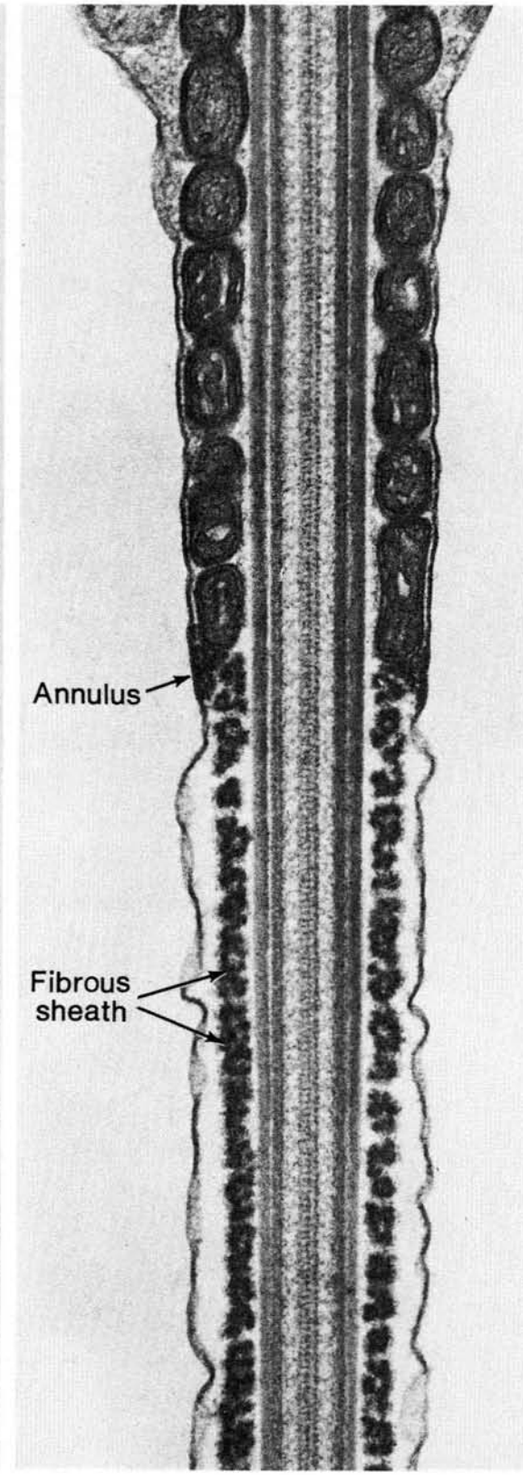


Figure 333. Longitudinal section of the junction of midpiece and principal piece in spermatozoon of chinchilla, *(Chinchilla laniger)*.

Figure 334. Comparable region of the spermatozoon of the suni *(Nesotragus moschatus)*.

Figure 333, *left* **Figure 334,** *right*

The organization of the axoneme of sperm flagella appears to be identical to that of cilia. Visualization of its components is facilitated by aldehyde fixation in the presence of tannic acid, which serves as a mordant for subsequent staining with osmium and uranyl acetate (Mizuhira, 1971). This preparative procedure improves the contrast of the image but appears to result in some thickening of the doublets, arms, and spokes. The protofilaments in the walls of the microtubules are seen in negative image. Subunits of similar size are seen in negative image in the satellite fibrils (at arrows). This has led to the suggestion that these may also be composed of tubulin, but this requires verification.

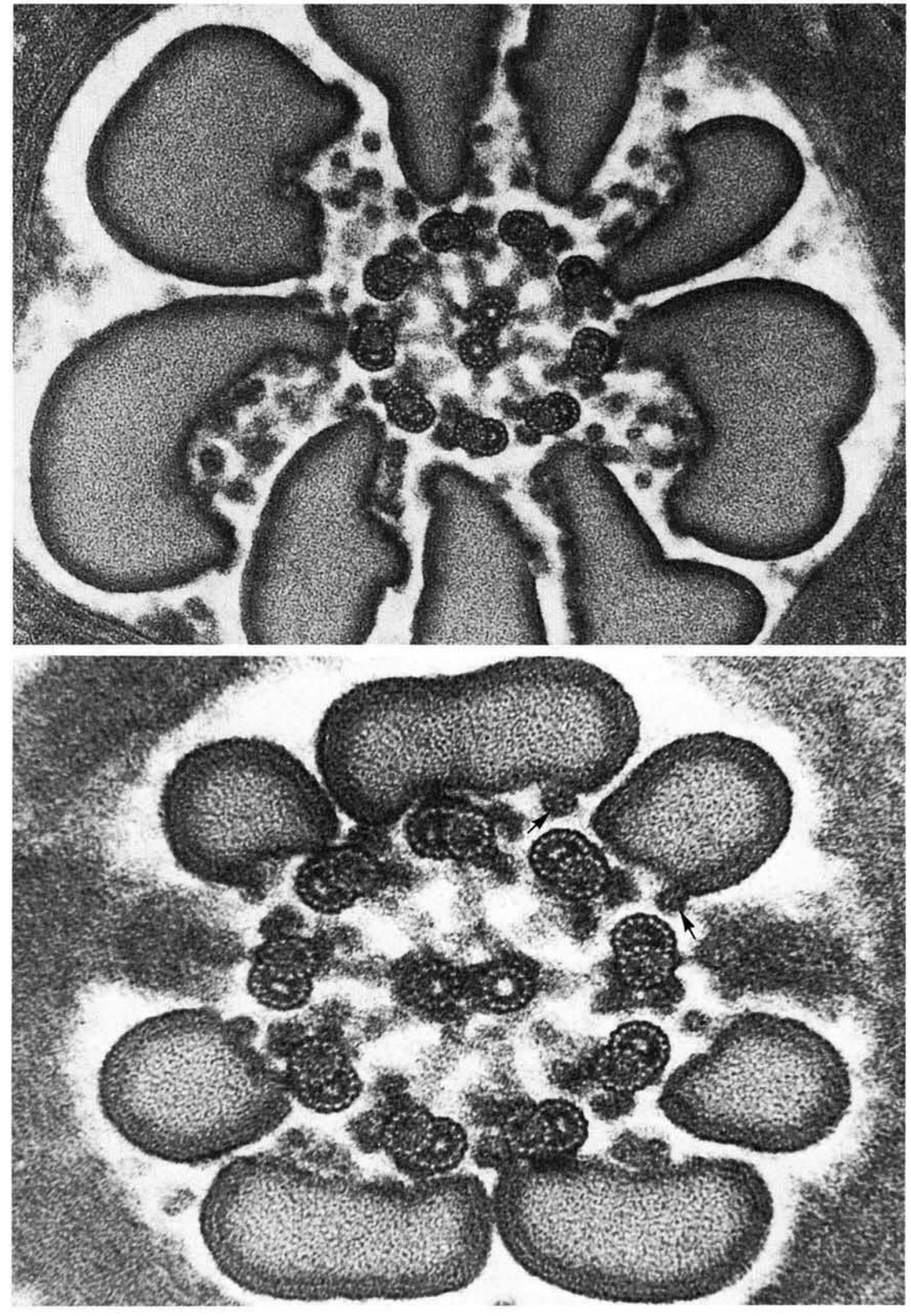
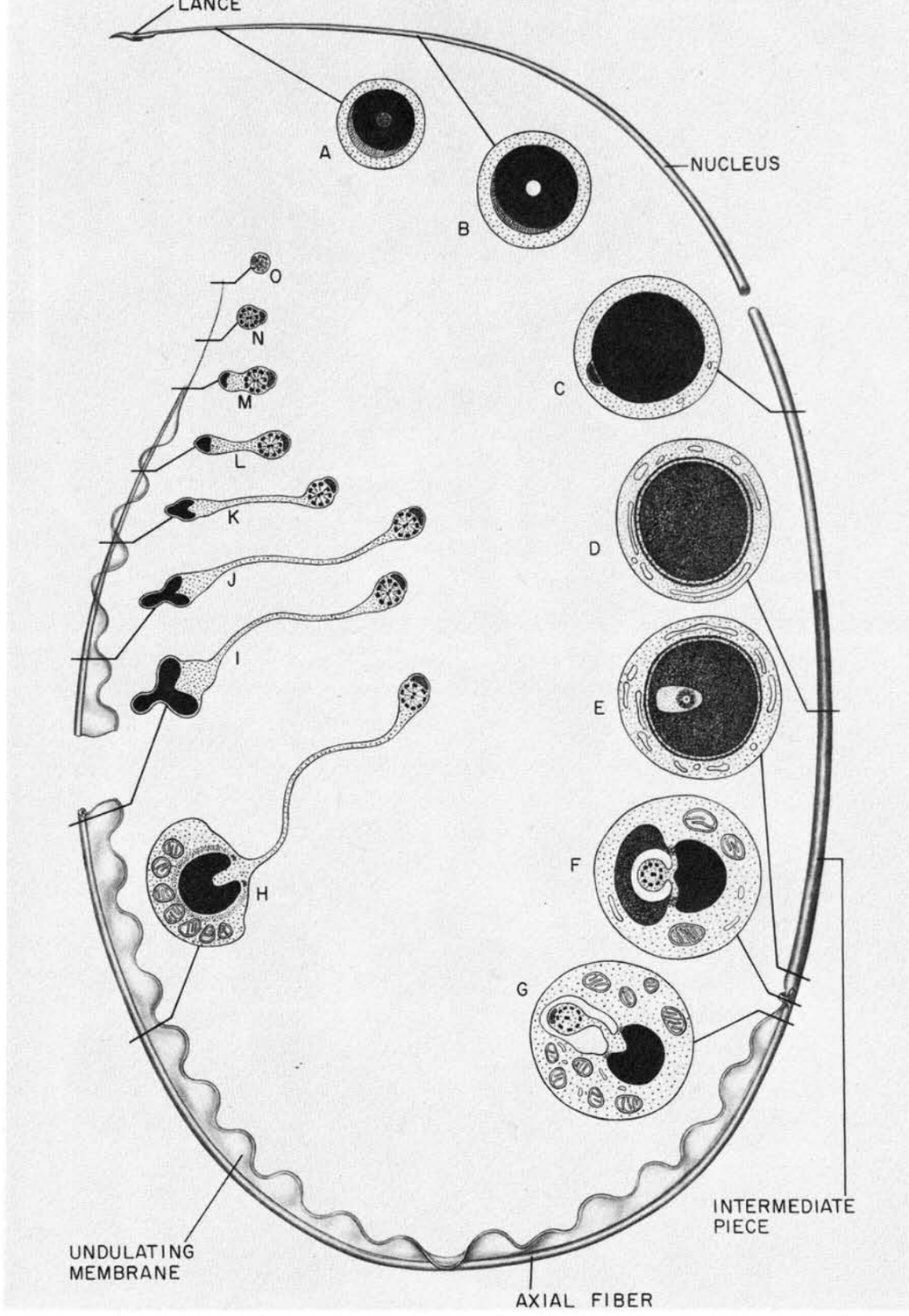


Figure 335. Cross-section of guinea pig sperm tail at the level of the midpiece, treated with tannic acid. (Micrograph courtesy of Daniel Friend.)

Figure 336. Cross-section of sperm tail at the level of the principal piece. Tannic acid preparation. (Micrograph courtesy of David Phillips.)

Figure 335, *upper* **Figure 336,** *lower*



Drawing of a urodele spermatozoon and the appearance of cross-sections at various levels, based upon studies of *Notophalmos viridescens*. (From D. W. Fawcett, Biol. Reprod. Suppl. 2, 90–127, 1970.)

URODELE SPERM FLAGELLUM

In the sperm tail of urodele amphibians, a large dense fiber or rod continues caudally in the axis of the nucleus and intermediate piece. This *axial fiber* is homologous with one of the nine outer dense fibers (number 8) of mammalian sperm tails. It tapers gradually toward the tip of the tail and its cross-sectional outline changes from horseshoe shaped (H) to a trefoil (J, K) and finally a thin crescent (L, M). Throughout the long middle piece of the spermatozoon, mitochondria are closely applied to the convex surface of the axial fiber (H).

The plasmalemma is attached to the axial fiber along the two ridges that form the heels of the horseshoe-shaped cross section, but between these lines of attachment it converges to form the *undulating membrane*, a thin fold that encloses the axoneme in its thickened free margin. A slender *marginal fiber* with a crescentic cross section is closely associated with doublet 3 of the axoneme. In the living spermatozoon, the thick axial fiber is immotile, but waves of bending rapidly propagated along the axoneme result in rapid undulations of the membrane that propel the sperm slowly forward.

Thus in urodeles, the 9+9+2 formula has been modified to 2+9+2. In toads, there is no marginal fiber and the axial fiber is thinner and more flexible than in urodeles. The activity of the axoneme not only produces rapid undulations of the membrane but results in low amplitude secondary bends in the tail as a whole. Motility resides entirely in the axoneme.

Urodele sperm occur in the testis in bundles, and a section through one of these at the level of the midpiece provides micrographs of the kind shown here. The cytoplasm surrounding the axial fiber contains numerous mitochondria. In both mammalian and urodele spermatozoa the mitochondria that provide the energy for locomotion are more closely associated with the dense fibers than they are with the motile axoneme. This relationship encouraged the speculation that the thick fibers or rods were accessory contractile elements. It is now known that they are non-contractile components of the sperm tail endowed with limited flexibility.

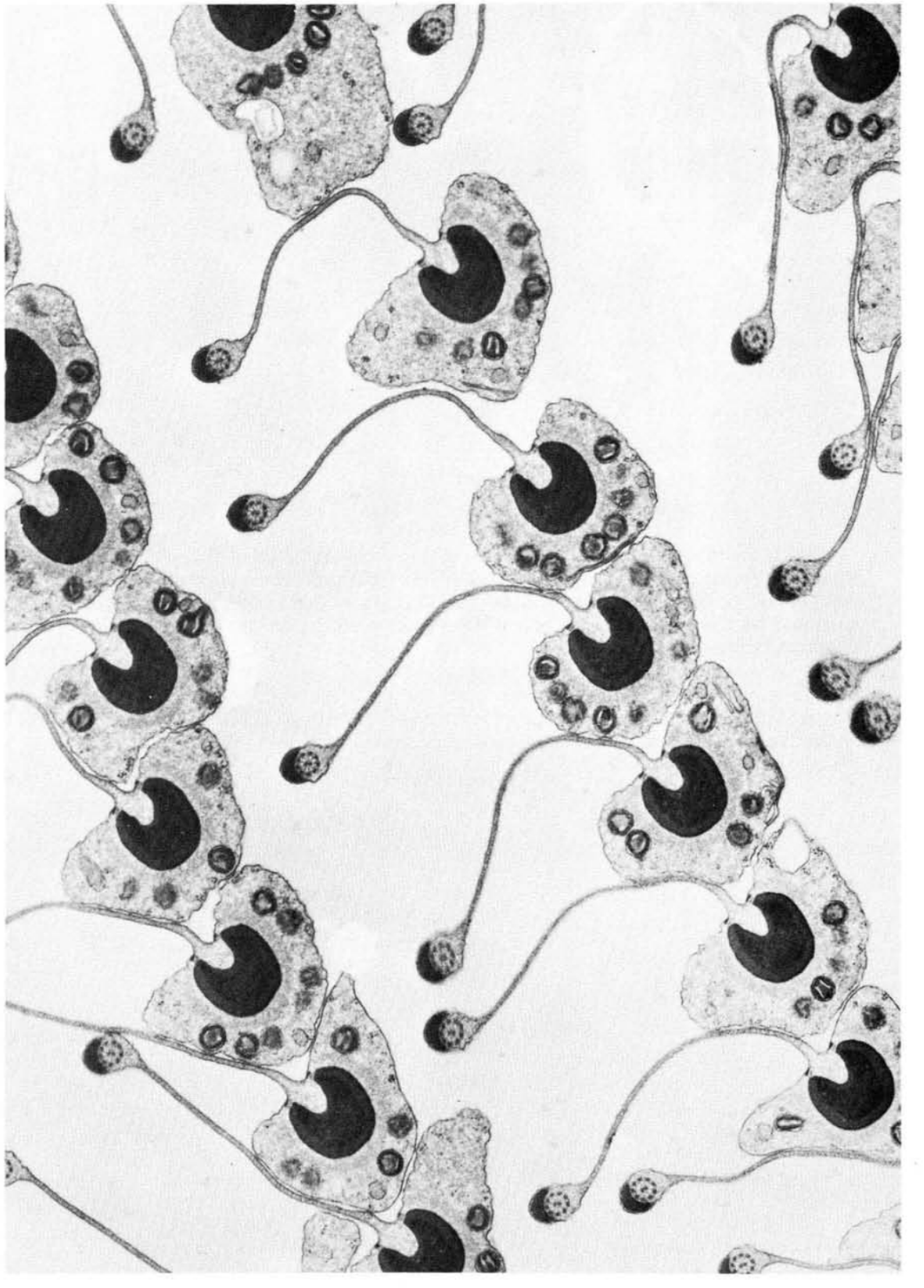


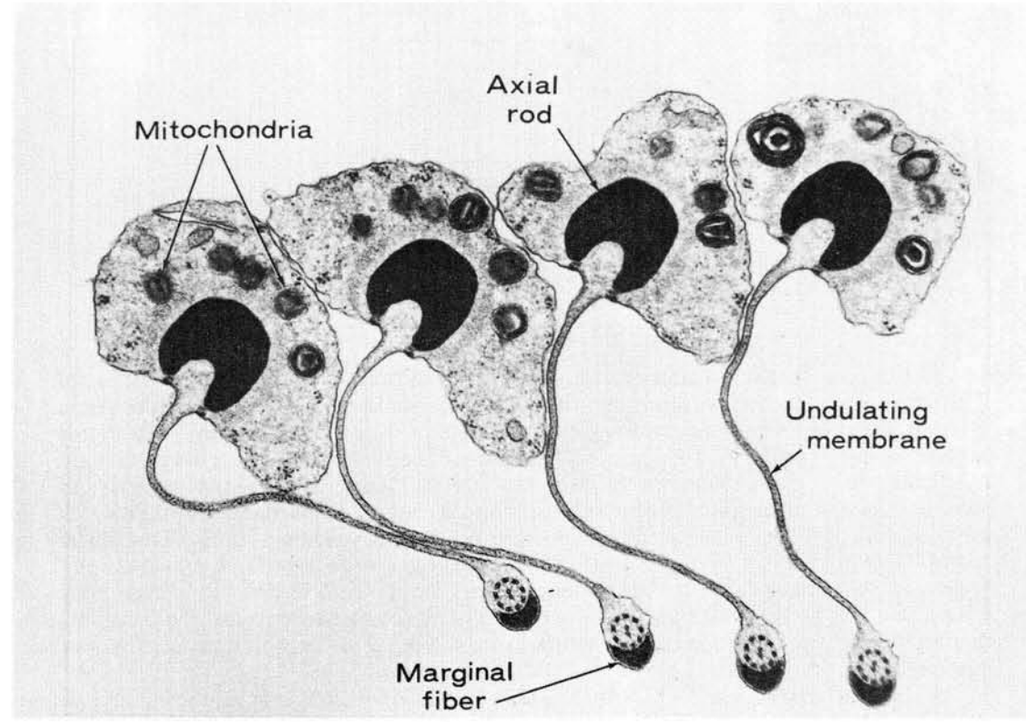
Figure 337. Transverse section through a portion of a sperm bundle of the newt, *Notophthalmos viridescens.*

Figure 337

The accompanying micrographs present transverse sections of the midpiece and principal piece of salamander sperm. Even though the flagellar membrane appears to be attached to parallel ridges of the axial fiber or rod, this evidently does not prevent ATP generated by the mitochondria from diffusing between the leaves of the undulating membrane to the axoneme.

In the relatively short principal piece, the axial rod assumes the form of a trefoil and the membrane is closely applied to its surface without intervening cytoplasm, except at the base of the undulating membrane. The axial fiber and marginal fiber evidently correspond to outer fibers 3 and 8 of the mammalian sperm tail.

Figure 338 and **339.** Electron micrographs of transverse sections of the midpiece and principal piece of several sperm from *Notophthalmos viridescens.* (From Fawcett, Biol. Reprod. [Supp. 2, 90–127, 1970.])



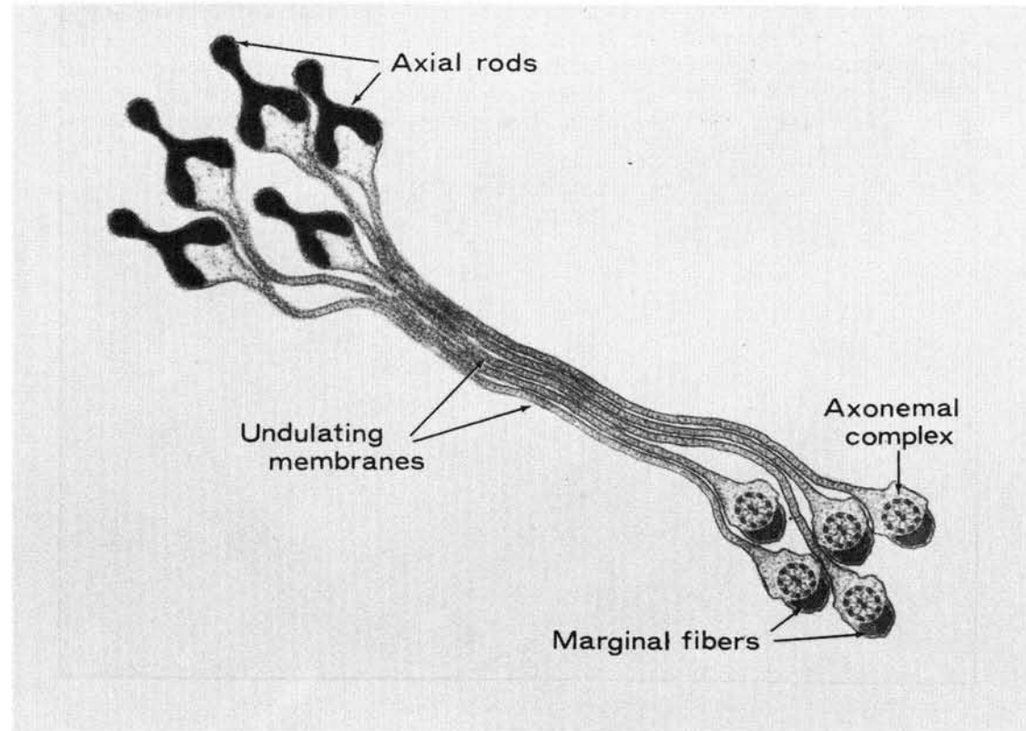
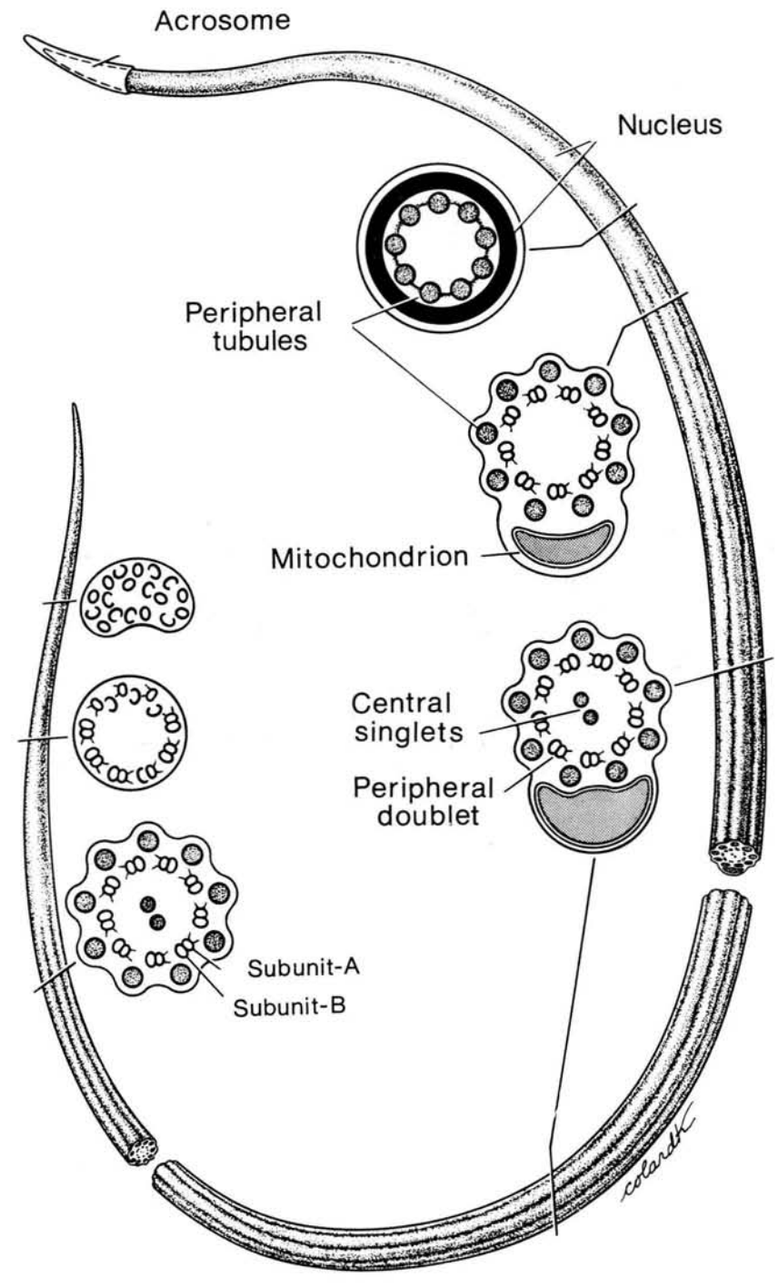


Figure 338, *upper* **Figure 339,** *lower*

INSECT SPERM FLAGELLUM

The flagella of insect spermatozoa vary greatly in their length and in the details of their cross-sectional organization, but the great majority have certain common features that are illustrated in the accompanying drawing based upon a caddis fly. The motor apparatus is a typical 9+2 axoneme, but in place of the nine outer dense fibers that are characteristic of mammalian sperm tails, the insects have nine *accessory tubules*. In cross section, these resemble other microtubules in having discernable protofilaments in their wall, but they vary in diameter from species to species with the number of protofilaments ranging from 13 to 16. Although composed of tubulin, these do not appear to be accessory motor elements, for they possess no dynein arms, and they would seem to be too far apart to interact in a sliding tubule mechanism. It is assumed that they serve as elastic stiffening structures analogous to the outer dense fibers of mammalian sperm flagella.

Instead of a helical mitochondrial sheath, insect sperm flagella have one or two modified mitochondria that run longitudinally along one side of the axoneme for nearly its entire length, which may be several hundred microns in some species. In the course of spermiogenesis, electron dense material accumulates in the mitochondrial matrix and forms one or more extensive paracrystalline structures that replace the usual pattern of membranous cristae. These *mitochondrial derivatives* are subject to considerable interspecific variation, and usually bear little resemblance to conventional mitochondria.



Drawing of an insect spermatozoon and representative cross sections, based upon studies of a caddis fly. (From D. Phillips, J. Cell Biol. 44:243–277, 1970.)

Sperm flagella of insects also differ from those of mammals in the position of the nine outer elements in relation to the doublets of the axoneme. In the mammal, each of the outer dense fibers remains directly radial to the corresponding doublet. In insect sperm, the accessory tubules arise as extensions from the wall of subunit B of each doublet, but when they detach, they take positions opposite the spaces between the axonemal doublets.

The position of the mitochondrial derivative is constant with respect to the components of the axoneme. It is centered on doublet number 3. A line passing through the centers of the central pair of microtubules is about 15 degrees oblique to the axis of symmetry passing through the mitochondrial derivative and between doublets 7 and 8.

Figure 340. Cross sections of sperm tails of a caddis fly. (Micrograph courtesy of David Phillips.)

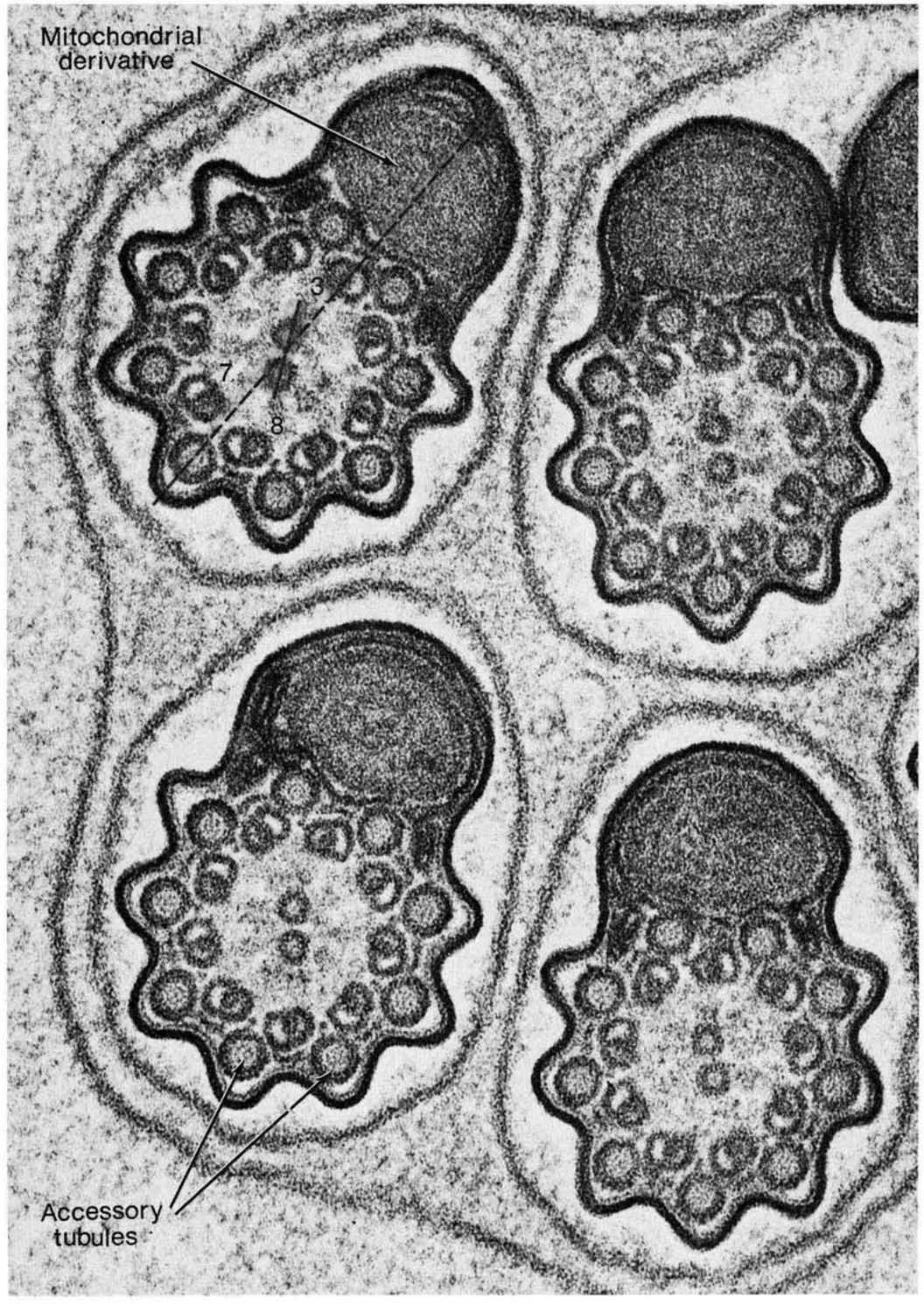


Figure 340

In some insect species, the lumen of the accessory tubules is filled with a dense material which gives them the appearance of dense fibers, but at higher magnification the protofilaments of the tubule wall can be resolved. The central pair of microtubules may also have a dense core.

The flagellar membrane of many Lepidopteran spermatozoa is decorated by radiating projections with a highly regular periodic structure. The chemical nature and significance of these appendages are obscure.

Figure 341. Cross sections of spermatozoa in the testis of a moth. (Micrograph courtesy of David Phillips.)

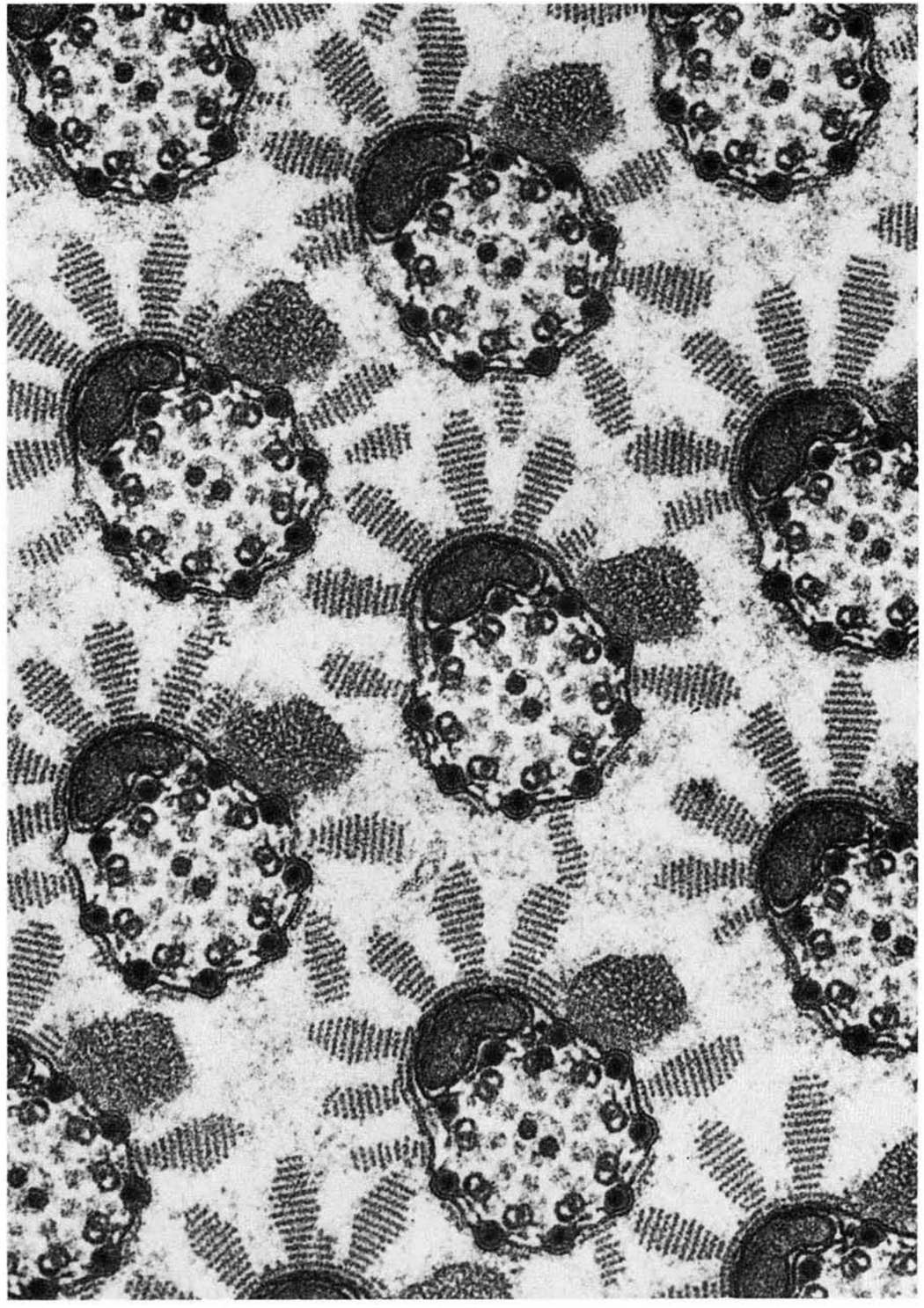
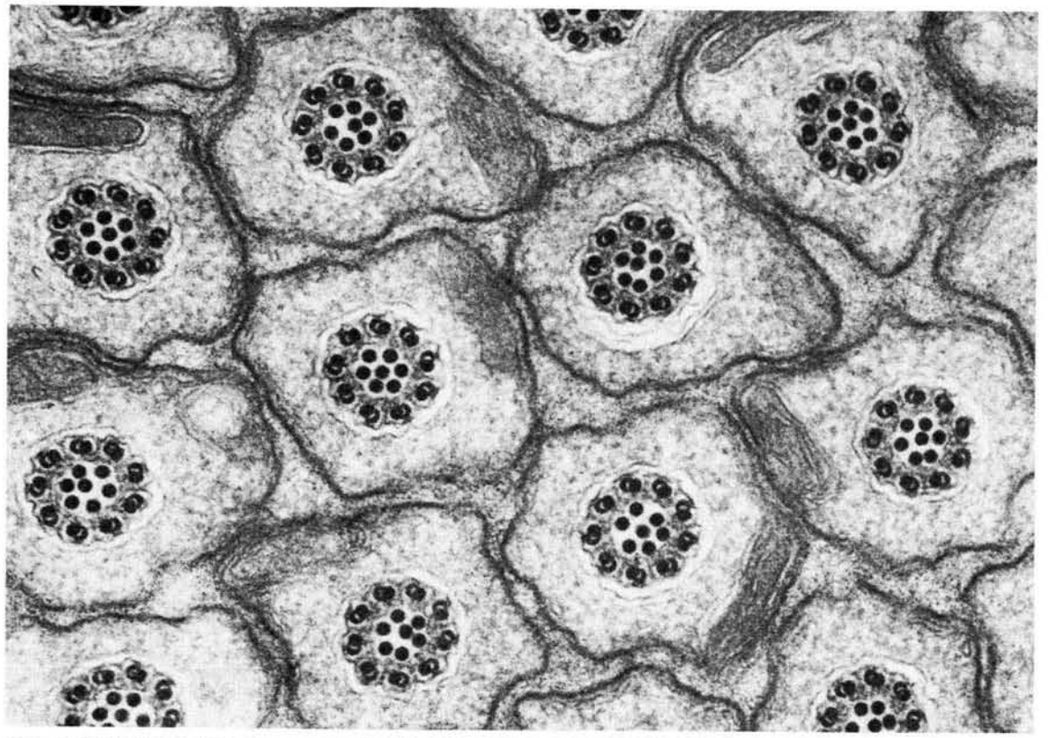


Figure 341

Sliding of the doublets of the axoneme has been conclusively shown to provide the motive force for flagellar motility. There is suggestive evidence that the regulatory function needed to convert doublet sliding to propagated waves of bending may involve some form of interaction between the radial spokes and the central pair of microtubules.

The nearly universal occurrence in motile flagella of the 9+2 or 9+9+2 patterns of axonemal components, and the observation that flagella with genetic absence of the central pair are immotile, has fostered the belief that the presence of two central microtubules is essential for motility. However, comparative studies on insect spermatozoa indicate that the number 2 is certainly not a *sine qua non*. The spermatozoa of a caddis fly in the upper figure on the facing page have a 9+7 formula while those of the mosquito, illustrated below, exhibit a 9+9+1 pattern. The spermatozoa of both species are motile, but detailed analysis of the tail movements has not been carried out to detect possible qualitative differences attributable to these unusual patterns.



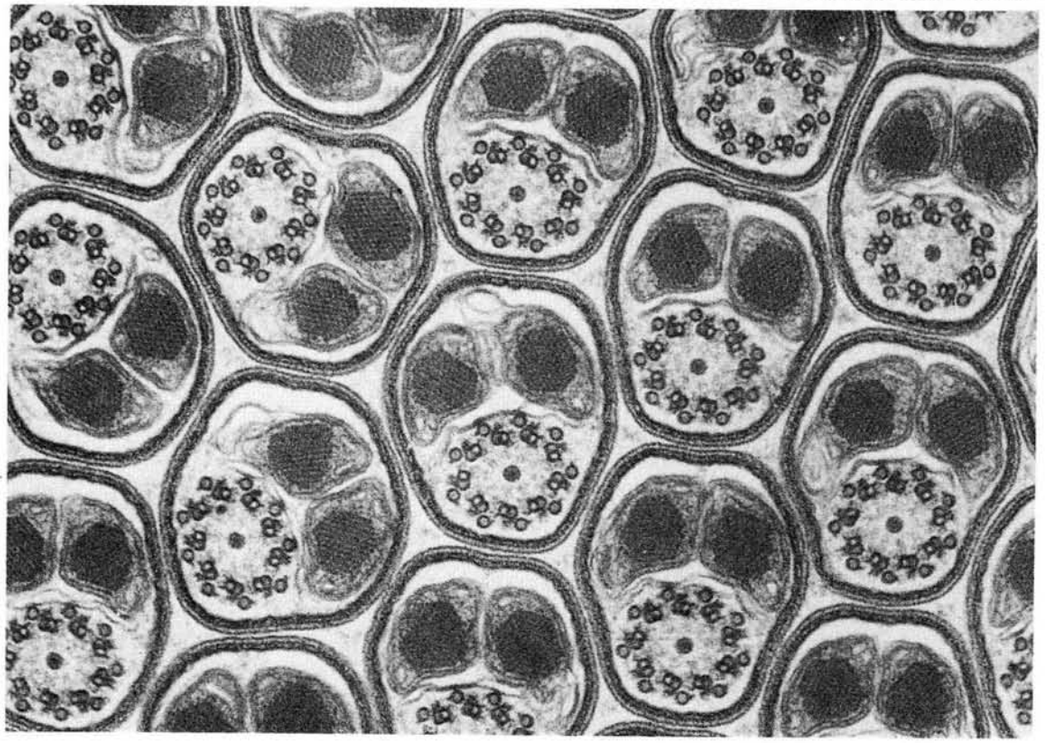


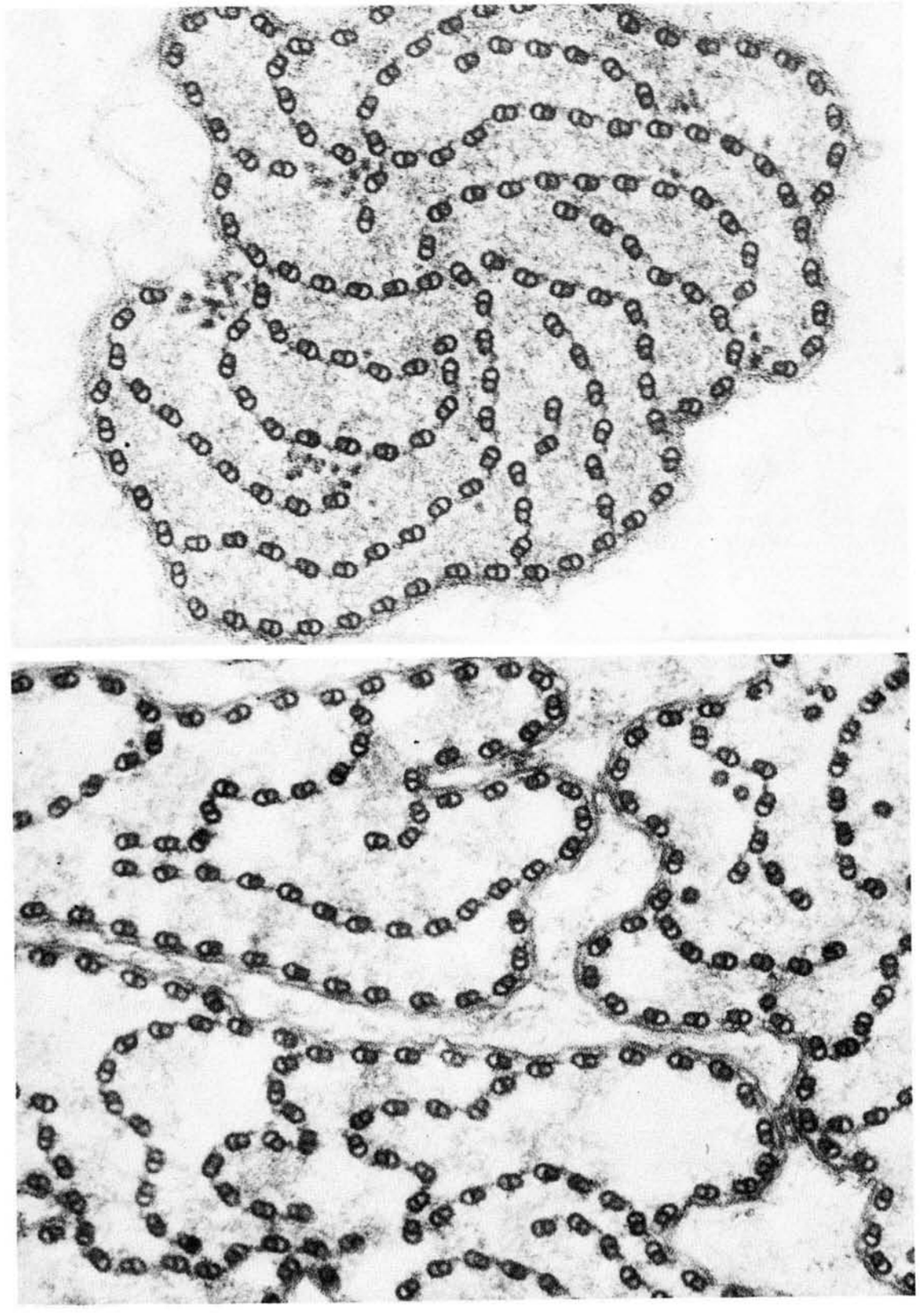
Figure 342. Cross sections of spermatozoa from the caddis fly *Polycentropus*. (Micrograph courtesy of David Phillips.)

Figure 343. Cross sections of spermatozoa of a mosquito of the genus *Culex*. (Micrograph from Phillips, J. Cell Biol. 40:28–43, 1969.)

Figure 342, *upper* **Figure 343,** *lower*

The most invariable components of motile flagella are the nine doublets. Rare exceptions are found among gall midges in which the sperm flagellum in some species has an extraordinary and varying pattern consisting of rows of doublets without any structure corresponding to the central pair in conventional axonemes. These spermatozoa exhibit a short period of vibratile motility in the female tract but do not show propagated waves of bending — an observation consistent with the view that the spokes and central pair in conventional 9+2 flagella are involved in translation of sliding into waves of bending.

It is evident in the lower figure that the 170 doublets are spaced at the normal intervals along the rows, but they possess only the outer dynein arm and no obvious spokes or other appendages.



Figures 344 and 345. Cross sections of the sperm tail of the Cecidomyid fly, *Monarthropalpus buxi*. (Micrographs courtesy of R. Dallai, from Baccetti et al., Tissue Cell 6:269–278, 1974.)

Figure 344, *upper* **Figure 345,** *lower*

One of the most extraordinary of the exceptions to the prevailing 9+9+2 pattern of insect sperm flagella is that of another cecidomyid fly illustrated in the upper micrograph on the facing page. The lobulated, elliptical cross section of the sperm tail contains mitochondria scattered among about 1000 closely packed doublet microtubules arranged in poorly defined rows.

In a related genus shown in the lower figure, approximately 100 doublets are arranged in parallel concentric rows or a spiral. These spermatozoa are believed to have a vibratile motion like that of other cecidomyid flies, but their motility has not been observed.

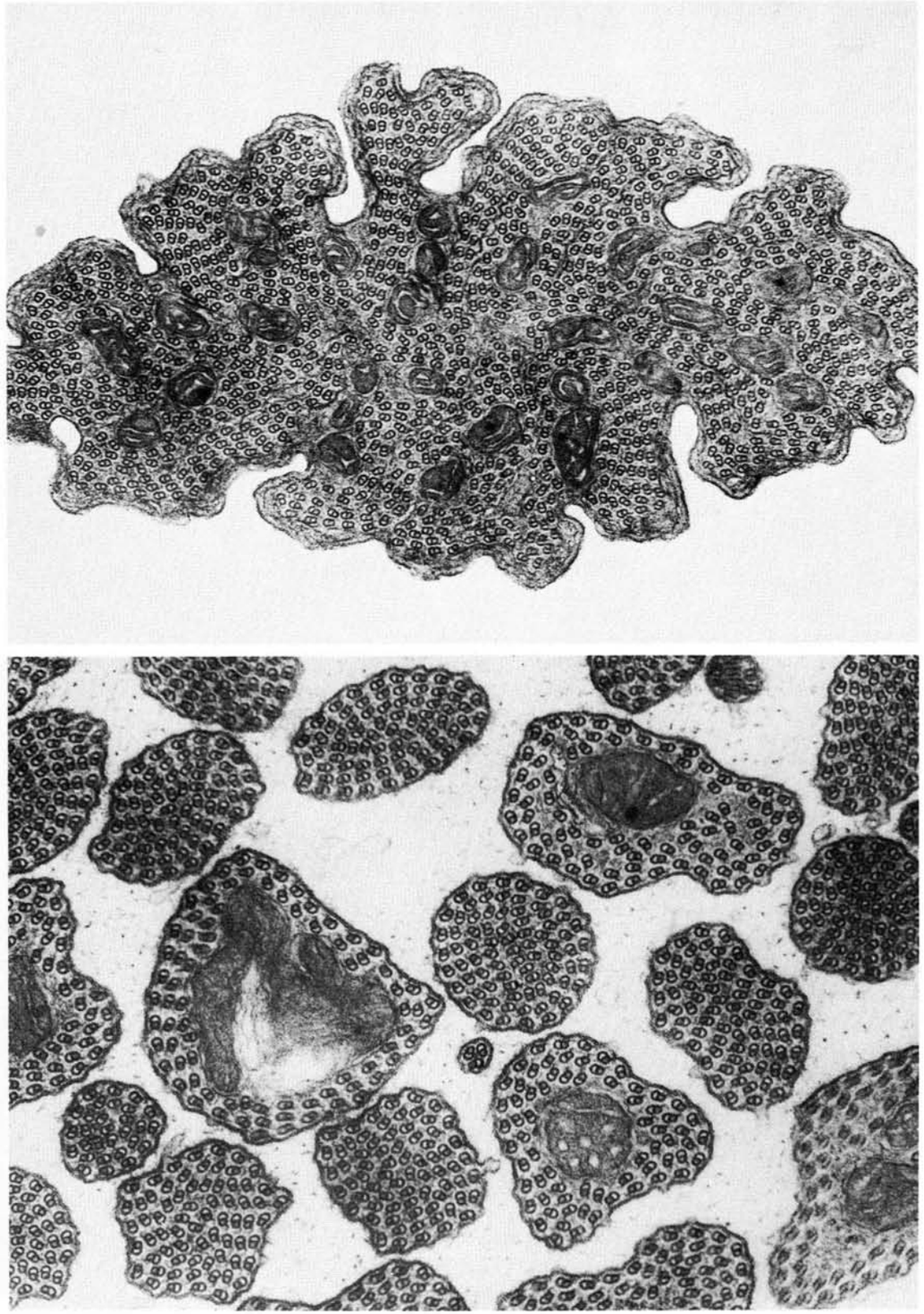


Figure 346. Cross section of sperm tail of the cecidomyid fly, *Diplolaboncus tumorificus*. (Micrograph from Baccetti and Dallai, J. Ultrastr. Res. 55:50–69, 1976.)

Figure 346

At higher magnification, it is apparent that the doublets have only the outer dynein arm (see at arrows). The arms on doublets in the same row are consistent in their orientation, but those in neighboring rows may either point in the same or in opposite directions.

The motor apparatus is still more aberrant in scale insects (coccids). It consists of singlet microtubules arranged in concentric rings around the nucleus or an amorphous central core.

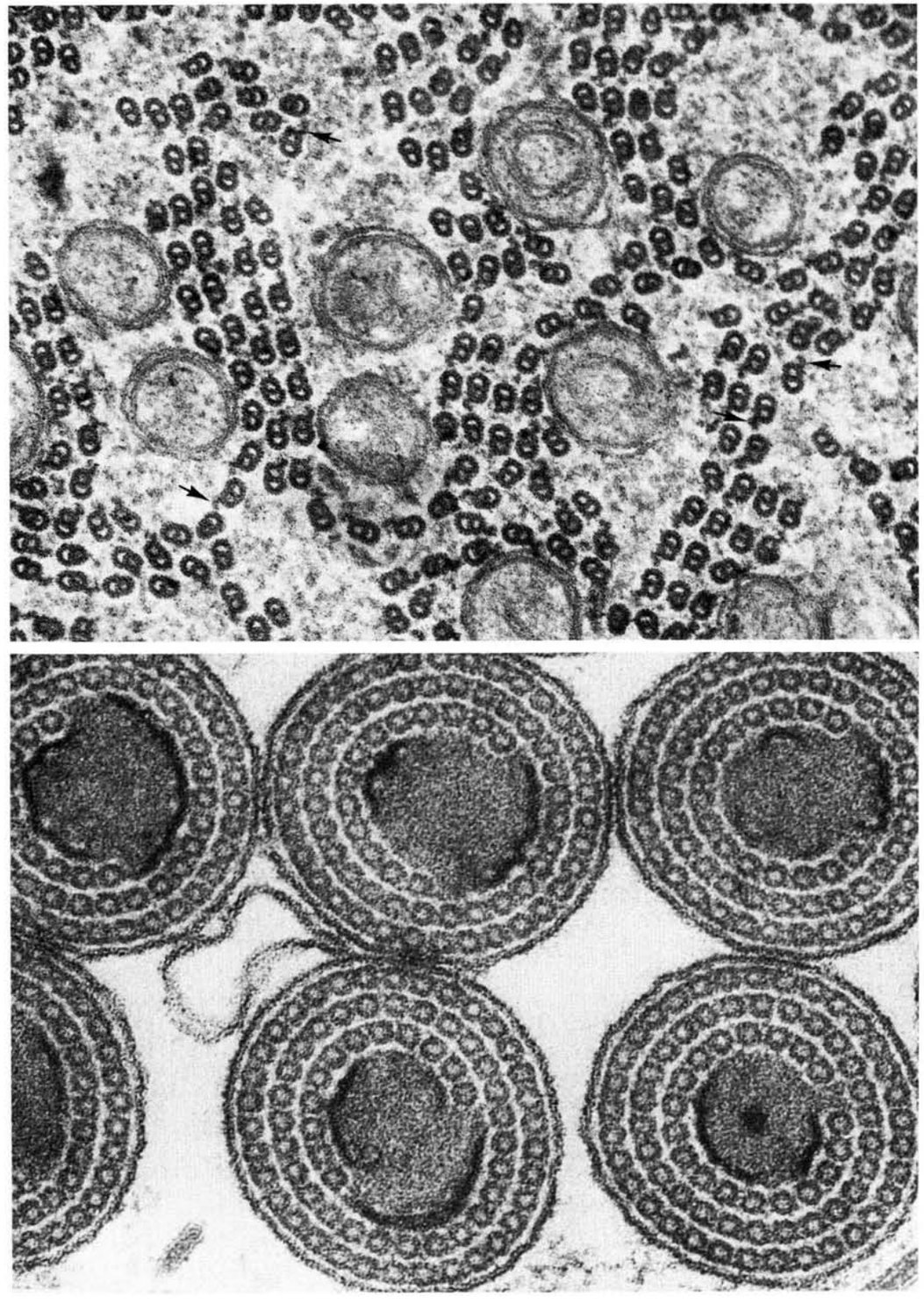


Figure 347. An area from a cross section of sperm tail from the cecidomyid fly, *Diplolahoncus tumorificus.* (Micrograph courtesy of Romano Dallai.)

Figure 348. Transverse sections of spermatozoa of the oyster shell scale insect, *Lipidosaphes.* (From Phillips, Spermiogenesis, Academic Press, New York, 1974.)

Figure 347, *upper* **Figure 348,** *lower*

REFERENCES

Cilia and Flagella and Sperm Flagellum

Afzelius, B. Electron microscopy of the sperm tail. Results obtained with a new fixative. J. Biophys. Biochem. Cytol. 5:269–278, 1959.

Afzelius, B., R. Eliasson, O. Johnson et al. Lack of dynein arms in immotile human spermatozoa. J. Cell Biol. 66:225–232, 1975.

Albrecht-Buehler, G. and A. Bushnell. The ultrastructure of primary cilia in quiescent 3T3 cells. Exp. Cell Res. 126:427–437. 1980.

Allen, R. D. A reinvestigation of cross sections of cilia. J. Cell Biol. 37:825–831, 1968.

Amos, L. A. and A. Klug. Arrangements of subunits in flagellar doublet microtubules. J. Cell Sci. 14:523–549, 1974.

Amos, L. A., R. S. Linck and A. Klug. Molecular structure of flagellar microtubules. *In* Cell Motility, Book C, (R. D. Goldman, T. D. Pollard, and J. L. Rosenbaum, eds.), pp. 847–867, Cold Spring Harbor Laboratory, New York, 1976.

Andre, J. and J. P. Thiery. Mis en evidence d'un sous-structure fibrillaire dans les filaments axonematiques des flagelles. J. Miscrosc. 2:71, 1963.

Bacetti, B., R. Dallai, F. Giusti and F. Bernine. The spermatozoon of Arthropoda XXV. A new model of tail having up to 170 doublets: *Monarthropalpus buxi*. Tissue Cell 6:269–278, 1974.

Baccetti, B., V. Pallini and A. G. Burrini. The accessory fibers of the sperm tail. III. High sulfur and low sulfur components in mammals and cephalopods. J. Ultrastr. Res. 57:289–308, 1976.

Baccetti, B. and R. Dallai. The spermatozoon of Arthropoda XXVII. Uncommon axoneme patterns in different species of cecidomyid flies. J. Ultrastr. Res. 55:50–69, 1976.

Baccetti, B. and B. A. Afzelius. The Biology of the Sperm Cell. S. Karger, Basel, 1976.

Ballowitz, E. Untersuchunger über die Struktur der Spermatozoön zugleich ein Beitrag sur Lehre von feinerem Base der contraktilen Elements. Arch. Mikroskop. Anat. Entwicklungsmech. 32:401–473, 1888.

Barnes, B. G. Ciliated secretory cells in the pars distalis of the mouse hypophysis. J. Ultrastr. Res. 5:453–467, 1961.

Bishop, D. W. and H. Hoffmann-Berling. Extracted mammalian sperm models. I. Preparation and reactivation with ATP. J. Cell. Comp. Physiol. 53:445–466, 1959.

Bishop, D. W. Sperm motility. Physiol. Rev. 42:1–59, 1962.

Brinkley, B. R., E. J. Stubblefield and T. C. Hsu. The effects of colcemid inhibition and reversal on the fine structure of the mitotic apparatus of chinese hamster cells in vitro. J. Ultrastr. Res. 19:1–18, 1967.

Brokaw, C. J. Movement of flagella of Polytoma uvella. J. Exp. Biol. 40:149–156, 1963.

Brokaw, C. J. Non-sinusoidal bending waves of sperm flagella. J. Exp. Biol. 43:155–169, 1965.

Brokaw, C. J. Bend propagation by a sliding filament model for flagella. J. Exp. Biol. 55:289–304, 1971.

Brokaw, C. J. Computer simulation of flagellar movement. Biophys. J. 12:564–586, 1972.

Chasey, D. Observations on the central pair of microtubules from the cilia of *Tetrahymena pyriformis*. J. Cell Sci. 5:453–458, 1969.

Chasey, D. Further observations on the ultrastructure of cilia from *Tetrahymena pyriformis*. Exp. Cell. Res. 74:471–479, 1972.

Dallai, R. An overview of atypical spermatozoa in insects. *In* The Spermatozoon (D. W. Fawcett and J. M. Bedford, eds.), pp. 253–265, Urban and Schwarzenberg, Baltimore, 1979.

Fawcett, D. W. Cilia and flagella. *In* The Cell, vol. 2 (J. Brachet and A. E. Mirsky, eds.), pp. 217–298, Academic Press, New York, 1961.

Fawcett, D. W. The anatomy of the mammalian spermatozoon with particular reference to the guinea pig. Z. Zellforsch. 67:279, 1965.

Fawcett, D. W. A comparative view of sperm ultrastructure. Biol. Reprod. 2, Suppl. 90–127, 1970.

Fawcett, D. W. The mammalian spermatozoon. A review. Dev. Biol. 44:394–436, 1975.

Fawcett, D. W. and S. Ito. The fine structure of bat spermatozoa. Am. J. Anat. 116:567, 1965.

Fawcett, D. W. and D. M. Phillips. Recent observations on the ultrastructure and development of the mammalian spermatozoon. *In* Comparative Spermatology (B. Baccetti, ed.), Academic Press, New York, 1969.

Fawcett, D. W. and K. R. Porter. A study of the fine structure of ciliated epithelia. J. Morphol. 94:221–281, 1954.

Gibbons, I. R. The relationship between fine structure and beat in the gill cilia of a lamellibranch mollusc. J. Biophys. Biochem. Cytol. 11:179–205, 1961.

Gibbons, I. R. Studies on the protein components of cilia from *Tetrahymena pyriformis*. Proc. Nat. Acad. Sci. 50:1002–1010, 1963.

Gibbons, I. R. Chemical dissection of cilia. Arch. Biol. (Liège) 76:317–352, 1965.

Gibbons, I. R. Studies on the adenosine triphosphatase activity of 14S and 30S dynein from cilia of *Tetrahymena*. J. Biol. Chem. 241:5590–5596, 1966.

Gibbons, I. R. The biochemistry of motility. Ann. Rev. Biochem. 37:521–546, 1968.

Gibbons, B. H. and I. R. Gibbons. Flagellar movement and adenosine triphosphatase activity in sea urchin sperm extracted with Triton X-100. J. Cell Biol. 54:75–97, 1972.

Gibbons, I. R. and A. V. Grimstone. On flagellar structure in certain flagellates. J. Biophys. Biochem. Cytol. 7:697–716, 1960.

Gibbons, I. R. and A. J. Rowe. Dynein: A protein with adenosine triphosphatase activity from cilia. Science 149:424–426, 1965.

Greenwood, A. D., I. Manton and B. Clarke. Observations on the structure of the zoospores of Vaucheria. J. Exp. Bot. 8:71–86, 1957.

Grimstone, A. V. and A. Klug. Observations on the substructure of flagellar fibres. J. Cell Sci. 1:351–362, 1966.

Jensen, O. S. Untersuchunger über die Samenkörper der Saugethiero, Vögel, und Amphibian. I. Saugetheire. Arch. mikroskop. Anat. Entwicklungsmech. 301:379–425, 1887.

Linck, R. W. Flagellar doublet microtubules: Fractionation of minor components and α-tubulin from specific regions of the A-tubule. J. Cell Sci. 20:405–439, 1976.

Linck, R. W. Advances in the ultrastructural analysis of the sperm flagellar axoneme. *In* The Spermatozoon (D. W. Fawcett and J. M. Bedford), Urban and Schwarzenberg, Baltimore, 1979.

Manton, I. The fine structure of plant cilia. Symp. Soc. Exp. Biol. 6:306–319, 1952.

Manton, I. and B. Clarke. Observations with the electron microscope in the internal structure of the spermatozoid of Fucus. J. Exp. Bot. 7:416–432, 1956.

Ogawa, K., T. Mohri and H. Mohri. Identification of dynein as the outer arms of sea urchin sperm axonemes. Proc. Nat. Acad. Sci. 74:5006–5010, 1977.

Olson, G. E., D. W. Hamilton and D. W. Fawcett. Isolation and characterization of the fibrous sheath of rat epididymal spermatozoa. Biol. Reprod. 14:517–530, 1976.

Olson, G. E., and R. W. Linck. Observations of the structural components of flagellar axonemes and central pair microtubules from rat sperm. J. Ultrastr. Res. 61:21–43, 1977.

Pease, D. C. The ultrastructure of flagellar fibrils. J. Cell Biol. 18:313–326, 1963.

Pedersen, H. and H. Rebbe. Absence of arms on the axoneme of immobile human spermatozoa. Biol. Reprod. 12:541–544, 1975.

Phillips, D. M. Exceptions to the prevailing pattern of tubules (9+9+2) in the sperm flagella of certain insect species. J. Cell Biol. 40:28–43, 1969.

Phillips, D. M. Fine structure of *Sciara coprophila* sperm. J. Cell Biol. 30:499–517, 1966.

Phillips, D. M. Insect sperm: Their structure and morphogenesis. J. Cell Biol. 44:243–277, 1970.

Phillips, D. M. Spermiogenesis. Academic Press, New York, 1974.

Picheral, B. Les éléments cytoplasmique au cours de la spermiogenèse du Triton Pleurodeles waltlii. III. L'evolution des formation caudales. Zeitsch. Zellforsch. 131:399–416, 1972.

Picheral, B. Structural, comparative and functional aspects of spermatozoa in urodeles. *In* The Spermatozoon (D. W. Fawcett and J. M. Bedford, eds.), pp. 267–287. Urban and Schwarzenberg, Baltimore, 1979.

Purkinje, J. F. and G. Valentin. Entdeckung kontinuierlicher durch Wimperhaare erzengter Flimmerbewegungen als eines allgemeinen Phänomens in der Klassen der Amphibien, Vögel, und Sangethieren. Arch. Anat. Physiol. 1:391–400, 1834.

Rieder, C. L., C. G. Jensen and L. Jensen. The resorption of primary cilia during mitosis in a vertebrate (Ptk_1) cell line. J. Ultrastr. Res. 68:173–185, 1979.

Rumery, R. E. and E. M. Eddy. Scanning electron microscopy of the fimbriae and ampullae of rabbit oviducts. Anat. Res. 178:83–102, 1974.

Sale, W. S. and P. Satir. The direction of active sliding in *Tetrahymena* cilia. Proc. Nat. Acad. Sci. 74:2045–2049, 1977.

Satir, P. Studies on cilia. The fixation of the metachronal wave. J. Cell Biol. 18:345–365, 1963.

Satir, P. Structure and function in cilia and flagella, pp. 1–52. Protoplasmatologia III E, 1965.

Satir, P. Studies on cilia. II. Examination of the distal region of the ciliary shaft and the role of the filaments in motility. J. Cell Biol. 26:805–834, 1965.

Satir, P. Studies on cilia. III. Further studies on the cilium tip and a "sliding filament model" of ciliary motility. J. Cell Biol. 39:77–94, 1968.

Scherft, J. P. and W. T. Daems. Single cilia in chondrocytes. J. Ultrastr. Res. 19:546–555, 1967.

Sleigh, M. A. Cilia and Flagella. Academic Press, London, 1974.

Sorokin, S. Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. J. Cell Biol. 15:363–377, 1962.

Stephens, R. E. Thermal fractionation of outer fiber doublet microtubules into A- and B-subfiber components: A- and B-tubulin. J. Molec. Biol. 47:353–363, 1970.

Stephens, R. E. Isolation of nexin — the linkage protein responsible for the maintenance of the ninefold configuration of flagellar axonemes. Bio. Bull. 139:438, 1970.

Summers, K. E. and I. R. Gibbons. Adenosine-triphosphate-induced sliding of tubules in trypsin-treated flagella of sea urchin sperm. Proc. Nat. Acad. Sci. 68:3092–3096, 1971.

Warner, F. D. New observations on flagellar fine structure. The relationship between matrix structure and the microtubule component of the axoneme. J. Cell Biol. 47:159–182, 1970.

Warner, F. D. and D. R. Mitchell. Structural conformation of ciliary dynein arms and the generation of sliding forces in *Tetrahymena* cilia. J. Cell Biol. 76:261–277, 1978.

Warner, F. D. and P. Satir. The structural basis of ciliary bend formation. Radial spoke positional changes accompanying microtubule sliding. J. Cell Biol. 63:35–63, 1974.

Witman, G. B., J. Plummer and G. Sander. *Chlamydomonas* flagellar mutants lacking radial spokes and central tubules. Structure, composition, and function of specific axonemal components. J. Cell Biol. 76:729–747, 1978.