

THE FINE STRUCTURE
AND ARRANGEMENT OF MICROCYLINDERS
IN THE LUMINA OF FLAGELLAR FIBERS
IN CRICKET SPERMATIDS

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ABSTRACT

Flagellar structure in spermatids of several species of cricket was studied with the electron microscope. The flagella of mid-spermatids contain the usual 9 + 2 set of fibers and a set of nine accessory fibers. At first all are hollow, then the lumina become filled with an electron-opaque matrix material in which narrow electron-lucent microcylinders are embedded. The accessory fibers and one central fiber become filled first, then the B subfibers and the other central fiber, and finally the A subfiber. In all but the B subfibers, microcylinders are arranged in a circular or oval group that lies against the wall of the lumen and encloses one or several additional microcylinders. In accessory fibers there are 9-11 microcylinders in the outer group and 4-5 in the inner group. In the central fibers and the A subfibers there are 7-9 microcylinders that enclose one or two more. In the B subfibers there is a crescentic group of 6-7 microcylinders that partially encloses 2-3 more. Microcylinders become packed as though they are independent units; the matrix appears to be an amorphous substance that fills the spaces around the microcylinders. The mean diameter of the microcylinders is 36 A, and they have a center-to-center distance of 56 A. In both respects they resemble wall subunits of flagellar fibers and microtubules and they may be similar structures but with a different localization. The diameter of accessory fibers is about 350 A, which is 25% greater than that of the other flagellar fibers and of cytoplasmic microtubules. Rotation tests suggest that the accessory fibers have 16 wall-subunits.

INTRODUCTION

Studies of the flagellar fibers of sperm have revealed many cases in which the fiber lumen is not empty. In some organisms, a narrow hollow rod has been resolved in the center of the lumen (Cameron, 1965; Phillips, 1966), the rest of the lumen being apparently empty; more commonly, material of high electron opacity seems to extend throughout the lumen (André, 1961; Phillips, 1969). In a previous study of flagella of house cricket spermatids (Kaye, 1964), dense material

was noted in some flagellar fibers and was found to have a complex substructural organization. Long, narrow, electron-lucent structures, termed microcylinders, run lengthwise in the lumen, embedded in dark matrix material. In longitudinal section where microcylinders are seen in side view, fibers display a series of alternating dark and light linear profiles. In transverse section, the microcylinders are seen end-on as light circular areas surrounded by dark material. In appearance

and size, these microcylinders are similar to the filamentous units that have been found in the walls of microtubules (André and Thiéry, 1963).

It can be seen in transverse sections that a number of microcylinders occur within the lumen of a fiber and that they are regularly arranged. In the present work, attention has been given to determining what complements and arrangements of microcylinders occur in the different types of fibers. Microcylinders have now been found in the lumens of all fibers of the axonemal complex, and specific arrangements of them have been found to characterize each fiber type. The observed variations in arrangement indicate that the microcylinders act as distinct units; hence they are not a part of the matrix.

Microcylinders are about 36 A in diameter, which is very similar to the size of the wall units seen in negatively stained microtubules (Pease, 1963; Behnke and Zelander, 1967). Electron-lucent structures similar to microcylinders have been demonstrated in the walls of cricket flagellar fibers. Their center-to-center spacing in the wall is about the same as the spacing of microcylinders in the lumen.

MATERIALS AND METHODS

Testes were taken from specimens of the house cricket *Acheta domestica*, which had been raised in the laboratory; from collected specimens of crickets that were tentatively identified as the common field cricket *Acheta assimilis*, and from collected specimens of tree crickets of the genus *Oecanthus*. Some of the latter were identified as *O. quadripunctatus*, which may be a color variation of *O. nigricornus* (Blatchley, 1920). The substructure and dimensions of flagellar fibers are so similar in the three species of cricket used in the present work that distinctions among species will not be made. The particular species used for each micrograph is, however, noted in the appropriate figure legend. There are some species differences in other components of the flagella and these are noted where pertinent.

All fixatives were used ice-cold. Testes were fixed in 6% glutaraldehyde and postfixed in 2% osmium tetroxide, both buffered to pH 7.2-7.4 with Sorensen's phosphate buffer. Testes were also fixed in 2% osmium tetroxide buffered to pH 7.2-7.4 with Veronal buffer, and then postfixed in formalin buffered to the same pH with the Sorensen buffer. Following fixation the testes were rinsed with ice-cold Sorensen buffer and dehydrated quickly in ethanol solutions beginning with 70%. They were embedded in Epon according to the method of Luft (1961). Sections were

TABLE I
Fiber Diameters

Fiber type	Mean	Range	N*
	A	A	
Accessory fibers			
Hollow	344	310-370	10
Filled	356	320-390	40
Central singlets			
Hollow	279	230-290	5
Filled	285	260-300	9
Cytoplasmic microtubules	285	270-310	10

The outer diameters of flagellar fibers, both hollow and filled with microcylinders and matrix, and also of various cytoplasmic microtubules which occur in spermatids.

* N represents the number of fibers observed with each arrangement.

stained with 1% uranyl acetate followed by lead citrate (Reynolds, 1963).

Sections were examined, and micrographs made, with a Siemens Elmiskop I electron microscope at 80 kv and at direct magnifications up to 120,000 X.

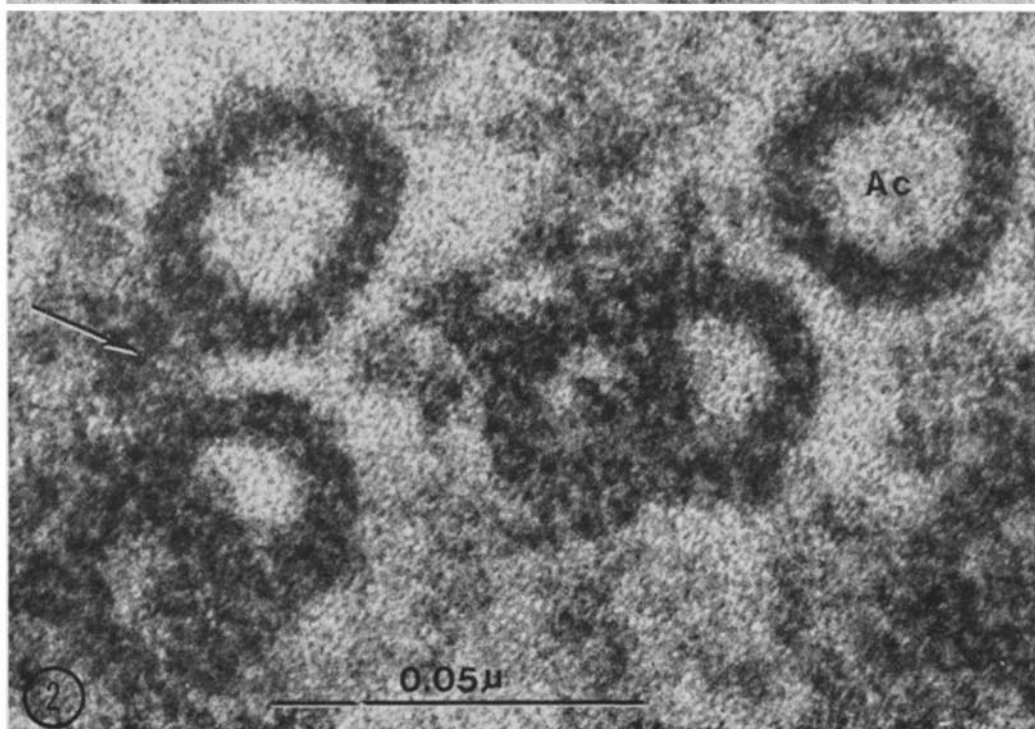
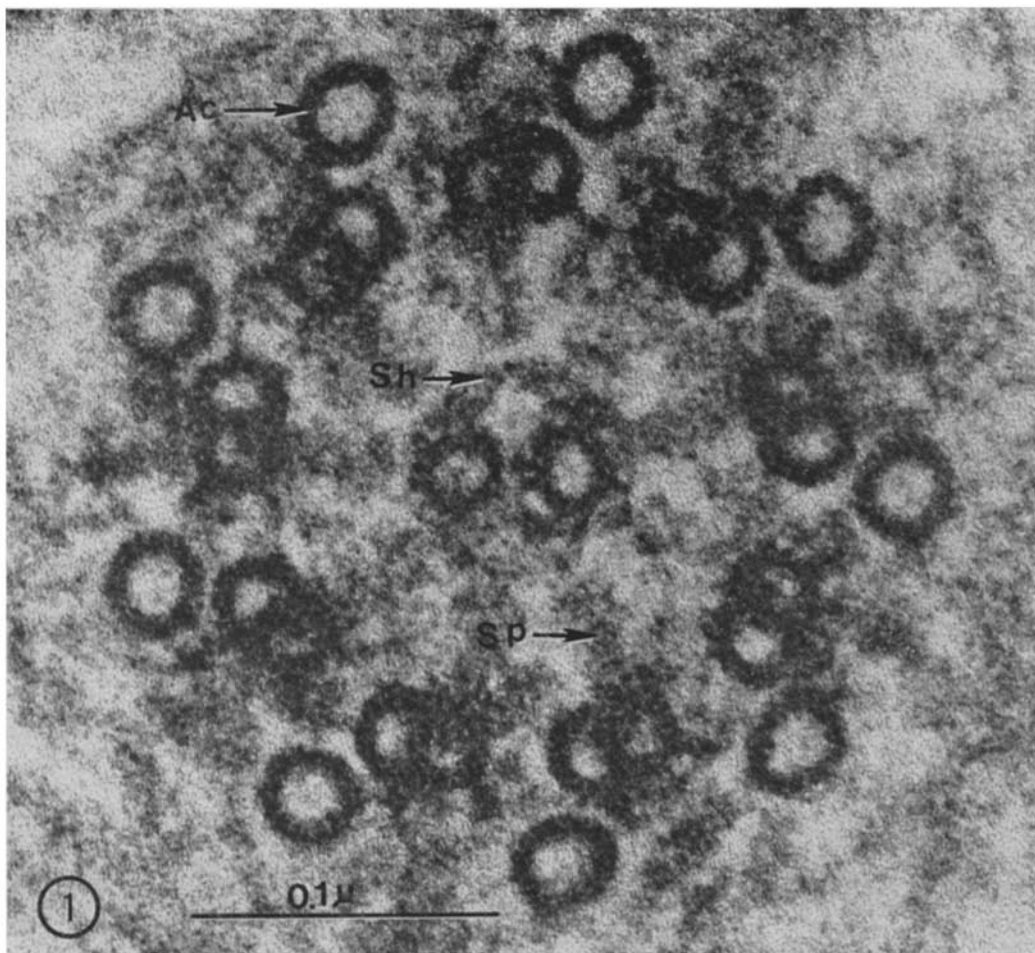
The magnification of the electron microscope was determined by the following two methods: (a) by the use of the internal calibration system of Siemens wherein the image of a hole of known diameter is projected onto standard circles on the viewing screen, and (b) by the use of a diffraction grating-replica with 2160 lines/mm.

All measurements of the size of flagellar components were made on enlarged photographic prints with final magnifications up to about 500,000 X. The scale used was a reticle divided into tenths of a millimeter, which was viewed through a 7 X magnifying lens.

The magnification of the photographic enlarger was determined from prints made of a calibration plate that were processed along with prints of micrographs to minimize errors due to swelling or shrinkage of the photographic paper.

OBSERVATIONS

Flagella of early spermatids contain the usual complement of nine doublet and two singlet fibers. A set of nine additional singlet fibers, the accessory fibers, appears subsequently. These fibers are typical of most insect sperm (André, 1961; Kaye, 1964; Phillips, 1969) and appear to originate from an extension that grows from each doublet, then curves around to form an additional fiber (Cameron, 1965). In crickets the accessory fibers are considerably larger in diameter than



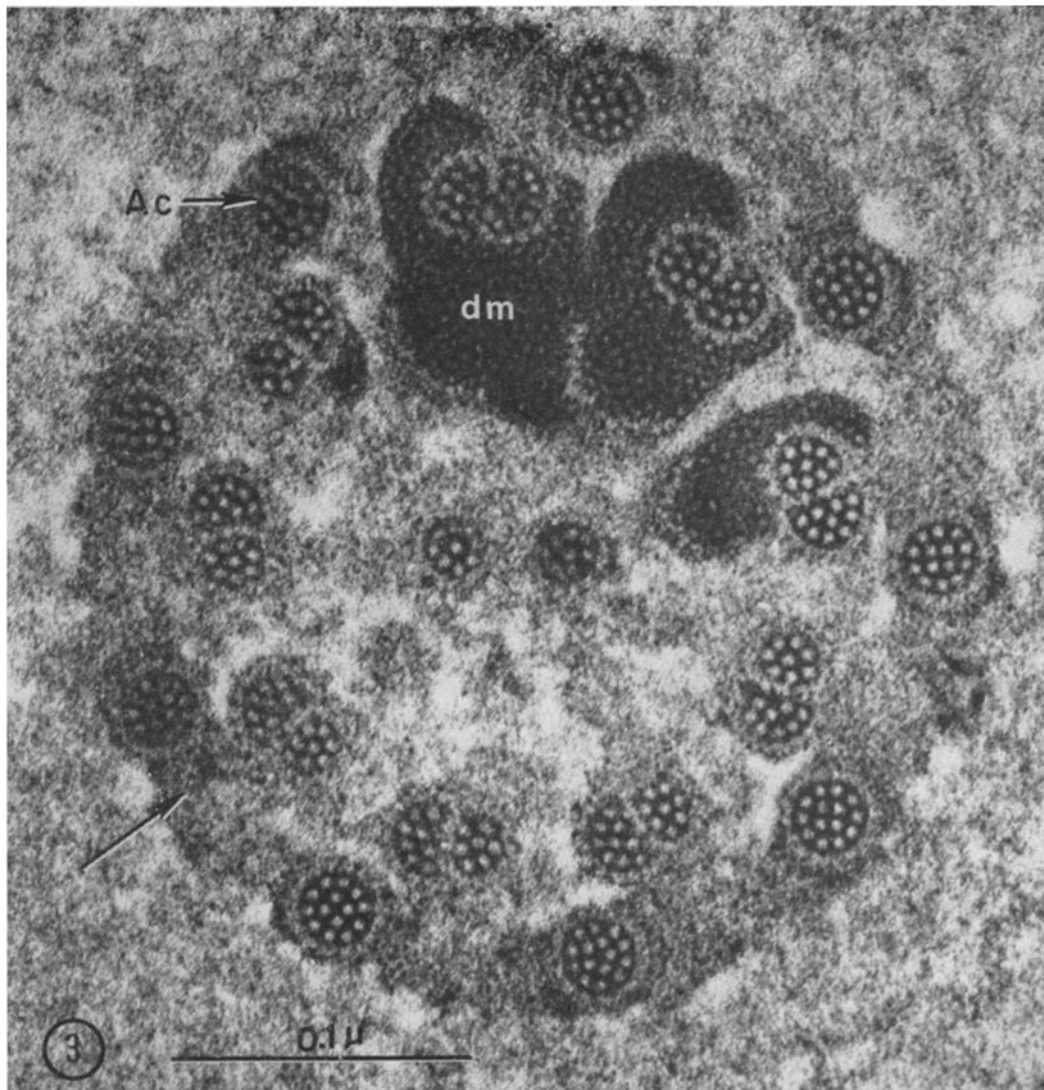


FIGURE 3 A transverse section through the flagellum of a late spermatid of a tree cricket. Accessory fibers (*Ac*) are connected by diffuse material (arrow). Dense material (*dm*) partially envelops several doublet fibers. All fibers are filled with matrix and microcylinders. $\times 390,000$.

FIGURE 1 A transverse section through the flagellum of a mid-spermatid of a field cricket, which shows the 9 + 2 set of fibers, accessory fibers (*Ac*), a sheath (*Sh*) associated with the central pair, and spokes (*Sp*), which run from each A subfiber to the sheath. $\times 410,000$.

FIGURE 2 A portion of a flagellum similar to that in Fig. 1, showing the accessory fibers (*Ac*) and their associated doublet fibers. No organized structure is visible in the walls of the fibers. One doublet and its associated accessory fiber are connected by a short arm (arrow). $\times 980,000$.

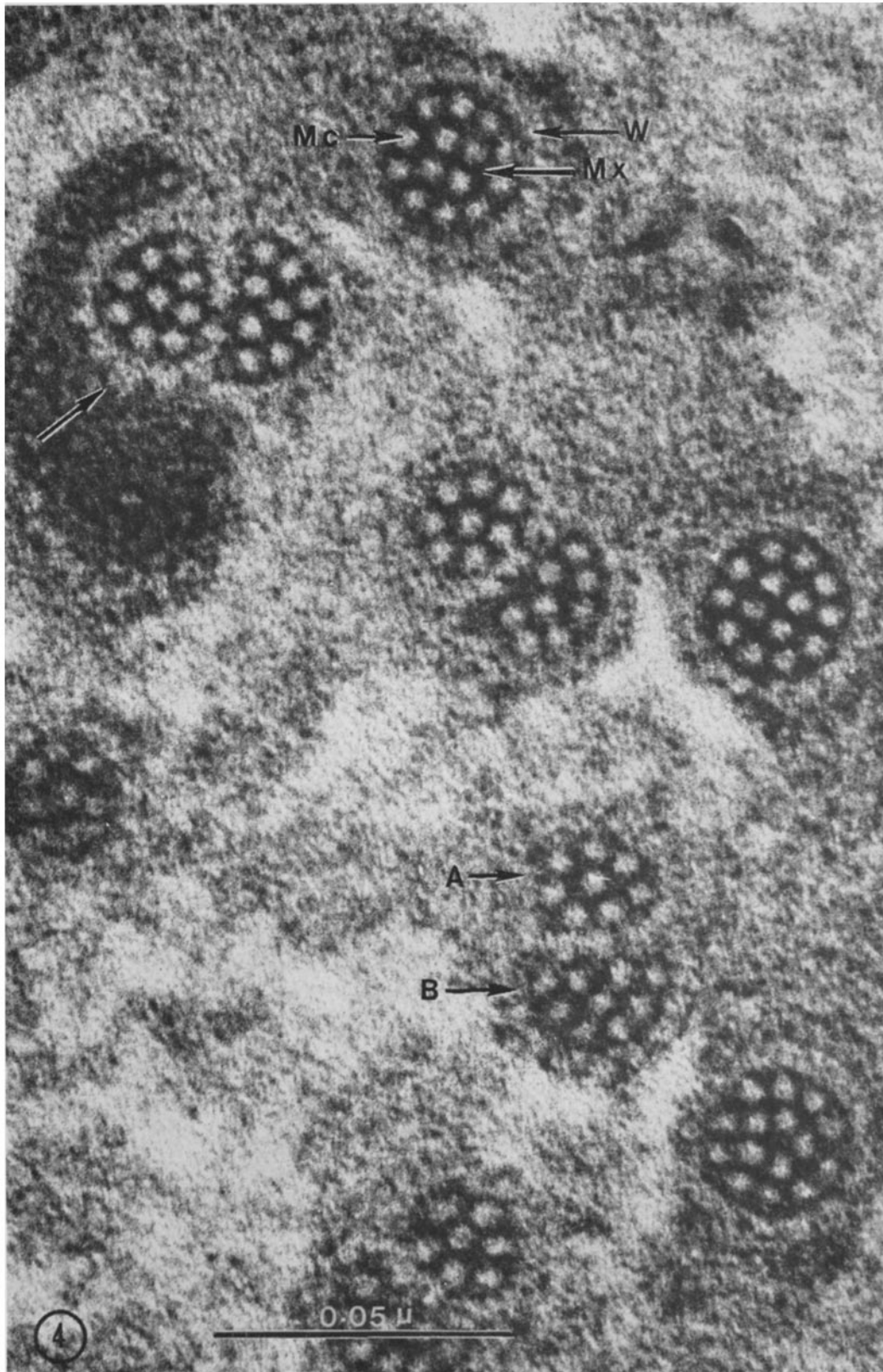


FIGURE 4 A portion of the flagellum in Fig. 3. Microcylinders (*Mc*) appear as light circular areas against the dark matrix (*Mx*). Accessory fibers with microcylinder complements of the 10 + 4 and the 10 + 5 types are shown together with a variety of complements in the A (*A*) and B (*B*) subfibers of the doublets. The electron opacity of the walls (*W*) of the fibers is low relative to that of the matrix. The border of the dense material appressed to the doublet fiber wall is scalloped (arrow) in a manner that suggests wall subunits are impressed in it. $\times 920,000$.



FIGURE 5 A transverse section through a flagellum in the tailpiece of a late tree cricket spermatid. The outline of the flagellum is oval, accessory fibers are absent, the central pair (*Ce*) is displaced toward the periphery, and the uppermost peripheral fiber is a singlet. Dense material (*dm*) is associated with most of the peripheral fibers. $\times 310,000$.

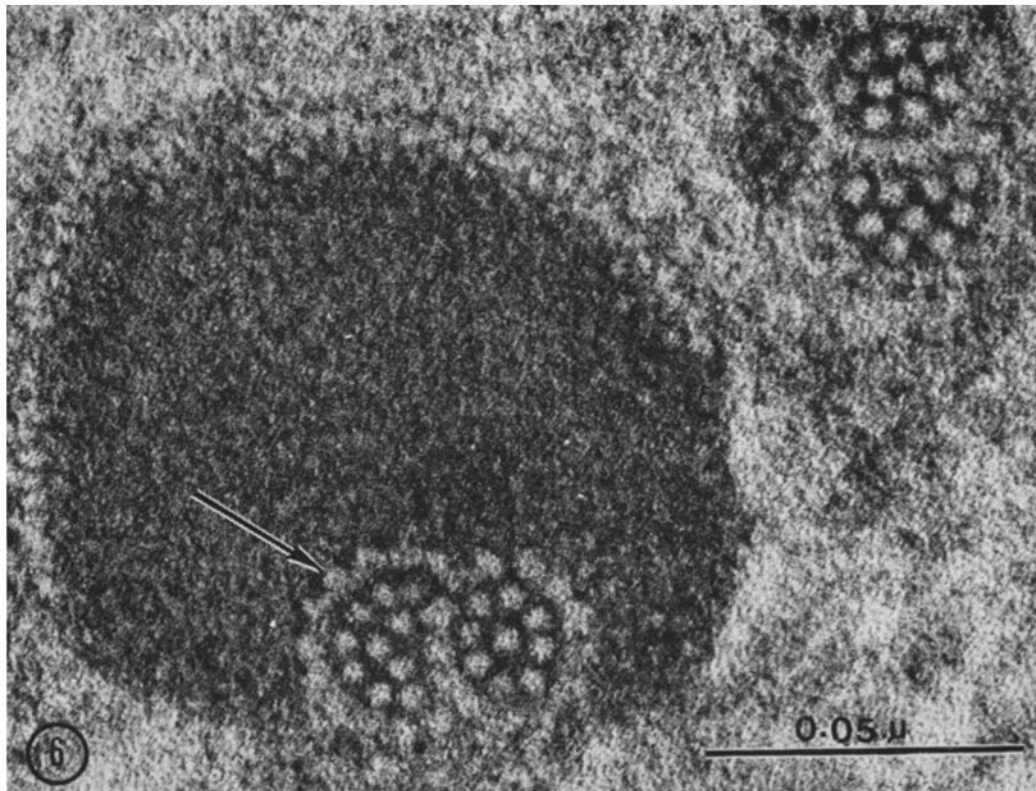


FIGURE 6 An enlargement of one of the doublet fibers in the preceding figure. The border of the dense material is scalloped (arrow) where it is appressed against the walls of the fibers. $\times 830,000$.

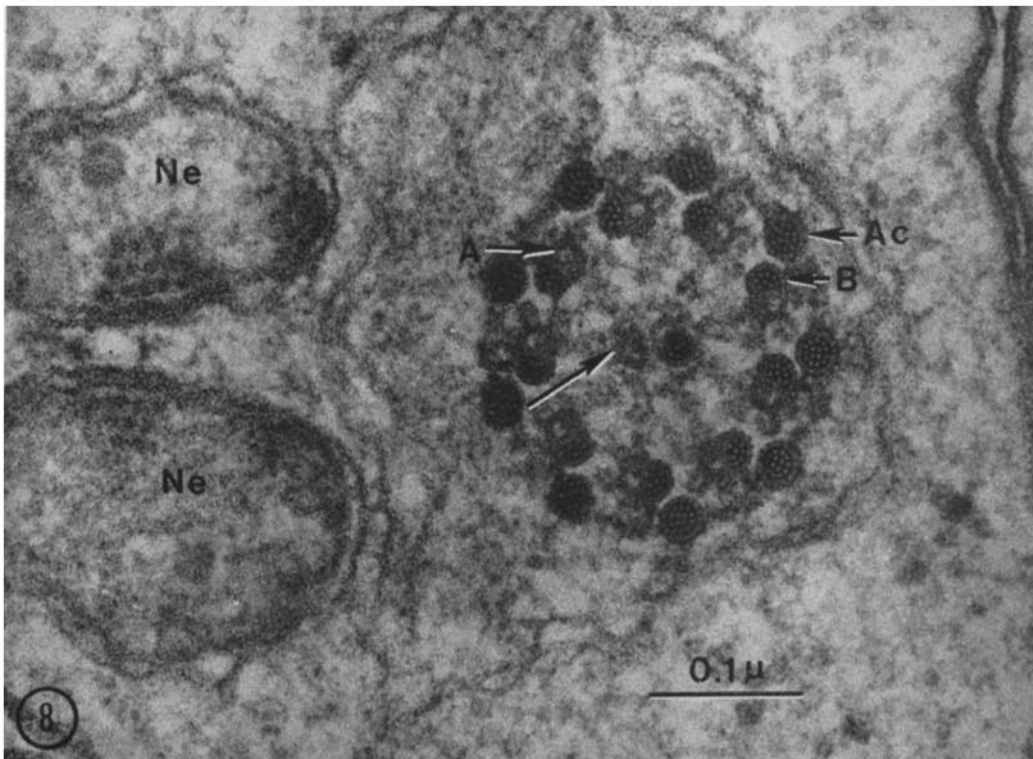
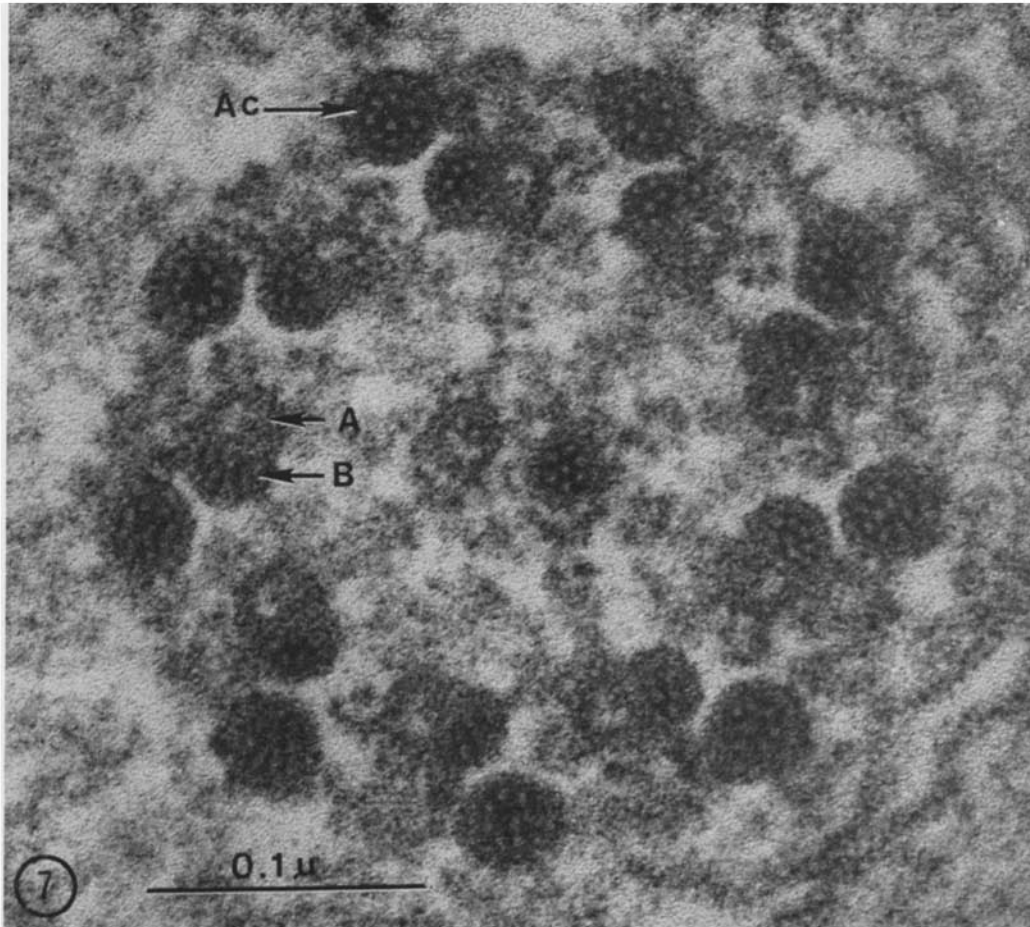
the central singlets (Table 1). The following components of flagella in mid-spermatids can be seen in Figs. 1 and 2: central singlet fibers that have a sheath associated with them, the outer doublet fibers, spokes going from the A subfibers toward the central fibers' sheath, and the accessory fibers in a circle just peripheral to the doublets. Each accessory lies close to the B subfiber of a doublet. Some of the doublets display a short

arm extending toward the adjacent accessory fiber.

Some additional components occur in flagella of late spermatids, and can be seen in Figs. 3 and 4. A diffuse material has appeared that forms connections between the accessory fibers and also sends projections to the doublets. Masses of another kind of material, of high electron opacity, partially envelop doublet fibers in some cases, as seen in

FIGURE 7 A transverse section through a flagellum of a field cricket spermatid in an intermediate stage of development. Microcylinders and matrix occur in the lumens of accessory fibers (*Ac*), B subfibers (*B*), and one central fiber, but in lower numbers than at the end of development. The A subfibers (*A*) and one central fiber are still hollow. $\times 370,000$.

FIGURE 8 A transverse section through a flagellum of a field cricket spermatid in which the A subfibers (*A*) and the central fiber (arrow) closest to the nebenkern (*Ne*) are hollow. The accessory fibers (*Ac*) and the B subfibers (*B*) have microcylinders and matrix in their lumens. $\times 200,000$.



Figs. 3-6. The material has a spongy appearance, with thin lines of high opacity surrounding areas of lower opacity. It occurs in the endpiece of house cricket spermatids, and in both the mid- and endpieces of tree crickets, spermatids, but has not been seen in field cricket spermatids.

Flagellar fibers have empty lumens in mid spermiogenesis; during late spermiogenesis, the lumens become filled with material. Flagella of late spermatids with some fibers filled and others still hollow (or containing only a trace of disordered material) have been observed, and they presumably are in intermediate stages of development. Two intermediate conditions have been observed, and they suggest that the accessories and one central fiber fill in relatively early, the other central fiber and the B subfibers sometime later with no set sequence of precedence, and the A subfibers last. The flagella shown in Figs. 7 and 8 illustrate a condition that has been seen fairly often. All their accessory fibers, one central singlet, and the B subfibers are filled. The member of the central pair that is filled is always that one which is farther from the nebenkern, as shown in Fig. 8. Another intermediate condition is also common and was pictured previously (Kaye, 1964); in this case, all accessories, both central singlets, and some of the B subfibers are filled, while other B subfibers and all A subfibers are empty. It was suggested previously that this latter condition, which was the most advanced developmental state that had been seen then, represented the state of the flagellar fibers at maturity. Subsequently, however, flagella with all fibers filled have been observed.

In late spermatids (Figs. 3-6) the lumina of all three types of flagellar fibers are filled and display a regular arrangement of microcylinders in a surrounding matrix. An extremely high electron opacity is the outstanding characteristic of the matrix, which appears structureless and without definite organization. The strikingly regular patterns seen in the lumen result from the orderly arrangement of electron-lucent areas, approximately circular in profile, which represent transverse sections of the microcylinders. The very high contrast between the matrix and the microcylinders gives the impression that the microcylinders are simply holes of no electron opacity within the matrix; however, close inspection shows that their opacity is definitely higher than that of empty areas in the section. Nonetheless,

observation of the microcylinders is dependent on the presence of the surrounding matrix. Similarly, the walls of fibers are inconspicuous in contrast to the opaque matrix; but the inner boundary of the fiber wall is outlined clearly by the underlying matrix. Where both subfibers of a doublet are filled (Figs. 3-6), it is especially clear that they share a common wall.

When material first appears in the lumen, its organization is different from that typical of later stages, there being a higher proportion of matrix and a lower number of microcylinders relative to the condition in later spermatids. This can be seen by comparing the intermediate stages shown in Figs. 7 and 8 with the later stages that are described in detail below; the B subfibers of Fig. 8 and all types of filled fibers in Fig. 7 have fewer microcylinders than is typical for the later stages.

In the most advanced flagella observed, when all fibers are filled, each kind of fiber is characterized by a particular arrangement and complement of microcylinders, both of which show only slight variation. In the accessory fibers (Figs. 3 and 4), the central singlets (Figs. 3 and 5), and the A subfibers (Figs. 3-6), the microcylinders are arranged in a ring near the periphery of the lumen, enclosing one or several more microcylinders. The B subfibers (Figs. 3-6) are slightly different, their peripheral group forming an incomplete ring. The number of microcylinders in the ring and the number in the center depend largely on the type of fiber; there is very little variation otherwise. All the combinations that have been observed, and their frequencies, are listed in Table II.

The accessory fibers are the largest fibers (Table I) and contain the largest complement of microcylinders. There are 9, 10, or 11 microcylinders in their outer ring; the ring is approximately circular. The central area contains a cluster of either four microcylinders in a square arrangement or five in a pentagonal arrangement. Fibers with a circle of 10 around a cluster of 4 are seen most frequently; a circle of 10 around a cluster of 5 is next most frequent. Both types are shown in Figs. 3 and 4. A relatively rare arrangement, a circle of 11 around a cluster of 4, is also included in the same figures.

Very similar complements of microcylinders are found in the central singlets and in the A subfibers. The most common arrangement in both is

TABLE II
Microcylinder Complements of Flagellar Fibers

Fiber type	Microcylinders		N
	Number	Arrangement	
Accessories	13	9 + 4	1
	14	10 + 4	14
	15	10 + 5	4
	15	11 + 4	1
			—
			20
Central	7	6 + 1	2
	8	7 + 1	6
	9	8 + 1	1
			—
			9
A subfibers	7	6 + 1	1
	8	6 + 2	1
	8	7 + 1	15
	9	7 + 2	3
	9	8 + 1	7
			—
			27
B subfibers	8	6 + 2	7
	9	6 + 3	12
	9	7 + 2	2
	10	7 + 3	3
			—
			24

The total number of microcylinders in completely filled flagellar fibers of various types. "Arrangement" indicates the number in the outer circles and inner groups, or in the enclosing arc and inner group for the B subfibers N is the number of fibers observed with each arrangement.

a seven membered ring around one central microcylinder. Rings with six and eight microcylinders have also been found in both kinds of fibers. The shape of the ring differs in the two fibers, however. It is circular in the singlet fibers and oval in the A subfibers, corresponding with the circular and oval shapes of the lumina in the two cases.

The lumen of the B subfibers is concave in shape where it lies beneath the free wall of the fiber and is irregularly flattened or slightly concave where it abuts the wall that separates the A and B subfibers of the doublet. As can be seen in Figs. 4 and 6, the arrangement of microcylinders reflects

this shape. There is a peripheral arc of six or seven microcylinders beneath the free wall, and two or three more microcylinders lie between this arc and the wall that separates subfibers A and B. Although the B subfibers lack a complete ring at the periphery and have fewer microcylinders, the arrangement is otherwise notably similar to the arrangement in accessory fibers. When tracings of B subfibers were superimposed on pictures of accessories, many cases were found in which microcylinders in B subfibers and in accessories coincide perfectly with each other. The radius of curvature of the B subfiber is approximately equal to that of the accessories, and the B subfibers correspond to a segment of an accessory.

The diameter and center-to-center spacing of the microcylinders in accessory fibers were measured. The boundary of a microcylinder was taken to be the edge of the surrounding matrix. There is a narrow electron opacity gradient at the boundary, caused by diffraction and lens aberrations, and a part of this was arbitrarily included in the diameter of the microcylinder. An average diameter of 36 A was found (and a range of diameters from 27 to 45 A for a sample of 22). The center-to-center spacing in the outer circle averaged 56 A (with a range of 49–66 A for a sample of 12 pairs of adjacent microcylinders). The mean center-to-center spacing was reported earlier (Kaye, 1964) to be about 47 A. The present measurements are presumably more reliable because they include a larger sample and were made on micrographs of considerably higher resolution.

In this study attention has been given primarily to the lumina rather than the walls of fibers. As is evident from Figs. 2 and 4, the walls display no organized substructure, although the procedures used and the resolution permitted clear demonstration of lumen substructure. Micrographs taken some years ago of material prepared differently (methacrylate embedding and lead hydroxide staining) do show wall substructure, as seen in Figs. 9–11, although the resolution is relatively low. Electron-lucent structures, similar to microcylinders in appearance, can be seen in the walls of the two accessory fibers that are sectioned transversely. They form a circle which is peripheral to the lumen with its microcylinders. Rotation tests, as shown in Figs. 10 and 11, considerably enhance the visibility of the wall units,

with strongest reinforcement at $n = 16$. Although no direct demonstration of wall units has resulted from methods used recently, appearances are consistent with their occurrence, and sometimes are suggestive, as seen in Figs. 4 and 6: the dense material that surrounds the doublet is scalloped along its inner border where it is closely appressed to the doublet's wall in just the manner expected if wall subunits impressed their outlines on the material. Hollow fibers have presented no indications of wall units, even in rotation tests.

DISCUSSION

Although the microcylinders look like empty holes in the matrix, their arrangement indicates that they are discrete, independent units. If microcylinders were simply channels in a holey matrix material, then the pattern presented by microcylinders would be expected to be similar in all of the fibers, just as the pattern of holes is the same in any piece of a wire screen, irrespective of its size and shape. The patterns seen in the lumina of accessory fibers and of B subfibers are the same in some cases, but basically different arrangements of microcylinders also occur in various other fibers. The matrix appears to be a structureless substance, which fills the space not taken up by microcylinders in the fiber lumen. The low electron opacity of the microcylinders presumably indicates that they were poorly fixed or were not stained by the methods used here.

It is clear that there is a fairly definite number of microcylinders in the lumen of a fiber and that they are arranged in a definite pattern rather than at random. The size of the microcylinders, as indicated by their measured diameters, can hardly be the explanation for the particular number characteristic of a given fiber, since the lumina are large enough to accommodate considerably more than occur. Nor do microcylinders appear to be derived from a source that provides a fixed number; for each type of fiber the number of microcylinders varies over a small but definite range.

Fig. 12 shows models that were built out of pennies, in an attempt to reproduce the situation seen in accessory fibers and thereby gain some insight into the probable significance of the arrangements. The models suggest that the arrangements observed reflect maximal packing of structures into limited spaces. The most common arrangement in accessory fibers is a circle of 10

around 4 central microcylinders. As seen in Fig. 12 *a*, when 4 pennies are grouped in a square, the maximum number that can lie on the circle circumscribing the square is 10. Further, as seen in Fig. 12 *b*, the maximum number that can lie on the circle circumscribing the circle of 10 is 16; this agrees with the finding of 16 wall units in accessories.

In the case of a pentagonal cluster of 5 in the central group, the circle that circumscribes the pentagon will accommodate 11 pennies (Fig. 12 *d*); this circle is obviously wider than the circle of 10 pennies around a square of 4 and requires more space. A smaller circle made up of only 10 pennies will fit around a cluster of 5, however, if the enclosing group is positioned in one particular way relative to the pentagonal group. They are arranged in this way so that squares are formed by a pair of outer pennies plus a pair of inner pennies (Fig. 12 *c*). In this arrangement, no member of the outer group lies on the same radius as a member of the inner group, and the circle of 10 pennies around 5 is only 2% wider than the circle of 10 pennies around a group of 4. This particular arrangement was always found in accessories having 5 in the center; it can be seen in Figs. 3 and 4.

It is not impossible that the dark matrix material around microcylinders prevented detection

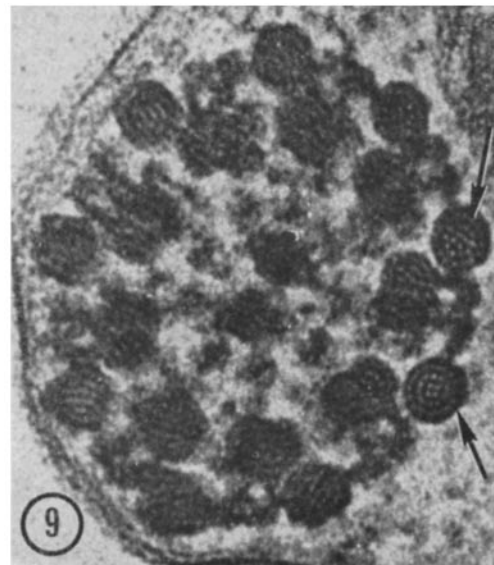
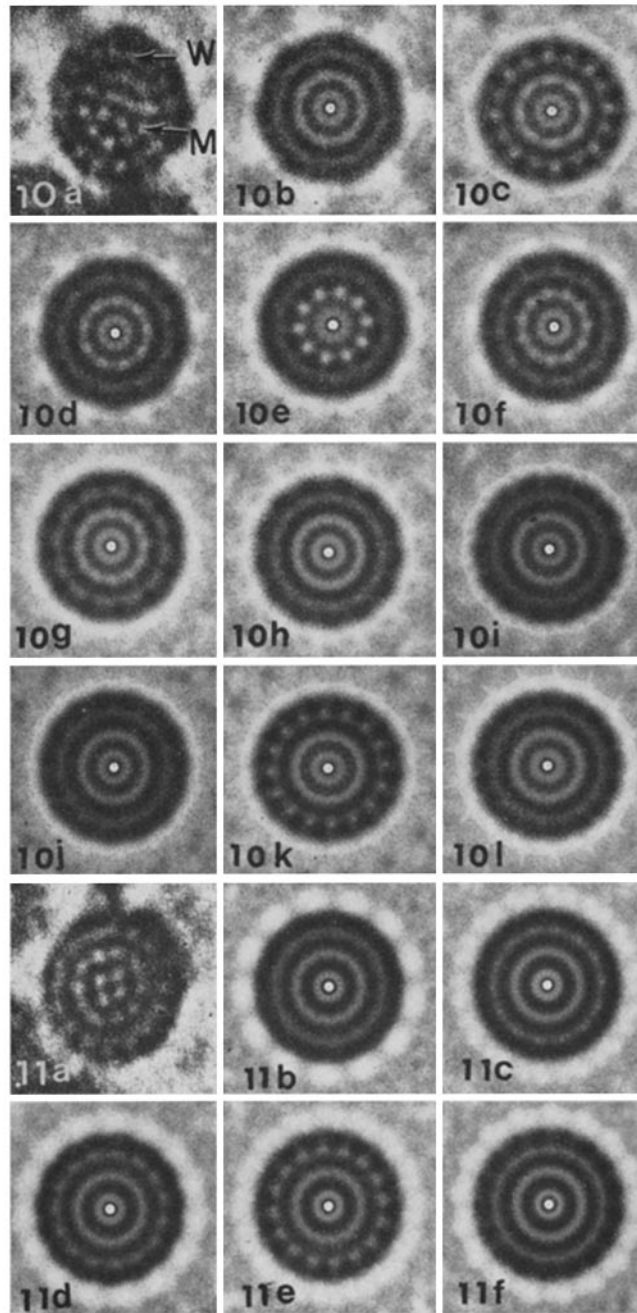


FIG. 9

FIGURE 9 A transverse section through a flagellum of a late house cricket spermatid. Subunits are visible in the walls of two accessory fibers (arrows), which resemble the microcylinders in appearance. (Methacrylate embedding; lead hydroxide staining; RCA EMU-3E electron microscope.) $\times 250,000$.

FIGURE 10 A rotation test series of one of the two accessory fibers pointed out in Fig. 9. Fig. 10 (a) is the original fiber showing the wall units (*W*) and the outer circle of microcylinders (*M*). The rotations range from $n = 7$ for Fig. 10 (b) to $n = 17$ for Fig. 10 (l), where n is a fractional increment of an arc. Strong reinforcement of the wall units occurs at $n = 8$ and $n = 16$ (Figs. 10 (c) and (k)). Reinforcement of the outer circle of microcylinders occurs at $n = 10$ (Fig. 10 (e)). $\times 580,000$.

FIGURE 11 A part of a rotation test series for the second accessory fiber in Fig. 9. Fig. 11 (a) is the original fiber. The rotations range from $n = 13$ for Fig. 11 (b) to $n = 17$ for Fig. 11 (f). Strong reinforcement of the wall units occurs at $n = 16$ (Fig. 11 (e)). $\times 580,000$.



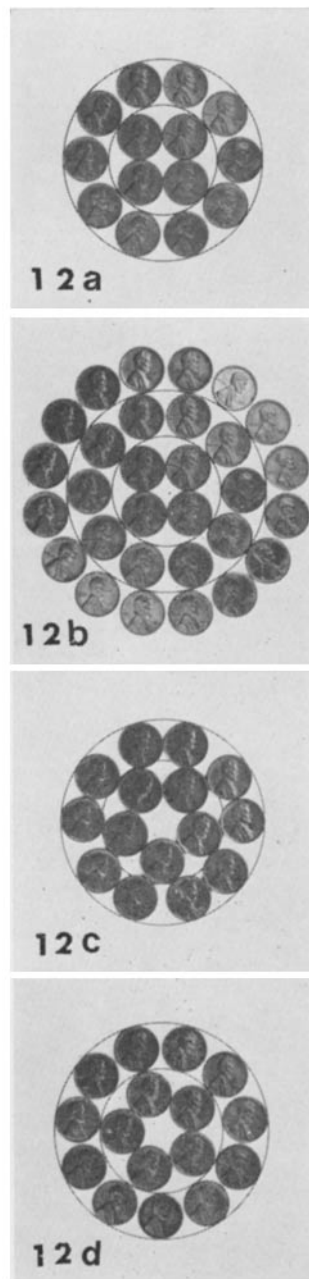


FIGURE 12 *a-d* Models that show the maximum number of circular units (pennies) that can be packed in successive concentric circles. FIG. 12 (*a*). 10 pennies lie on a circle that circumscribes a square grouping of four; the packing resembles that of the microcylinders in accessory fibers with the 10 + 4 complement. FIG. 12 (*b*). 16 pennies lie on the circle that circumscribes the ten in Fig. 12 (*a*). They are to be compared with the 16 wall units detected in rotation tests of accessory fibers.

TABLE III
Frequencies of the Sum of Microcylinders in Doublet Fibers

Total numbers of microcylinders	Expected frequency	Actual frequency	N
15	0.02	0	0
16	0.19	0.22	4
17	0.46	0.45	8
18	0.29	0.28	5
19	0.046	0.056	1
			18

The data in Table II on microcylinder numbers in A and B subfibers were used to compute the frequencies with which various total numbers of microcylinders in a doublet would be expected to occur if the number in one of the subfibers was independent of the other. This is shown as expected frequency. The actual frequencies were determined from doublets where the microcylinder number in both subfibers could be determined. The number of such doublets is given as N.

of some part of the microcylinders that extended beyond the observed translucent region; in this case, the diameter measurements presented here would give an underestimate of the size. A larger microcylinder size is suggested by the indications that closest packing produces the particular arrangements seen; hence the structures should extend over a distance that is equivalent to their spacing, 56 A. However, this characteristic spacing of microcylinders could conceivably be produced by unobserved extensions that project, from a 36 A core, only at infrequent points along their length and make contact with adjacent microcylinders; it is unnecessary to assume that the microcylinders are really 56 A thick, with a

FIG. 12 (*c*). A pentagonal cluster of 5 pennies encircled by 10, so grouped that squares are formed by pairs from the outer circle plus pairs from the inner cluster. This is the arrangement seen in the 10 + 5 type of accessory fiber. The outer pennies lie inside the circle that circumscribes the inner cluster and the over-all diameter is only slightly larger than that of the 10 + 4 arrangement. FIG. 12 (*d*). 11 pennies fit on a circle that circumscribes a pentagonal cluster of 5. This arrangement was never seen in accessory fibers. The over-all arrangement is considerably larger than the 10 + 4 arrangement.

solid layer of unseen material around them. The electron-opaque matrix material seen in the lumen of cricket fibers gives the appearance of a continuum and no sign of being part of the microcylinders. Since it occurs over relatively large areas that are free of microcylinders in intermediate stages (Fig. 7), it must be a separate component.

In size and spacing, the microcylinders are similar to the filamentous units found in the walls of flagellar fibers and microtubules after negative staining; microcylinders are about 36 Å wide and their spacing is about 56 Å, remarkably close to the 35–40 Å diameter and 50–60 Å spacing reported for units of the wall (André and Thiéry, 1963; Pease, 1963; Gall, 1966; Behnke and Zelander, 1967). The electron-lucent structures in the walls of cricket fibers look similar to the microcylinders, and, in addition, rotation tests indicate that there are 16 wall units in the accessory fibers, which is the number expected if the wall units have the same dimensions, and are packed the same way as microcylinders. These correspondences between microcylinders and wall units suggest that they are the same structures, but have different localizations.

The microcylinders clearly differ in some respects from the wall units described from sectioned material. Several studies (Phillips, 1966; Silver and McKinstry, 1967) have shown wall units having the appearance of tubular structures, with opaque material forming the tubes and a translucent region inside. The diameter of the translucent region is approximately that of microcylinders and of filamentous units in negatively stained preparations. However, the over-all diameter of the tubes is 60–75 Å, which is considerably larger than either.

An important difference between microcylinders and wall units is that microcylinders are relatively easy to demonstrate in sections, while wall units, in the cricket cells studied here and in other cells in general, are only rarely demonstrable. The relative ease with which microcylinders can be detected may be due to their surrounding matrix and not to any inherent feature of the microcylinders. The matrix seems to function as a negative stain, outlining the microcylinders, and conceivably keeps them in place during the fixation and embedding procedures as well. Such a material is not seen as a normal component of microtubule walls in general. It is noteworthy

that the clearest demonstration of wall units in sections (Ledbetter and Porter, 1964) was obtained with microtubules that showed "atypical staining patterns"; the wall units stood out as electron-lucent areas in an enveloping electron-opaque material, very much like microcylinders in the surrounding matrix material. The unusual opacity around the microtubule walls, which made the wall units visible, may have reflected the presence of some material that is not usually present in cells, or is not usually preserved, rather than being an aberration of staining, as Ledbetter and Porter suggested.

It was proposed by Ringo (1967) that the microcylinders found in the lumina of cricket flagellar fibers are actually displaced wall subunits. He proposed that during spermiogenesis the arrangement of subunits in a ring, forming the wall, is broken down and that the subunits "condense" to form a closely packed group. Ringo discounted the conclusion (Kaye, 1964) that 14 microcylinders most frequently occur in accessory fibers, and assumed that there were just 13 microcylinders present, which could then be equated with the 13 wall subunits that have been reported for some microtubules (Ledbetter and Porter, 1964). While Ringo's suggestion is an interesting one, it is based on a misconception of data and it also conflicts with several further observations. The number of microcylinders present depends on the type of fiber, and 13 is neither a characteristic nor a frequent number for any. Fibers have the same diameters after microcylinders have appeared as they did when still hollow (Table I), whereas a decrease in diameter would be expected if the microcylinders appeared as the result of the wall's collapsing. Further, wall units are demonstrable in fibers that have a full complement of microcylinders.

Finally, if microcylinders were displaced wall subunits, the number available to each of the subfibers of a doublet should be limited by the total number of wall subunits in the doublet. In that case, the more microcylinders there are in one subfiber of a particular doublet, the fewer there should be in the other. Just the opposite is true; the data in Table III show that the number of microcylinders in the two subfibers of particular doublets varies independently. The frequencies with which various complements of microcylinders occur in the A and B subfibers

were determined separately. They were then used to calculate the total numbers per doublet and their frequencies, which would be expected if the various kinds of A and B subfibers combined with each other at random. The total numbers per doublet which actually occur, determined from those doublets where both subfibers could be analyzed, correspond almost perfectly with the expected values.

It is quite obvious in the cricket that singlet fibers are not all alike. The accessory fibers appear to contain 16 wall units rather than the 13 sometimes assumed to be generally present. They are clearly larger than the central fibers or the cytoplasmic microtubules, which occur in the cytoplasm of the cricket flagellum. These size differences are evident in single micrographs that contain all three types of singlet fibers, so there is no possibility that variations in specimen preparation or errors in calibration of magnification are responsible. Behnke and Forer (1967) have distinguished four different kinds of microtubules in flagella of crane fly spermatids on the basis of microtubule reactions to various physical and chemical treatments. They grouped accessory and central fibers together in one class and placed cytoplasmic microtubules, B subfibers, and A subfibers each in separate classes. In the crane fly, accessories are the same size as the other singlets. The large size of the accessory in cricket indicates not only that there is a distinction be-

tween it and the central singlets, but also that the characteristics of homologous fibers may vary in different organisms.

A single cylinder centrally located in the lumen has been found in flagellar fibers of sperm in several insect species (Cameron, 1965; Phillips, 1966) in contrast to the many cylinders found in crickets. Cameron (1965) has presented evidence that the appearance of the lumen in the two cases may be determined by the electron stains used. When flagella of a beetle sperm were stained with lead only, the lumen of the accessory fibers gave some of the appearances of microcylinders embedded in a matrix, though, as Cameron pointed out, the pictures are not clear; if sections were stained with uranyl and lead, the fibers displayed a single, central cylinder. However, in our study of crickets, the appearance of the lumen has been the same with either stain; and in some other orthopterans examined (unpublished observations), lumina have displayed a single rod after the staining procedures that yielded many microcylinders in the cricket. No explanation for the staining effect noted by Cameron has been suggested by the present study.

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