

## THE FINE STRUCTURE OF FLAGELLA IN SPERMATIDS OF THE HOUSE CRICKET

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### INTRODUCTION

The basic components of flagella in most organisms are two central fibers surrounded by a ring of 9 outer double fibers, (4); the fibers usually have the appearance of hollow tubes. A variation of this structure has been reported for several cases of sperm flagella in which the fibers appear to be nearly solid (2, 8). The following observations of the flagella of cricket sperm show that their fibers are of the dense rather than the hollow type, and have a regular substructure not seen heretofore in dense fibers.

Another variation of the basic structure of flagella exists in cricket spermatids. There is an extra circle of 9 fibers which encloses the flagellum proper; these fibers show essentially the same fine structure as the two central fibers.

### MATERIALS AND METHODS

Testes of the house cricket, *Acheta domestica*, were fixed, as described previously (6), in an osmium tetroxide-dextran mixture, postfixed in formalin, and embedded in a methacrylate mixture. Sections were stained with lead hydroxide (9), and "sandwiched" with carbon film.

### OBSERVATIONS

The flagellum of a cricket spermatid in an early stage of spermiogenesis is shown in transverse sec-

tion in Fig. 1. It has the usual 9 outer double fibers, with arms on subfiber A, and two central fibers surrounded by a sheath, as described for other types of cilia and flagella (5). One difference from the norm is notable: subfiber B of each of the outer pairs has a long arm projecting from the outermost part of the fiber.

At a later stage, as shown in Fig. 2, an additional fiber is attached to each of the outer double fibers at the junction of the two subfibers. These new fibers will be called accessory fibers. All fibers appear hollow in early spermatids.

In later spermatids the interiors of the accessory fibers, the two central fibers, and several of the outer double fibers become electron opaque. In the late spermatid of Fig. 3 and in the nearly mature sperm of Fig. 4, several accessory fibers are oriented with their long axes nearly perpendicular to the plane of the section, and it can be seen that these fibers have a well ordered fine structure. Small circular areas of low density, bounded by dense material, are closely packed in the interior of the fiber. The circles are of fairly uniform size: measurements of 10 circles, including one-half of the wall at each end of a diameter, indicated a range of 39 to 47 Å with a mean of 43 Å. The number and arrangement of the circles in the

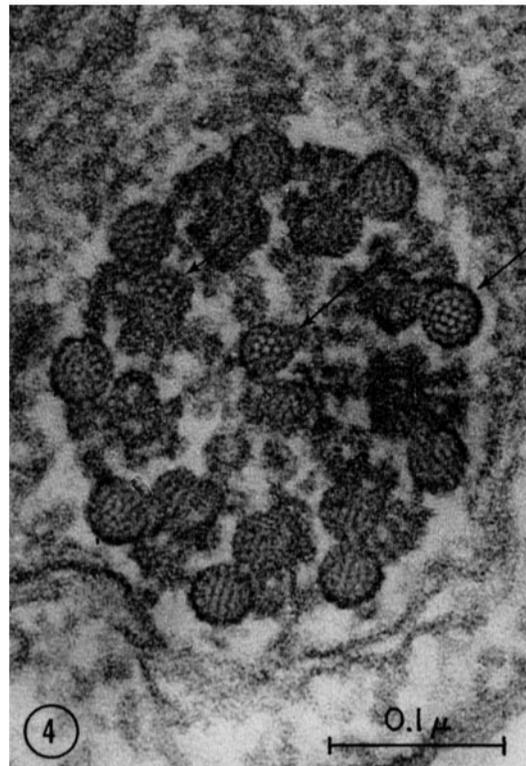
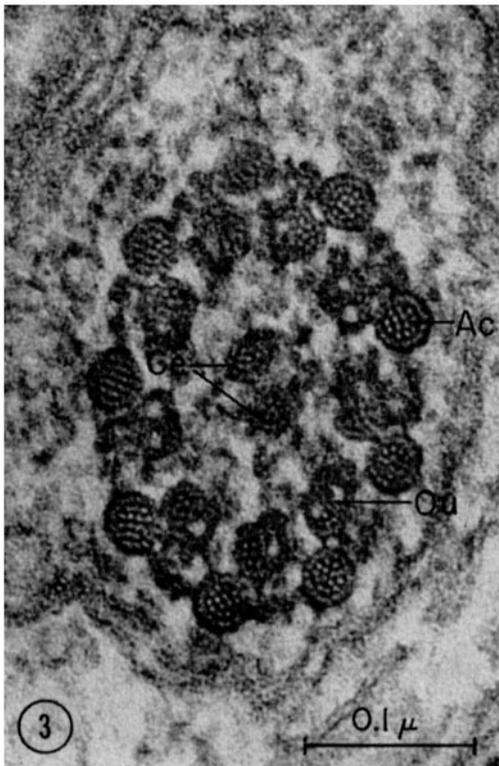
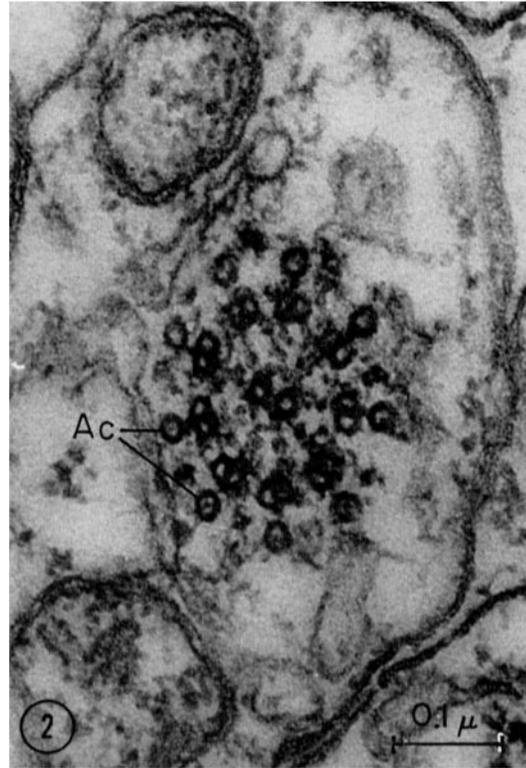
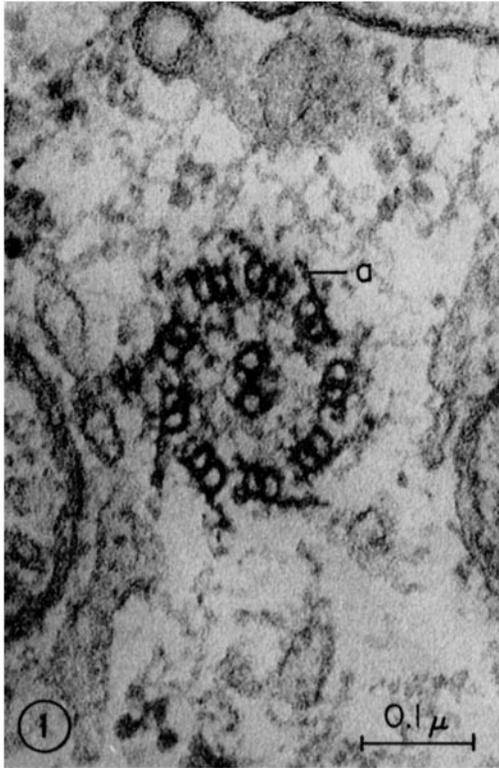
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FIGURE 1 A transverse section through the flagellum of an early spermatid of the house cricket. The usual components of flagella are present: 2 central fibers surrounded by 9 outer double fibers. An unusual feature is the extra arm (*a*) on subfiber B of each outer double fiber.  $\times 150,000$ .

FIGURE 2 A transverse section through the flagellum of a later spermatid. An additional circle of 9 "accessory" fibers (*Ac*) is present.  $\times 150,000$ .

FIGURE 3 A transverse section through the flagellum of a late spermatid. The accessory fibers (*Ac*) and the two central fibers (*Ce*) are no longer hollow. Several accessory fibers have a definite substructure in the form of circular areas of low electron opacity surrounded by layers of high opacity. Some of the subfibers of the outer double fibers (*Ou*) are hollow; others are not. The accessory fiber at 9 o'clock shows the pattern which results from a slight tilt in the plane parallel to the rows of microcylinders.  $\times 230,000$ .

FIGURE 4 A transverse section through the flagellum of a nearly mature spermatid. Circular areas are evident in the fibers indicated by arrows. Patterns which result from a slight tilt are evident in the other fibers.  $\times 230,000$ .



accessory fibers appears to be relatively constant. Five such fibers were found to contain an inner cluster of 4, surrounded by a cirlet of 10. One accessory fiber contained 13 circles. The entire group of circles is separated from the outer boundary of the fiber by a clear layer. The distance from the center of the dense material bounding the group of circles to the center of the fiber boundary is 40 to 60 Å.

The central fibers have a structure similar to that of the accessory fibers, but contain a smaller number of circles. In two analyzable cases they were found to contain a single central circle surrounded by a cirlet of 7 more, for a total of 8. One of the central fibers of Fig. 3 shows a few circles; 6 circles are especially clear in one central fiber in Fig. 4.

The outer double fibers are variable in appearance. In some of those shown in Figs. 3 and 4, both subfibers are hollow; in others, one subfiber of each pair is fully or partially filled with material. Most of the dense subfibers are indistinct. Circular areas are visible in many of the dense subfibers, but they are never so distinct or well arranged as in the accessory fibers. About 8 circles are seen in some of the subfibers, fewer in others, but in no case have reliable counts been obtained.

An oblique and a longitudinal section through flagella of nearly mature sperm are shown in Figs. 5 and 6. In Fig. 5, the central fibers are free of over- or underlying fibers and within them several fine lines which parallel the long axis of the fiber can be seen. They appear regularly spaced with a mean center-to-center spacing of about 47 Å. Fine lines can be seen in most of the other fibers as well, but these are partially obscured by overlying structures. In the longitudinal section of Fig. 6, an accessory fiber appears which is free of obscuring structures. Here, too, the fiber displays a number of fine dense lines with a center-to-center spacing of about 47 Å. In particularly straight segments of flagella, such as that shown in Fig. 6, some of the

fine lines can be traced for considerable lengths, up to 0.2 to 0.4  $\mu$ .

#### DISCUSSION

The foregoing observations indicate that in flagella of house cricket spermatids the interior of dense fibers consists of cylinders, of low electron opacity and circular cross-section, in a material of high electron opacity. The dense material appears to be a continuum; it separates cylinders from each other but there is no hint of its organization into discrete membranes around individual cylinders. The cylinders are closely packed, in a regular arrangement, parallel to the long axis of the fiber, and their number and arrangement appears to be specific for a given type of fiber.

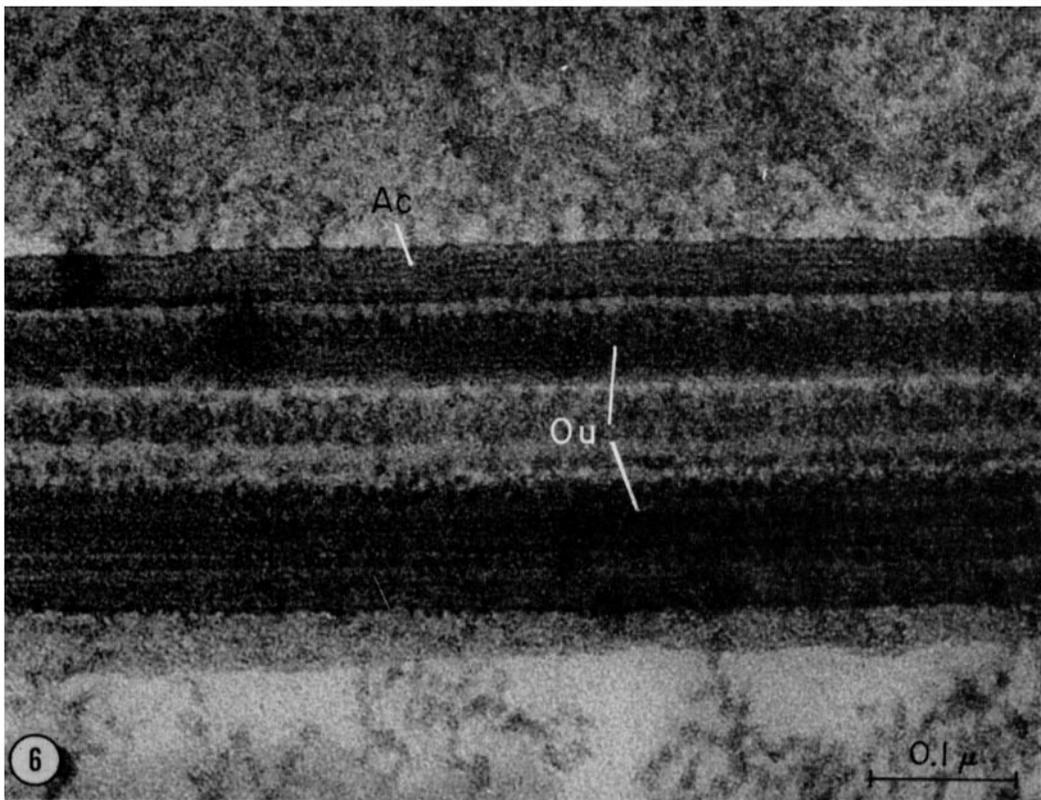
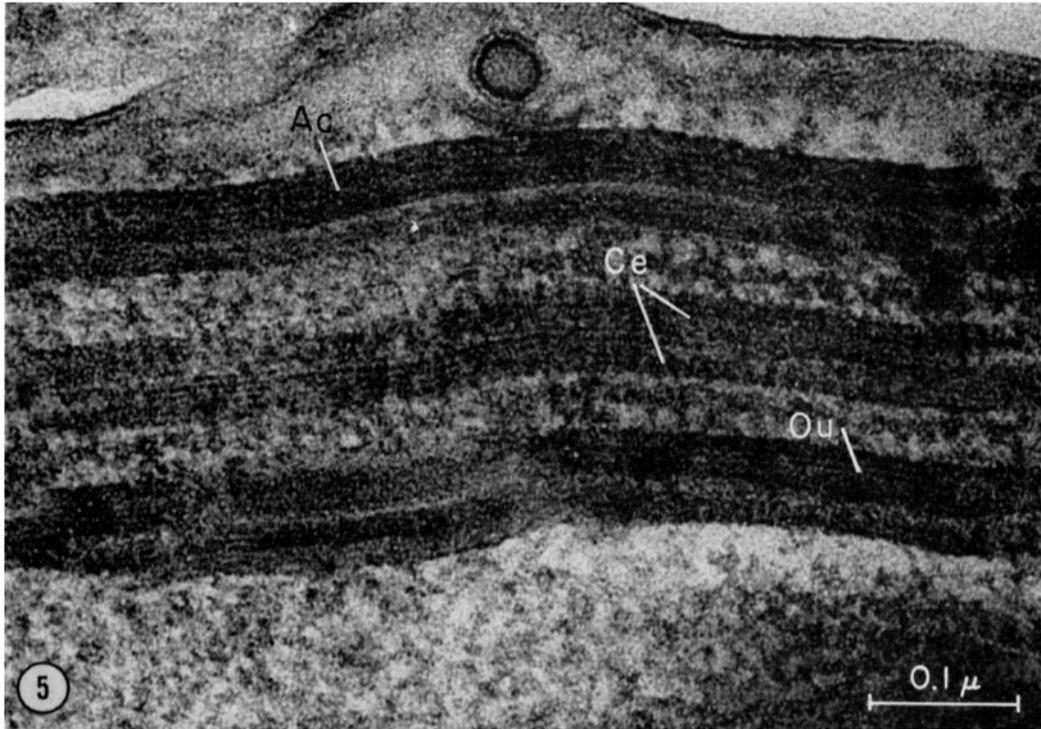
The dense parallel lines seen in longitudinal sections represent layers of dense material between cylinders, and have a spacing of 47 Å, essentially the same as the 43 Å spacing found with transverse sections. The diameter of the cylinders is too small to be measured accurately in the micrographs obtained in the present study, but can be estimated from the following considerations. One repeating unit of about 45 Å consists of a cylinder and a dense layer. The diameter of a cylinder seems about twice the thickness of dense material between adjacent cylinders at their closest point; this indicates a cylinder diameter of 30 Å and a 15 Å thickness of dense material.

In transverse sections of a flagellum, fibers of several different appearances are found. The rarest is that of circles with a regular arrangement. Only a rare, perfect, end-on-view of a fiber should show the microcylinders as circular areas; a tilt of a degree or two would blur the view of individual cylinders. More frequently, the fibers show a pattern of several conspicuous dense lines or no regular pattern at all. These fibers are probably tilted and their particular appearance depends on the plane of the tilt. As seen in Fig. 7, the cylinders fall into 4 rows parallel to the plane indicated on the

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**FIGURE 5** An oblique section through the flagellum of a nearly mature spermatid. An accessory fiber (*Ac*), several outer double fibers (*Ou*), and two central fibers (*Ce*), are shown. In the central fibers fine, dense, parallel lines are visible. Similar fine, dense lines can be seen in some of the other fibers as well, but not so clearly.  $\times 200,000$ .

**FIGURE 6** A longitudinal section through the flagellum of a nearly mature spermatid. An accessory fiber (*Ac*), and several of the outer double fibers (*Ou*) are visible. Fine, dense, parallel lines are especially clear in the accessory fiber.  $\times 200,000$ .



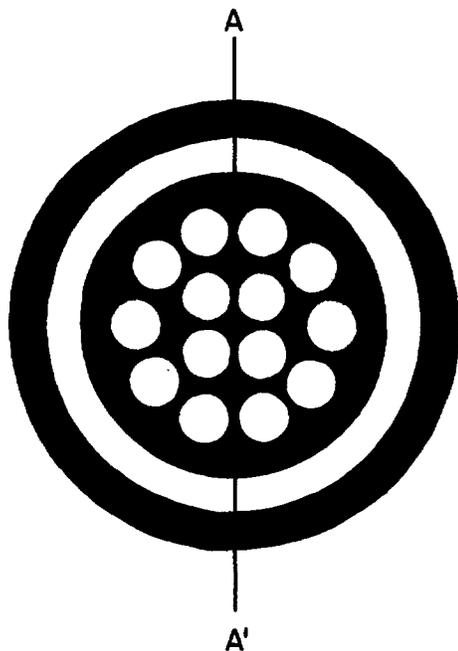


FIGURE 7 A diagram showing the idealized arrangement of microcylinders in an accessory fiber as seen in transverse section. The plane (A-A'), indicated on the diagram, divides the microcylinders into two groups of two rows each. The microcylinders fall into 4 nearly parallel rows only in the direction parallel to plane (A-A').

diagram. If the axis of a fiber is tilted in a plane parallel to the rows, the layers of dense material separating the rows of cylinders will be seen as 3 dense lines. A slight tilt of this kind does not noticeably affect the circularity of the dense outer boundary of the fiber or of the dense material around the outer circlet of cylinders. Several fibers with this appearance can be seen in Figs. 3 and 4. Tilts in any other plane should result in complicated arrangements of short segments of dense lines and ellipses. Fibers which have such appearances also occur in Figs. 3 and 4.

In several respects, the flagella of cricket spermataids resemble those which occur in the moth, *Macroglossus*, described in detail by André (1). The major points of similarity are the presence of 9 accessory fibers attached to the outer double fibers, and the occurrence of electron-opaque material

in the central pair and the accessory fibers. Although most of the filled fibers appeared uniformly electron opaque, André showed one accessory fiber (André's Fig. 9) with several dense concentric layers alternating with lighter layers. Close inspection of this micrograph suggests that the light layers may be subdivided into small compartments. It seems possible that these compartments are microcylinders similar to those found in cricket.

With the possible exception of moth sperm flagella noted above, observations of fixed and sectioned material of other organisms have detected nothing resembling microcylinders in the usual, hollow fibers of flagella. However, two recent studies of negatively stained preparations of some mammalian sperm (3, 7) have demonstrated a pattern of fine parallel lines which run lengthwise in all eleven fibers. These linear elements have been called protofibrils by André and Thiéry (3). The protofibrils have a width similar to the spacing found for cricket microcylinders; André and Thiéry (3) report them to be 35 Å thick, and Pease (7) reports 35 to 40 Å. Any conclusions about homologies of microcylinders and protofibrils will have to await a description of the protofibrils in sections of flagella.

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