THE FINE STRUCTURE OF *HAEMATOLŒCHUS* SPERMATOZOAN TAIL

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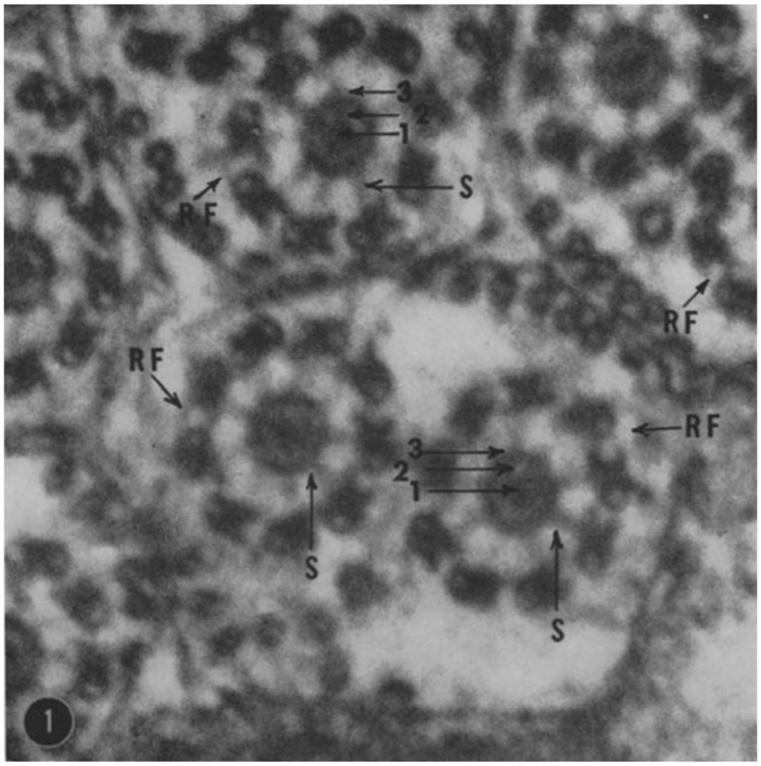
For a number of years investigators generally agreed that electron optical studies had established a basic pattern for the axial fiber bundle of sperm tails. This consisted essentially of two single filaments centered within a ring made up of nine doublet filaments. It was further established that this structure possessed bilateral symmetry, *i.e.*, one plane and only one plane would divide the



Explanation of Figures

All of the electron micrographs are from spermatozoa of the flatworm *Haematolœchus medioplexus* fixed in 1 per cent veronal-buffered osmium tetroxide. The micrographs were taken at an instrumental magnification of 9,500 and were further enlarged photographically.

FIGURE 1

Cross-section through a sperm tail to show the general configuration of the axial fiber bundles. The single central unit with its core (*1*), cortex (*2*), and sheath (*3*); the peripheral doublet filaments and the radial spokes (*S*) can be observed. The arrows (*RF*) indicate possible connections between the rim elements. × 166,000.

bundle into mirror portions. The general agreement of this pattern with that found for similar structures in cilia and flagella lent considerable justification to such an interpretation (Fawcett, 1958). Afzelius (1959) using a modification of the classical osmium tetroxide technique with sea urchin spermatozoa, noted some new features which he attributed to improved fixation and preservation. On the basis of these findings, he offered a new interpretation of the structure of the doublet filaments which led him to conclude that the axial bundle is an asymmetrical unit. This note deals with the structure of the sperm tail of *Haematolœchus medioplexus*, a flatworm which is parasitic in the lungs of *Rana pipiens*. It substantiates many of the observations made by Afzelius and introduces a significant morphologic variation in the basic pattern.

METHODS

Living specimens of *Haematolœchus medioplexus* were removed from the lungs of *Rana pipiens* (which had been killed by decapitation), washed in saline, and fixed in veronal-buffered osmium tetroxide, following the method of Palade (1952). Since the specimens selected for study had dorsoventral thickness of less than 1 mm., fixation *in toto* provided no obstacle to the penetration of tissue by the fixative. Testes were dissected out of the fixed specimens and embedded in *n*-butyl methacrylate which was then polymerized at 47°C. Dissection of desired tissues from previously fixed specimens avoided the postmortem changes associated with prolonged manipulation of cytological material prior to treatment. Sections were prepared using glass knives and a Porter-Blum ultramicrotome. An RCA EMU-2B electron microscope was used for this work. The micrographs were taken at an initial magnification of 9,500 and were enlarged photo-

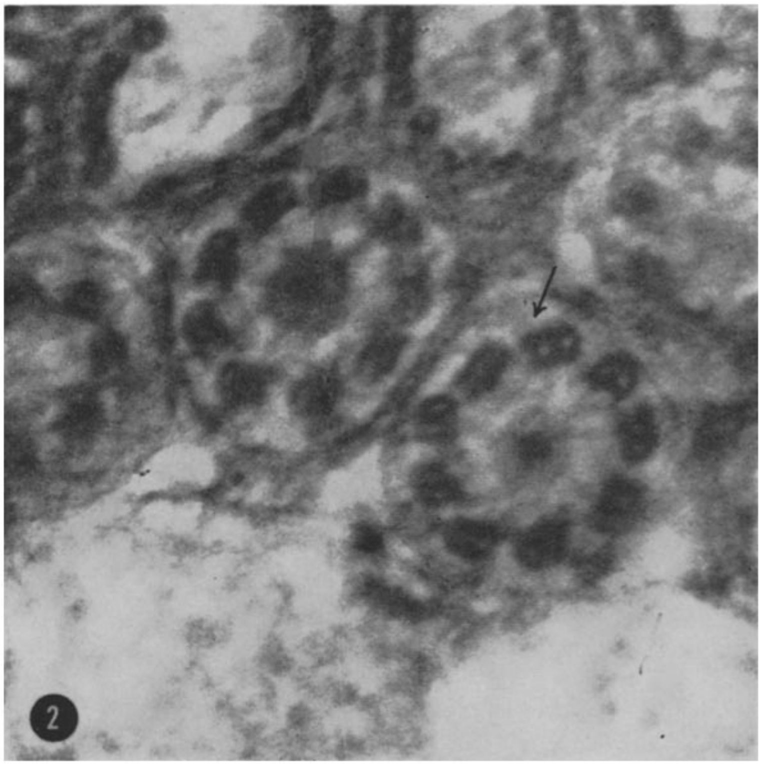


FIGURE 2

Cross-section through two sperm tails, each with a single axial fiber bundle. The arrow indicates a doublet filament with the two openings and the arms extending from one in the counter-clockwise direction. × 210,000.

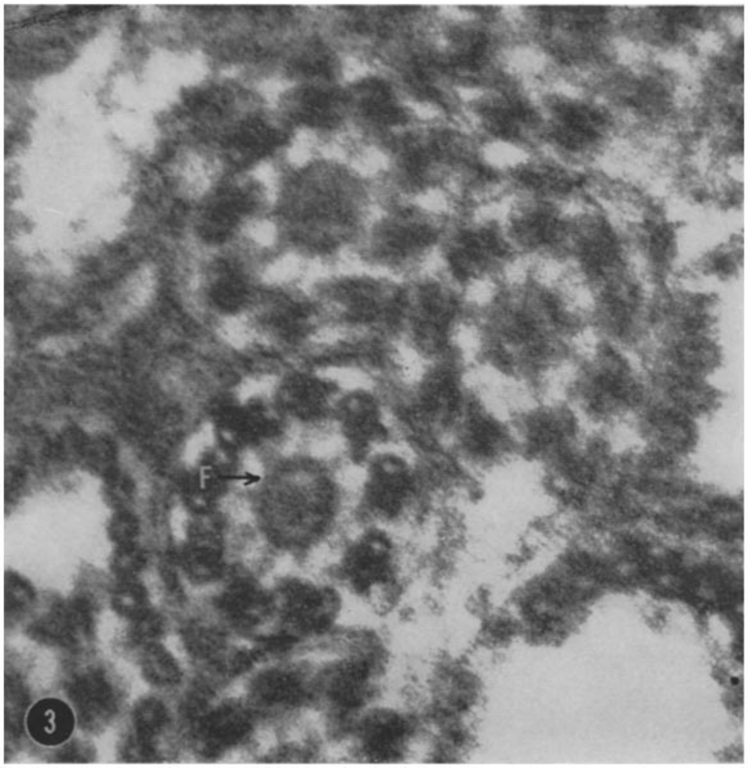


FIGURE 3

Cross-section through axial fiber bundles. A suggestion of fibrillar texture in the sheath of the central unit can be observed at *F*. × 200,000.

graphically to the desired size. The magnifications at the settings of the microscope were calculated by calibration using a diffraction grating ruled at 28,800 lines to the inch.

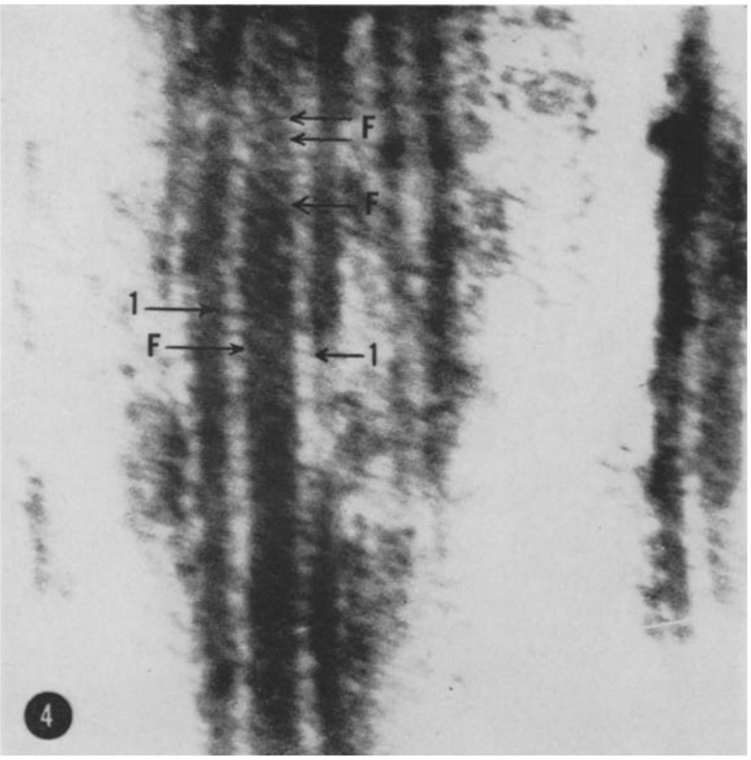
RESULTS

The axial fiber bundle of the *Haematolœchus* sperm tail consists of a single filament centered within and attached to a ring of nine doublet filaments by numerous ladder-like connections along the longitudinal axis. This structure shows a cross-sectional pattern resembling a wheel; the hub is the central filament which is connected to the rim of nine doublet filaments by spokes, the ladder rungs (*S*, Fig. 1).

(*a*) *Single Central Unit:* This unit has a diameter of 50 mμ which is about one-third that of the entire axial fiber bundle. In cross-section, three distinct areas can be recognized. The center is a dense core enveloped by an apparently homogeneous cortex (matrix substance), which shows a lesser density. These two areas are encased by a sheath, the density of which approximates that of the core. The central unit shows a fibrillar structure (*F*, Figs. 3 to 5) and is described further below.

(*b*) *Doublet Filaments:* The outline of each of the nine doublet filaments, which make up the rim, approaches that of a rectangle, the longer axis of which is in an approximate tangential position. A slight tilt of each rim element from the true tangential plane, however, places one end closer to the central filament than the other. Each rim element has two circular openings approximately equal in size. Open arms, unequal in length, extend from the end of the doublet nearer the central axis (Fig. 2). The rim units appear to be held in fixed relationship to each other by fine fibrils circumferentially distributed (*RF* in Fig. 1).

(*c*) *Spokes:* Longitudinal sections of the axial fiber bundle show the spokes extending between the central unit and the doublet filaments in a vertical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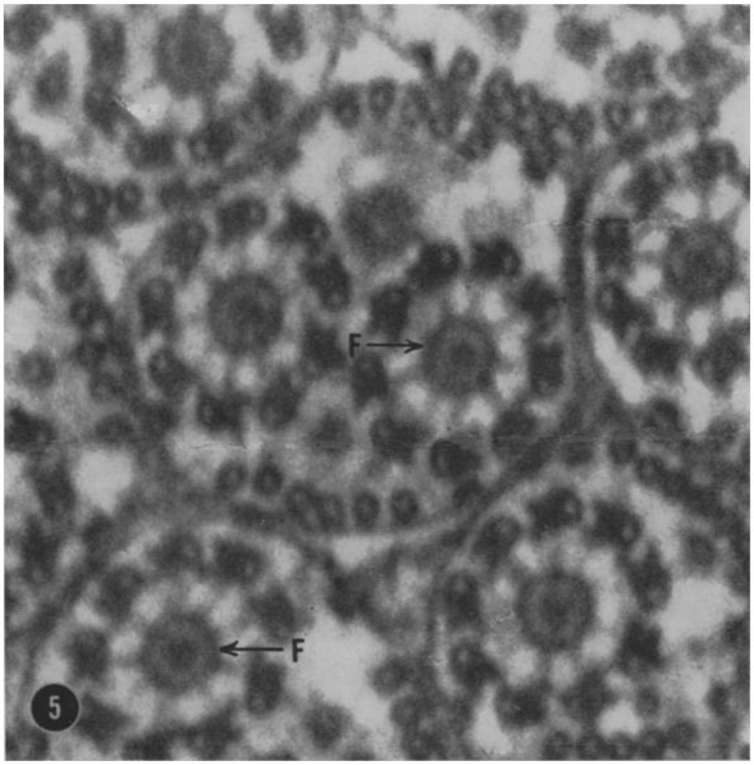
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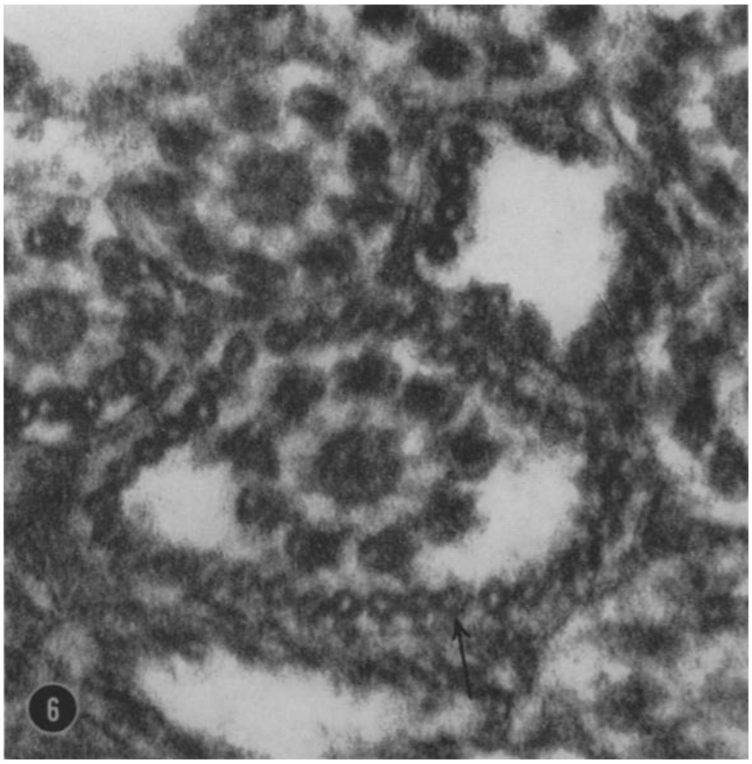


FIGURE 6

Cross-section of a sperm tail showing a tail sheath containing 36 fibrils, (one of them indicated by an arrow). × 178,000.

fashion, like rungs on a ladder, *i.e.*, at right angles to the long axis of the rim units (Fig. 4). At intervals of 220 A, diagonal and parallel striations in the longitudinal section of the central unit of the axial fiber bundle suggest a connection between spokes on one side with spokes at different levels on the other.

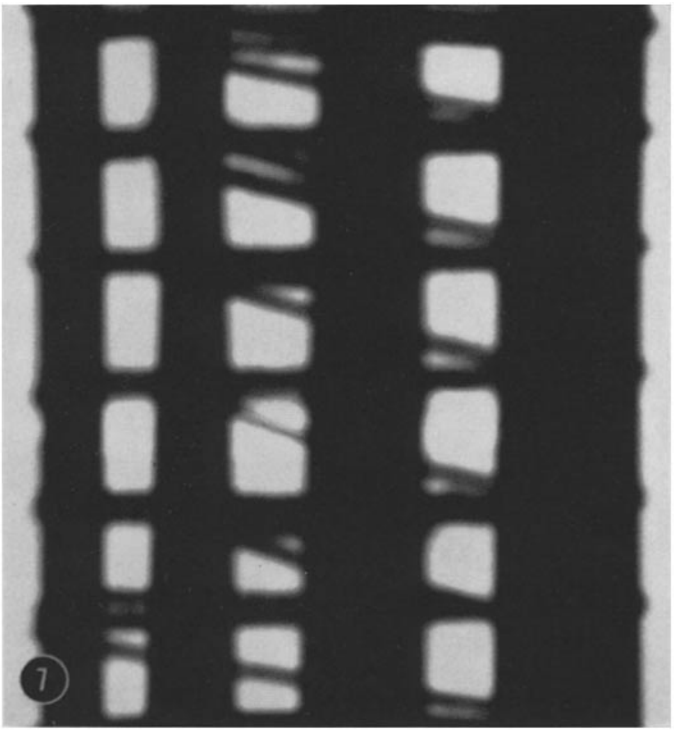
In cross-section the spokes appear as single radii extending to the doublet filaments. Since the spokes are vertically spaced, in those cross-sections taken at a level between spokes, they seem to be absent. The connection between spoke and doublet filament cannot be clearly defined except that it is topographically nearer to the end titled inward. At the junction of spoke and the sheath of the central unit, however, the spoke appears to spiral within the sheath at an angle. This is suggested by the fibrillar structures observable in some of the

FIGURE 4

Longitudinal section of an axial fiber bundle. The spokes connecting the peripheral elements to the central unit (arrow *I*), and the diagonal slope of the fibrils (*F*) in the central unit, can be observed. × 130,000.

FIGURE 5

Cross-section similar to Fig. 1, showing the fibrillar structure (*F*) of the sheath of the central unit. The apparent absence of spoke fibers is due to sectioning at a level above or below the fiber. × 170,000.



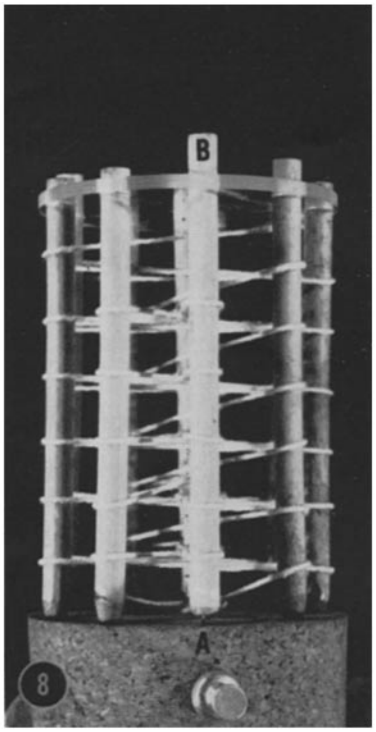


FIGURE 7

Shadowgraph of a suggested model of the axial fiber bundle.

FIGURE 8

Photograph of the suggested model of the tail filament showing a single fiber extending the length of the bundle from points *A* to *B*.

cross-sections (Figs. 3 and 5), as well as the striations noted in longitudinally cut specimens (Fig. 4). It is conceivable that the core sheath is thus a structure created by joining of the spoke fibrils.

(*d*) *Terminal Portion of the Spermatozoan Tail:* It has been described as a tuft of exceedingly fine fibrils. In the early observations, widely varying numbers of fibrils were reported. The numbers suggested are inconsistent with the more recent electron optical findings with regard to the fibrils observable in sperm tails. Random fraying of the axial fiber bundle itself, for example, could not yield as many as the 48 reported by Randall and Friedlander (1950). The tail sheath of *Haematolœchus* is made up of 36 fibrils (Fig. 6). Fraying of this sheath, at the most distal portion along with the fibrils of the axial bundle, might account for the large number of fibrils reported for sperm tails in earlier works.

DISCUSSION

(*a*) *Structure and Symmetry:* These observations establish that the axial fiber bundle of the sperm tail of at least one animal species has a *single* central unit. This finding, so diametrically opposed to the almost universally accepted concept of two central filaments in sperm tails (and cilia) of animals and plants, suggests that another basic pattern exists. Further studies with flatworms and their close relatives, not only with sperm tails, but with the cilia of miracidia and coracidia should be rewarding.

Afzelius (1959) clearly demonstrated the existence of spoke filaments (which has been reported by others in different forms) in sea urchin spermatozoa. The observations with *Haematolœchus*, using classical osmium-fixation techniques, confirm the presence of these spokes as definitive structures in some sperm. This is evident from their occurrence in both longitudinal and cross-sections as well as the fact that they are of uniform dimension and clearly extend between the central and rim elements. Some additional information concerning their interconnections is provided. This study indicates that rows of these spokes join the doublet filaments to the central and perhaps to each other. A suggestion as to their role in the movement of the tail is given below.

The doublet filaments of *Haematolœchus* appear to be similar to those reported in *Psammechinus miliaris* by Afzelius in so far as number of openings and arms is concerned. Whether the openings of any doublet filament are equal or unequal in diameter cannot be established.

The presence of two central filaments in an axial fiber bundle serve as useful markers in any determination of symmetry. Afzelius on the basis of these and certain peculiarities of the individual doublet filaments reported that this bundle unit is asymmetrical, a departure from the previously accepted belief of bilateral symmetry. In *Haematolœchus*, there appear to be several planes of section which would yield mirror portions. It, therefore, is suggested that the axial fiber bundle of this form is radially symmetrical.

(*b*) *Functional Aspects:* Although several explanations for the movement of the sperm tail have been suggested, none has gained general acceptance. The organization of the structural elements in the sperm tail of flatworms, as described in this paper, offers an uninterrupted pathway for impulse transmission and coordination along the axial fiber bundle. If, in fact, the spokes interconnect *via* the core elements, as they appear to do, there is then continuity from the proximal to the most distal portion of the tail.

Gibbons and Grimstone (1960) described secondary fibers, disposed circumferentially between the central element and the rim units, in approximately the position of Afzelius's (1959) spokes and suggested that these radial structures were in fact the secondary fibers and that continuity between the peripheral and central elements was illusory. Our evidence strongly supports their existence as integrated structures. We have attempted to reconstruct the axial fiber bundle from the electron micrographic evidence and find that a single fiber could distribute itself along the length of the bundle giving the patterns found in sections of this structure. The three dimensional model is shown in Figs. 7 and 8. Fig. 7 is a shadowgraph of the model and Fig. 8 is a photograph depicting a portion of the axial fiber bundle as we visualize it. An uninterrupted fiber extends from point *A* to point *B* on the photograph and could extend the length of the entire structure in the axial fiber bundle.

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