ELECTRON MICROSCOPE STUDY OF SPERMIOGENESIS IN A FIRE-BRAT INSECT, *THERMOBIA DOMESTICA* PACK

I. Mature Spermatozoon

S. R. BAWA, Ph.D.

From the Department of Anatomy, Cornell University Medical College, New York. Dr. Bawa's present address is Department of Biophysics, Panjab University, Chandigarh, Panjab, India

ABSTRACT

The fine structure of the mature spermatozoon of the insect *Thermobia domestica* has been investigated. This flagellate spermatozoon is unique with respect to the relative positions of the centriole, nucleus, mitochondrial nebenkern derivatives, and acrosome along the length of the cell. The acrosome lies at the posterior end of the nucleus. Unlike spermatozoa of most animals, the *Thermobia* spermatozoon has a nucleus that reveals an unusual lamellar pattern of the material inside of it. This flagellate spermatozoon is also unusual in its tendency to intertwine with other spermatozoa, and during movement the intertwined portion of two (double) spermatozoa is always directed forwards. In the axial filament complex, one of the fibrils of each peripheral double fibril bears a hook-shaped process. An indistinct granular material is seen between the 9 outer coarse fibers. Cytoplasmic tubules, probably corresponding to the "microtubules" of other investigators (67), have been observed around the nucleus, mitochondrial nebenkern derivatives, axial filament complex, and acrosome. A description is given of a complex membrane system which surrounds and separates the main organelles of the cell from each other.

INTRODUCTION

Spermatogenesis in Thysanuran insects, *e.g. Lepisma domestica*, *Lepisma saccharina*, *Ctenolepisma urbana* and *Pterobius maritimus*, has been studied by many light microscopists (18, 21, 33–36, 52–55), but their opinions differ regarding the positions of the centriole and the acrosome in the mature spermatozoon. An earlier study (10) with phase microscopy and cytochemical methods revealed that the *Thermobia* spermatozoon is atypical since it possesses a centriole towards the anterior end and an acrosome at the base of the nucleus. Since further elucidation of the morphological details of these thread-like spermatozoa is beyond the limits of resolution of light optics, an electron microscope study of the mature spermatozoon of *Thermobia domestica* was undertaken. The present observations supplement our earlier findings (11–13) and clearly demonstrate that the *Thermobia* spermatozoon differs in many respects from the flagellate spermatozoa of other animals examined to date. This spermatozoon is unique with respect to the relative positions of the centriole, axial filament, nucleus, mitochondrial nebenkern derivatives, and acrosome along the length of the cell. Furthermore, it

is unusual in its motility and in its specific associations with other spermatozoa in the testes and at various regions along the reproductive tract. A description of the fine structure of the mature spermatozoon of *Thermobia* is presented here. Observations dealing with the morphogenesis of the acrosome, sperm tail, nucleus, and mitochondrial nebenkern will be described in subsequent papers.

MATERIAL AND METHODS

Adult males of *Thermobia domestica* Pack were used in this study. The insects were reared in the laboratory according to the directions of Dr. A. J. Adams (1).

Live male insects were placed on a clean glass slide; their thoracic region was then cut off, and the reproductive organs together with the viscera were gently squeezed out directly into the ice-cold fixatives for electron microscopy. For phase microscopy, the material was obtained in a similar manner and the testis lobes and the seminal vesicles were placed in a freshly prepared Carlson's fluid (19).

The living spermatozoa were studied with phase optics, and photomicrographs were made with Adox KB14 35-mm film using a dark contrast 10/0.22 dry objective. Movement of the spermatozoa was recorded on a 16-mm Kodak plus X reversal film using a Zeiss Standard phase contrast microscope equipped with an Airflex 16 camera in a microcinematographic set up.

Feulgen-stained (69) squash preparations of the seminal vesicles, fixed in Carnoy's solution, proved useful in determining the approximate extent of the nucleus in the mature spermatozoon.

For electron microscopy, the material was fixed either in ice-cold buffered (57) 2 per cent osmium tetroxide containing sucrose (20) for 30 minutes to 1 hour, or in unbuffered 2.5 per cent potassium permanganate (50) for 10 minutes. After fixation, the tissue was rapidly dehydrated in graded ethanols and embedded in Araldite[1], Epon 812 (51), or in butyl-methyl methacrylate (72). Sections were cut on a Porter-Blum microtome with glass or diamond knives, and were picked up on 200-mesh copper grids covered with Parlodion and carbon. Sections from Araldite-embedded tissue were transferred to bare 150-mesh copper grids. To enhance contrast, sections were routinely stained with an aqueous solution of uranyl acetate (73). The electron micrographs in Figs. 6 and 9 were taken with Siemens Elmiskop I; all other micrographs were taken with an RCA EMU 3B.

OBSERVATIONS

Light Microscopy

Examination of the squash preparations of the fresh testis lobes revealed double as well as single spermatozoa, the latter measuring approximately 600 to 650 μ in length. Single live spermatozoa are immotile, but those obtained from the seminal vesicles exhibit active movement and frequently appear intertwined in pairs (Figs. 1 and 2). The intertwining of two spermatozoa seems restricted to about one-third of their length; the remaining two-thirds of each spermatozoon is free from the other member of the pair. The posterior two-thirds of the spermatozoon appears highly refractile, and in this region its maximum diameter is approximately 1.3 μ. Measurements indicate that the diameter of the spermatozoon in the intertwined region is approximately 0.5 μ. Feulgen-stained

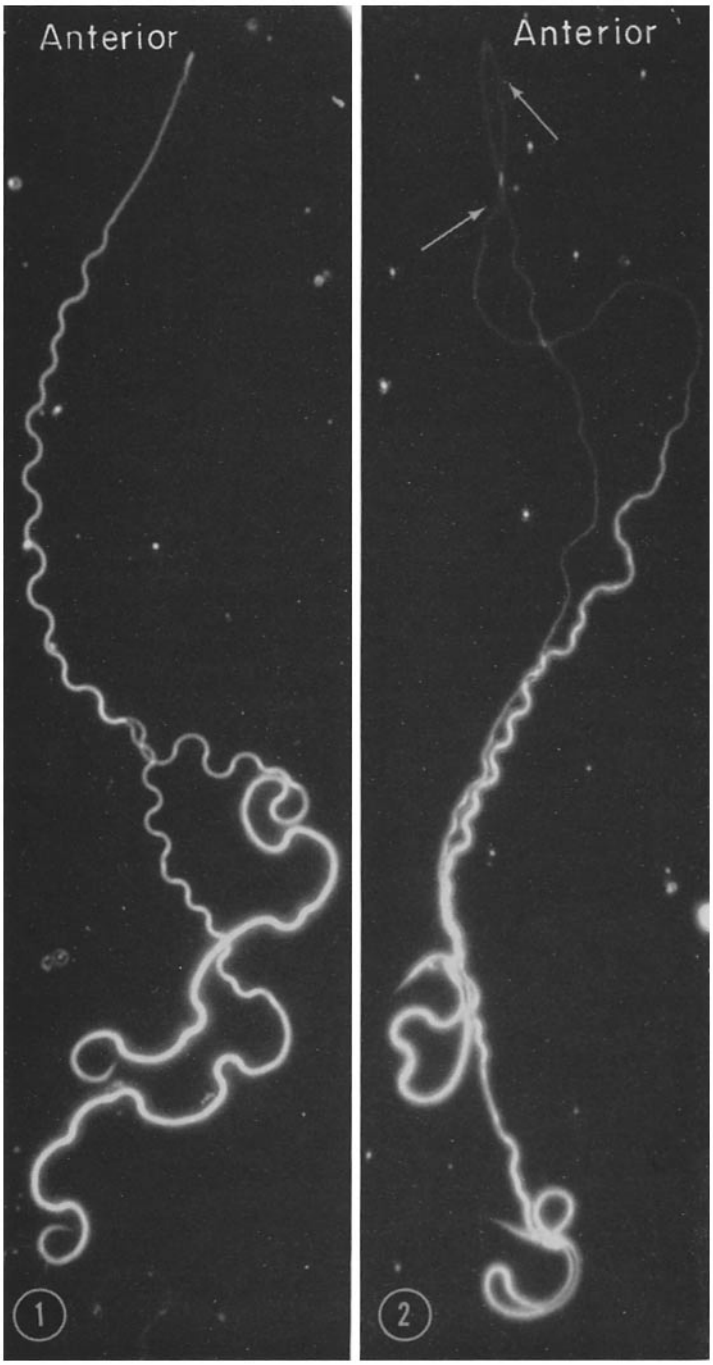
[1] Supplied by R. P. Cargille Laboratories, Inc., New York City.

Legends

A, Acrosome
AF, Axial filament complex
CF, Coarse fibers
Cf, Central fibrils
CT, Cytoplasmic tubules
HP, Hook-shaped process
MN, Mitochondrial nebenkern derivatives
MS, Cytoplasmic membranous sheath
N, Nucleus

FIGURE 1 Photomicrograph of live pair of spermatozoa of *Thermobia* obtained from the seminal vesicle. Note intimate intertwining of the two spermatozoa along approximately one-third of their length. Phase, dark contrast. × 400.

FIGURE 2 Photomicrograph of a pair of spermatozoa obtained from the seminal vesicle. The dissociation of the intertwined zone of the two spermatozoa is apparent (arrows). Note that the posterior two-thirds of the spermatozoa is highly refractile. Phase, dark contrast. × 400.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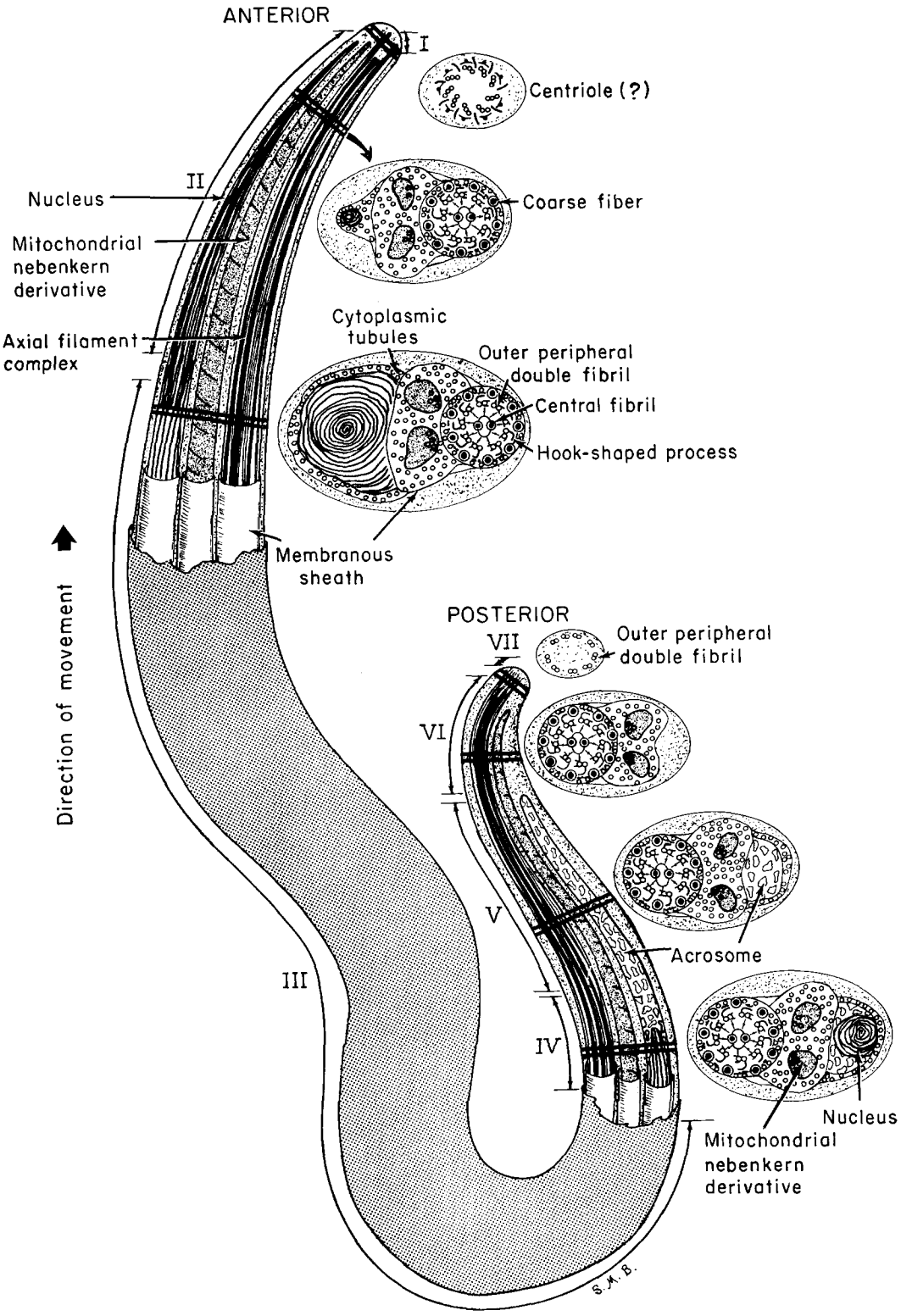

FIGURE 3 A schematic diagram of a mature spermatozoon of *Thermobia*. It has been divided into segments *I* to *VII*. Figs. 4 to 14 depict the fine structural details of these segments.

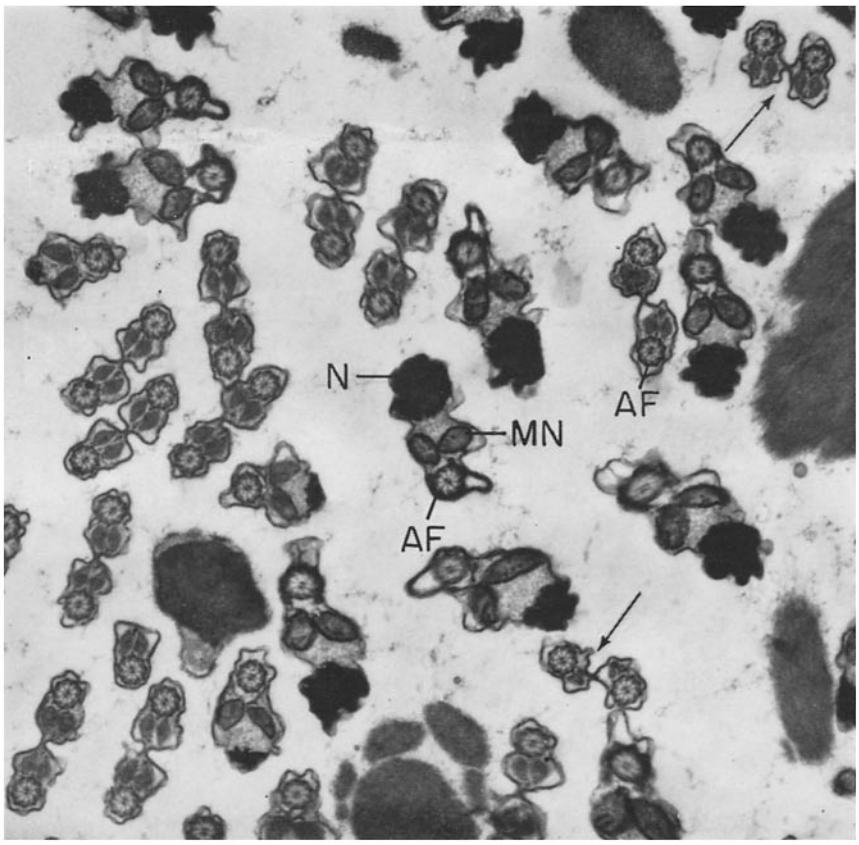

FIGURE 4 Electron micrograph showing sections of spermatozoa through segment *II*. The nucleus (*N*), mitochondrial nebenkern derivatives (*MN*), and the axial filament complex (*AF*) are clearly visible. The spermatozoa are connected specifically at places where their nuclei oppose each other, except in certain instances (arrows). Osmium tetroxide. Epon. × 17,000.

(69) spermatozoa reveal that the nucleus runs nearly the whole length of the sperm except for about 10 to 20 μ towards its acrosomal (posterior) end.

Electron Microscopy

Before describing the *Thermobia* spermatozoon, it is pertinent to determine its anterior and posterior ends. A cinephotographic record of their movement shows that the paired spermatozoa move violently with their intertwined part in the forward direction, a wave of contraction passing backwards. By definition, then, the intertwined zone represents the anterior portion of the spermatozoon. Additional observations regarding the polarity of these spermatozoa will be presented in the following sections of this report. The fine structure of the mature spermatozoon of *Thermobia domestica* is schematically illustrated in Fig. 3.

Nucleus

The nucleus of the spermatozoon is delimited posteriorly by an acrosome, while anteriorly it tapers into a thin filament. Cross-sections depicting the disposition of the nucleus and its submicroscopic organization are schematically represented in segments *II*, *III*, and *IV* in Fig. 3, and the corresponding electron micrographs are presented in Figs. 4, 9, and 13. Longitudinal sections of the nuclei are shown in Fig. 10. Without exception, and irrespective of fixation and embedding methods employed in the present study, the nucleus reveals a highly organized pattern. In favourably sectioned nuclei through segment *III*, electron-

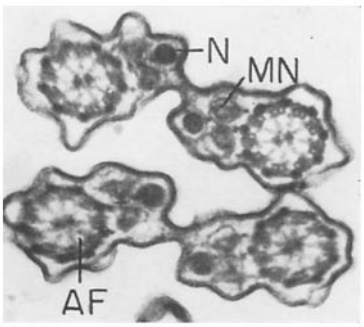

FIGURE 5 Sections of spermatozoa through segment *II*. There is indication of a continuous membrane around both spermatozoa. Osmium tetroxide. Epon. × 53,000.

opaque lamellae, about 60 A thick, separated by less dense interlamellar material are clearly visible (Figs. 10 and 11). Such a regular nuclear pattern is retained even in the spermatozoa obtained from the *receptaculum seminis* of the female insect (unpublished). The anterior one-third of the nucleus appears quite dense, this density probably resulting from the crowding together of the lamellae seen in the posterior two-thirds of the nucleus. In the spermatozoa in Fig. 12, the nuclear envelope appears in section as a single dense line, whereas in early spermatocytes it is clearly double.

Acrosome

The acrosome appears somewhat like a conical cap sitting on the nucleus and extending over it on one side for a short distance. Posteriorly, the acrosome, which gives a positive reaction with PAS technique (10, 46), extends for about 7 to 10 μ alongside the mitochondrial nebenkern derivatives (Fig. 3). The acrosome seems to consist of electron-opaque granular material which is riddled with lighter regions (Fig. 13).

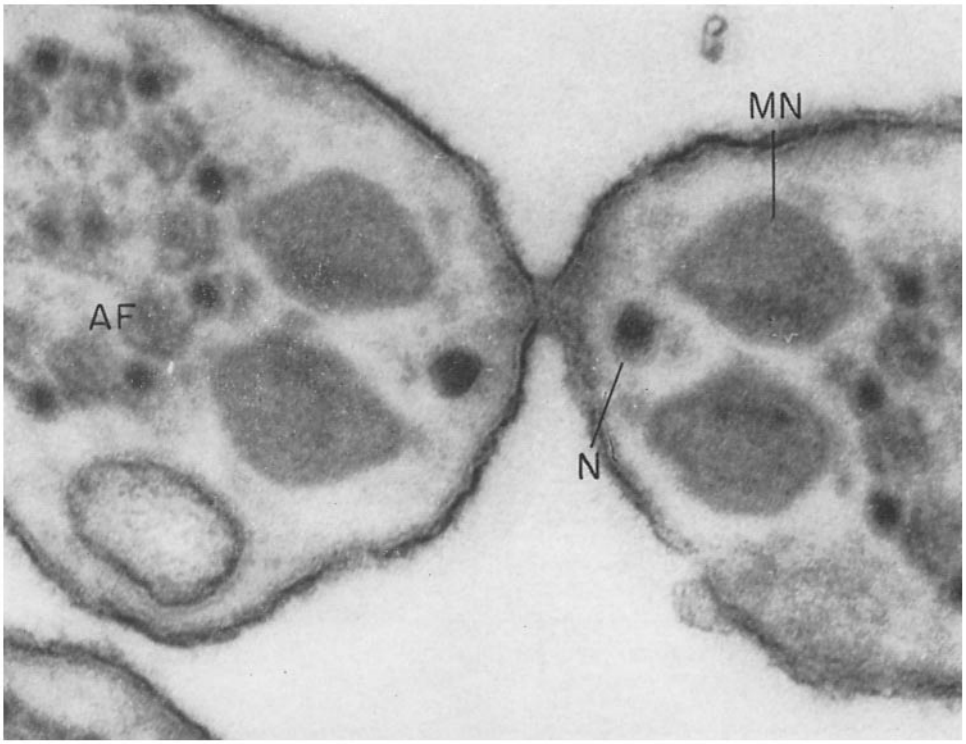

FIGURE 6 High resolution electron micrograph of a section of spermatozoa through segment *II*. Note electron-opaque material sandwiched between distinct plasma membranes of the opposing cells. Osmium tetroxide. Araldite. × 240,000.

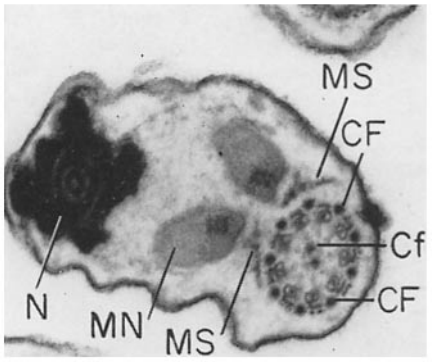

FIGURE 7 Electron micrograph of section of a spermatozoon through segment *III*. Note the mitochondrial nebenkern derivatives (*MN*) revealing the dense elements in their matrix. One of the fibrils of each of the peripheral double fibrils bears a hook-shaped process. Some granular material is also seen between the outer coarse fibers (*CF*). Note the fragments of the cytoplasmic membranous sheath (*MS*) around the axial filament. Osmium tetroxide. Araldite. × 64,000.

Mitochondrial Nebenkern Derivatives

In insects the mitochondria undergo complex morphological changes, and in the spermatid they fuse into a mitochondrial nebenkern (7). In late spermatids of *Thermobia*, the mitochondrial nebenkern divides into two components about equal in diameter. The two components of the mitochondrial nebenkern extend almost the entire length of the spermatozoon (Fig. 3) and henceforth will be referred to as MN derivatives. Since the "cristae" of the MN derivatives project perpendicular to their longitudinal axis, they could not be discerned in the cross-sections (Figs. 11, 13, and 14). The position of the MN derivatives relative to the nucleus, acrosome, and axial filament complex can be seen in Figs. 7, 9, 11, and 12. Very often, the matrix of the MN derivatives contains certain dense elements which are particularly clear in that region which faces the axial filament complex (Figs. 7 and 8). Somewhat comparable dense structures have been described previously in the mitochondrial elements of spermatozoa in the insect (7, 48, 59) and gastropod mollusc (7, 39, 43).

Axial Filament Complex

The axial filament complex extends alongside the MN derivatives from the anterior to the posterior end of the spermatozoon. Cross-sections of the spermatozoa through segments *I* to *VII* (Fig. 3) illustrate the changes in the organization of the axial filament complex. The fibrils are numbered according to the method of Afzelius (3). Though the position of the two central fibrils of the axial filament complex in relation to the nucleus is not fixed, the central fibrils frequently lie in a plane parallel to the nucleus and flagellum (Figs. 11 and 14). The two central fibrils are about 300 A apart, and each fibril measures about 220 A in diameter. Some indistinct granularity is visible around the central fibrils, and radiating from around the latter (Figs. 5, 11, and 14) are nine electron-opaque

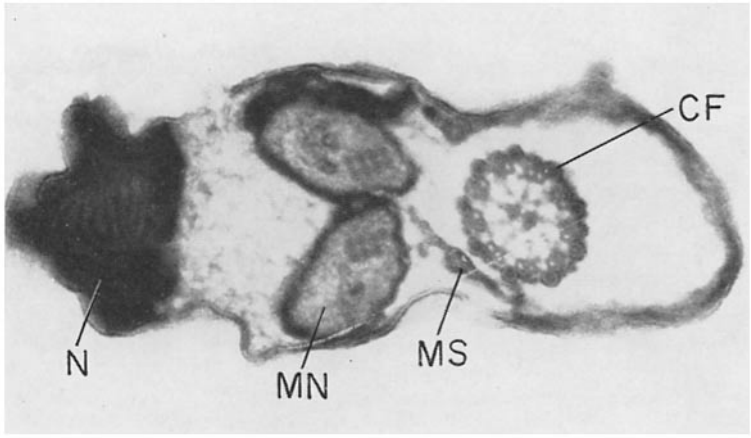

FIGURE 8 Section of a spermatozoon, depicting the dense elements in the matrix of the mitochondrial nebenkern derivatives (*MN*). Osmium tetroxide. Araldite. × 75,000.

"spokes" of Afzelius (3, 4). Each spoke shows slight thickening of granular material somewhat comparable to the "secondary fibers" (38). Almost touching the outer tips of the radial spokes are 9 peripheral double fibrils. Each of the double fibrils, in cross-section, is oval with a long axis approximately 400 A and a short axis about 350 A. From one of the fibrils of each of the peripheral vertebrate and vertebrate species (30, 31, 56, 70). Each of the coarse fibers is about 260 A in diameter and, like the two central fibrils, has an electron-opaque core and a less dense envelope. Some electron-opaque granular material can be discerned between the outer coarse fibers (Fig. 7; and Fig. 11, arrows). In certain cross-sections, in the vicinity of 9 coarse fibers, one to three additional dense bodies

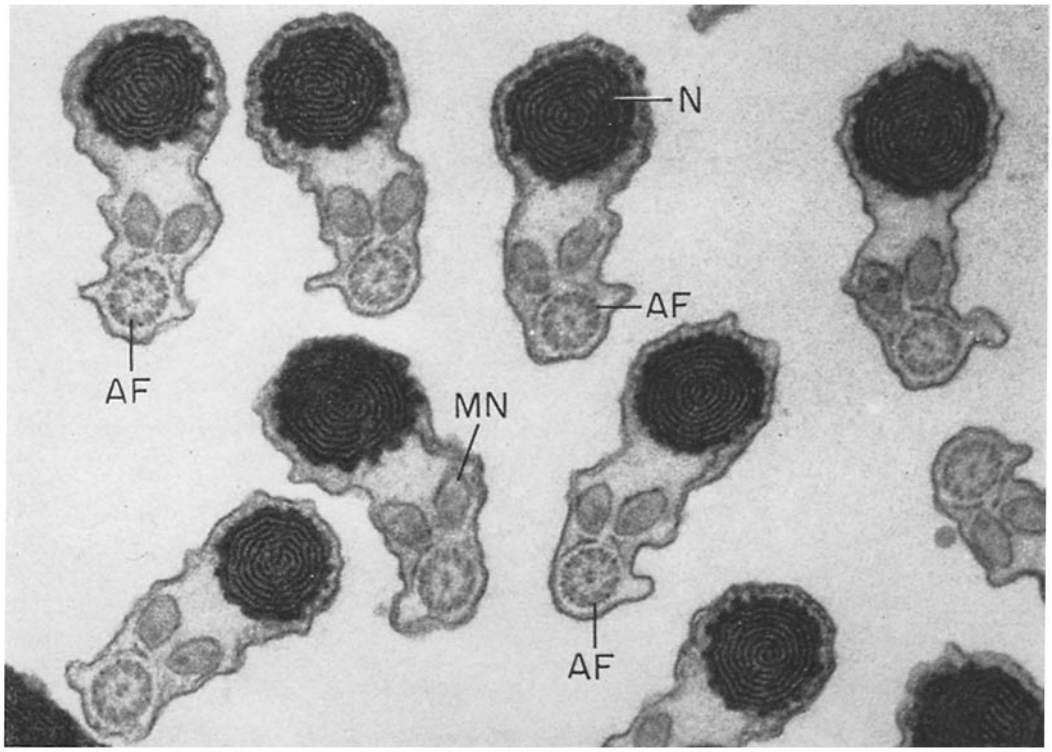

FIGURE 9 Electron micrograph showing cross-sections of the spermatozoa through segment *III*. An unusual structural pattern of the nuclear material is demonstrated in each section. Osmium tetroxide. Araldite. × 63,000.

doublets extend two arm-like processes which are especially clear in the spermatid tail. The other fibril of the peripheral doublet, which lacks arm-like extensions, bears a hook-shaped process somewhat resembling the third arm described in the sperm tail of *Macroglossum stellatarum* (6). The hook-shaped processes project radially and sometimes touch the outer coarse fibres of the axial filament complex.

Surrounding the 9(2) + 2 fibrillar complex is an additional set of 9 longitudinal fibers comparable to the "coarse fibers" observed in many in- of unknown significance can be recognized (Fig. 14, arrows). In potassium permanganate–fixed tissue, the coarse fibers as well as the central fibrils can be identified, but the peripheral double fibrils, "hook-shaped" processes, and radiating spokes are no longer visible (Fig. 12). Such a solubility reaction of these fibrils with potassium permanganate is similar to that found in the human sperm tail (14).

Though the morphogenesis of the kinetic apparatus of *Thermobia* will be described elsewhere, it is evident from the present study that the two cen-

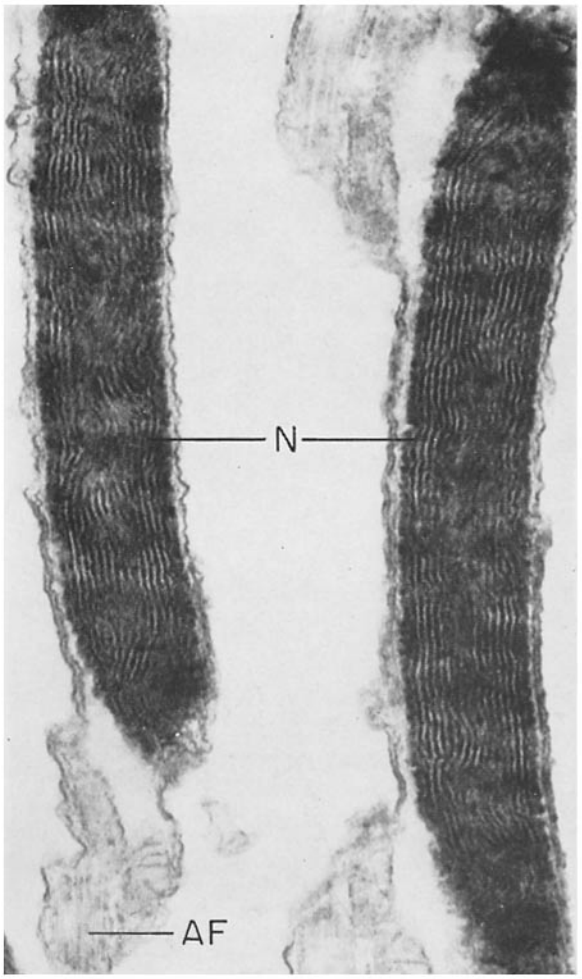

FIGURE 10 Electron micrograph of longitudinal sections of spermatozoa through segment *II* revealing lamellar arrangement of the material inside of the nucleus. Osmium tetroxide. Araldite. × 40,000.

tral fibrils of the axial filament complex tend to disappear first, followed by a progressive tapering off of the coarse fibers along the posterior (acrosomal) end of the spermatozoon. The peripheral double fibrils are the last to disappear since they may still be recognized as round, dense elements in transverse sections after the acrosome and the MN derivatives are no longer visible. The diameter of the spermatozoon in this region is approximately 4000 A (Fig. 13, stars). Finally, the characteristic pattern of these fibrils is lost and they disappear, probably at different levels towards the posterior end of the spermatozoon.

Membrane System

A complex membrane system, especially clear in potassium permanganate–fixed material (Fig. 12), surrounds and separates the nucleus, MN derivatives, axial filament complex, and acrosome from each other. Sometimes the membrane enclosing the axial filament complex seems fragmented. Two such fragments are visible in Figs. 7 and 11. These membrane fragments in *Thermobia* spermatozoa seem to correspond to the "tail membrane" of the honey bee spermatozoon (63).

Cytoplasmic Tubules

Discrete cytoplasmic tubules measuring approximately 200 A in diameter are seen around the nucleus, mitochondrial nebenkern derivatives, axial filament complex, and acrosome (Fig. 14). Cytoplasmic tubules of varying dimensions (120 to 270 A) have been encountered in diverse type of cells of many other organisms (7, 8, 39, 47, 65,

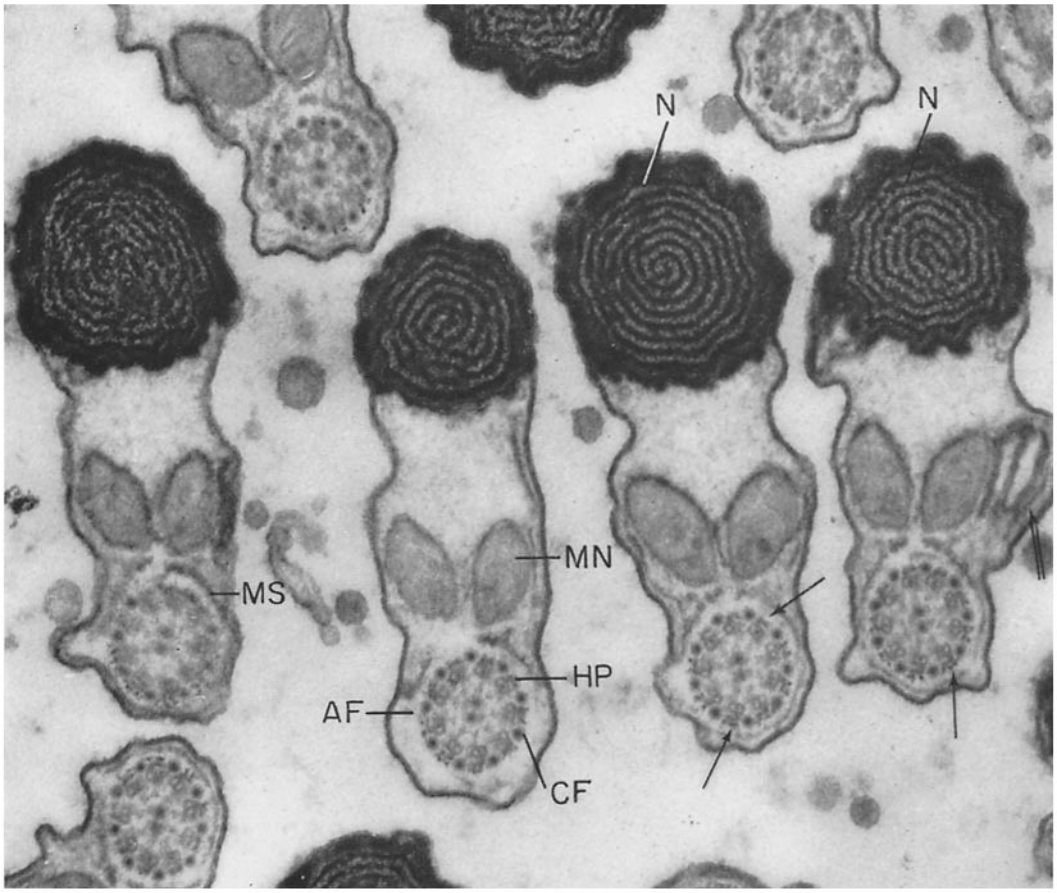

FIGURE 11 High resolution electron micrograph of transversely sectioned spermatozoa through segment *III*. Note the characteristic disposition of the nucleus (*N*), mitochondrial nebenkern derivatives (*MN*), and the axial filament complex (*AF*). Note a ring of 9 coarse fibers around 9(2) +2 axial filament unit, and the indistinct radiating "spokes." A "hook-shaped" (*HP*) process projects from one of the fibrils of each peripheral double fibril. A segment of the complex cytoplasmic membranous sheath is seen adjacent to one of the *MN* derivatives (double arrow). Osmium tetroxide. Araldite. × 62,000.

67). As the longitudinal sections of these tubules were not found, it was difficult to judge the extent and their possible association with the endoplasmic reticulum, the achromatic spindle, the Golgi elements, and the centriole (see Slautterback (67) for references). Whether the above-mentioned structures should be called "tubules" or "filaments" is trivial, as recently pointed out by Slautterback (67). Though the significance of these tubules in the *Thermobia* spermatozoon is obscure, nevertheless the approximation of the size of these tubules with that of a single fibril of the peripheral double fibrils of the axial filament complex and their solubility reaction with potassium permanganate suggest that both of the cytoplasmic elements may perform similar functions.

Intertwining of the Spermatozoa

One of the most remarkable features of *Thermobia* spermatozoa is their tendency to intertwine in pairs. The two spermatozoa are usually joined in such a manner that their nuclei are opposed to each other (Figs. 4 and 6), but there are exceptions to this arrangement (Fig. 4, arrows). At the sites of the adhesion of the two associating spermatozoa, the plasma membrane of each appears as two electron-opaque lines, approximately

350 A, thick with an interspace 40 A wide. The distance between the opposing plasma membranes is about 95 A, which apparently is much less than the distance frequently observed for other intercellular spaces. Though the two spermatozoa are intimately associated with each other, the plasma membranes of the two units remain separate. However, at certain places it seems that there is a continuous membrane around both spermatozoa (Fig. 5). Furthermore, at places of contact of the two intertwining spermatozoa, an electron-opaque substance fills the gap between the opposing cell membranes (Fig. 6). This dense material persists even in the potassium permanganate-fixed tissue. Due to the minuteness of this dense substance, its finer structure has been difficult to resolve.

DISCUSSION

The main points of interest in the study of the fine structure of the mature spermatozoa of *Thermobia domestica* are: the location of the acrosome at the "wrong" end, *i.e.* at the base of the nucleus; the means of adhesion of paired spermatozoa, and the lamellar structure of the nucleus which extends, along with the mitochondrial nebenkern derivatives and the axial filament complex, almost the entire length of the cell.

Although tubules, filaments, fibrils, and lamellae (2, 26, 39, 44, 60, 66) have been observed inside of the nucleus of the spermatids of a wide variety of organisms, none of the mature spermatozoa examined to date have revealed any submicroscopic structure inside of the nucleus. However, in *Thermobia* the lamellar pattern of the spermatid nucleus is retained even in the nucleus of the mature spermatozoon. But it is not clear whether these lamellae are some sort of lipid material or whether such an organisation reflects the arrangement of the chromsomes and the nuclear sap (62, 75). Further, it is difficult to account for the failure of the chromatin material to condense into a homogeneous dense structure often reported in other species (30, 60).

The flagellate spermatozoa can be grouped into two categories. The first category includes those animal spermatozoa which have an acrosome at the anterior tip of the nucleus. The second category includes spermatozoa of the spider-beetles *Ptinus tectus* and *P. hirtellus* (28, 42), iceryine coccids (40), and certain fish (37, 58). *Thermobia* spermatozoa do not fall into either of these categories because the mature spermatozoa of this species do

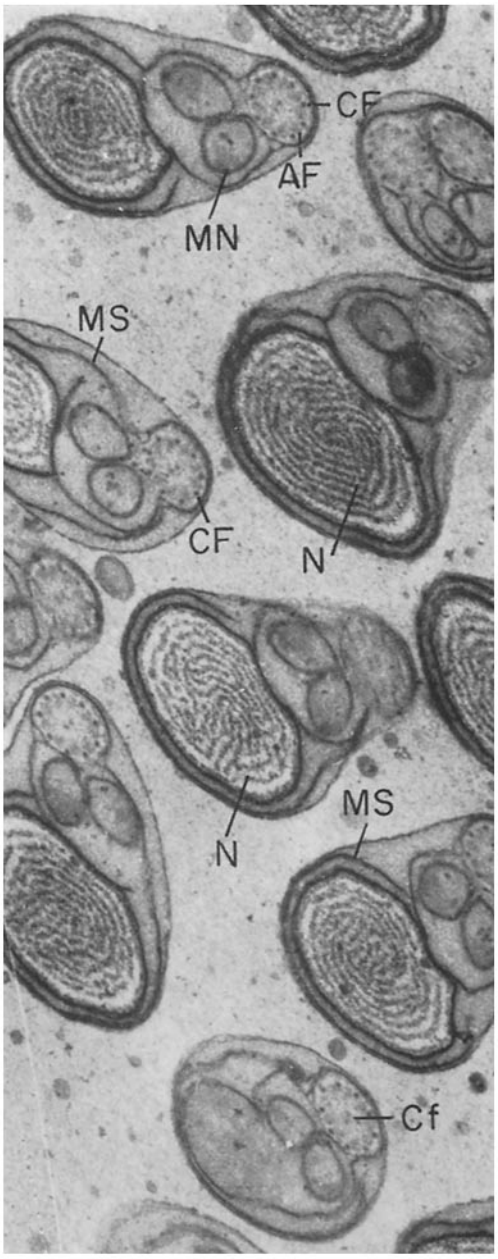

FIGURE 12 Sections of spermatozoa through segment III. The nucleus (*N*), mitochondrial nebenkern derivatives (*MN*), and the axial filament complex (*AF*) of each spermatozoon is enclosed in a cytoplasmic membranous sheath (*MS*). Note the clarity of the 9 coarse fibers (*CF*) and the 2 central fibrils (*Cf*) as compared to 9(2) fibrils which are barely visible. Potassium permanganate. Araldite. × 21,000.

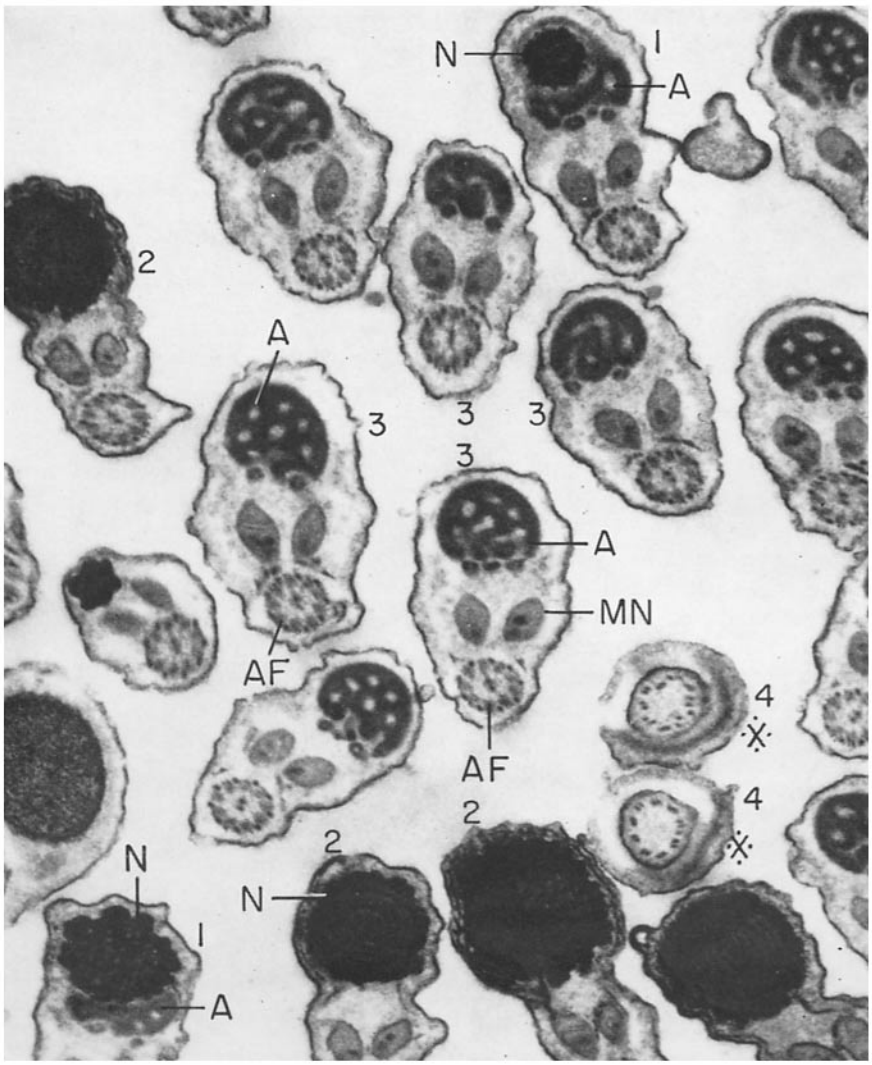

FIGURE 13 Electron micrograph shows sections of spermatozoa through segments *III*, *IV*, *V*, and *VII*. Sections numbered *1* are from region *IV* where the nucleus (*N*) is delimited by an acrosome (*A*). Sections numbered *2*, *3*, and *4* represent segments *III*, *V*, and *VII* of the spermatozoon as illustrated in the schematic diagram. At starred areas the diameter of spermatozoon is 4000 A. Osmium tetroxide. Araldite. × 33,000.

possess an acrosome but it is located at the "wrong" end, that is, at the basal end of the nucleus.

Because the acrosome commonly has an apical position, it is usually implicated in playing an important role in the fertilization of the egg (74). Bowen (17) proposed that the acrosome probably contains certain enzymes which initiate the physico-chemical reaction of fertilization. Leuchtenberger and Schrader (49) presented evidence of the possibility of a relationship between the PAS-positive reaction of the acrosome (46) and its possessing the enzyme hyaluronidase. The acrosome of certain echinoderms and molluscs is considered to contain lysin, which probably dissolves the egg membrane and helps in the penetration of the spermatozoon into the egg (22–24, 71). In *Thermobia* spermatozoa, however, it is not known

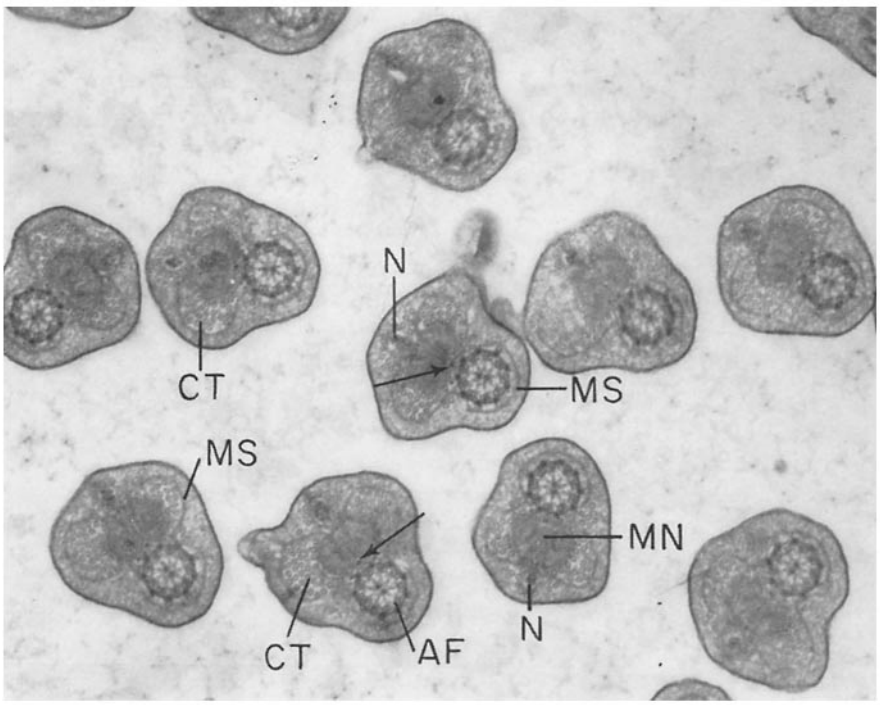

FIGURE 14 Electron micrograph of sections of spermatozoa through segment *II*. The nucleus (*N*), mitochondrial nebenkern derivatives (*MN*), and the axial filament complex (AF) are separated from each other by a membranous sheath (*MS*). Circular profiles of transversely sectioned cytoplasmic tubules (*CT*) can be recognized around the main organelles of the cell. Also note one or two electron-opaque elements at places indicated by the arrows. Osmium tetroxide. Epon. × 35,000.

whether the anterior end bearing the centriole perched at its tip or the posterior end bearing the acrosome enters first during the act of fertilization. Obviously, further study is required to elucidate the role, if any, of the basilar acrosome in fertilization of the egg in *Thermobia*.

In most animal spermatozoa, the tail-forming centriole lies in the immediate vicinity of the nuclear membrane (5, 15, 27) and the axial filament complex arises from the centriole (68). But in *Thermobia*, neither the centriole nor the axial filament apparatus seems to bear such spatial relationship with the nucleus.

The coarse fibers identified in the *Thermobia* sperm tail are by no means uncommon, as such fibers have been described in all mammalian spermatozoa (14, 31, 56, 70), in other vertebrates (68) and also in invertebrate species (39, 63, 68). However, in *Thermobia* these fibers appear round throughout their length, in contrast to the varying shape and size described for these fibers in the mammals. Radial connections between various constituents of the sperm tail, *i.e.* central fibrils, peripheral double fibrils, and the coarse fibers, have been demonstrated in the flagellar axis of the spermatozoa of the lepidopterous insect *Macroglossum stellatarum* (6). In the swimming-plate cilia of the Ctenophore *Mnemiopsis leidyi*, Afzelius (4) found connections between two of the outer 9 double fibrils and the ciliary membrane. Recently, Lang (45) described a complex pattern of fibrils in cross-sections of the flagellar base of the colorless flagellates *Polytoma obtusum* and *P. uvella*. Such distinct connections amongst outer coarse fibers were not encountered in *Thermobia*; instead, indistinct granular material could always be discerned. It is known that the coarse fibers of the axial filament complex not only decrease in size as they proceed away from the centriolar end but disappear one after the other at different levels of the sperm tail (30, 31, 70). Obviously, sections nearer the centriolar region of spermatozoa should reveal, as

they do, an axial filament composition of 9 + 9 (2) + 2 elements. On the other hand, as expected, sections closer to our proposed posterior end of the spermatozoa show fewer fibrils. Consequently, sections numbered 4 in Fig. 13 are from the posterior region, and Figs. 4 to 6 represent sections through the anterior intertwined segments of the spermatozoa.

In the present study it has been repeatedly observed that single live spermatozoa are motionless, whereas double spermatozoa show active movement. Why *only* the double spermatozoa of *Thermobia* should reveal movement is not clear. The biological significance of these anomalous spermatozoa is also obscure. Regarding the formation of double spermatozoa in *Thermobia*, two possible phenomena need consideration. Firstly, the "pairedness" of these spermatozoa may be due to a pre-existing morphological difference inasmuch as the two conjoined metamorphosing spermatids fail to separate later into single units. If it were so, the syncytial clusters containing as many as 4 to 20 spermatids should transform themselves, with their intercellular bridges (32) intact, into syncytial clusters of mature spermatozoa. But we do not find any such configurations of spermatozoa. On the other hand, there is evidence that almost all the interconnected spermatids during spermiogenesis, separate and develop into single units. Subsequently, these spermatozoa intertwine along a portion of their length. Therefore, it is more likely that the "pairedness" of *Thermobia* spermatozoa is a secondary phenomenon. Precisely what forces impel the mature spermatozoa of *Thermobia* to intertwine is an unsolved puzzle.

A number of investigators using light optics have described conjugate or double spermatozoa in the insect *Dytiscus* (9), the gastropod mollusc *Turritella* (41, 61), and the American opossum *Didelphys* (16, 25, 64). In these organisms, the spermatozoa associate in pairs by the approximation of their heads, while their middle pieces and tails remain free from each other. Recently, Fawcett (29) obtained elegant electron micrographs of *Didelphys* spermatozoa which illustrate the adhesion of two spermatozoa along their acrosome surfaces. In *Dytiscus* and *Didelphys*, the pairing of the spermatozoa is a transitory phenomenon; they remain associated only in the vas deferens, disassociating later into single units. In *Thermobia*, the spermatozoa remain intertwined not only in the male reproductive ducts but also in the *receptaculum seminis* of the female. But it could not be ascertained whether the intertwined spermatozoa unwind themselves into single units before fertilization of the egg. Curiously, the intertwining of these spermatozoa is confined to approximately one-third of their length. Though in the majority of electron micrographs (Fig. 6) the identity of the opposing plasma membranes of the intertwined spermatozoa is demonstrable, Fig. 5 suggests the possibility of fusion of the cell membranes at certain levels along the zone of adhesion between the two cells. The fine structure of the dense material which is interposed between the opposing plasma membranes of the intertwined spermatozoa has not been resolved. Certainly the dense material is not discernible while the spermatozoa exist in a single state, and its source is unknown.

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