STUDIES OF SPERMIOGENESIS IN A NEMATODE, *NIPPOSTRONGYLUS BRASILIENSIS*

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ABSTRACT

The fine structure of the developing spermatids and the mature sperm of *Nippostrongylus brasiliensis* was investigated. Immature spermatids are found at one end of the tubelike testis, and the mature sperm at the other. The spermatid has a prominent nucleus, with the chromatin clumped at the margin. It also contains a pair of centrioles, located near the nucleus. The cytoplasm is filled with ribosomal clusters, but it lacks an organized Golgi area or endoplasmic reticulum. Besides the normal mitochondria, the spermatid has specialized mitochondrionlike inclusions with dense matrix, few broad cristae, and a crystalloid structure always facing the nucleus. As spermiogenesis proceeds, the nucleus elongates, comes to lie at one end, and later evaginates to form a separate head structure, leaving the mitochondria and other cytoplasmic organelles in a broad cytoplasmic region. The nuclear material becomes filamentous and spiral, and the centrioles come to lie at one end near the junction of the head and the cytoplasmic portion of the sperm. Microtubules are found in the cytoplasmic region extending from the tubelike nucleus. The specialized mitochondria are about eighteen in number, and are arranged in rows in staggered groups of three around the microtubules in the cytoplasmic region. The mature sperm is aflagellate and lacks an acrosome. No movement of the sperm was ever observed.

INTRODUCTION

Nematode sperm are usually described as nonflagellate, ameboid cells containing a large refringent body, possibly an acrosome. This description is, however, based largely on the study of ascarid sperm (10, 25, 35), and most species in this vast phylum are as yet unstudied. In all but a few cases, nematode sperm have been considered to be nonflagellate, but one free-living species, *Trilobus longus*, was thought by Chitwood (6) to possess a true flagellum. Other nematode sperm have head and tail-like structures, but are in actuality nonflagellate. In *Oxyuris ambigua*, for example, the "tail" contains only a single long mitochondrion (4), and in the present study the narrow and wavy portion of *Nippostrongylus* sperm was found to contain the nucleus, with mitochondria concentrated in the broader "headlike" region.

The lack of flagella in nematode sperm is probably associated with the apparent complete lack of cilia or flagella in the entire phylum. In one case, that of the tick *Amblyomma*, the absence of a true flagellum seems to have been compensated for by a microtubular system (29) which apparently provides for sperm motility. Christensen (7) also described the presence of similar tubules in the aflagellate but motile sperm of the flatworm *Plagiostomum*. Although a number of sperm lack typical flagella, they nevertheless appear to possess centrioles. The absence of flagella may thus be related to functional and morphological modifica-

tions in the centriole, as suggested by Sotelo and Trujillo-Cenoz (34), rather than in their absence. The present paper describes spermiogenesis in the nematode *Nippostrongylus brasiliensis*, with these problems in mind. These sperm, although they possess microtubules, are aflagellate and probably nonmotile. They possess two centrioles of atypical structure, possibly functional, after fertilization, in the cleavage of the egg.

MATERIALS AND METHODS

Nippostrongylus brasiliensis was maintained in the laboratory by cultivating larvae from the feces of the infected rats and transferring them periodically into normal rats (14). Adult worms were collected from the intestine by their positive thermotropism to a light bulb, as described by McCue and Thorson (17).

For Light Microscopy

For light microscopy the worms were fixed in hot formaldehyde-acetic alcohol. After the usual dehydration procedure through the graded series of alcohols, they were embedded in paraffin (mp 56°C). To show the presence of DNA in sperms, the Feulgen reaction was used. The testis was isolated from the worm and was fixed in acetic alcohol for 30 to 60 min. It was then transferred to an albuminized slide and covered with a cover slip. By gentle tapping, the tissue was dispersed and was frozen on dry ice. The cover slip was removed and the Feulgen reaction was carried out, with light green as a counterstain. For the detection of RNA, 2 μ sections of Epon-embedded material were stained for ½ hr in azure B solution (0.025% in McIlwain buffer at pH 4.0) at 60°C (12). A control slide was treated for 1 hr with RNase (0.2 mg/ml, adjusted to pH 6.5). The PAS reaction was also carried out in 2 μ sections, using as control one slide treated with saliva.

Two different methods were used to investigate the presence of basic proteins in sperm. (*a*) Fast green–histone stain (1). Fragments of the testis were fixed in 10% neutral formaldehyde for 1 hr and squashed on a slide. The cover slip was removed with dry ice. After washing in running water for 1 hr, the tissues were treated for 15 min in 5% trichloroacetic acid in a boiling water bath. The slides were then washed in three changes of 70% ethanol and stained in 0.1% alkaline fast green at pH 8.1 for 1 hr, differentiated in 95% ethanol, dehydrated, and mounted. (*b*) *Modified Sakaguchi reaction* for argine (18). The tissues were fixed in 10% neutral formaldehyde. The staining solution was prepared as follows: to 47 ml of 1% NaOH solution was added 1 ml of 1% solution of 2,4-dichloronapthol, and the mixture was vigorously shaken. The slides were placed in this solution for 1 hr. They were then washed in a large volume of 1% NaOH solution and finally mounted in glycerine at pH 11.0 adjusted with NaOH.

To detect the presence of mitochondria, living sperm were placed in a dilute solution of Janus green B (1:100,000) made up in physiological saline. A drop of suspension was placed on a slide and immediately examined under the microscope. Using Nitro-BT, succinic dehydrogenase activity was also demonstrated in the mitochondria of the sperm. Buffered succinate was prepared by mixing equal volumes of 0.2 M Na-succinate and 0.2 M phosphate buffer at pH 7.6. The slide was incubated in the mixture containing equal amounts of the above stock solution and aqueous Nitro-BT (1 mg/ml) for 5 to 20 min at 37°C. It was washed in saline and fixed in 10% formaldehyde-saline for 10 min. After rinsing in 15% ethanol for 5 min, it was mounted in glycerine

FIGURE 1 Phase photomicrograph of mature sperm. Some mitochondria have been lost from the cytoplasm, apparently because the sperm has been damaged by contact with the slide. × 2000.

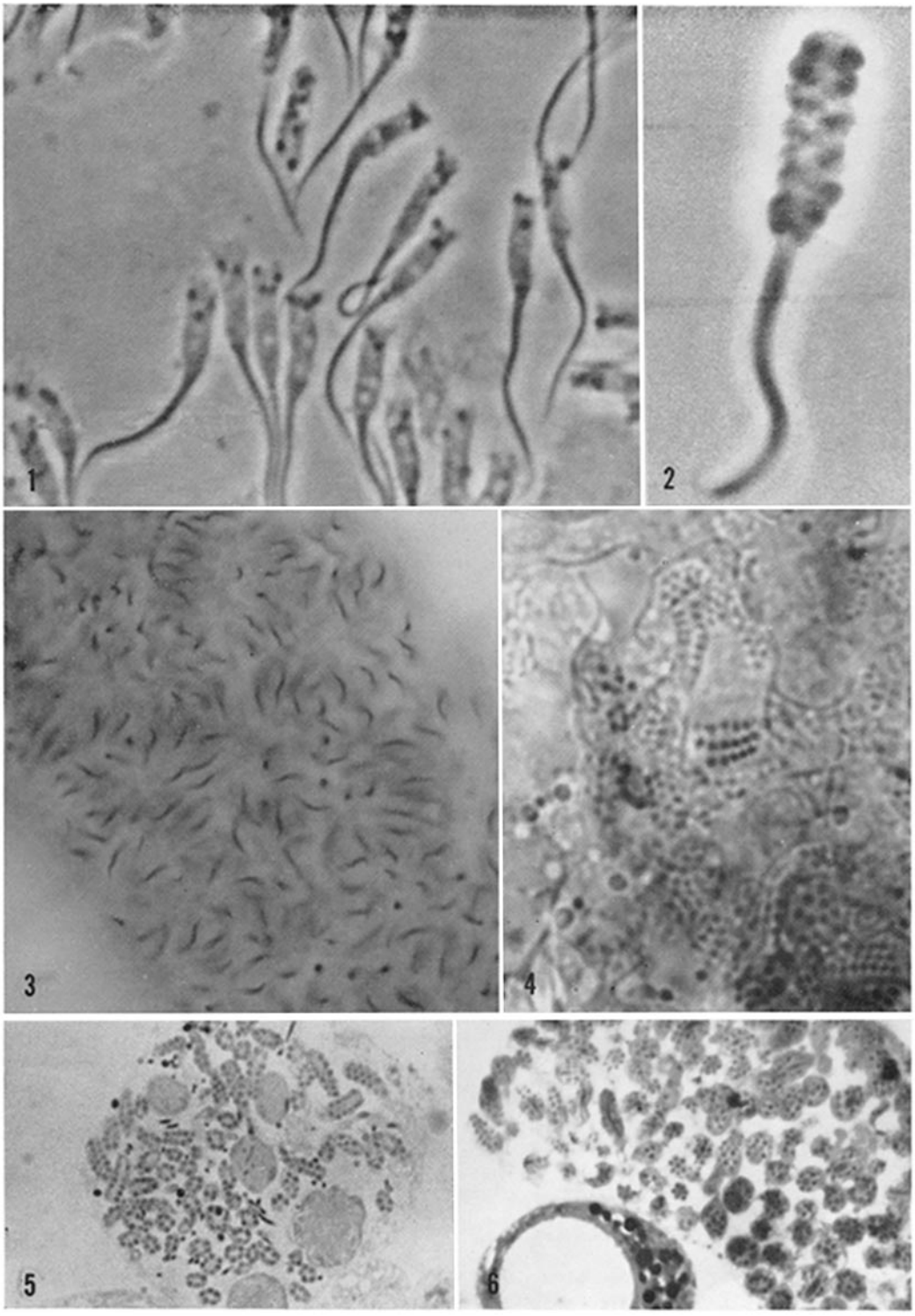
FIGURE 2 Phase photomicrograph of mature sperm at higher magnification. Note the broad cytoplasmic region packed with dense mitochondria. × 4500.

FIGURE 3 Photomicrograph of the testis showing Feulgen-positive head of the mature sperm. × 1400.

FIGURE 4 Photomicrograph of the mature sperm stained with janus green B. Mitochondria arranged in row in the broad region of the sperm can be seen as dark granules. × 1600.

FIGURE 5 Photomicrograph of OsO_4-fixed testis showing PAS-positive granules in the sperm. × 1400.

FIGURE 6 Photomicrograph of OSO_4-fixed testis stained with azure B. × 1600.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jelly. Control slides incubated without succinate were negative, indicating that blue formazan crystals represented the sites of succinic dehydrogenase activity. Sperm were also fixed in Regaud's fluid, with the slide being transferred to a potassium permanganate solution for brief rinse and then bleached in oxalic acid. After being washed in distilled water, it was stained with Altmann's aniline fuchsin with gentle heating. Excess of stain was drained off and methyl green was used as a counterstain.

For Electron Microscopy

The worms were fixed in freshly prepared 1% osmium tetroxide in 0.2 M phosphate buffer at pH 7.6 at 4°C for 1 hr. They were cut into small pieces as soon as they were immersed in the fixative, because otherwise the presence of a thick cuticle hindered the proper fixation of the internal structures. The tissues were washed twice with double-distilled water and dehydrated through a graded series of ethanols, allowing 30 min in each change. After treatment with absolute alcohol, they were brought to room temperature and transferred to propylene oxide. After two changes of propylene oxide, the worms were embedded in Epon 812 (16).

In some cases, testes were isolated from the worms and fixed separately in two ways:

1. 10% formaldehyde for 15 to 30 min with postfixation in 1% OsO_4 at pH 7.6 for 1 hr.
2. 6% glutaraldehyde for 1½ hr with postfixation in 2% OsO_4 at pH 6.8 for 1 hr.

The material was embedded either in Epon 812, or in a mixture of Epon and Araldite (20). In the case of the Epon-Araldite mixture, the polymerization procedure was slightly altered. The capsules were placed at 37°C overnight. They were then transferred to 50°C and left there for 24 to 48 hr. Materials embedded in Epon-Araldite mixture were much easier to cut as compared to those in Epon 812. Sections were stained either with lead hydroxide (15) for 12 to 15 min or with 3% saturated solution of uranyl acetate (36) for 12 to 24 hr.

RESULTS

Light Microscopy

The testis of *N. brasiliensis* consists of a single slender tubelike structure, the anterior end of which is folded back on itself to form a small loop. The testis is telogenic, i.e., the immature spermatocytes are at one end and the mature sperm at the other. Under the phase-contrast microscope, one can see the youngest spermatocytes as a cluster of cells at the anterior end, and posterior to them the developing spermatocytes, arranged in a single row. The mature sperm are found at the posterior end of the testis, stored in the seminal receptacle. The sperm is aflagellate with a 15 to 20 μ–long head which frequently shows a wavy structure suggesting a loose spiral. The remainder of the sperm consists of a cytoplasmic portion, approximately 6 μ wide, with about 18 prominent mitochondrionlike inclusions (Figs. 1 and 2). In all the living specimens (more than fifty) examined, movement of sperm was never observed. The cytoplasmic region of the sperm has a firm shape, and there is no evidence for ameboid movement. A large number of mature sperm are also found in the female uterus. The mature worms live in an environment where male and female worms are close together, most of the time entangled with each other. These worms copulate, and during this process the sperm are transferred to the female.

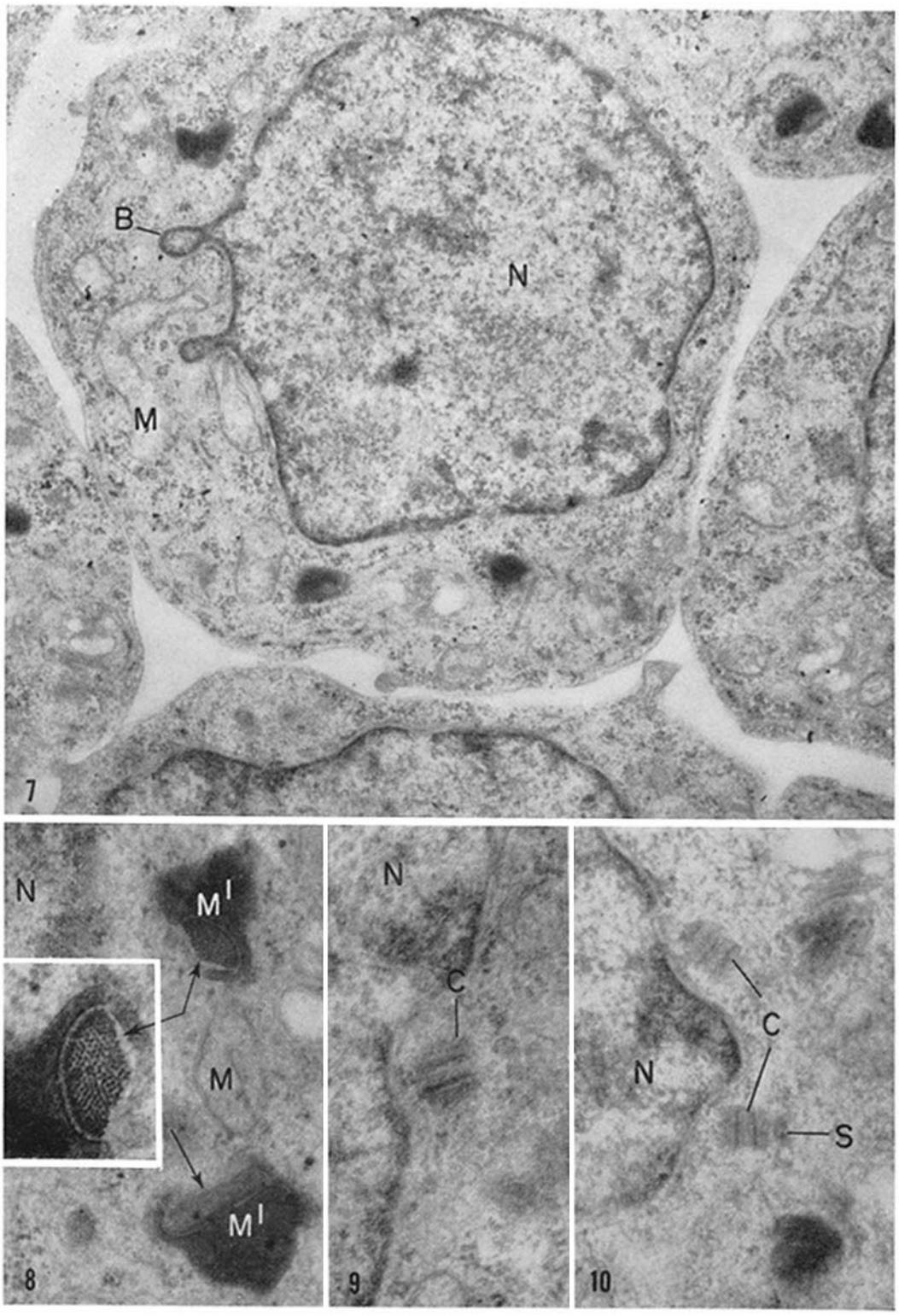
Squash preparations of the testis stained with the Feulgen reaction showed that the elongated cylindrical region of the sperm was strongly Feulgen positive and that the broad region was negative (Fig. 3). This clearly indicated the presence of the nucleus in the elongated region. Control slides treated with DNase did not stain. When the mature sperm were stained for basic proteins by the

FIGURE 7 Electron micrograph of spermatid showing nucleus (*N*) with bleblike protrusion (*B*), and mitochondria (*M*). × 24,000.

FIGURE 8 Electron micrograph of spermatid showing mitochondria (*M*) and a part of the nucleus (*N*). Also, note a few specialized mitochondria (M^1) with crystalloid structure, in both longitudinal and transverse sections (arrows). × 36,000.

FIGURE 9 Section of spermatid showing a part of the nucleus (*N*) and one centriole (*C*). × 46,000.

FIGURE 10 Section of spermatid showing a part of the nucleus (*N*) and a pair of centrioles (*C*), each with a procentriolelike satellite (*S*). × 37,000.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method of Alfert and Geschwind (1), the nucleus was positive, demonstrating the presence of trichloroacetic acid–insoluble histonelike protein. The basic protein contains a significant arginine content as shown by the Sakaguchi test (18).

Living sperm were stained with Janus green B which showed the presence of small granules, probably mitochondria, in the broad cytoplasmic region (Fig. 4). These granules also stained with Altmann's acid fuchsin method, and gave a positive reaction with nitro-BT, indicating the probable presence of succinic dehydrogenase activity. Thus, although most of these cytoplasmic inclusions possess a specialized structure as demonstrated below, they can probably be identified as mitochondria on the basis of their histochemical properties. Since these bodies have a high density, however, as shown by their phase image, it is possible that they may stain nonspecifically. This seems unlikely, since they were demonstrable with Janus green when no other organelles within the cell were noticeably colored. The spermatocytes were strongly basophilic with azure B, indicating high concentration of RNA. Tissues extracted with RNase demonstrated only nuclear (DNA) staining. The granules in the sperm gave a PAS-positive reaction (Fig. 5), while the unhydrolyzed control slides were negative. Both granules and sperm heads stained when OsO_4-fixed Epon sections were treated with concentrated (0.25%) azure B (Fig. 6). Under these conditions, azure B was not specific for nucleic acids, demonstrating the basophilia of both nucleic acid and acidic proteins. We have concluded that the mitochondrionlike inclusions contain both proteins and polysaccharides.

Electron Microscopy

The spermatids possess only a small amount of cytoplasm. The nucleus is sometimes so deeply indented that sections may appear to contain two nuclei. The nuclear envelope is typical, with an inner and outer membrane; a narrow ring of chromatin lines the inner membrane. The envelope has numerous annuli, and at some places it bulges out into the cytoplasm to form blebs (Fig. 7). There is no well organized nucleolus, but dark patches of chromatin can be seen scattered in the nucleoplasm. The cytoplasm lacks any organized endoplasmic reticulum. Instead, the cytoplasmic matrix is filled with many dense ribosomal clusters, and a few scattered cisternae. No well developed Golgi area is present, although a few smooth cisternae are often visible. This is in contrast to spermatids of other forms in which the Golgi area is often well organized and takes part in acrosome formation.

A striking feature of these cells is the development of characteristic mitochondrionlike inclusions. These inclusions have a dense matrix, and one side is bounded by a double membrane which has two to three infoldings resembling mitochondrial cristae, only being somewhat wider. On the other side, the double membrane is indented, and, within the indentation on the outer surface of the membranes, is found a crystalloid structure which in longitudinal section appears as parallel rods; in cross-section these rods give the appear-

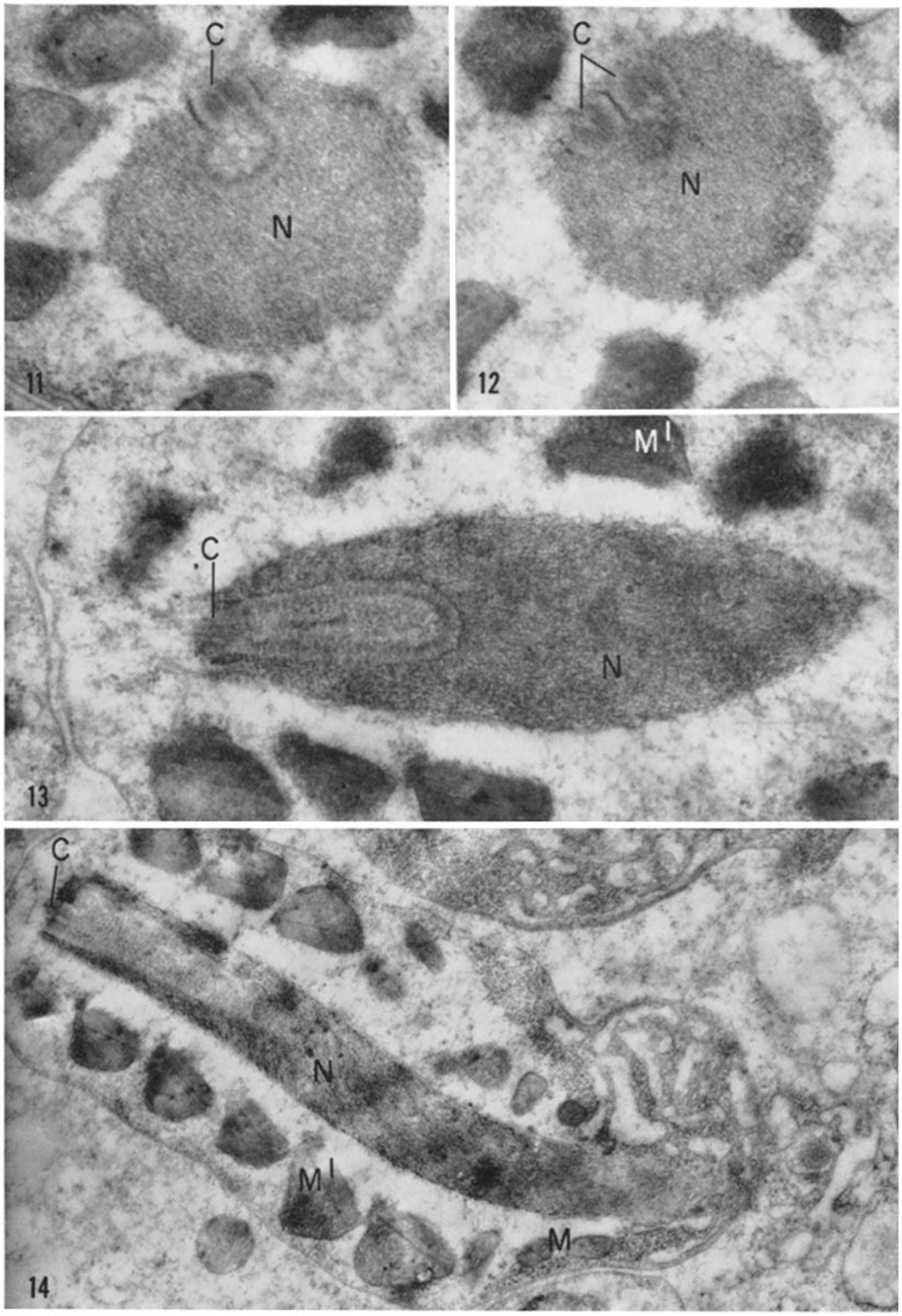
FIGURES 11 to 14 This series of electron micrographs shows the changes in the nucleus during spermatogenesis.

FIGURE 11 Intermediate spermatid; the centriole (*C*) is shown at the nuclear margin; the nuclear material (*N*) is not yet filamentous. × 25,000.

FIGURE 12 A stage similar to that shown in Fig. 11. The two centrioles (*C*) are side by side at the margin of the nucleus (*N*). × 25,000.

FIGURE 13 The nucleus (*N*) is more elongated, and mitochondria (M^1) are arranged around it. × 25,000.

FIGURE 14 The nucleus (*N*) has elongated further and become hollow at its base, where the centrioles (*C*) are located. The chromatin is filamentous in structure. Note the modified mitochondria (M^1) arranged linearly, with their crystalloid always facing inside. A few normal mitochondria (*M*) are present. The tip of the nucleus is associated with endoplasmic reticulum. × 25,000.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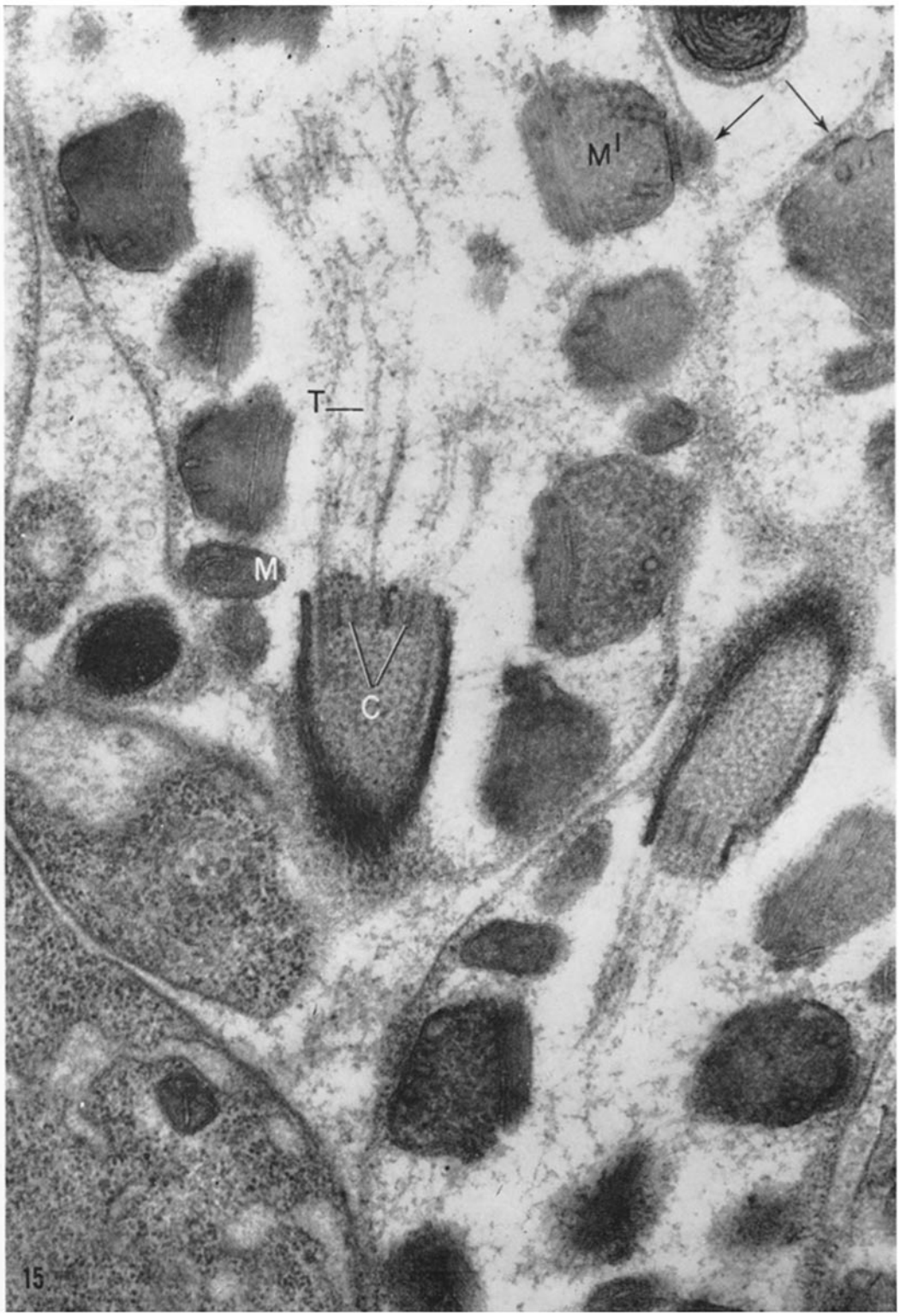

FIGURE 15 Oblique section of two mature sperm showing a pair of centrioles (*C*) at the rim of the tube-like nucleus, and microtubules (*T*) extending from it. Note that the majority of mitochondria (M^1) are of a modified kind and seem to be attached to the cell membrane (arrow). A few normal mitochondria (*M*) can also be seen. × 35,000.

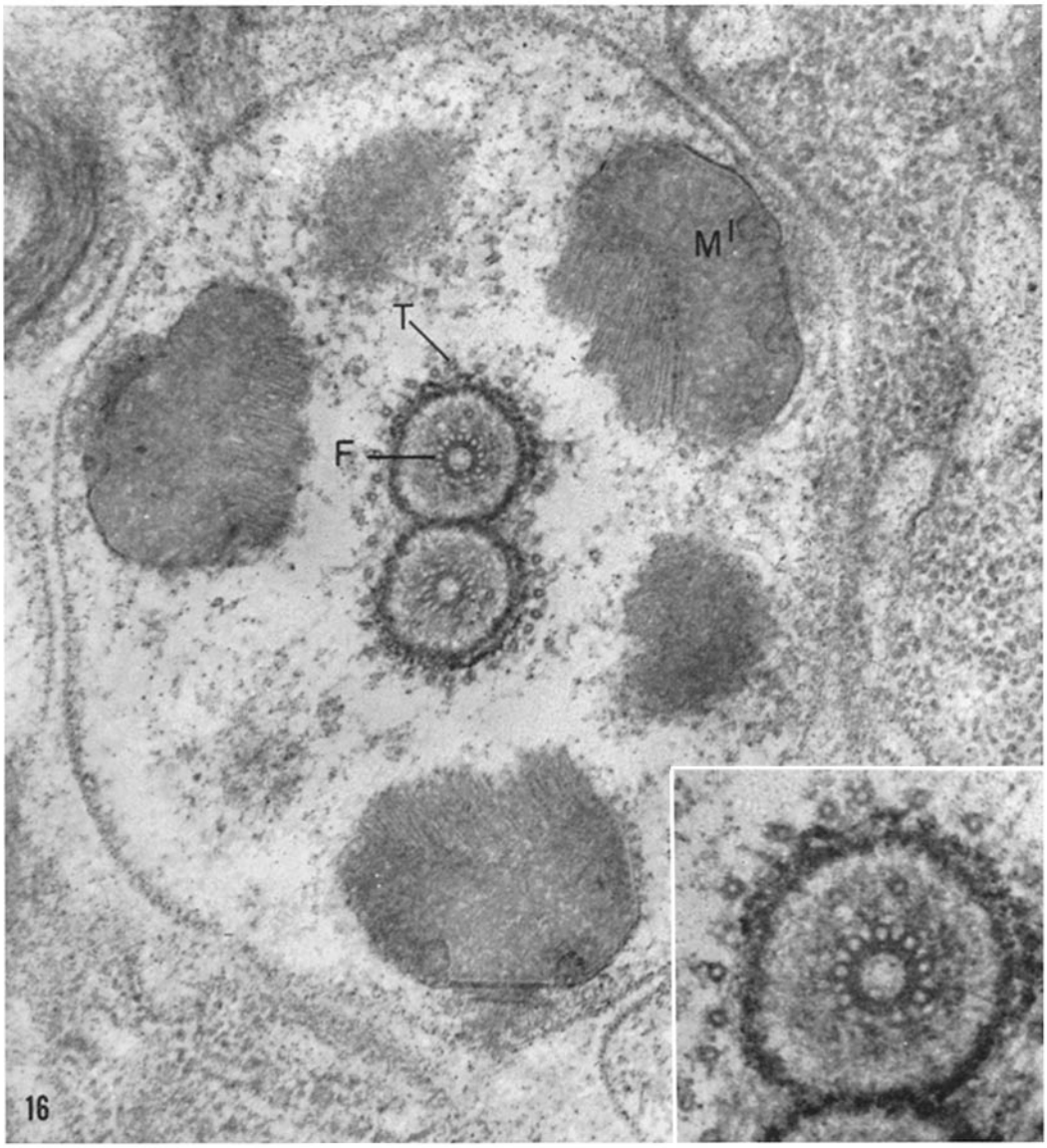

FIGURE 16 Cross-section of sperm passing through centriolar region. Each centriole has a central ring of dense material surrounded by 18 filaments (*F*). Note the microtubules (*T*) around the centriole, and mitochondria (M^1) with a few small cristae. One of the centrioles is shown at higher magnification (inset). Fig. 16, × 49,000; inset, × 84,500.

ance of hexagonal packing (Fig. 8). The outer surface of the crystalloid apparently is not membrane bounded. These mitochondrionlike bodies become more abundant in the mature sperm and are linearly arranged along the side of the broad region of the sperm (Fig. 15). Characteristically, the crystalloid side of these inclusion bodies always faces inside towards the nucleus of the developing spermatid, and towards the microtubules in the mature sperm (Fig. 15). In the spermatid there are also several mitochondria which have a more typical structure and less dense matrix. The mature sperm also contains a few typical mitochondria interspersed with the specialized inclusion bodies (Figs. 15 and 17).

A centriole, about 160 mμ long and 100 mμ

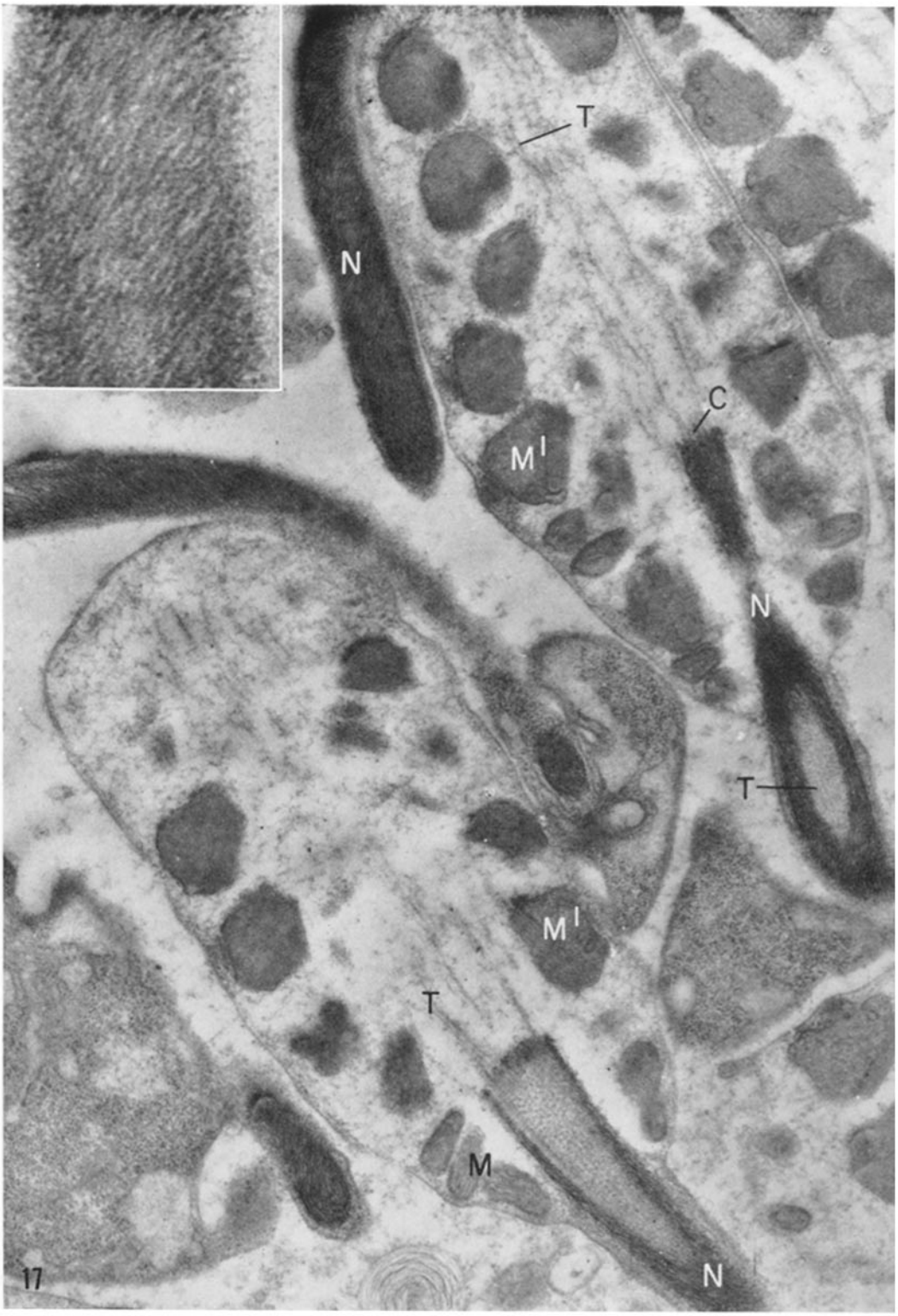

FIGURE 17 Longitudinal section of sperm showing elongated nucleus (*N*) containing filamentous chromatin apparently arranged in a spiral form, and a broad cytoplasmic region containing normal mitochondria (*M*), specialized mitochondria (*M*[1]), and microtubules (*T*). Also note the centriole (*C*) at the junction and few microtubules inside the hollow nucleus. Inset shows the chromatin at higher magnification. Fig. 17, × 19,500; inset, × 100,000.

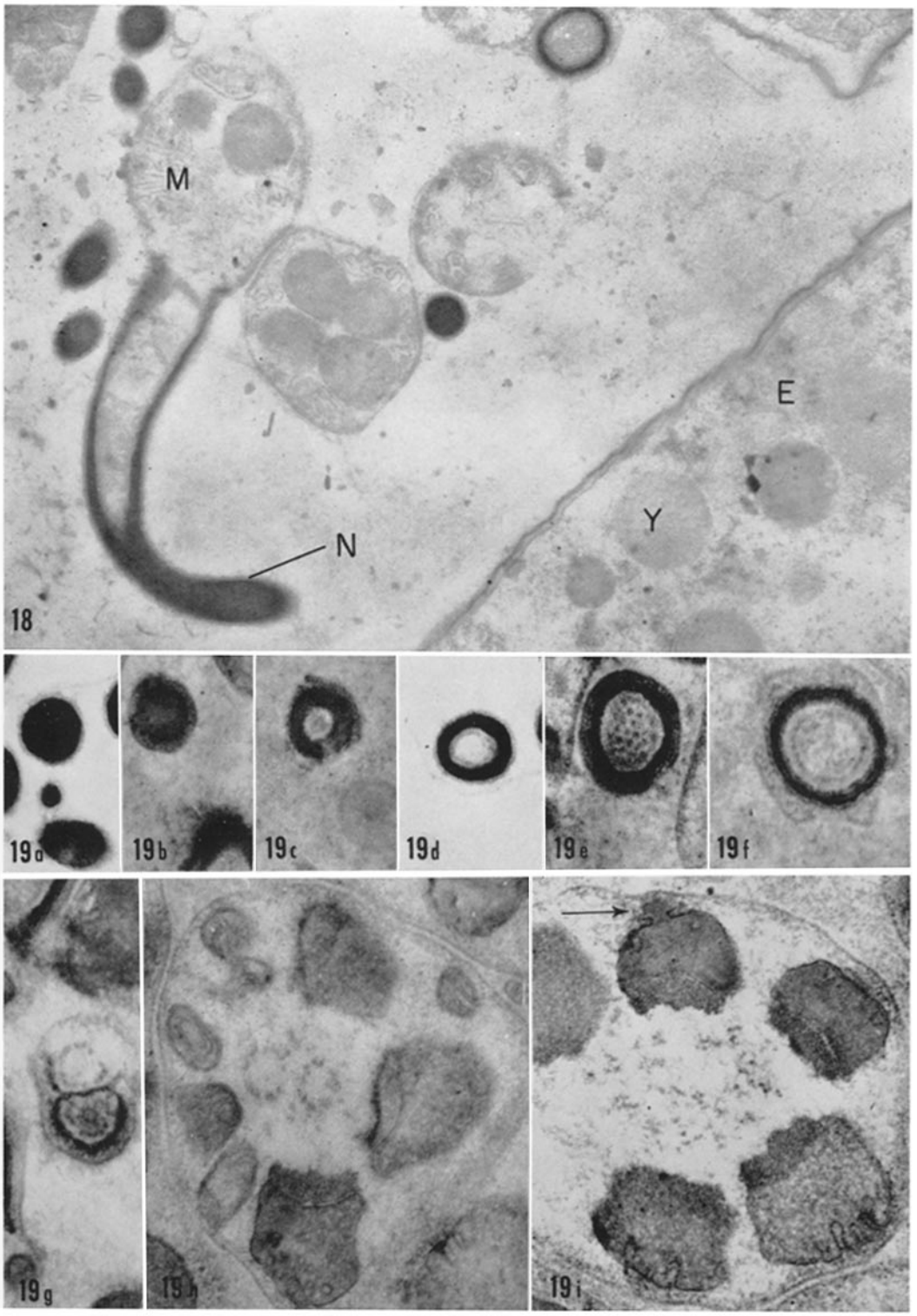

FIGURE 18 Electron micrograph of sperm inside the uterus of female *Nippostrongylus* showing elongated nucleus (*N*) and broad cytoplasmic region containing mitochondria (*M*). A part of the egg (*E*) can also be seen with yolk granules (*Y*). × 19,500.

FIGURE 19 *a* to *i* A series of cross-sections of mature sperm. The approximate locations of the sections along the sperm axis are shown on the diagram (Fig. 20). Fig. 19 *i* shows the protusion from the mitochondrion (arrow). × 24,500.

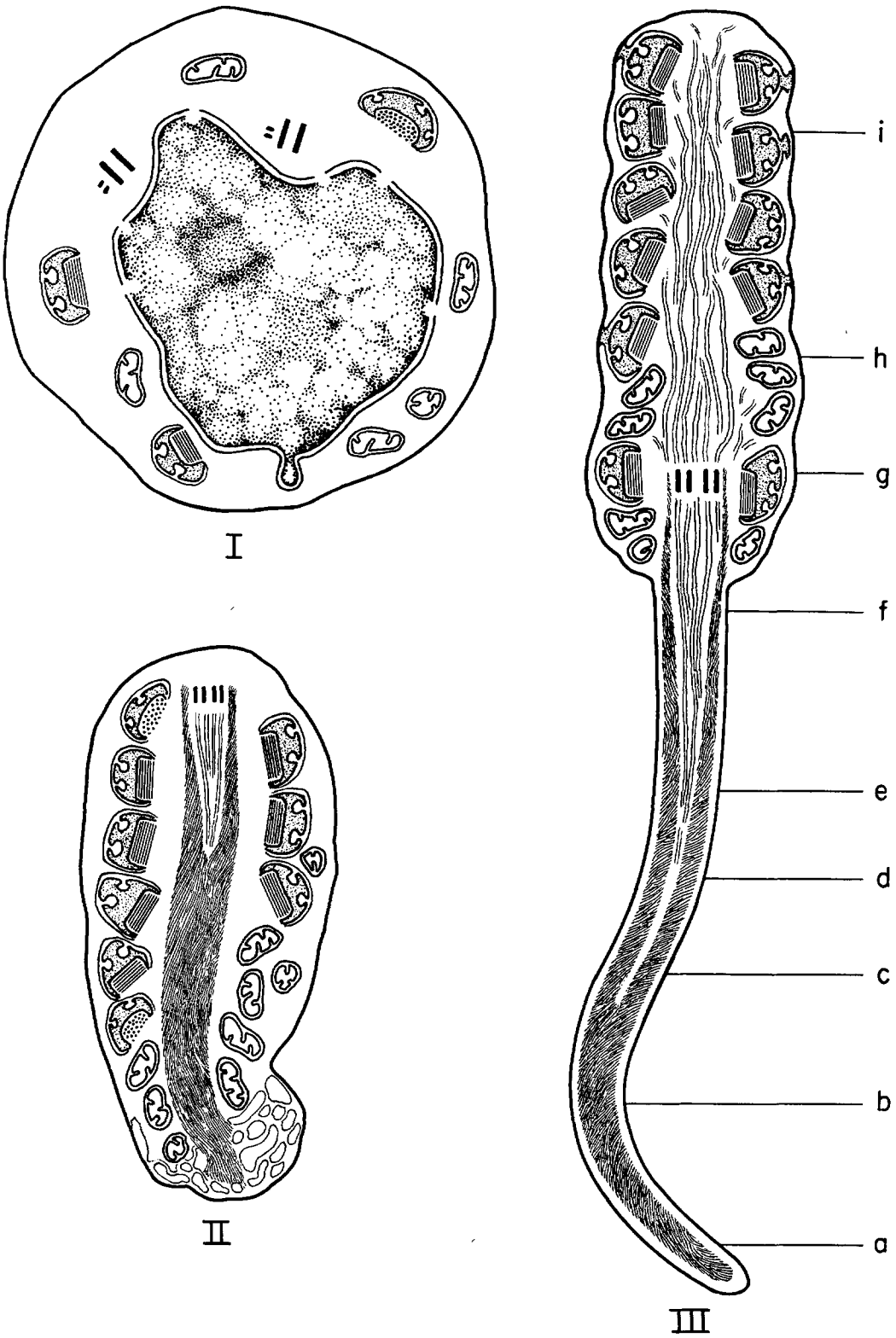

FIGURE 20 Schematic diagram showing the development of a spermatid into mature sperm of *Nippostrongylus*. I. Diagram of spermatid based on Figs. 7 to 10. II. Diagram of developing sperm, cf. Fig. 14. III. Diagram of mature sperm; *a* to *i* represent the approximate regions of the cross-sections in Fig. 19, *a* to *i*.

wide, is visible outside the nuclear envelope (Fig. 9). In some sections, two centrioles are visible, situated 1 or 2 μ apart (Fig. 10). In the close proximity of each centriole, a small satellite body is sometimes apparent.

As the development of the spermatid proceeds, the nuclear material becomes more filamentous in appearance, with a longitudinal orientation (Figs. 11 to 14). In the later stages, the bundle of filaments becomes twisted into a spiral. At higher magnification, the filaments can be resolved as being about 25 mμ in thickness (Fig. 17). The nuclear envelope is no longer visible, and the two centrioles come to lie within a cone-shaped indentation at the base of the nucleus. This indentation also contains a dense granular material, and a series of microtubules, which extend past the centrioles into the cytoplasmic portion of the spermatid (Figs. 15 to 17). A cross-section through the nearly mature sperm at the base of the nucleus (Fig. 16) shows that the centrioles contain a low density core surrounded by a dense cylinder with a wall thickness of about 180 A. Outside this layer is a circle of 18 short rods, each about 200 A in diameter and 1600 A long. Alternate rods appear slightly larger in diameter, and more distinct. The centrioles are encircled by material of medium density, containing a few scattered microtubules and ending in a dense double margin, possibly an extension of the nucleus. Each pericentriolar region is about 340 mμ in diameter. These two pericentriolar areas are together bordered by a circle of about 36 microtubules.

As the development proceeds further, the nucleus elongates, comes to lie at one end of the cell, and later evaginates to form a separate head structure, surrounded by a thin, enveloping cytoplasmic layer. The nucleus is a solid structure at the tip and takes a tubular shape towards the main body of the sperm (Figs. 17 and 18). During this process, the mitochondria and the other cytoplasmic components remain in the cytoplasmic portion of the sperm cell. There is a small dense region, possibly an area of attachment, between the cell membrane and the mitochondrionlike inclusions. In some micrographs (Figs. 15 and 19), this appears to be a protusion from the organelle. Cross-sections of the cytoplasm, perpendicular to the sperm axis, pass through either three or six mitochondrionlike inclusions, indicating a regular arrangement in staggered groups of three.

A series of micrographs is shown in Fig. 19, showing cross-sections through various regions of the mature sperm. A diagram of sperm morphology, reconstructed from micrographs, is shown in Fig. 20.

DISCUSSION

The lack of studies on nematode spermatogenesis by modern techniques of electron microscopy has made it difficult to provide a generalized structure of a typical nematode sperm. Early light microscope studies have described the nematode sperm as nonflagellate or ameboid, with a large cytoplasmic inclusion, the refractile body, or "acrosome" (23, 25, 35). The only fine structure study of nematode sperm has been made on *Ascaris*, and stages in meiosis and sperminogenesis were described in detail (10). In the present study on *Nippostrongylus*, no structure similar to an acrosome or refractile body was observed. Other sperm in which the acrosome has been thought to be absent are those of certain decapods, some trematodes (23), and some isopods (30). Since acrosomal function has been associated with the penetration of the sperm into the ovum during fertilization (8), it would be interesting to study the process of fertilization in *Nippostrongylus*. During fertilization in *Ascaris* the entire spermatozoan has been described as entering the egg (19, 27). The broad region containing the nucleus enters first and the triangular refractile body is incorporated later into the egg cytoplasm. The refractile body has been considered to be an acrosome by Nath and Singh (25), because of its relation with the Golgi apparatus of the spermatocyte. It is, however, PAS negative, being largely made up of basic protein (26, 27), and enters the egg cytoplasm intact, where it later disappears. It clearly does not take part in the production of acrosomal filaments or in the formation of a fertilization cone. For these reasons, it seems doubtful that the refractile body of *Ascaris* sperm is homologous to the acrosome of flagellated sperm, and *Ascaris* may also be considered as lacking a typical acrosome with the function of the refractile body still obscure, as also suggested by Favard (10). In *Ascaris*, the refractile body or the refringent cone forms by the coalescence of numerous cytoplasmic inclusions called "*granules d'ascaridine*" which were thought to originate from endoplasmic reticulum (10). It is of considerable interest, in relation to the present study, that each of these bodies contains a crystalloid, somewhat resembling that of the mitochon-

drionlike inclusions in *Nippostrongylus*, and also is associated with a membranous portion containing folded cristalike structures. In *Ascaris*, crystalloids or "*bâtonnets*" were interpreted as products of Golgi material and become fused to the *granules d'ascaridine*. As sperm matures, the crystalloids disappear and the membranous components, which Favard considers to be Golgi dictyosomes, line the margin of the refringent cone. The resemblance between the inclusions, which form the refringent cone in *Ascaris*, and the mitochondrionlike bodies in *Nippostrongylus* seems close enough to suggest that the dense material in the one species is homologous with that in the other, but is not coalesced into a central mass in *Nippostrongylus*.

In the present paper, we have used the term "mitochondrionlike inclusion" for the dense, specialized structures described here, because they are superficially similar to mitochondria and stain with Janus green, and because mitochondria in other sperm frequently become modified to possess dense inclusions and crystalloid components (2). We have not, however, been able to determine the initial origin of the mitochondrionlike inclusions. If they indeed arise from normal mitochondria, then *Nippostrongylus* is an unusual case in which certain mitochondria undergo a marked modification whereas others in the same cell do not. However, if they arise from Golgi material in *Ascaris*, as suggested by Nath and Singh (25), or partly from Golgi material and partly from endoplasmic reticulum, as shown by Favard (10), these inclusions must be considered products of Golgi material and endoplasmic reticulum of unusual complexity. Also, the number of typical mitochondria in mature sperm of *Nippostrongylus* is markedly reduced, so that one must conclude either that the sperm have little need for mitochondrial enzymes, or that this function is taken over by the dense inclusions. Mitochondria are almost universally present in the sperm of different animals, possibly associated with the flagellar processes in the supply of energy. However, mitochondria have been reported to be entirely absent in some isopod sperm (30) and dragonfly sperm (24).

Although no motility of the sperm was ever observed, this cannot rule out the possibility of some active movement within the uterus. If there is any motility, it could be either ameboid or oscillatory, like flagellar movement. The ameboid movement could be achieved in *N. brasiliensis* only if the broad tail region moves in an ameboid fashion, either pushing the elongated end forward or dragging it behind. Ameboid motion has been described for the sperm of *Ascaris* (25). Oscillatory movement would seem to require a flagellum, although a few motile spermatozoa are known which do not have the 9 + 2 filamentous structure. In some tick spermatozoa, the motile structure is made up of 200 to 250 fibrillar units of 60 to 90 A in diameter (29). Christensen (7) also described the presence of similar tubules in the flatworm, *Plagiostomum*, which lacks a flagellum; he regarded these as structures aiding in sperm motion. In the case of some nematodes, sperm act only in activation of cleavage, with the egg developing parthenogenetically (22). This possibility seems improbable for *Nippostrongylus*, since mature sperm are usually found in the uterus of the female.

Although the sperm of *N. brasiliensis* lack a flagellum, they have a pair of centrioles arranged parallel to each other, each composed of eighteen short rods and associated with several microtubules which extend into the broad cytoplasmic region. In *Ascaris*, on the other hand, the spermatocytes have a pair of centrioles arranged at right angles to one another, but no centriole was found in the mature sperm (10). Microtubules also are of common occurrence in flagellate sperm. In the firebrat insect, microtubules were found around the nucleus of the spermatozoa (3). Microtubules have been reported in spermatozoa of flatworms (31, 32), in which these structures are very well developed beneath the plasma membrane. A "manchette" of microtubules is also present in the tail of cat sperm, but it is, however, lost before the sperm is released from the seminiferous tubule (5). In these cases a structural role for them has been suggested. The microtubules in the sperm of *N. brasiliensis* are not situated under the plasma membrane, but they extend from the hollow, tubelike nucleus into the axial cytoplasm. They may help in sperm motility or protoplasmic streaming, possibly in relation to obtaining energy from the mitochondria which are present around the microtubules in the broad region of the sperm; but, like the manchette, they could also be considered as merely "skeletal" in maintaining the body form or for reinforcement of the attachment of head to body.

The nucleus of *N. brasiliensis* has an oriented structure arranged in a spiral fashion with a pitch of about 30°. The head shape is also spiral, possessing approximately one gyre, probably reflecting

the structural orientation of the nuclear contents. In several other invertebrate sperm, the chromatin has been described as composed of filamentous or lamellar material, as in flatworms (32), in grasshopper (9, 13, 37), in crayfish (21), in snail (28), and in sea urchin (33). Some orientation of nuclear material has also been reported in certain vertebrate sperm as well (5, 11). The fine structure of the nucleus of *Ascaris*, however, shows a compact mass of chromatin material (10), and is spherical in shape, quite unlike the elongated and loosely spiraled head structure in *Nippostrongylus*.

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