SPERMATOGENESIS IN ANIMALS AS REVEALED BY ELECTRON MICROSCOPY

I. Formation and Submicroscopic Structure of the Middle-Piece of the Albino Rat*

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By ordinary light microscopy (2, 3, 13) and recently by phase microscopy (1, 14), the course of formation of the middle-piece of sperm cells in various mammals has been observed to proceed as follows: the fine axial fibril of the tail flagellum develops from the centriole; as the spermatid nucleus becomes elongated or ovoid, the axial fibril bends along the peripheral margin of the cell; the mitochondria migrate to the periphery of the cell, arrange themselves along the long axis of the axial fibril, form a string of beads by chain-like association, and further coil themselves around the axial fibril to form a helix.

Recently Watson (24) and Burgos and Fawcett (6) have sectioned mammalian testes, and obtained noteworthy electron micrographs of the transformations undergone by the head and neck portions during spermatogenesis. Yasuzumi and Minamino (27) have studied thin sections of the seminal epithelium cells of the testis of the adult albino rat in the electron microscope, and have provided a general description of spermatogenesis.

The present paper presents new information derived from electron microscopic studies of sections of adult albino rat testis and epididymis concerning the existence of two sets of axial fibrils in the tail flagellum. Another problem which has been studied and is here discussed is the mechanism by which the mitochondria are disposed around the developing axial fibrils of the tail flagellum to form the mitochondrial sheath.

Experimental

Blocks 0.5 to 1 mm. in thickness of fresh testis and epididymis of an adult albino rat were fixed in buffered osmium tetroxide according to the method of Palade (16). After 2 hours of fixation, all tissues were washed briefly in distilled water, dehydrated in increasing concentra-

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tions of ethyl alcohol, and embedded in *n*-butyl methacrylate according to the procedure of Newman, Borysko, and Swerdlow (15). A Shimadzu microtome with a glass knife (11), and a Nippon microtome with an Eversharp razor blade were used for cutting sections. These were then examined in a Siemens electron microscope model ÜM-100, or an electron microscope of the Japan Electron Optics Laboratory Company model JEM-T4, or a Shimadzu electron microscope model SM-1. The embedding medium was not removed. Selected fields were photographed at initial magnifications of 7,000 to 13,000 and enlarged optically as desired.

OBSERVATIONS

The observations on the metamorphosis of the middle-piece will be described from electron micrographs. After the formation of the acrosome and head cap, the caudal sheath makes its appearance in the cytoplasm around the caudal pole of the nucleus. In a slightly oblique, longitudinal section the caudal sheath shows an elongated profile with the opening directed posteriorly (Fig. 1). The mitochondria are located in the peripheral area of the cytoplasm, not inside the caudal sheath. Their profiles are oval in shape. The cristae appear to have various shapes such as round (M_1), crescentic (M_2), or triangular (M_3), and seem to be disposed either crossing (M_4), or parallel (M_5) to one another. Occasionally the mitochondria appear "empty" (M_6). The limiting membrane of such mitochondria appears to be thicker than that of mitochondria provided with numerous cristae. As the metamorphosis of the spermatid nucleus progresses, a primitive tail flagellum appears in the postnuclear region, which is composed of a pair of filaments in the center and nine filaments in a circle 0.3 μ in diameter. The primitive flagellum is enclosed by a membrane sheath (Fig. 1).

Fig. 2 represents a small field in the cytoplasm of an elongated spermatid, in which the mitochondria appear clustered around the axial fibrils of the developing tail flagellum. Each mitochondrion is enclosed by a thick limiting membrane about 30 mμ in thickness. In Fig. 3, the plane of section happens to approximate the axis of a developing middle-piece. The mitochondria are not yet arranged in such regular order as in the more advanced spermatid. In this stage the cristae appear to be variously disposed; they cross each other (M_1), form rings (M_2), or curve along the limiting membrane (M_3, M_4). Only one mitochondrion (M_5) contains cristae that traverse it from side to side. The mean mitochondrial diameter is about 0.3 to 0.5 μ, which measurement is smaller than that for mitochondria found in several other cells (17, 18, 21–23).

The tail flagellum develops in the postnuclear region, as seen in the transverse section of the elongated spermatid (Fig. 4). With the differentiation of the tail flagellum the spermatid elongates, and only a little space is left between the tail flagellum and the cell membrane (Figs. 5 to 7). In sufficiently thin, transverse sections of late stages in this development, four or five mitochondria are seen closely surrounding the bundle of axial fibrils (Figs. 4 and 6). In a more advanced stage, the bundle of the axial fibrils is enclosed by one

or two mitochondria which are markedly transformed and assume a horseshoe shape (Fig. 7).

In a cross-section of the middle-piece of the mature sperm, no typical mitochondria are visible, but two membranous structures can be seen separated by a space about 0.13 to 0.2 μ in width (Fig. 8). The axial fibrous components are clearly visible in cross-sections. In the center, nine filaments 30 mμ in diameter with a low density are arranged around a pair of central filaments. These filaments are surrounded by nine fibrils of much greater density and special morphology; their cross-section has the shape of a daisy petal.

The structure of various portions of the middle-piece sheath in relation to the axial fibrils can be conveniently followed in slightly oblique longitudinal sections. Figs. 9 and 10 illustrate the appearance of the axial fibrils and their sheath in such sections. It is clear from longitudinal sections that the axial fibrils are continuous in the middle-piece and main-piece, as is to be expected.

When the mitochondrial sheath is cut longitudinally it looks like a series of alveoli of various shapes and sizes. Each alveolus has an osmiophilic covering approximately 100 A in thickness and a central space occupied by a material of lesser density. In tangential and oblique sections the mitochondrial sheath gives the appearance of a banded structure. The thin cell membrane 70 A is distinctly visible, being closely applied to the mitochondrial sheath (Figs. 9 and 10). In longitudinal sections of a mature sperm the central filaments and the dense peripheral fibrils can be seen running parallel through the middle-piece and main-piece. The sheath of the main-piece is composed of a layer of dense material with numerous short interruptions (Fig. 10).

DISCUSSION

Inside the caudal sheath of the late spermatid is found the intracellular portion of the tail flagellum. The number, size, and arrangement of the tail filaments are the same as reported in the cat spermatid (6). These filaments form a central set of axial fibrils. In the tail flagellum of the mature sperm, an additional, peripheral set of filaments is found which is composed of nine dense fibrils with an asymmetrical, wedge-like profile in cross-section.

The arrangement of axial fibrils in the mature sperm tail flagellum is therefore quite different from that found in kinocilia encountered elsewhere in the animal kingdom (7, 19, 28). The tail flagellum has two sets of fibrils, a central set and a peripheral one. In the central set, nine filaments with a low density are arranged around a pair of filaments. The peripheral set is composed of nine fibrils of much greater density and special morphology.

Kinocilia lash to and fro in the same plane operating on the principle of the paddle (8, 10), while sperm tail flagella have a helical undulating motion which functions in the manner of a propeller or screw (5, 12). The quite different movements of kinocilia and tail flagella seem due to the structural difference not only in the sheath, but in the axial fibrils.

The details of development of the main-piece in the spermatid are being studied further and will be reported in the near future.

In spermatogonia and Sertoli's cells the mitochondria are scattered in the cytoplasm and their cristae lie parallel to one another at more or less regular intervals, as indicated in a previous study (27). In the young spermatids of the rat (24, 27), they are generally seen in the peripheral area of the cytoplasm, whereas in the same cells and at a comparable stage in mouse testis they are scattered throughout the cytoplasm (26). The mitochondria found in the developing spermatids of the albino rat are similar in structure and size to those present in rat spermatocytes (18). In both cases the arrangement of the cristae differs from that encountered in spermatogonia in that the mitochondria are no longer filled with regularly arranged lamellae or ridges but contain a highly irregular array of cristae. The present investigation shows that a number of changes occur in the organization of mitochondria during spermatogenesis as indicated by the following observations: (*a*) the lamellar internal structure is very different in spermatogonia and spermatids, in spite of the fact that these cells are present in the same specimen and are prepared in exactly the same manner; (*b*) the limiting membrane appears thick when the mitochondrion does not clearly show well arranged cristae; (*c*) in the middle-piece of mature sperms, the helical sheath originating from the mitochondria is devoid of cristae.

The mitochondria are disposed around the developing axial fibrils of the sperm tail flagellum, as has been observed by means of the light microscope (2, 3, 5) and recently by means of the phase microscope (1, 14). In a review of mammalian spermatogenesis, Gresson (9) has emphasized that there is no general agreement as to the nature of the mitochondrial sheath of the middle-piece. The points at issue seem to be whether it is helical in structure and whether the mitochondria form a discontinuous or a continuous band. Some electron microscopists have claimed a helical structure (4, 20, 25) in studies of smear preparation subjected to various treatments. The observations reported in the present study suggest that the mitochondria coalesce in a horseshoe and then in ring structures (Figs. 5 to 8) during the formation of the sheath which apparently assumes a helical, canalicular configuration at maturity.

In the late, elongated spermatid, the Golgi complex can be seen in the region of the cell opposite the developing axial fibril. However, the function of such a complex and its role in this stage are not clear in the present study.

SUMMARY

The submicroscopic structure of the middle-piece and its formation has been studied in the electron microscope in thin sections of seminal epithelia and mature sperms of an adult albino rat. The mitochondrial sheath, laid down during metamorphosis, has a helical configuration. It has a central canal

0.13 to 0.2 μ in width. This sheath is enclosed by a thin membrane $\sim$ 70 A thick which is continuous with the cell membrane. The axial fibrous components of the tail flagellum consist of two sets: the central set is composed of nine filaments 300 A in diameter, with a relatively low density and surrounding a pair of filaments; the peripheral set comprises nine fibrils of much greater density with an asymmetrical wedge-like profile in cross-section.

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BIBLIOGRAPHY

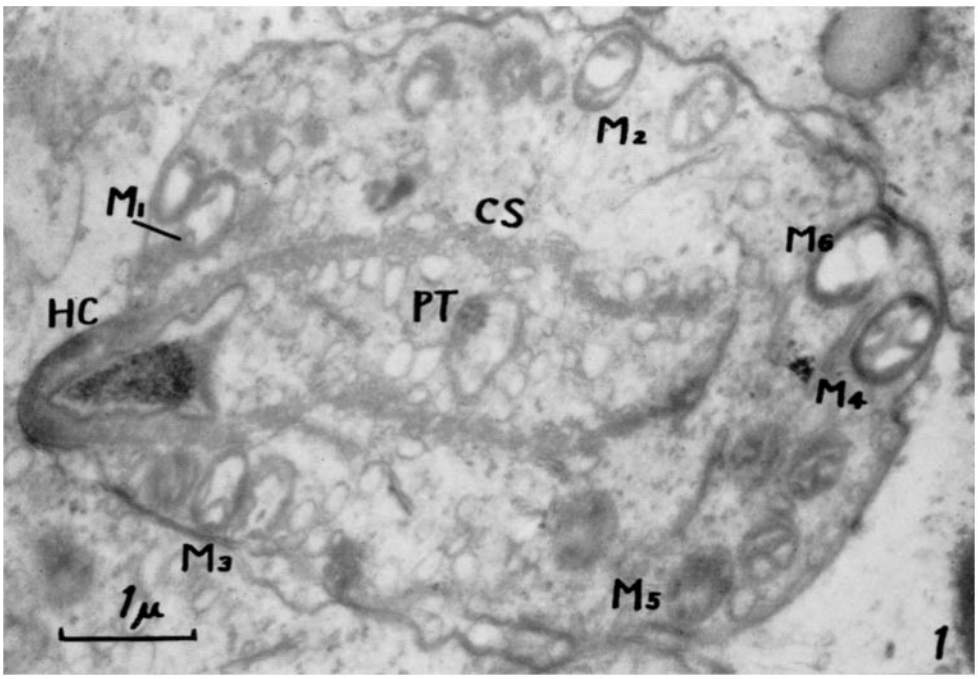
1. Austin, C. R., and Sapsford, C. S., *J. Roy. Micr. Soc.*, 1951, **71,** 397.
2. Bell, L. G. E., *Quart. J. Micr. Sc.*, 1953, **94,** 37.
3. Benda, C., *Arch. mikr. Anat. u. Entwcklngsmechn.*, 1887, **30,** 49.
4. Bretschneider, L. H., *Proc. K. Nederl. Akad. Wetensch.*, 1949, **52,** 301.
5. Brown, H. P., *Ohio J. Sc.*, 1945, **45,** 247.
6. Burgos, M. H., and Fawcett, D. W., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 287.
7. Fawcett, D. W., and Porter, K. R., *J. Morphol.*, 1954, **94,** 221.
8. Gray, J., Ciliary Movement, New York, The Macmillan Company, 1928, 162.
9. Gresson, R. A. R., *Cellule*, 1951, **54,** 81.
10. Krijgsman, B. J., *Arch. f. Protistenk.*, 1925, **52,** 478.
11. Latta, H., and Hartmann, F., *Proc. Soc. Exp. Biol. and Med.*, 1950, **74,** 436.
12. Lowndes, A. G., *Proc. Zool. Soc. London, Series A*, 1941, **111,** 111.
13. McGregor, J. H., *J. Morphol.*, 1899, **15,** suppl., 57.
14. Nakakishi, Y., *Japan. J. Genet.*, 1955, **30,** 128.
15. Newman, S. B., Borysko, E., and Swerdlow, M., *J. Research Nat. Bureau Standards*, 1949, **43,** 183.
16. Palade, G. E., *J. Exp. Med.*, 1952, **95,** 285.
17. Palade, G. E., *Anat. Rec.*, 1952, **114,** 427.
18. Palade, G. E., *J. Histochem. and Cytochem.*, 1953, **1,** 188.
19. Potts, B. P., and Tomlin, S. G., *Biochim. et Biophysica Acta*, 1955, **16,** 66.
20. Randall, J. T., and Friedlaender, M. H. G., *Exp. Cell Research*, 1950, **1,** 1.
21. Rhodin, J., Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney, Karolinska Institutet, Stockholm, Aktiebolaget Godvil, 1954.
22. Sjöstrand, F. S., *Nature*, 1953, **171,** 30.
23. Sjöstrand, F. S., and Rhodin, J., *Exp. Cell Research*, 1953, **4,** 426.
24. Watson, M. V., *Biochim. et Biophysica Acta*, 1952, **8,** 369.
25. Yasuzumi, G., *Electronmicroscopy*, 1950, **1,** 25.
26. Yasuzumi, G., unpublished data.
27. Yasuzumi, G., and Minamino, T., International Congress of Electron Microscopy London, 1954, in press.
28. Yasuzumi, G., and Wakisaka, I., *Cytologia*, Tokyo, in press.

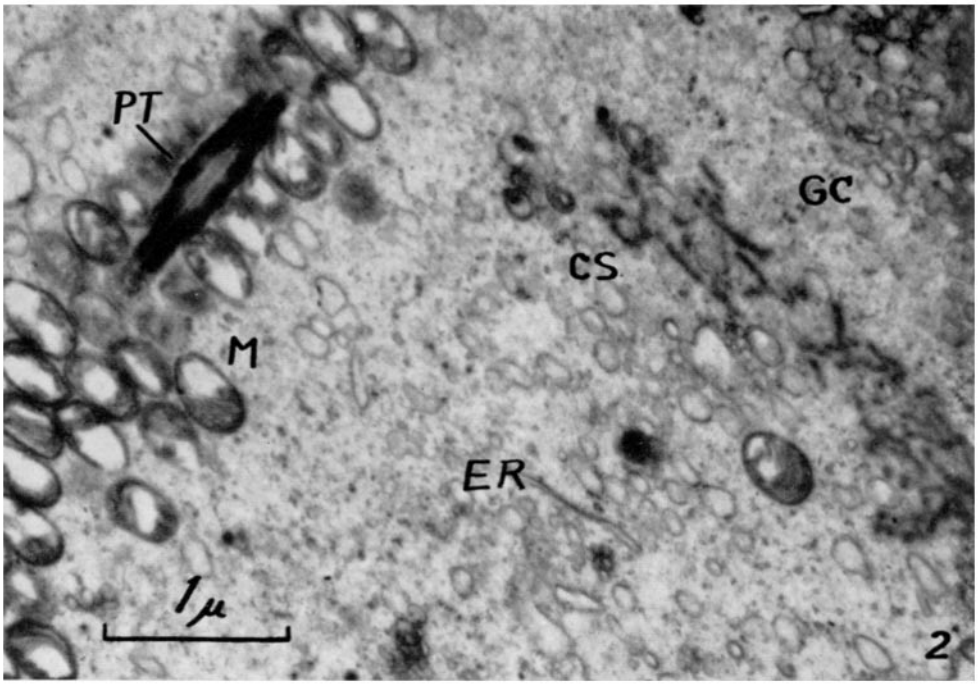
EXPLANATION OF PLATES

PLATE 120

FIG. 1. A section through a spermatid in which the caudal sheath (*CS*) and the primitive tail filaments (*PT*) are developing. The compact nucleus shifts towards the peripheral part of the cell and finally part of the nucleus is projected outside the cytoplasm. The space between the dense material of the nucleus and the internal layer of the nuclear membrane is of irregular width. The nucleus is enclosed by the head cap (*HC*). The mitochondria (M_1–M_6) are situated in the peripheral region of the cytoplasm in this stage. The cristae mitochondriales are arranged irregularly. The micrograph was taken with a ÜM-100 model at 7,000 diameters and enlarged optically to 17,000.

FIG. 2. A section through a spermatid at the level of the developing middle-piece. The oblique section of the axial filaments can be seen. The mitochondria (*M*) are piled together, showing oval shaped profiles. In the right upper corner, the Golgi complex (*GC*) consists of an aggregation of small vacuoles. At the right upper part of the figure, a group of filaments with higher density is visible, suggesting the so called caudal sheath (*CS*). The cross- and oblique sections of the endoplasmic reticulum (*ER*) and microsomes are clearly visible. The micrograph was taken with a ÜM-100 model at 7,000 diameters and enlarged optically to 25,000.



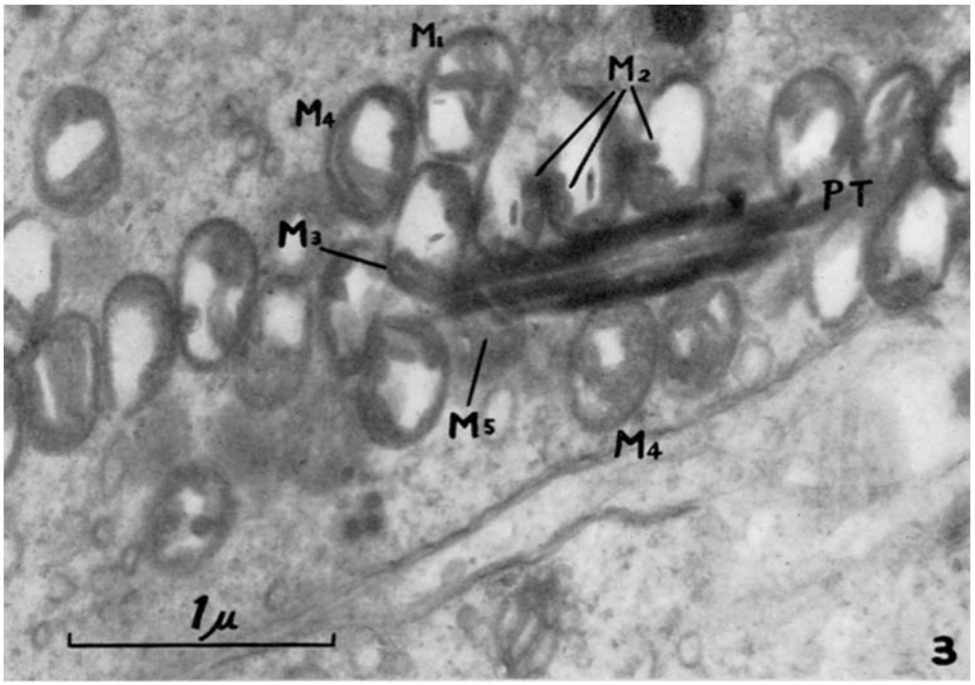


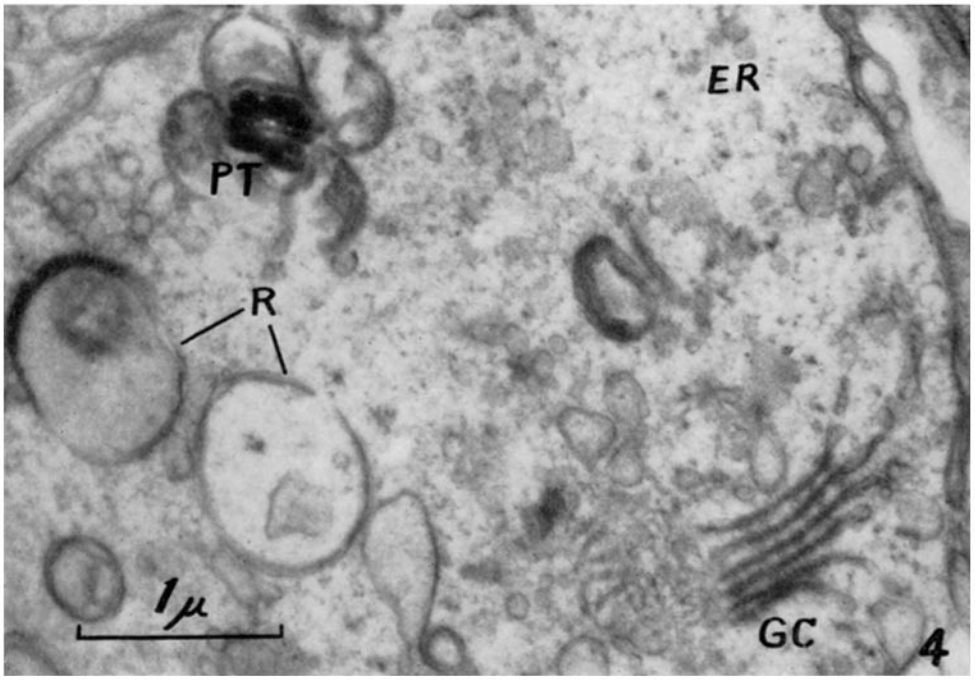
(Yasuzumi: Spermatogenesis revealed by electron microscopy. I)

PLATE 121

FIG. 3. The mitochondria are situated close to the axial fibrils of the primitive tail flagellum (*PT*). The mitochondria are no longer filled with regularly arranged cristae. They seem instead to be adherent to the wall of mitochondria (M_1–M_5). The limiting membrane of each mitochondrion is well preserved. The micrograph was taken with a ÜM-100 model at 13,000 diameters and enlarged optically to 35,000.

FIG. 4. Transverse section through the postnuclear region of an advanced spermatid, showing a cross-section of the primitive tail flagellum (*PT*) surrounded by at least four mitochondria in the peripheral portion of the cell. The Golgi complex (*GC*) is clearly visible in the region opposite the primitive tail flagellum (*PT*). The Golgi complex (*GC*) consists of membranes arranged parallelly in six layers and of small vacuoles. In the left lower portion can be seen large, complete or incomplete, ring-shaped profiles (*R*) from 0.8 to 1.0 μ in diameter. The ring figures are surrounded by double membranes about 0.54 μ thick. Within each ring figure is a cloud of less opaque material. Numerous vesicular elements belonging to the endoplasmic reticulum (*ER*) are scattered in the cytoplasm. The micrograph was taken with a JEM-T4 model at 7,000 diameters and enlarged optically to 37,000.





(Yasuzumi: Spermatogenesis revealed by electron microscopy. I)

PLATE 122

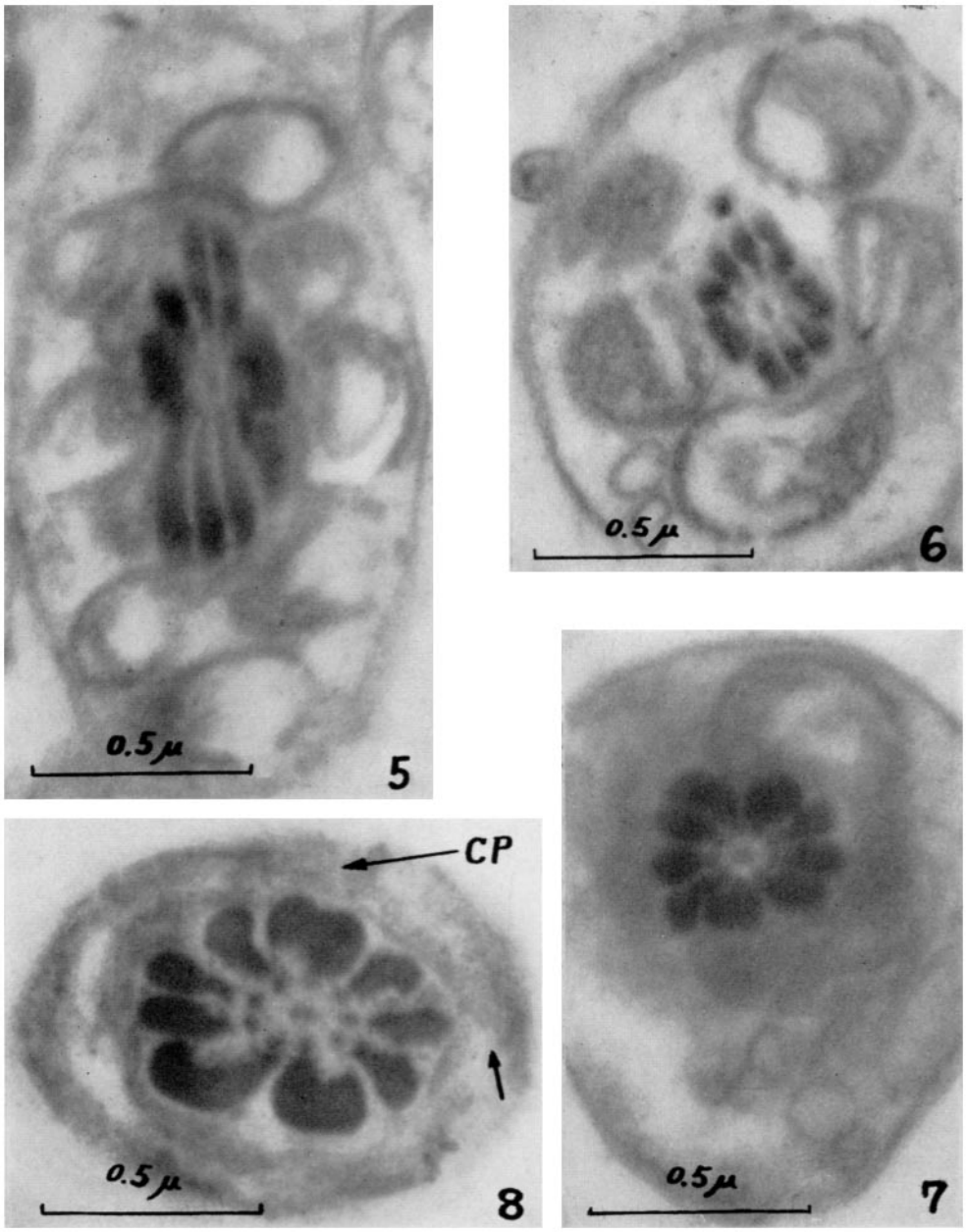
FIGS. 5 to 7. Electron micrographs of oblique and cross-sections through the developing tail flagellum in the late, elongated spermatids. They illustrate the close association between the mitochondria and the peripheral axial fibrils. The micrographs were taken with a JEM-T4 model at 6,000 diameters and enlarged optically to 60,000.

FIG. 5. An oblique section through the developing tail flagellum. The axial fibrils are surrounded by a large number of mitochondria.

FIG. 6. The peripheral axial fibrils are surrounded by five mitochondrial profiles which possess only traces of cristae mitochondriales, but the limiting membrane is clearly visible.

FIG. 7. The peripheral axial fibrils are closely surrounded by a mitochondrial profile shaped like a horseshoe.

FIG. 8. A cross-section through the middle-piece shows nine filaments about 30 $m\mu$ in diameter arranged around a pair of filaments and surrounded by nine dense fibrils with an asymmetrical, wedge-like profile in cross-section. The sheath is apparently formed by double membranes which leave a space between them. At the point marked *CP* there is a suggestion of a crossing point in the canalicular helix. The arrows point to a curved region of the canalicular helix. The thin superficial membrane is hardly visible, being in close contact with the outside of the helical sheath. The micrograph was taken with an SM-1 model at 6,000 diameters and enlarged optically to 60,000.

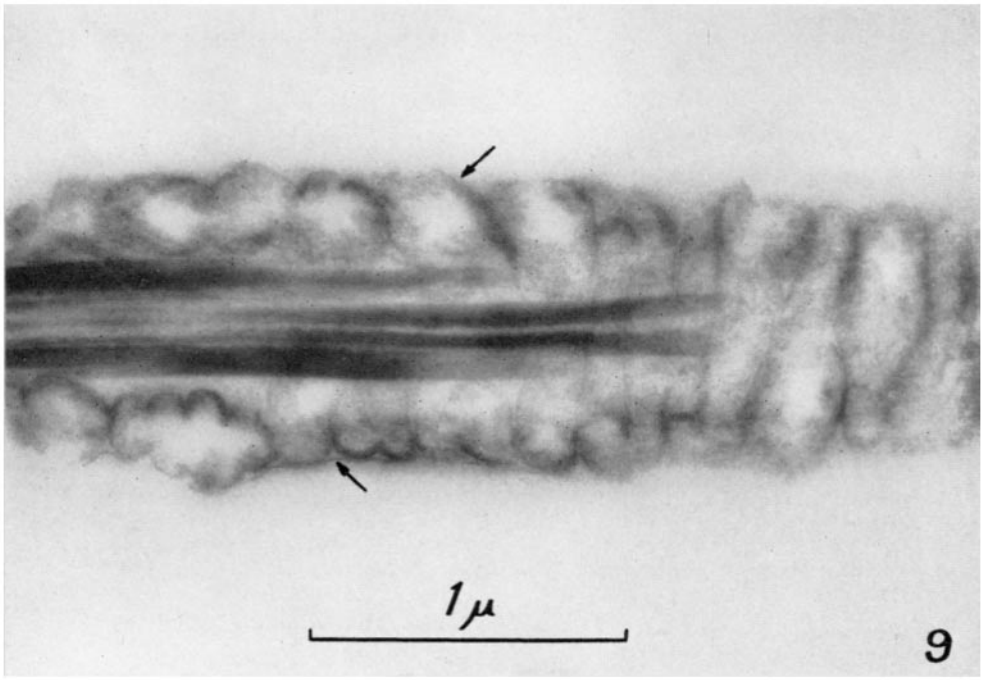


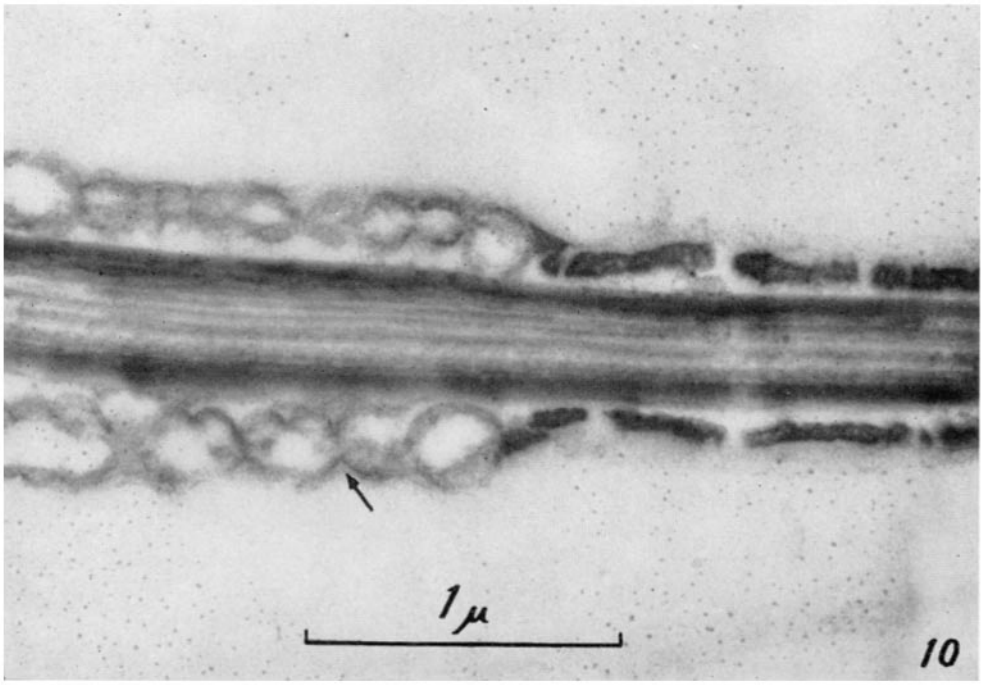
(Yasuzumi: Spermatogenesis revealed by electron microscopy. I)

PLATE 123

FIG. 9. Electron micrograph of a slightly oblique, longitudinal section through the middle-piece in the mature sperm. A few thin filaments of the central set and four peripheral dense fibrils run parallel along the longitudinal axis of the middle-piece. Because of the obliquity of the section the right end of the sheath appears as a continuous banded structure, though its actual form is that of a canalicular helix. The superficial membrane is visible at the points marked by the arrows. The micrograph was taken with a JEM-T4 model at 6,000 diameters and enlarged optically to 42,000.

FIG. 10. A longitudinal section through the middle-piece and main-piece of a mature sperm. Central filaments and dense peripheral axial fibrils run parallel through the middle-piece and main-piece. The mitochondrial sheath is composed of a series of cavities of various shapes and sizes. The sheath of the main-piece 0.06 to 0.1 μ in thickness shows discontinuities at short distances because the sheath is formed by a tape-like helix. The arrow points out the superficial membrane. The micrograph was taken with a JEM-T4 model at 6,000 diameters and enlarged optically to 42,000.





(Yasuzumi: Spermatogenesis revealed by electron microscopy. I)