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An Electron Microscope Study of Spermiogenesis in the Japanese Eel, *Anguilla japonica**

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Abstract

After injecting Synahorin, the fine structure of spermatid differentiation in the Japanese eel, *Anguilla japonica* is studied. In telophase of the last maturation division the chromatin material condenses to form the nucleus, followed by formation of nuclear envelope. The nucleus rapidly grows in size, and compared to early spermatid it increases about 28.9% in surface area by late spermatid stage, with the simultaneous transformation from oval to sickle shape. The nucleus of early and middle stage spermatid generally shows the presence of one wall-less vacuole, whereas late spermatid has more than one. The only mitochondrion, which is big and rounded, gradually moves down and encircles the flagellum to form middlepiece. Sperm head is devoid of any acrosome, and the tail shows 9+0 pattern.

In comparison to spermatogenesis and spermiogenesis more literature is available dealing with the ultrastructure of spermatozoa of teleost fishes. For instance, sperm of salmon[1] and carp[2] has already been investigated. Reports on the fine structure of the sperms of *Jenynsia ineata*,[3] goldfish,[4] *Pantodon buchholzi*[5] are also available. Recently the authors have reported ultrastructure of Japanese eel spermatozoon.[6] So far, ultramicroscopic observations have only been made upon spermatogenesis of guppy[7–9] and a scorpaenous fish.[10]

The information concerning the spermiogenesis is more scanty, and the phenomenon has been investigated in a few fish including *Porichthys notatus*,[11] *Oligocottus maculosus*[12] and *Squalus suckleyi*.[13] Though attempts have been made to gather some informations about spermatogenesis in Japanese eel by light microscopy,[14] any ultramicroscopic study is not yet to appear. In the present study the spermiogenesis of the Japanese eel has been investigated by ultramicroscopic methods, with an aim to clarify the fine morphological changes during the process.

Materials and Methods

Two to three years old eels, brought from Yaizu, were kept in fresh water ponds for 24 hours. Synahorin (Teikoku Hormone Mfg. Co.) of 50 RU in 0.6% NaCl was, then injected to them intramuscularly, after being anesthetized by

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1/5000 Tricaine methanesulphate (MS–222). They were, afterwards transferred to 1/3 sea water, kept there for one week, and one more Synahorin injection was given during this period. During next two weeks, they were kept successively in 1/2 and 3/4 sea water, with two injections of Synahorin per week. The final confinement in sea water lasted five weeks, with only two injections during the first seven days. The water temperature during those courses was kept 16 to 20°C.

Testes were removed after cutting open the viscera. Their pieces of approximately 1 mm^3 were overnight kept in the 2.5% Glutaraldehyde in Millonig's buffer of pH 7.4, at +4°C, followed by fixation in 1% osmium tetroxide in the same buffer for two hours. Occasionally only osmium tetroxide was also used as fixative. After dehydration in the grades of increasing alcohol concentration, the tissue blocks were embedded in Epon 812. About 500 Å sections, cut with the help of glass knives on a Porter-Blum microtome, were placed on naked copper grids, followed by staining with uranyl acetate for 20 minutes and lead citrate for 10 minutes[15]. The sections thus stained were, then, examined by a Hitachi HS–7 electron microscope.

Observations

As shown in Figure 1, during the telophase of the last maturation division of secondary spermatocyte chromatin condenses to a thick shapeless mass, surrounded by incomplete nuclear envelope. In their cytoplasm, which is rather dense are embedded ribosomes and a few small vacuoles. When nuclear envelope is fully formed, the chromatin mass assumes as oval shape. In the nucleus of early spermatid (Fig. 2), nuclear envelope is undulating with a few projections penetrating the cytoplasm. Nuclear pores are very few, congregating at a point where chromatin is lacking. Though, irregularly spaced, the two membranes of nuclear envelopes are altogether very close. Some flat vesicles, and ribosomes are distributed rather sparsely in the cytoplasm. The later usually clustered together to form polysome. The cell at this stage is spherical and measures about 1.60 μ in diameter. The spermatid shown in Figure 3 is nearly of the same stage as that of Figure 2. The very characteristic of early spermatid nucleus is the presence of one wall-less vacuole. The rest of the nucleus is composed of fine granules of high electron density. The average diameter of nucleus at this time measures about 1.10 μ.

Then, spermatids appear to become elongated and oval in shape. The nucleus itself is also elongated than that of the last stage and located at the end of one side of the cell, while in the opposite side of the nucleus there appears a large mitochondrion. Beside the mitochondrion, a few number of ribosomes, endoplasmic reticulum of vacuolar or tubular form are found dispersed in the cytoplasm of the spermatid (Fig. 4). The mitochondrion is in ball-shape and has

tubular cristae arranged irregularly. The matrix of the mitochondrion contains granules of various sizes and appears rather dark (Fig. 5). Its average diameter is 1.13 μ. A little more advanced stage of spermatid, many microtubules are visible around the caudal portion of the nucleus (Fig. 6), particularly concentrating in the region of future middlepiece. As the elongation of nucleus is over, the microtubules disappear. At this stage early spermatid tail also makes its appearance. The tail starts from the caudal portion of the spermatids and expand almost straight. It's length is about 1.96 μ and its diameter 0.08 μ. The flagellum seems to be composed of peripheral fibers enclosed by the plasma membrane.

During middle phase, the nucleus continue to increases in size and takes a sickle-like shape (Fig. 7). The mitochondrion is seen descending towards the basal portion to form future middlepiece. At this stage also there is only one wall-less vacuole, but the undulation of nuclear membranes shows a marked decrease. Structures leading to the formation of acrosome are virtually absent.

In the late stage spermatid, the nucleus almost takes the form of sperm head, and more than one wall-less vacuole are visible in it (Fig. 8). This growth of nucleus brings its sheath so close to the plasma membrane, that it looks to be third membrane (Figs. 8, 11). But at some place a few cytoplasmic vesicles still maintain their presence between the plasma and nuclear membranes. The nucleus diameter now is about 1.41 μ. In the posterior sleeve of cytoplasm surrounding the tail, lies the big mitochondrion (Fig. 9). The thin cytoplasmic layer closely conforms to the external surface of the mitochondrion, and comes down to its posterior side to form tubular sheath. It further extended to the junction of the basal body (distal centriole) and tail. The diameter of mitochondrion, at this stage, is reduced to 0.97 μ. There is no trace of any implantation fossa (Fig. 10).

The tail joins the head consistently at an oblique angle (Figs. 9, 10). As shown in the transverse section, 9+0 axonemal complex is enclosed in the plasma membrane of cell (Fig. 12). The general appearance of flagellar transverse sections is circuler, but the plasma membrane which is closely associated with axonemal doublets, is wavy occasionally, one or two peripheral doublets displace and can be seen taking a bit interior of central position (Fig. 12).

Discussion

The observed fact that in the early spermatid of eel, the nuclear membranes showed clear undulation and chromatin material condensed in the nuclear area, is consistent with the observations on the teleost.[3,9,12] The same is true for the following events, when undulation of nuclear membranes were reduced, the nucleus grew to occupy almost whole of the sperm head during late spermatid stage. But the characteristic feature of present species is the presence of one wall-less vacuole and the absence of nucleoli in the early spermatid, whereas Daled,[16]

Daoust and Clermont[17] in mammals and Grönberg and Telkkä[9] in guppy, reported the presence of more than one nucleolus, which disappeared during elongation phase. The Japanese eel spermatid has also no intercellular bridge between two spermatids as found in *Oligocottus maculosus*.[12]

During elongation of nucleus was marked the appearance of microtubules in its caudal portion, and their extension to the region of future middlepiece. This condition was similar to that of mammals. In both cases, the microtubules disappeared after the elongation was over, but their exact role, in either cases remained unknown.

Usually more than one mitochondrion are reported to unit to form middlepiece in fishes. For instance, in *Oligocottus maculosus* two mitochondria,[12] in goldfish 2–4 mitochondria[4] and in *Pantodon buchholzi*[5] 9 mitochondria have been reported to participate in the formation of the middlepiece. But in Japanese eel spermatid only one mitochondrion is involved. No submitochondrial net, like that of *Jeynsia lineata*[3] was discerned in the mitochondrial sleeve. Further, presence of acrosomal vesicles and acrosome has been reported in lungfish[18] and *Squalus suckleyi*[13], but the absence of these structures is more prevalent condition in the fishes[2][7][4]. Japanese eel spermatid followed this general pattern and was devoid of acrosome.

Though the function of wall-less vacuoles is not yet known, their number increased in the late spermatid. Normal nuclear vacuoles, however, were reported in goldfish[4] and lamprey[19]. The Japanese eel spermatid also deviated from the usual fish structural pattern in lacking implantation fossa. Like lamprey[19] and *Oligocottus maculosus*,[12] no Y-shape condensation of matrix between flagellar membrane and peripheral doublets, was noted in this species. The flagellum of the Japanese eel spermatid resembled that of *Lycodontis afer*[20] in showing 9+0 flagellar pattern. If the central fibers is assumed to add to stiffness of the tail, then its absence may be one reason for the oblique angled juncture of the tail with the head. The other reason may be the pressure created by descent of a single mitochondrion at the time of middlepiece formation.

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Explanation of Figures

PLATE I

Fig. 1. Secondary spermatocyte at telophase. Nuclear membranes are incomplete and cytoplasm contains many ribosomes and a few small vacuoles. ×20,000.

Fig. 2. An early spermatid. The nucleus has enlarged and the chromatin is more densely packed. A few nuclear pores are indicated by arrows. N=Nucleus, R=Ribosome, NM=Nuclear membrane, CV=Cytoplasmic vesicle. ×30,000.

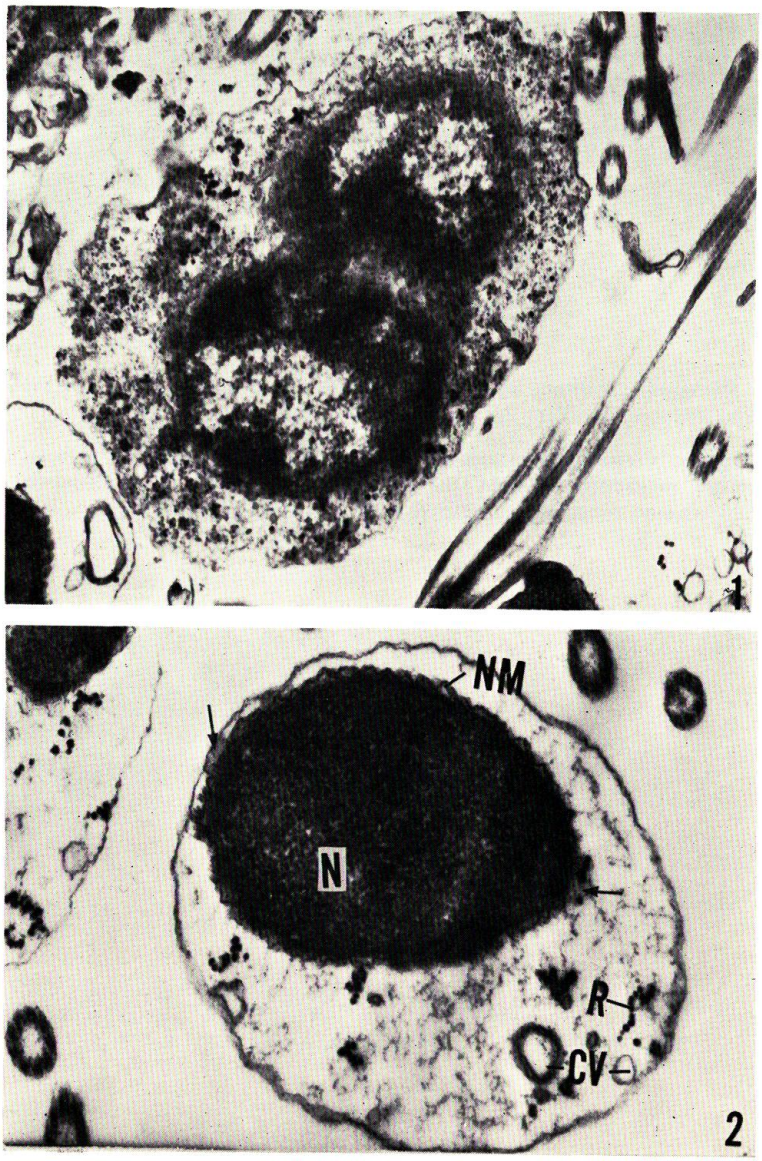

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PLATE II

Fig. 3. Micrograph showing an early spermatid with a nuclear wall-less vacuole. NP= Nuclear pore, V=Vacuole. ×20,000.

Fig. 4. An early spermatid whose nuclear membranes are confluent with smooth membranes extending throught the cytoplasm (Arrows). M=Mitochondrion, ER= Endoplasmic reticulum. ×26,250.

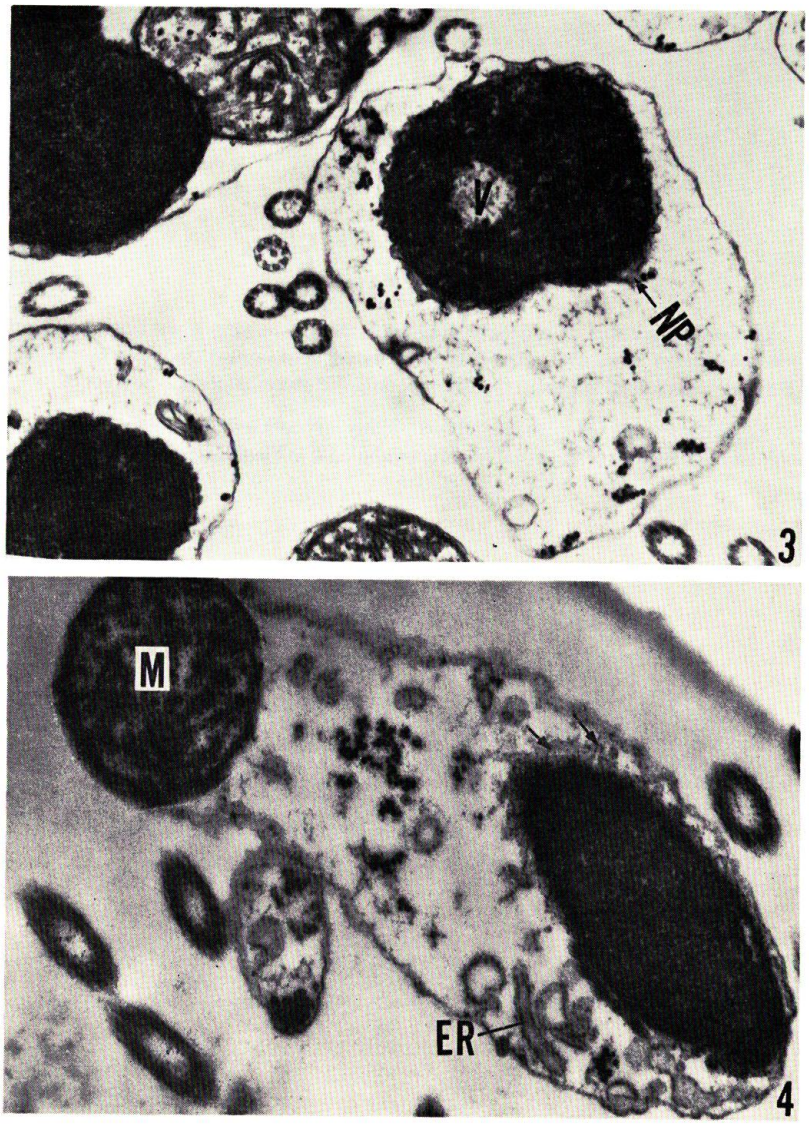

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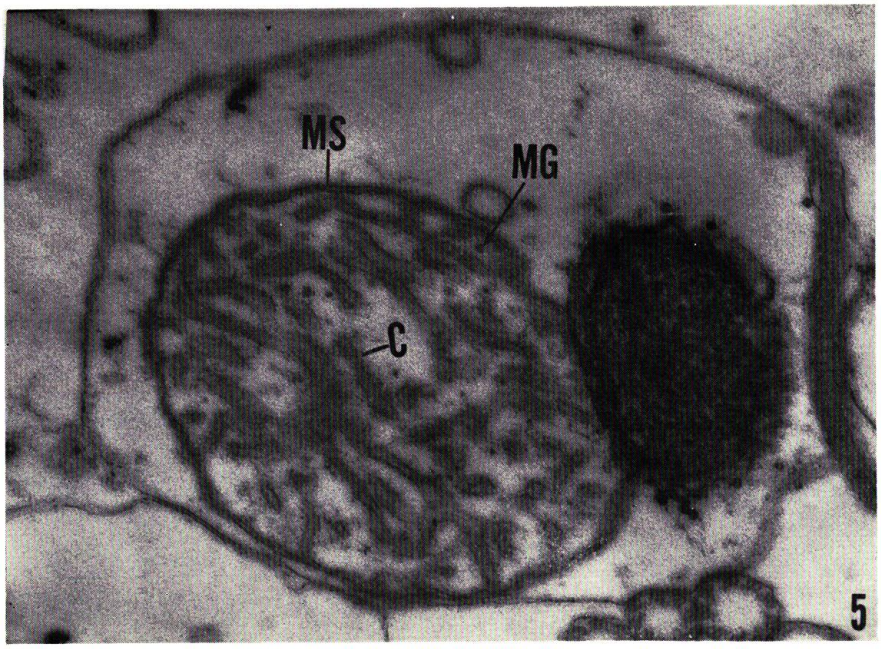
PLATE III

Fig. 5. Transverse section of an early stage spermatid showing very large and ball-shape mitochondrion with many irregularly situated cristae and granules of various sizes. MS=Mitochondrial sheath, C=Cristae, MG=Matrix granule. ×45,000.

Fig. 6. An early spermatid with flagellum. Many cytoplasmic microtubules are visible at the caudal region of cell. MT=Microtubule, F=Flagellum. ×22,500.



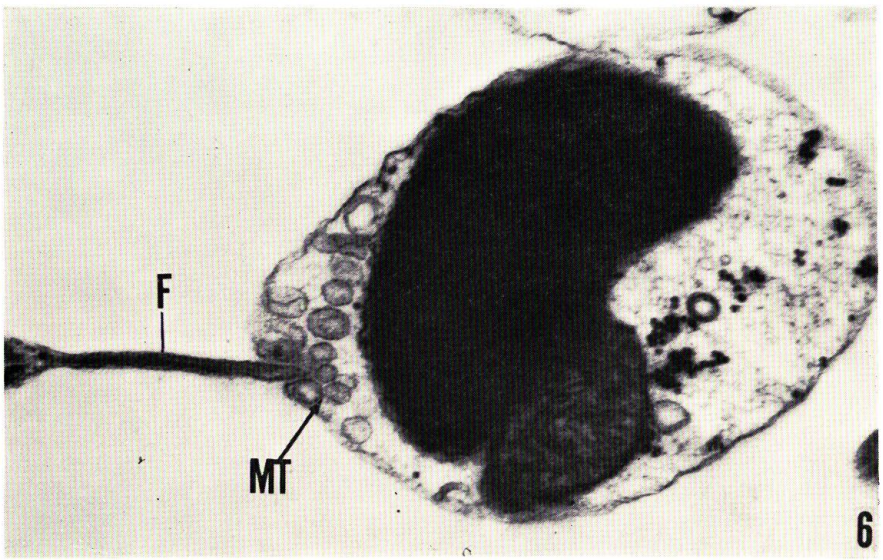


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PLATE IV

Fig. 7. Spermatid in middle phase of spermiogenesis. The nucleus shows almost sickle form. The mitochondrion already comes to basal portion to make future middle-piece. $\times 28{,}000$.

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PLATE V

Fig. 8. Transverse section of late spermatid. It is showing two nuclear wall-less vacuoles. Also some cytoplasmic vesicles are visible between plasma, nuclear membrane and mitochondrion. $\times 42{,}500$.

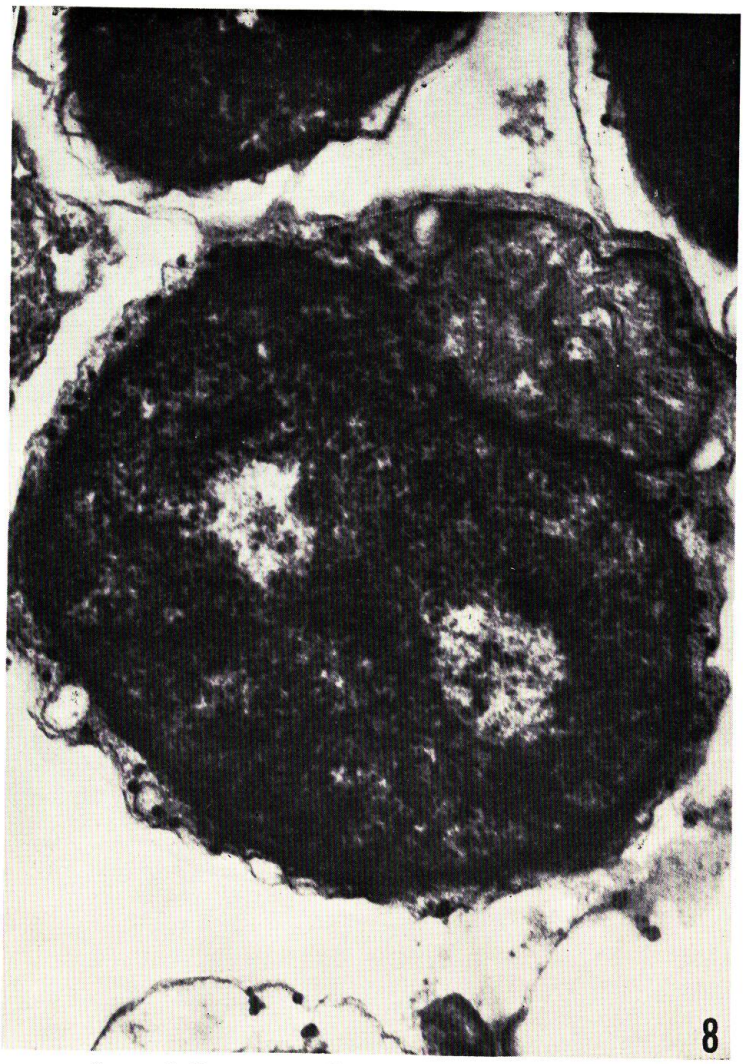

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PLATE VI

Fig. 9. Spermatid of late stage. The only large and ball-shaped mitochondrion is located in the posterior region of the cytoplasm. The flagellum show an oblique angle to the head. BB=Basal body. ×25,000.

Fig. 10. Micrograph showing tail, middlepiece and posterior part of the head. There is no implantation fossa where flagellum enters the posterior part of the head. ×40,000.

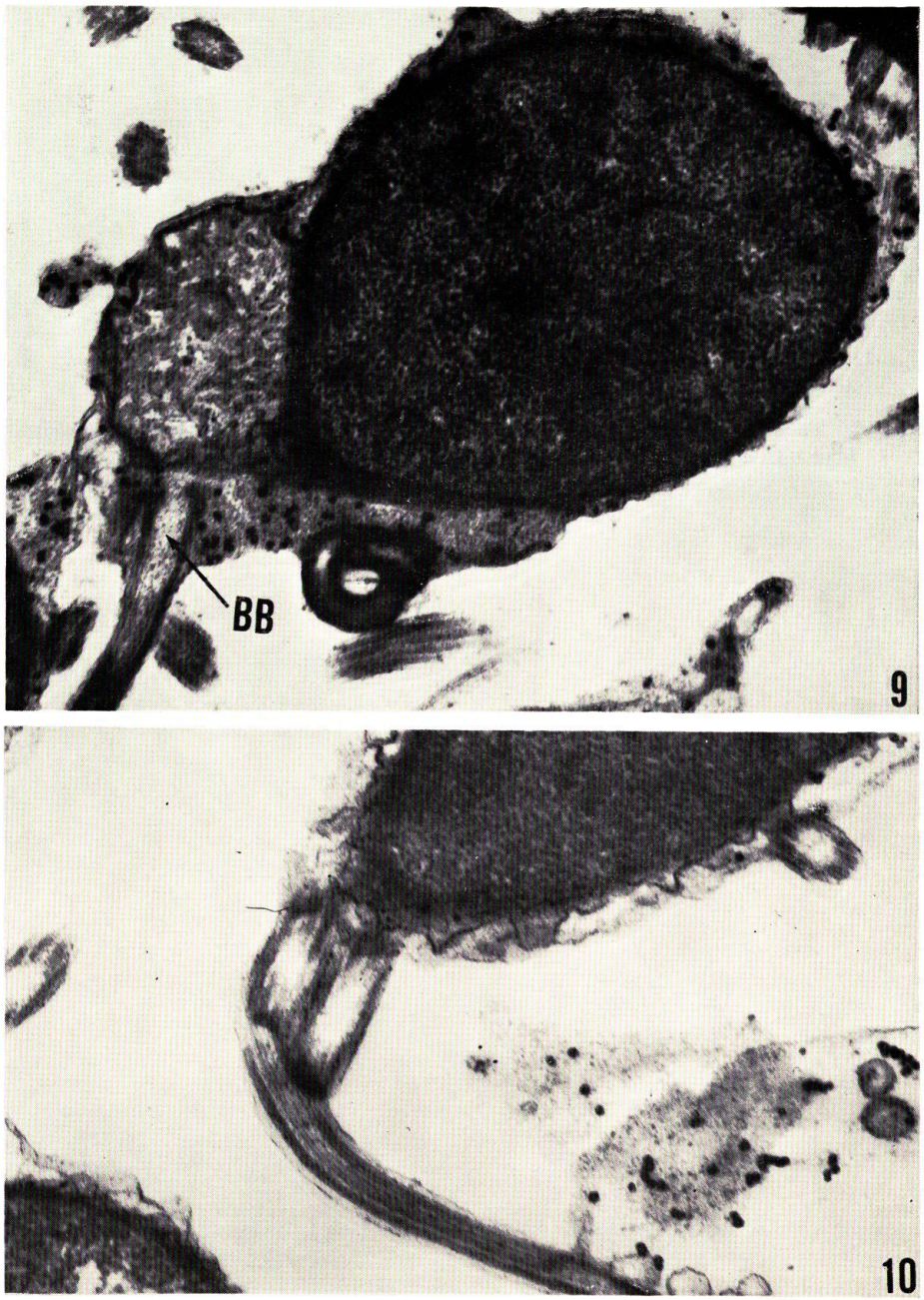

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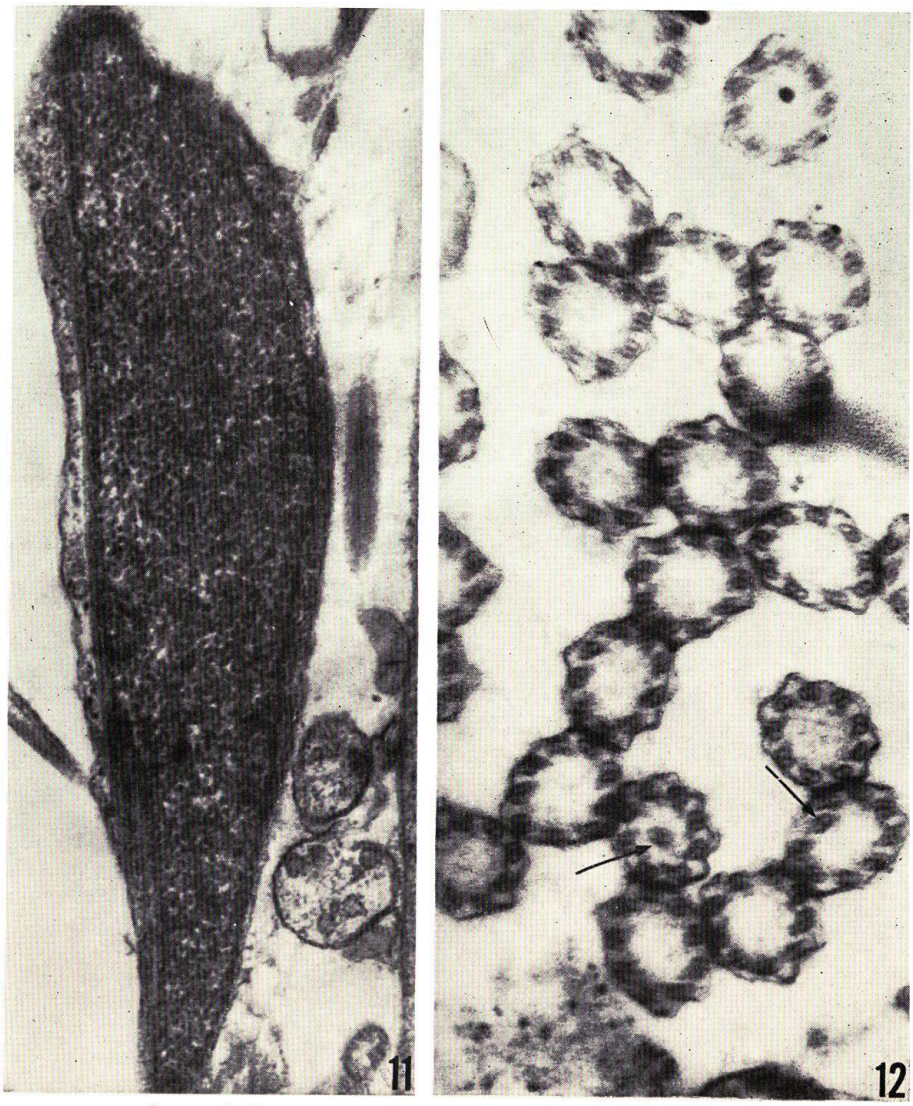
PLATE VII

Fig. 11. Longitudinal section of late spermatid. The head has become sickle form. No acrosome is visible. ×40,000.

Fig. 12. Transverse section of tail, showing nine double peripheral fibers and no central fiber. In a few displaced peripheral fibers are also visible (Arrows). ×67,500.



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