

Spermatogenesis in Animals as Revealed by Electron Microscopy

VIII. Relation between the Nutritive Cells and the Developing Spermatids in a Pond Snail, *Cipangopaludina malleata* Reeve

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PLATES 267 TO 274

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ABSTRACT

This paper deals with spermatogenesis in *Cipangopaludina malleata* Reeve, with special regard to the relation between the nutritive cells and the developing spermatids.

The nutritive cell gives rise to numerous, slender or broad, elongate pseudopodia which extend from its surface toward the seminiferous lumen. They are characteristically provided with rows of circular, oval, and elongate profiles identical in form and position with the profiles of the endoplasmic reticulum. As the elongate pseudopodia increase in number, they become more slender and more closely packed until they coalesce into a continuous sheet circumferentially disposed around the nucleus and the full length of the middle piece of the typical spermatid. Thus the mantle of the typical spermatozoon of the pond snail is formed by a thin fold of the cytoplasm of the nutritive cells. This wrapping appears to contain 16 to 18 elements of the smooth surfaced endoplasmic reticulum, which run parallel and helically (50 to 100 $m\mu$ apart). It is suggested that these constitute a conductor system for nutritional supply from the nutritive cells to the developing typical spermatids. The mantle is assumed to be a transient structure which disappears when the sperms are detached. The atypical spermatids develop while lodged in deep indentations of the surface of the nutritive cells.

INTRODUCTION

A current topic of major cytological interest is the means by which various cell organelles are formed. The Sertoli cell seems particularly appropriate for such an investigation in this regard, because therein can be studied a special development of the endoplasmic reticulum.

The very long history of the Sertoli cells started in 1865, when Sertoli (1) noted the presence of another cell type different from the germ cells in the human seminiferous tubule. They were tall, irregularly columnar cells which extended from the basement membrane to the seminiferous lumen. They have since been demonstrated in various species of vertebrates, and termed the Sertoli cells. It is well known that Merkel (2) designated them supporting cells, and in invertebrates, Gilson (3-5) called them the nutritive cells acting as nutritive agents for the spermatogenic cells. In Maximow

and Bloom's textbook of histology (6), they are referred to as the sustentacular cells of Sertoli. But in studies of the invertebrates they are in general thought of as the nutritive cells. Recently, electron microscopic observations on the relation between developing spermatids and the Sertoli cells in mammals have shown that four to seven membranes or layers are interposed between the nucleus of the late spermatid and the cytoplasm of the Sertoli cell (7, 8).

Spermatozoa of the pond snail, *Cipangopaludina malleata* Reeve are peculiar in that they occur as three types, eupyrene, oligopyrene, and apyrene (9). The sperm head of the eupyrene type, as described in the previous paper (9), is composed of a helically coiled, dense nuclear material which is covered by the double-layered mantle in which 16 to 18 fibrillar elements can be seen loosely winding along the major axis of the head. In the present

study on spermatogenesis of the same material, observations have been made relative to the origin of the double-layered mantle, and of the 16 to 18 fibrillar elements appearing within the mantle. It has been found that the double-layered mantle originates from the nutritive cells, and that the mature spermatozoa leave the mantle. It is further indicated that the 16 to 18 elements, which appear fibrillar at low magnifications, belong to the endoplasmic reticulum (10) which forms a continuous system in the thin layer of nutritive cell cytoplasm that surrounds the sperm head and middle piece.

Materials and Methods

The testes of *Cipangopaludina malleata* Reeve were fixed for 1 to 2 hours in 1 per cent osmium tetroxide buffered to pH 7.2 with the Michaelis veronal-acetate buffer. After fixation, the tissue was dehydrated directly, without washing in distilled water, in a graded series of ethyl alcohol. The tissue, embedded in a mixture of *n*-butyl and methyl methacrylate, was sectioned with glass knives on either a Shimadzu or a Porter-Blum microtome. All sections were examined in an Akashi electron microscope, model TRS-50, or the Siemens Elmiskop I, and micrographs were taken at magnifications ranging from 2,000 to 40,000 diameters.

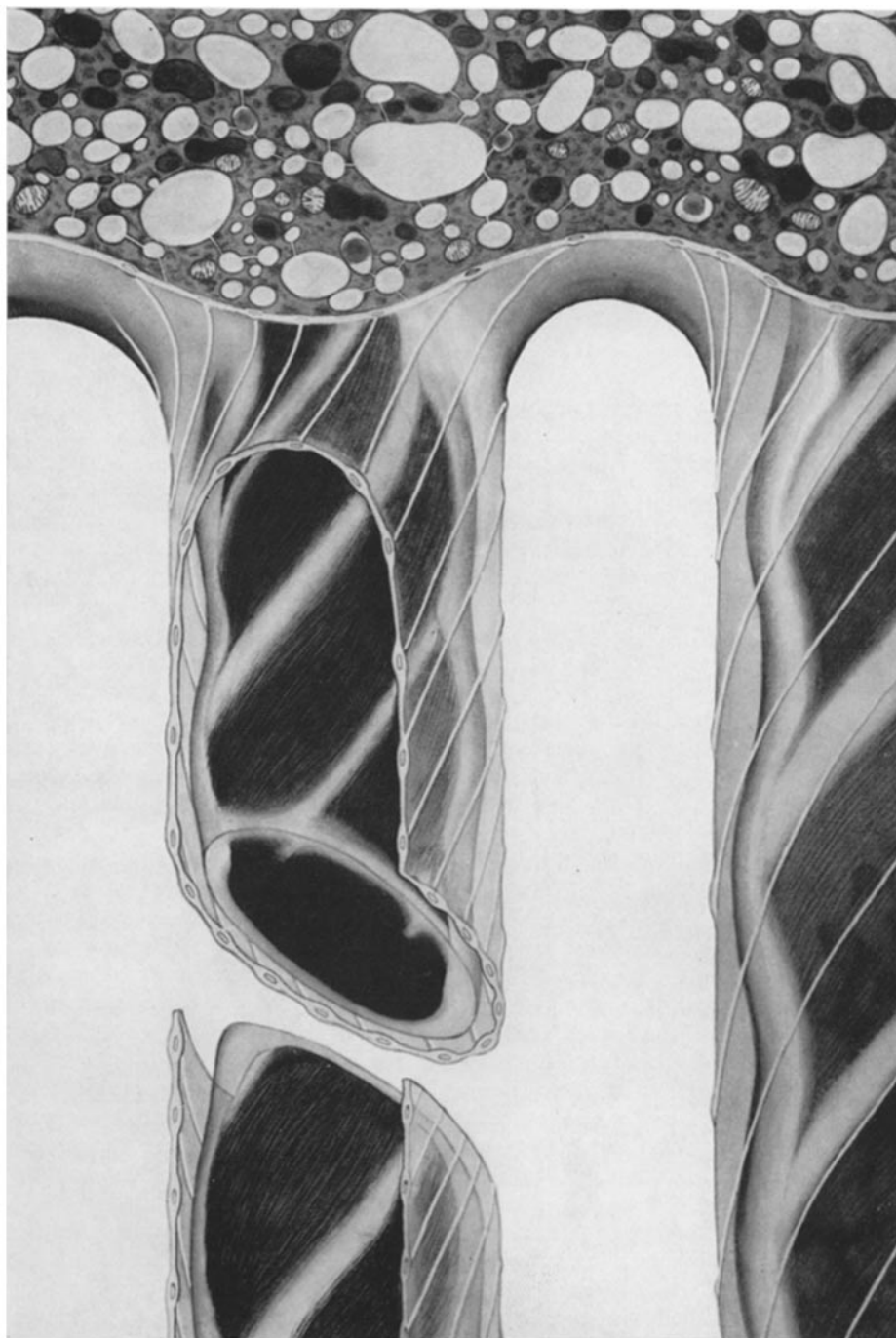
RESULTS

Immediately after the second division of the spermatocytes, spermatids are found along the inner surface of the epithelial lining of the seminiferous tubule. As a rule they are polyhedral, owing apparently to the pressure of the adjacent cells. Those spermatids lying nearest the lumen of the tubule appear less influenced by this factor and are often quite round. The cytoplasm is finely granular, and is outlined by a sharply defined cell membrane. The spherical nucleus is initially situated in the center, but as development proceeds, the whole spermatid becomes distally elongated toward the lumen of the tubule, and the nucleus shifts toward the proximal end of the cell. In the ovoid nucleus, it is possible to see dot-like or short fibrillar units 14 to 21 μ wide in slightly oblique cross-section. In the cytoplasm one can observe the Golgi complex, which consists of thin, flattened vesicles arranged in a parallel array, and a large number of small vesicles \sim 30 to 60 μ in diameter. Such small vesicles are frequently surrounded by a single membrane and similar clusters of vesicles have been referred to as unknown structures by Kaye (11), and as multivesicular bodies by Sotelo and Porter (12). They seem to belong to the Golgi com-

plex in so far as the pond snail spermatid is concerned, since they are always found in the Golgi zone and since the diameter of the vesicles is similar to that of the vesicles associated with the Golgi lamellae. The rest of the cytoplasm is filled with a considerable number of vesicles of different sizes which are taken to represent units of the endoplasmic reticulum (Fig. 1).

As the development of the typical spermatid proceeds, the nucleus increases in length and decreases in width, and concurrently with these changes in the shape of the nucleus, remarkable changes occur in the fine structure of the karyoplasm (9). The originally thin fibers aggregate into progressively thicker twisted strands (Figs. 2 and 3). This change seems to occur at first at the peripheral part of the karyoplasm, where a dense ring profile, about 30 μ wide, appears in cross-sections (Fig. 3). In the same sections other aggregates of the filaments appear separate within the nucleus whereas others appear attached to or continuous with the ring profile. Cross-sections of two nuclei in the middle stage of development are shown in Fig. 3. Here the nucleus shown at the right lower corner seems to be in an earlier developmental stage than that situated at the left upper corner, since the former shows many small bundles of chromosomal elements about 30 μ in width while the latter contains only thicker bundles over 65 μ in width. Although we cannot identify pairing of all the dense elements within the nucleus, some dense profiles seem to be associated in pairs. It would be of great interest to know the relation of these elements to chromosomes and whether they are arranged side by side or end to end. This question cannot be answered without some adequate method of serial sectioning. According to Inaba and Tanaka (13), the primary spermatocyte of *Cipangopaludina malleata* Reeve has 18 chromosomes in the diploid and 9 in the haploid. If all the chromosomes were lined up side by side in the spermatid nucleus, we might expect to find the number of bundles in some fashion related to the number of chromosomes. But the observed arrangement of dense profiles gives no suggestion of chromosome boundaries, such as Gall and Bjork (14) have already described in another animal.

The nucleus is surrounded by a narrow rim of spermatid cytoplasm. At this stage and level of sectioning, numerous elongate pseudopodia appear in the intercellular space around the spermatids. These are provided, in their central part, with a



TEXT-FIG. 1. Drawing designed to depict the structural relationship between maturing typical spermatids and nutritive cell in the testis of a pond snail. The helically coiled head part is enveloped by the mantle which arises from the nutritive cell where mitochondria, dense bodies, and a large number of vesicles of different sizes are found. The vesicles, apparently a vesicular form of the endoplasmic reticulum, are connected with one another. The endoplasmic reticulum in a canalicular form appears in the mantle. Here the elements run parallel and helically along the major axis of the head.

structure resembling the endoplasmic reticulum. They measure about 86 μ in diameter, and by virtue of this small diameter they are easily differentiated from the tail flagella of typical and atypical spermatozoa (Figs. 2 and 3).

It is at this stage that a considerable number of elongate pseudopodia of different sizes and forms grow out from the nutritive cell, project into the lumen of the seminiferous tubule, and achieve contact with the surface of the elongated spermatids. They seem never to divide into smaller branches (Figs. 4 and 5). They increase in length steadily until they come to surround the individual spermatids, and although their length cannot be accurately determined in thin sections, it is assumed that many of them reach the whole length of the middle piece.

The endoplasmic reticulum, in sections of nutritive cells, is characteristically represented by circular profiles of different sizes (Figs. 4 and 5), and it can be shown in serial sections that the circular profiles are connected by smaller tubules (Text-fig. 1). The circular and elongate profiles of the endoplasmic reticulum are also visible in the pseudopodia. In the early stages of the development of pseudopodia, mitochondria and dense bodies appear in small numbers in the nutritive cells (Figs. 4 and 5).

As spermatogenesis proceeds, the spermatids remain attached to the surface of the nutritive cells. The dense nuclear strands of the spermatids increase in diameter and the spaces between them are progressively reduced until the strands appear to fuse and the interspaces to disappear. At the same time the nuclear strands appear spirally coiled along the major axis of the nucleus. Thus, a longitudinal section through the nucleus shows a series of dense bodies surrounded by a less dense cytoplasm, enveloped by the apparently coalesced elongate pseudopodia of the nutritive cells (Figs. 6 and 7, and Text-fig. 1). In this stage also, the cytoplasm of the nutritive cell becomes more dense and more finely granular in character than at earlier stages. The endoplasmic reticulum is represented by an extensive system of vesicles of different sizes limited by a smooth surfaced, single membrane (Figs. 6 and 7).¹ Some vesicles have a content of lower density than the surrounding

cytoplasm and other vesicles show remarkably dense bodies (Fig. 7 and Text-fig. 1). An irregular mass of osmiophilic material is frequently found in the cytoplasm of the nutritive cell. This structure apparently corresponds to the chromatid body which has previously been reported by Yasuzumi and Tanaka (9) (Fig. 6). Mitochondria with a dense matrix are located close to the tip of the developing spermatid nucleus (Fig. 6). This atypically dense appearance of the mitochondria was also noted in the atypical spermatid of the pond snail (9) and does not appear to be a consequence of poor preservation.

In cross-sections through the almost mature spermatids, an array of round profiles about 86 μ in diameter, representing cross-sections through the elongate pseudopodia of the nutritive cells, appears surrounding the dense nucleus and the middle piece. At the level of the nucleus these pseudopodia are fused to form a mantle which envelopes the nucleus and to some extent the middle piece. The mantle appears as a ring-like profile in cross-section. Within the mantle (~ 86 μ in thickness) 16 to 18 dot-like elements, (75 μ wide on an average) can be observed (Fig. 8 A). In electron micrographs of greater magnification, it is observed that the dot-like elements correspond to a tubular form of the endoplasmic reticulum (Fig. 8 B). This cytoplasmic organelle is clearly visible in tubular form in the oblique sections of the mantle at the level of the nucleus and the middle piece (Figs. 9 and 10). In the cytoplasm surrounding the nebkern sheath of the middle piece, a large number of vesicular and tubular elements of the endoplasmic reticulum can be observed (Fig. 9). Cisternal elements are rarely present in the middle piece (Fig. 10).

Fig. 11 demonstrates a group of developing atypical spermatids and a small portion of the nutritive cell. The latter contains small mitochondria and numerous vesicles, some with dense granules. The atypical spermatid nucleus is reduced to a thin horseshoe-shaped profile. A bundle of tail filaments can be seen in the electron-transparent matrix of the head. Dense mitochondria appear among the developing tail filaments. The nucleus is lodged in a deep indentation of the surface of the nutritive cell, and is separated from the nutritive cytoplasm by the plasma membrane of the atypical spermatid and the nutritive cell. The cytoplasm of the atypical spermatid is filled with innumerable vesicles containing the dense granules

¹ It has been revealed at higher magnification that the smooth surfaced endoplasmic reticulum is limited by a double-layered membrane consisting of two opaque layers with a less opaque interspace (15).

already described by Yasuzumi and Tanaka (9) (Fig. 11).

When the typical spermatids have reached a certain degree of maturity and their cytoplasm has been sloughed off, they are set free, leaving the mantle projecting from the nutritive cell. At this stage, the ground substance of the cytoplasm of the nutritive cell becomes less dense and the endoplasmic reticulum in a vesicular form decreases in amount and appears shrunken. The mitochondria and the chromatoid bodies also decrease markedly in number (Fig. 12).

DISCUSSION

As the development of the typical spermatids proceeds, the nutritive cell becomes irregular in outline, and numerous elongate pseudopodia develop from its surface towards the lumen. As the pseudopodia increase in number, they become more slender and more closely packed until they coalesce into a continuous sheet. The greatly elongated pseudopodia approach the developing typical spermatids and become adherent to the surface of the head and middle piece of the typical spermatid. The sheet formed by the coalescence of the nutritive cell pseudopodia takes the shape of a thin walled mantle. Thus, the mantle of the advanced typical spermatid of the pond snail is not a membrane growing downward over the nucleus from the acrosomal granule appearing in the spermatid itself, as generally in vertebrates (7, 8, etc.), but is derived directly from the nutritive cell (Text-fig. 1).

A morphological difference between typical and atypical spermatids is shown in their relationship to the nutritive cell. The atypical spermatid is lodged in a deep indentation of the nutritive cell, and the substance of the atypical spermatid is separated from the surface of the nutritive cell by their cell membranes. Thus, the nutritive cell seems to be a sustentacular cell for the atypical spermatid. The ultimate fate of the mantle is unknown. Presumably it is a transient structure that disappears after the detachment of the sperms.

In the spermatogenesis of *Drosophila*, Guyénot and Naville (16), Geigy (17), and Aboim (18) have reported that the advanced spermatids form bundles and become implanted in groups of 50 or more within a giant nutritive cell having a vacuolated cytoplasm. In the pond snail nutritive cell, a vacuolar system, *i.e.* the endoplasmic reticulum, has been found whose appearance is similar to that observed in the light microscope (16-18).

The endoplasmic reticulum in the mantle was described in a previous report (9) as follows: the discontinuous fibrils with intermediate density within the mantle in a longitudinal section seem to correspond to 16 to 18 dots appearing in a cross-section; that the fibrils are not continuous in a longitudinal section and always identified as dots in a cross-section, suggests that they are helically arranged in the mantle. The "fibrils" appearing within the mantle, which in the present study are revealed to be tubular elements of the endoplasmic reticulum do in fact adopt a helical course, so that the tubular structure of the endoplasmic reticulum in a longitudinal section is not always demonstrable. In an oblique section through the mantle, the endoplasmic reticulum in a tubular form appears in circular or oblique profile in the same section, as can be seen in Figs. 9 and 10.

In early stages of the development of the elongate pseudopodia, tubular elements of the endoplasmic reticulum are not uniform in size or number, since each pseudopodium of a similar size reveals on cross-section one to five profiles. (Fig. 3). In cross-sections through the mantle of the mature sperm, the endoplasmic reticulum appears as single circular profiles 50 to 100 $m\mu$ apart (Fig. 8 A), and in longitudinal sections as a series of discontinuous elongate profiles (Fig. 7). The direction of coiling of the endoplasmic reticulum may be related to the coiling in the sperm head.

It is believed that the most important feature of the intracytoplasmic system presented here is a canalicular system that is continuous from the nutritive cell to the mantle surrounding the maturing spermatid. Under this circumstance, it seems appropriate to assign to the endoplasmic reticulum the possible role of conducting nutritive substance from the nutritive cell to the developing spermatids. It has already been suggested that the endoplasmic reticulum may function as an intracellular conductor (19, 20), as an apparatus for directing and facilitating intracellular diffusion, or as a segregation apparatus (21). The endoplasmic reticulum has also been considered a system involved in the import (22-24), export, and intracellular circulation of various substances (25). The present study seems to provide confirmative morphological evidence for these concepts.

During the study of this material one of the authors, G. Yasuzumi, had the opportunity to visit the Institute for Electron Microscopy at the Fritz-Haber-Institute of the Max-Planck-Gesellschaft, Berlin-Dahlem, and

the Anatomical Institute of Kiel University, Kiel, Germany, and was able to carry out research at these Institutes. The kindness and help of Prof. Dr. E. Ruska, Dr. S. Grund, and Dr. I. Wickel at the Max-Planck-Gesellschaft, and Professor W. Bargmann and Dr. A. Knoop at Kiel University are gratefully acknowledged.

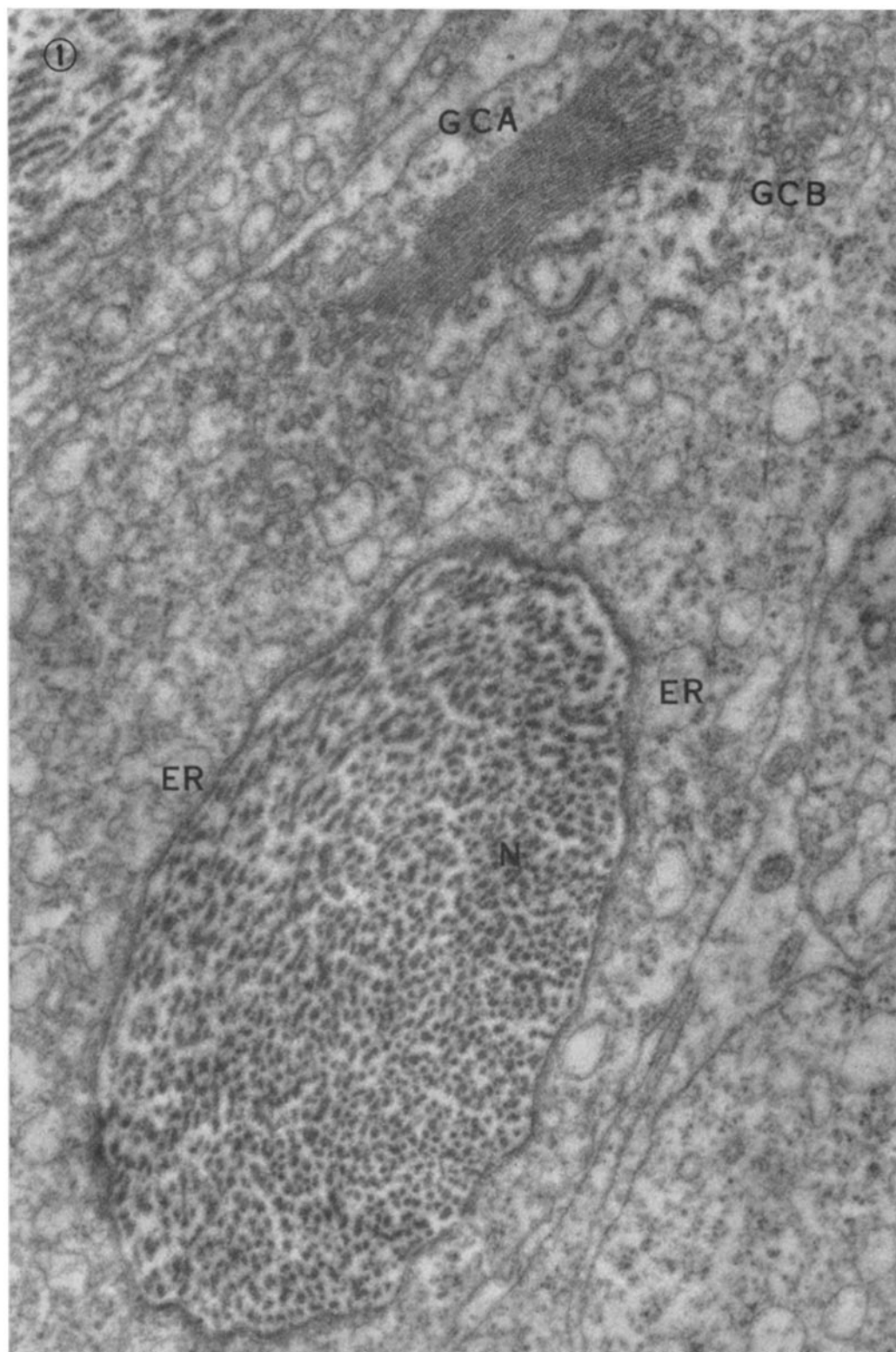
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EXPLANATION OF PLATES

PLATE 267

FIG. 1. Typical spermatids of *Cipangopaludina malleata* Reeve in a slightly oblique cross-section, showing a middle stage in the orientation of the chromosomal elements. The nucleus (N) is slightly deformed to an oval shape. The karyoplasm shows dot-like or short elongate bodies, which vary from 14 to 21 m μ in width. One type of Golgi structures (GCA) consists of thin, flattened vesicles in a parallel array and of small vesicles. Another type of Golgi structures (GCB) is found in the right upper corner of the figure, and is composed of a group of small vesicles surrounded by a single membrane. Smooth surfaced elements of the endoplasmic reticulum (ER) can be seen as circular profiles of different sizes in the finely granular matrix of the cytoplasm. At the left upper corner a slightly oblique longitudinal section of a spermatid nucleus is visible, showing its filamentous contents. $\times 55,000$.

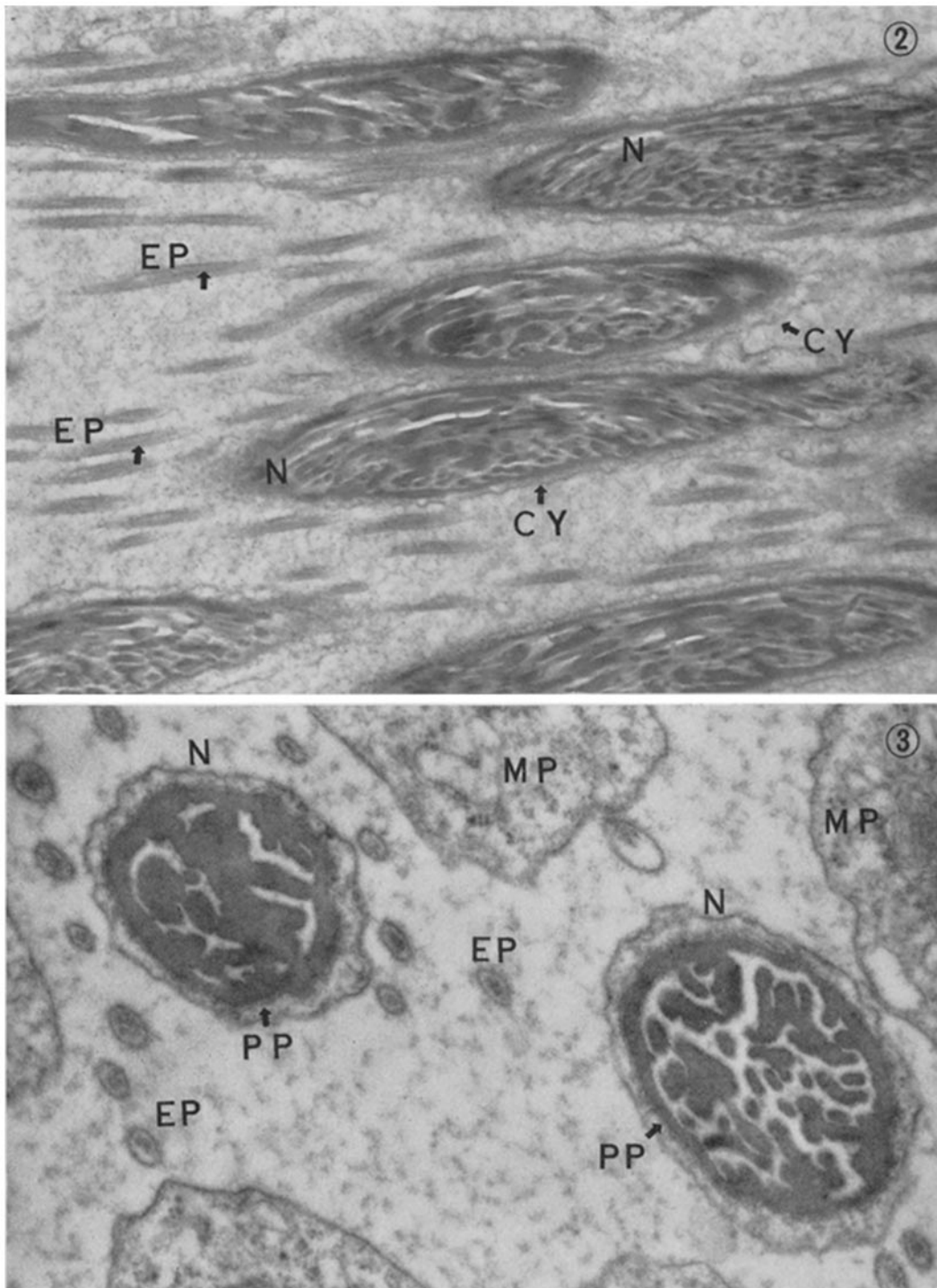


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PLATE 268

FIG. 2. Oblique longitudinal section through long conical nuclei (*N*) and elongate pseudopodia (*EP*) appearing in the intracellular spaces. The nucleus (*N*) shows twisted electron-opaque elements, 70 to 80 m μ in diameter. The nucleus (*N*) is enveloped by a thin, cytoplasmic layer (*CY*). It is difficult to differentiate the nuclear envelope from the internal nuclear elements. The elongate pseudopodia (*EP*) appearing in the intercellular spaces show a tubular structure (arrows) running along their major axis. $\times 22,000$.

FIG. 3. Cross-section through two typical spermatid nuclei (*N*), and a middle piece (*MP*) in late stages of differentiation, and elongate pseudopodia (*EP*) appearing in the intercellular spaces. The dense, round, oval, or irregularly shaped bodies seen within the nucleus are assumed to result from the coalescence of the fibrillar element seen at earlier stages. A ring of dense material can be seen at the peripheral portion (*PP*) of the nucleus (*N*). One to five small vesicular profiles are visible within the elongate pseudopodia (*EP*). $\times 50,000$.



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PLATE 269

FIGS. 4 and 5. Longitudinal section of nutritive cells (*NC*) of *Cipangopaludina malleata* Reeve at a late stage of spermatogenesis, showing numerous elongate pseudopodia (*EP*) from the nutritive cells (*NC*) into the intercellular space. Smooth surfaced elements of the endoplasmic reticulum (*ER*) appear as circular profiles of different sizes in the cytoplasm. The cavities of the endoplasmic reticulum (*ER*) contain finely granular substances of intermediate density, dense granules (*DG*), or fibrillar elements (*FE*). Circular or tubular profiles of the endoplasmic reticulum (*ER*) can be seen in the elongate pseudopodia (*EP*). $\times 23,000$.

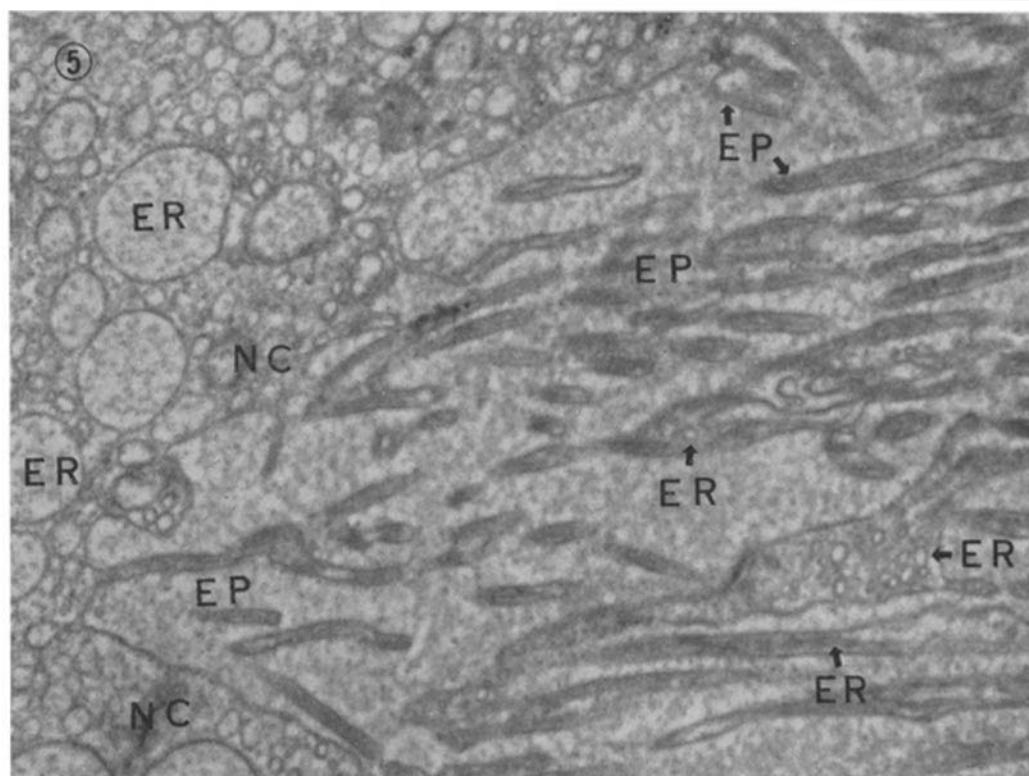
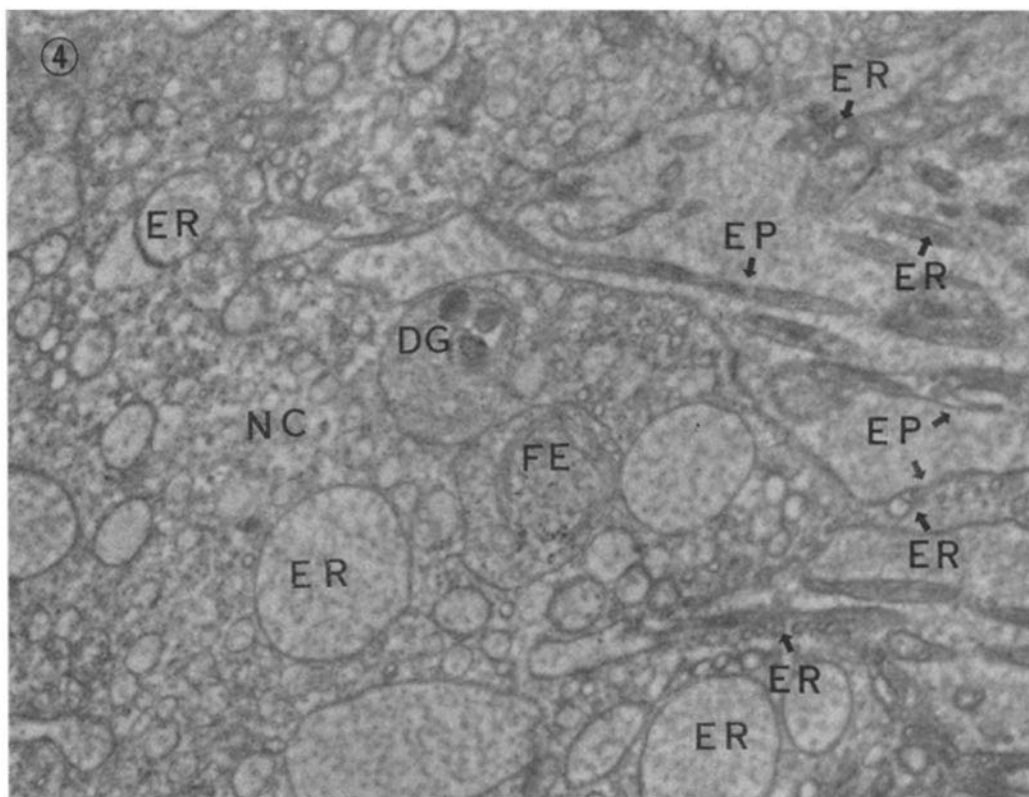
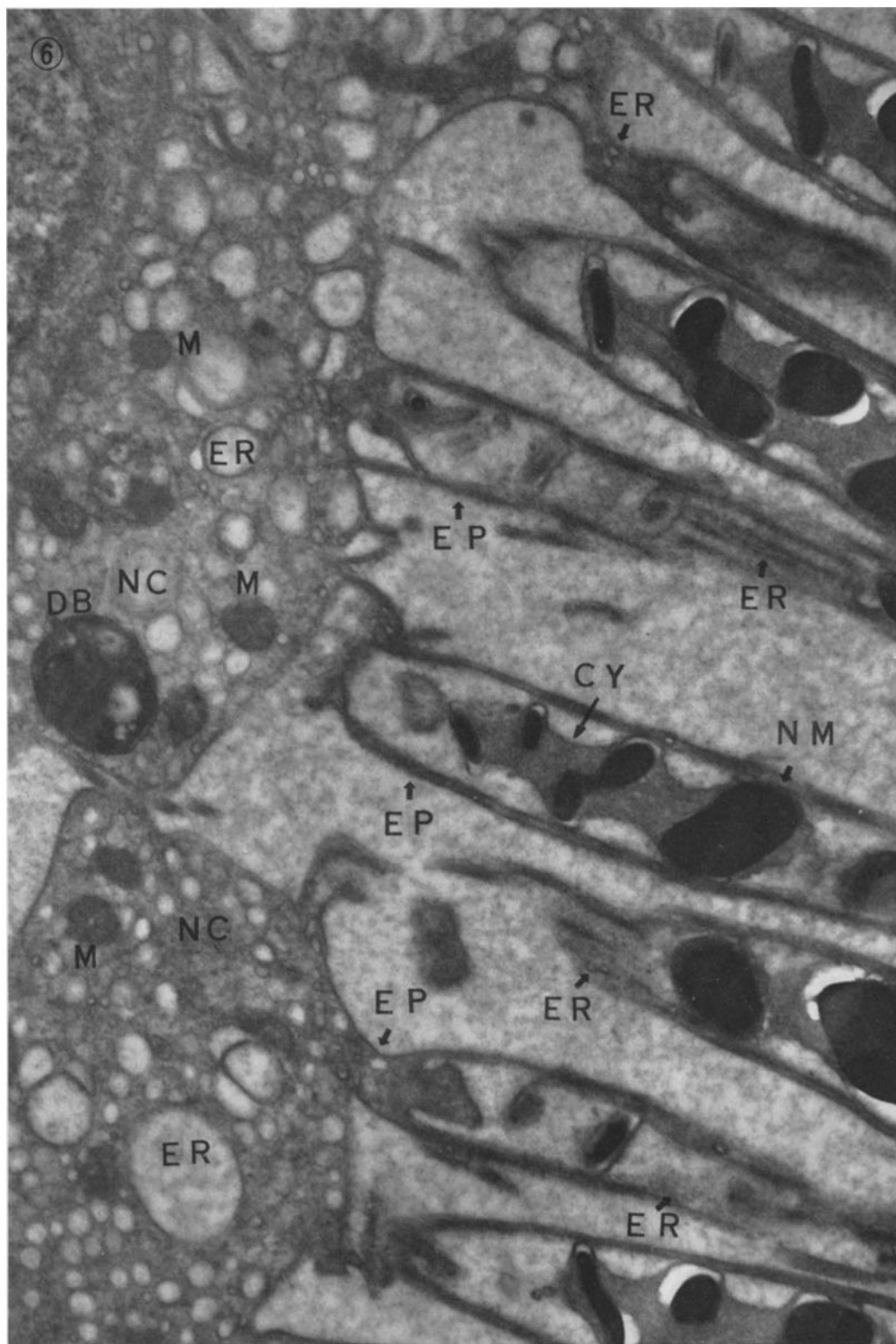


PLATE 270

FIG. 6. Longitudinal section through a group of maturing spermatids attached to two nutritive cells (*NC*). The masses of dense nuclear materials (*NM*) and of less dense cytoplasm (*CY*) in the spermatids are enveloped by the elongate pseudopodia (*EP*) from the nutritive cells (*NC*). The endoplasmic reticulum (*ER*) in the elongate pseudopodia (*EP*) appears in circular or long profiles. The nutritive cell (*NC*) contains small mitochondria (*M*) with a dense matrix, and a round dense body (*DB*) which is interpreted as part of a nutrient supply for the spermatids. The cytoplasm of the nutritive cell is rich in smooth surfaced endoplasmic reticulum (*ER*), a feature that gives it a loose reticular structure in the light microscope image. $\times 25,000$.



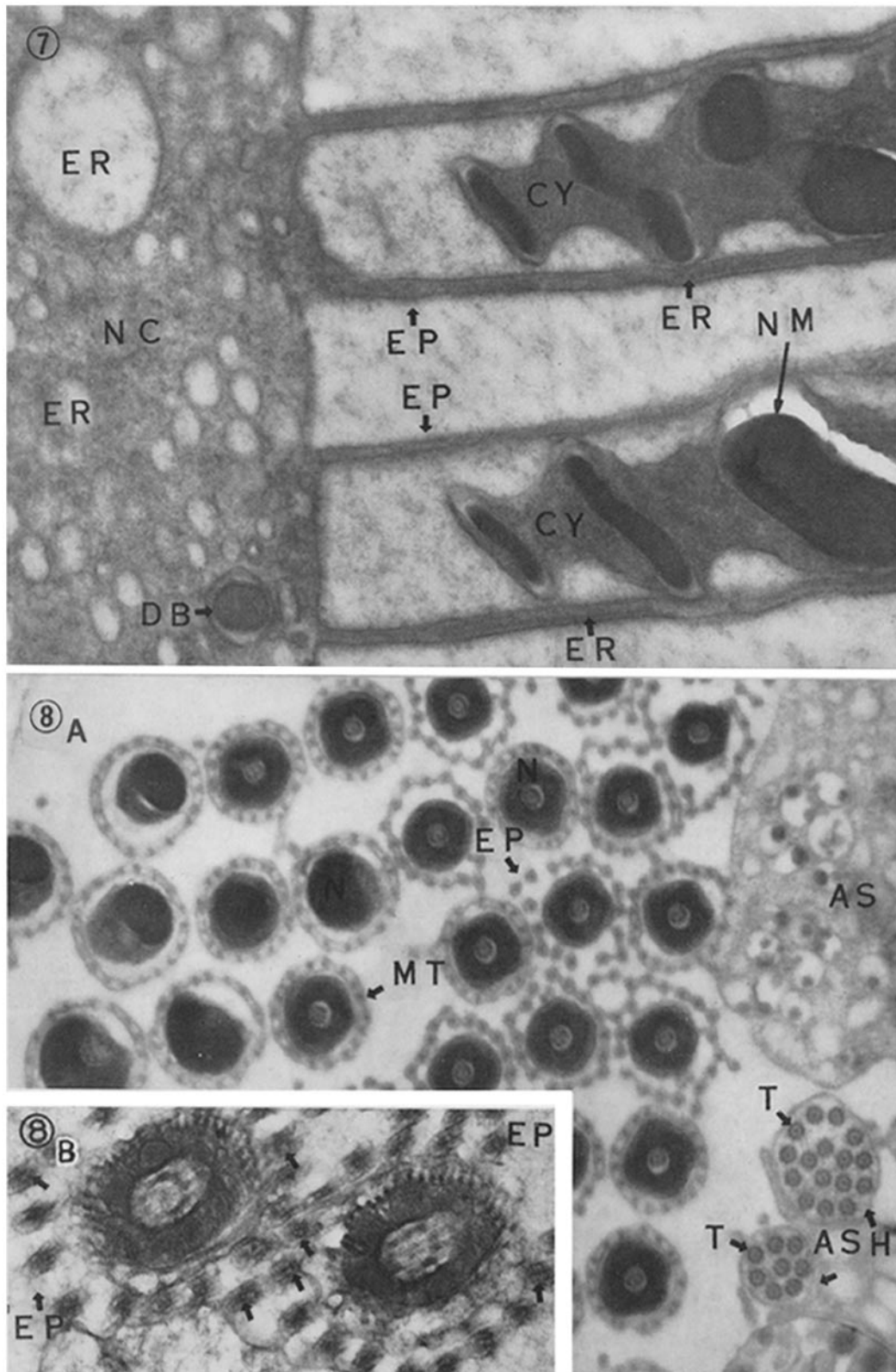
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PLATE 271

FIG. 7. Longitudinal section through two maturing typical spermatids attached to the nutritive cell (*NC*). A series of dense nuclear profiles enveloped by the less dense cytoplasm (*CY*) can be seen between the elongate pseudopodia (*EP*). Circular or elongate profiles of the endoplasmic reticulum (*ER*) appear in the elongate pseudopodia (*EP*). The nutritive cell cytoplasm (*NC*) shows a homogeneously fine granular matrix and elements of the endoplasmic reticulum (*ER*). The latter are represented by circular profiles of different sizes, one of which contains a dense body (*DB*). $\times 64,000$.

FIG. 8 *A*. Cross-section through distal portions of maturing typical spermatid nuclei (*N*) shows the centriole or proximal portion of the tail flagellum in the middle of the dense nuclear substance. The nucleus (*N*) is enveloped by numerous round profiles about $86\text{ m}\mu$ in width of the elongate pseudopodia (*EP*). In other parts the pseudopodia are fused to form a mantle (*MT*) which shows 16 to 18 dot-like profiles at low magnification. On the right upper side of the figure an atypical spermatid (*AS*) can be seen. Its cytoplasm contains numerous vesicles with dense small granules. On the right lower side 2 apyrene spermatid heads (*ASH*) with 8 and 16 tail flagella (*T*) are clearly visible. $\times 23,000$.

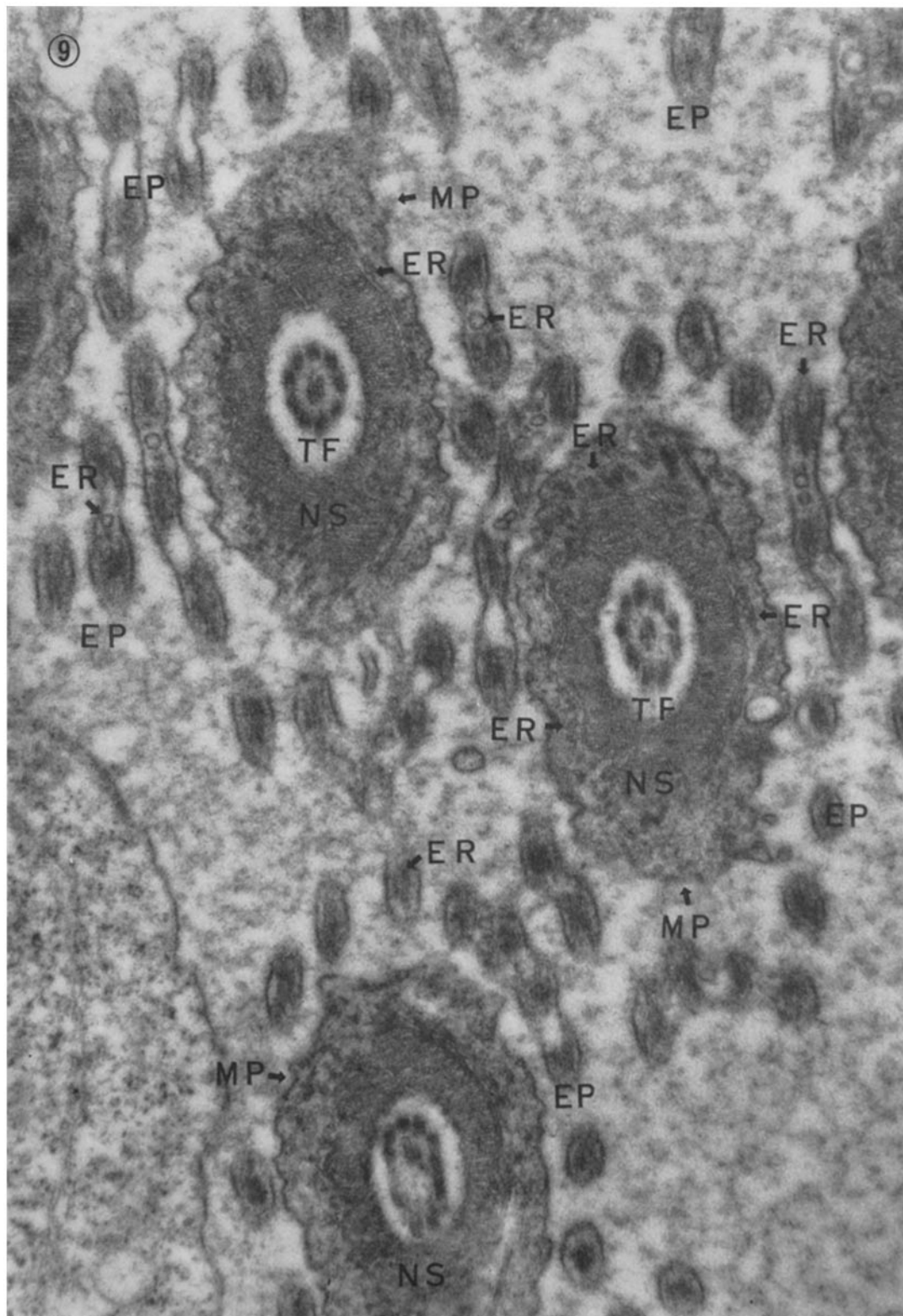
FIG. 8 *B*. Slightly oblique cross-section through the middle pieces of developing typical spermatids at higher magnification. They are surrounded by numerous elongate pseudopodia (*EP*) in which the endoplasmic reticulum in circular or tubular form is found at the points marked by the arrows. $\times 38,000$.



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PLATE 272

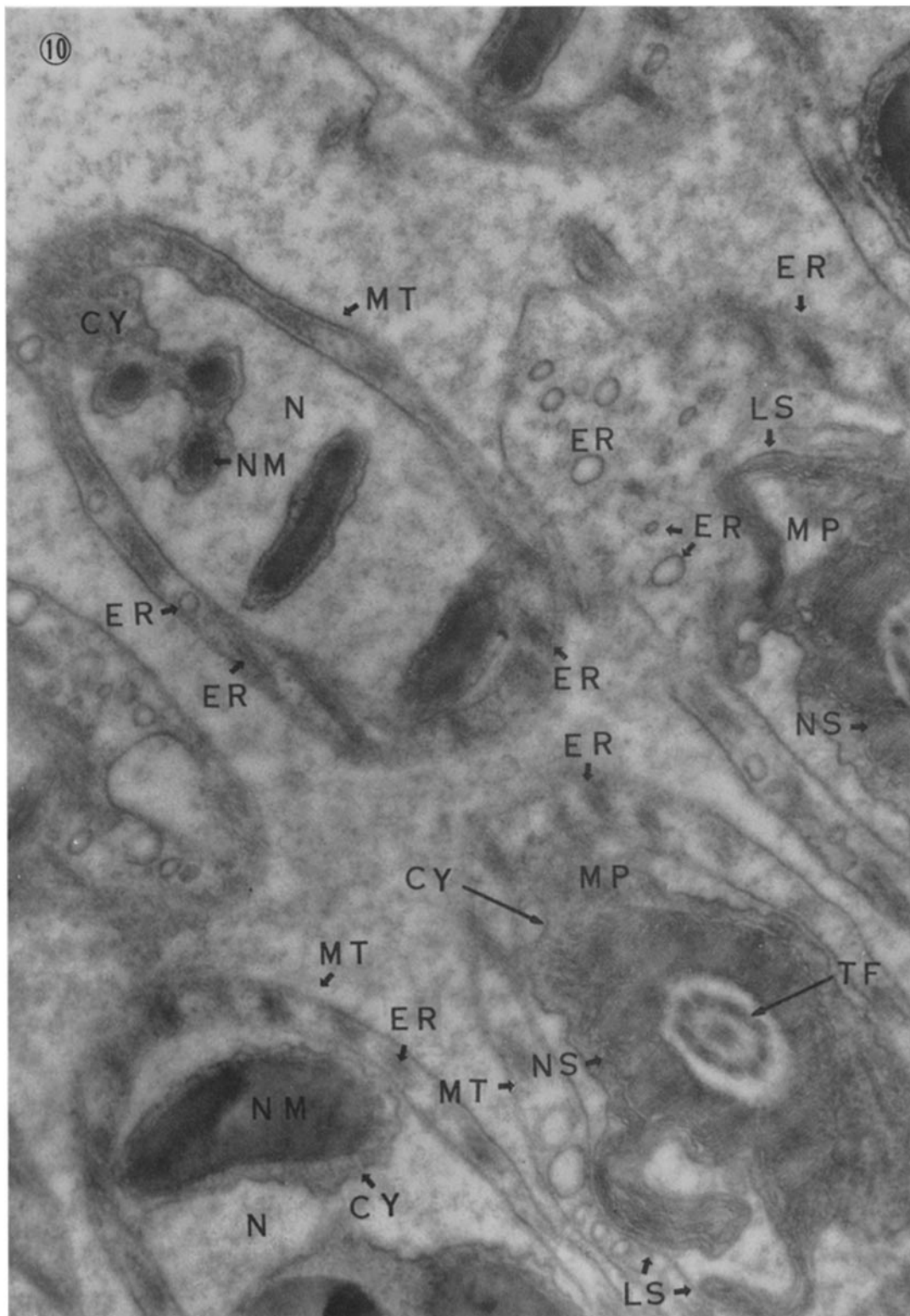
FIG. 9. Cross-section through the developing middle pieces (*MP*) of typical spermatids, showing the central set of tail filaments (*TF*), the nebenkern sheath (*NS*), and the narrow layer of cytoplasm in which circular or elongate profiles of the endoplasmic reticulum can be seen. The middle piece (*MP*) is surrounded by round or oval profiles of the elongate pseudopodia, each containing elements of the endoplasmic reticulum (*ER*). $\times 50,000$.



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PLATE 273

FIG. 10. Slightly oblique cross-section through maturing spermatid nuclei (*N*) and middle piece (*MP*), showing the mantle profile (*MT*) enveloping the nucleus (*N*) and the middle piece (*MP*). The dense nuclear material (*NM*) is surrounded by the less dense cytoplasm (*CY*). The bundle of the tail filaments (*TF*) is enveloped by the nebensheath (*NS*) which is surrounded by a layer of cytoplasm (*CY*) of irregular thickness, where a lamellar structure (*LS*) can be seen. The endoplasmic reticulum (*ER*) appearing in the mantle (*MT*) is clearly visible in cross-section, showing circular profiles, but not so clear in oblique longitudinal sections because it makes its way helically along the major axis of the mantle (*MT*). $\times 52,000$.



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PLATE 274

FIG. 11. Longitudinal section through developing atypical spermatids (*DAS*), showing an atypical spermatid head (*H*) lodged in a deep indentation of the surface of the nutritive cell (*NC*). The nucleus (*N*) is reduced to a thin arched profile which envelops a bundle of tail filaments (*TF*). The cytoplasm of the atypical spermatids (*AS*) is filled up with a large number of small vesicles containing dense granules. The Golgi complex (*GC*) is visible in the cytoplasm. The mitochondria (*M*) appear along the developing tail filaments (*TF*). The nutritive cell (*NC*) contains dense mitochondria (*M*), large vesicles with dense granules or less dense substances, and circular profiles of the endoplasmic reticulum (*ER*). $\times 9,000$.

FIG. 12. Longitudinal sections of mature typical spermatozoa (*MS*). The cytoplasm and mantle (*MT*) appear isolated from the spermatozoa (*MS*). At the left part of the figure can be seen the nutritive cell (*NC*). The middle pieces (*MP*), free of the mantle (*MT*), are visible at the right side of the figure. $\times 12,000$.

