

CYTODIFFERENTIATION DURING SPERMIOGENESIS IN *LUMBRICUS TERRESTRIS*

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ABSTRACT

The structural changes during spermiogenesis were studied on developing spermatids in seminal vesicles and receptacles of *Lumbricus terrestris* fixed in glutaraldehyde-osmium tetroxide and embedded in Epon-Araldite. The centriole plays a prominent role in the morphogenesis and organization of the microtubules of the manchette and flagellum. Microtubules arising from the centriole extend anteriorly to encase the developing middle piece, the nucleus, and the acrosome. The manchette not only provides a supporting framework for the cell during elongation, but also may provide the motive force for the elimination of both nucleoplasm and cytoplasm. The manchette participates in segregation and elimination of the nuclear vesicle that contains the nonchromatin nucleoplasm. Compartmentalization and conservation may also be a function of the manchette since those elements which remain within the framework of microtubules are retained, while all the cytoplasm outside the manchette is discarded. At maturation, the endoplasmic reticulum plays a key role in dismantling the manchette and reducing the cytoplasm external to it. During the early stages of middle-piece formation, six ovoid mitochondria aggregate at the posterior pole of the spermatid nucleus. Concurrent with manchette formation, the mitochondria are compressed laterally into elongate wedge-shaped components, and their outer limiting membranes fuse to form an hexagonal framework that surrounds the dense intramitochondrial matrices. Dense glycogen granules are arranged linearly between the peripheral flagellar tubules and the outer membrane of the mature sperm tail.

INTRODUCTION

In the earthworm, spermatogenesis begins in the testis with the production of primary spermatocytes, and continues in the seminal vesicles in which the spermatocytes proliferate synchronously to form morulae, each composed of 128 spermatids. By complex cytomorphogenetic processes, the spermatids which are attached to a central nucleate mass of cytoplasm differentiate into motile elongate spermatozoa (Chatton and Tuzet, 1941; Gatenby and Dalton, 1959). Cytodifferentiation of spermatids involves (1) cell elongation, (2) reduction of both nuclear and cytoplasmic volume, and (3) the formation of distinct cell compartments. The cytoplasmic alterations that

occur during spermatid differentiation provide a cell architecture adaptive for reproduction and eliminate nonessential cell components.

The role that various cell organelles play in directing spermatid differentiation has been the subject of numerous studies (Yasuzumi and Tanaka, 1958; Bawa, 1964; Bradke, 1963 *a, b*; Boisson and Mattei, 1965; Potswald, 1966; Tandler and Moriber, 1966). The Golgi apparatus, for example, is a key factor in acrosome formation in many species (Fawcett, 1966). The development of a system of microtubules that form the manchette is correlated with cell elongation during spermiogenesis (Burgos and Fawcett, 1955; Sil-

veira and Porter, 1964). According to these authors, the manchette provides a scaffold or supporting framework for the cell during elongation. The basic structural features of annelid spermiogenesis and acrosome formation were presented by Gatenby and Dalton (1959) and Cameron and Fogel (1963). Involvement of the manchette in spermatid differentiation was demonstrated by Bradke (1963 *a*). Improved techniques for electron microscopy, especially the use of glutaraldehyde-osmium tetroxide fixation, have disclosed many cytological details of spermatid differentiation that were not previously reported.

MATERIAL AND METHODS

Seminal vesicles and receptacles of *Lumbricus terrestris* were severed from the body wall, and small pieces of tissue, 1 to 2 mm², were placed for 1 hr in cold glutaraldehyde fixative that contained 5.6 ml of biological

grade glutaraldehyde (36.4%), 1.5 g of sucrose, 50 ml of 0.1 M sodium cacodylate, and 44.4 ml of distilled water at pH 7.3. Tissue blocks were rinsed rapidly in a mixture containing 5 g of sucrose in 100 ml of 0.1 M sodium cacodylate and post-fixed in two changes of 2% osmium tetroxide for 30 min each at 5°C. Following fixation, the blocks were dehydrated through 30, 50, 70, and 95% acetone-water solutions (10 min each) and through two 30-min changes of 100% acetone. The samples were agitated for 12 hr in a mixture of one part embedding medium with activator and one part acetone (1:1). After 1 hr of agitation in pure embedding medium plus activator, the samples were finally embedded in an Epon-Araldite mixture (Voelz and Dworkin, 1962). Final polymerization was carried out in 100% resin in BEEM capsules by overnight incubation at 60°C. Sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome. Thick (1- μ) sections were used for study with the light microscope. Thin sections (silver-gold) were mounted on uncoated cop-

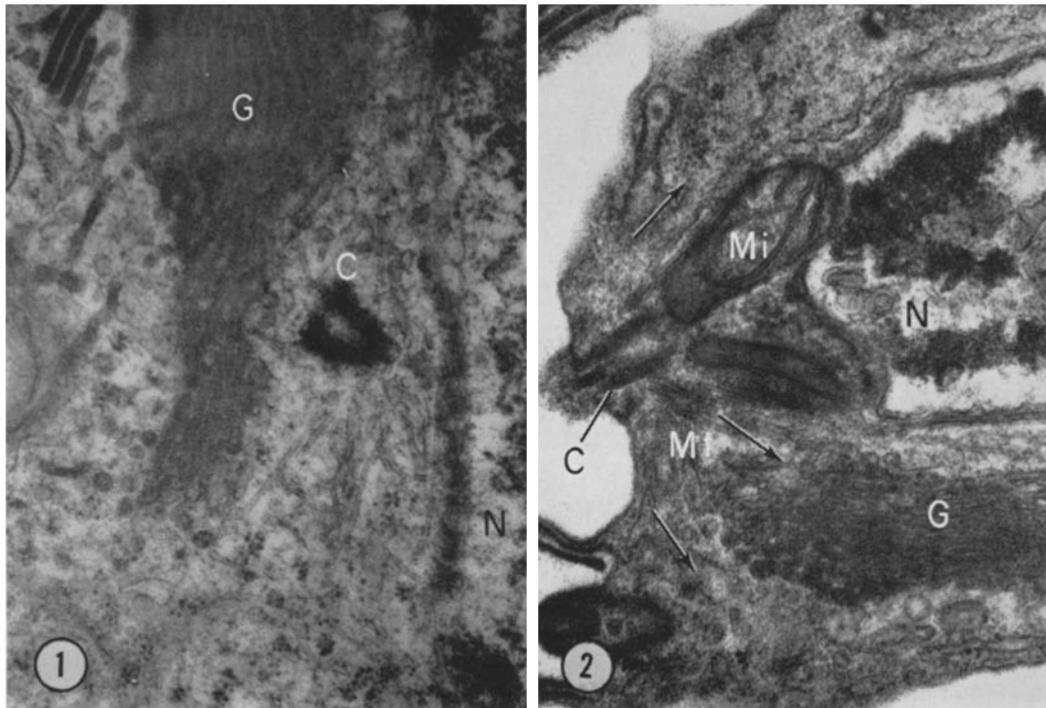


FIGURE 1 A centriole (*C*) and adjacent microtubules that extend randomly in the cytoplasm are located between the nucleus (*N*) and the Golgi membranes (*G*) of the early spermatid. $\times 36,000$.

FIGURE 2 A later stage in spermatid maturation reveals a condensation of the nuclear chromatin and mitochondria (*Mi*) at the posterior pole of the nucleus. As seen in longitudinal section, the microtubules of the manchette (arrows) diverge from the centriole (*C*) that forms the base of the flagellum. *G*, Golgi complex. $\times 36,000$.

per grids, stained first in a saturated solution of uranyl acetate in 40% ethanol for 3-10 min, rinsed in distilled water, and poststained for 5-10 min in an undiluted solution of lead citrate (Reynolds, 1963). The material was examined and photographed with RCA-EMU 3D and 3F electron microscopes.

OBSERVATIONS

Origin and Formation of the Manchette

In the early stages of spermatid development, a pair of centrioles is located at one pole of the nucleus where they are partially surrounded by the Golgi complex. One centriole migrates from this region to the peripheral cytoplasm of the spermatid where it is associated with the formation of the acrosome. Numerous microtubules are distributed randomly in the juxtannuclear Golgi region, and several of these radiate from the second, stationary centriole (Fig. 1). Concurrent with the condensation of chromatin and elongation

of the cell, microtubules converge upon the centriole at the distal pole of the nucleus (Figs. 2-4). The microtubules emanate from the centriole itself, not from the satellites that surround this centriole (Figs. 14 a and c). All the microtubules extend anteriorly to form the manchette which encases the middle piece and nucleus (Figs. 5-7 and 14 a). The distal end of the centriole, which is situated immediately beneath the cell surface, is involved with the formation of the flagellum (Figs. 2 and 3). Satellites that project laterally from the distal end of the centriole anchor this organelle in the distal cytoplasm and to the cell membrane where the flagellum emerges from the cell (Figs. 14 a and b).

Nuclear Elongation and Condensation of Chromatin

The full sequence of nuclear transformations during spermiogenesis is illustrated in Figs. 5-8,

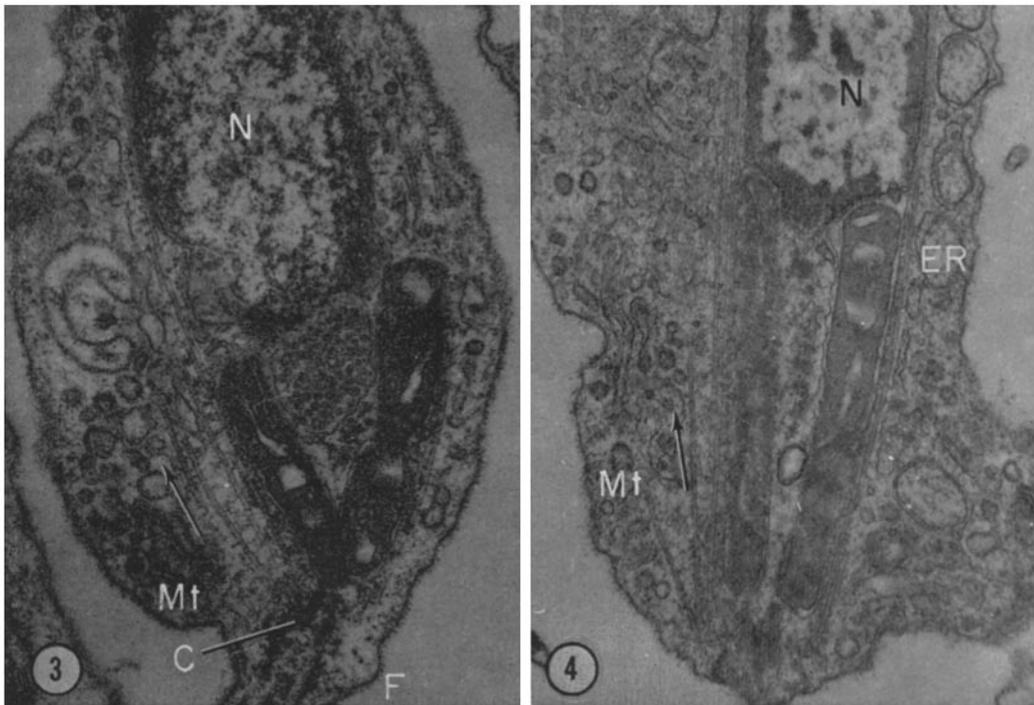


FIGURE 3 Microtubules of the manchette (*Mt*) extend anteriorly, beside the mitochondrial middle piece and the elongating nucleus, from the centriole (*C*) at the base of the flagellum (*F*). $\times 38,500$.

FIGURE 4 Dilated cisternae of the endoplasmic reticulum (*ER*) are observed beneath the plasma membrane in this longitudinal section of an elongating spermatid. *Mt*, microtubule. $\times 30,000$.

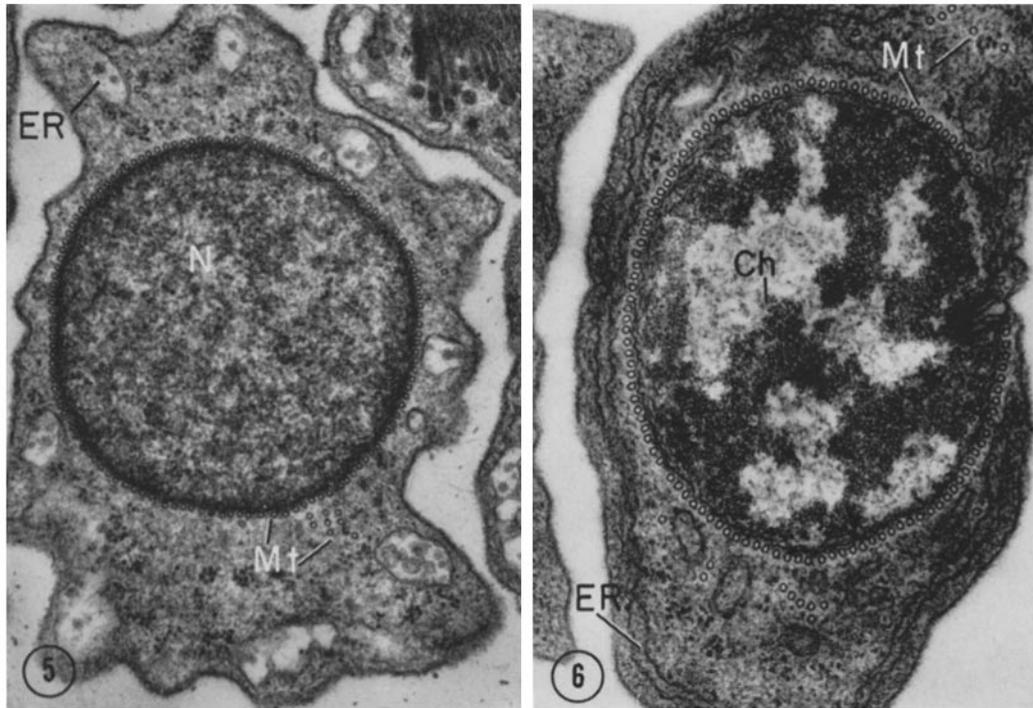


FIGURE 5 This cross-section of the spermatid nucleus (*N*) shows that its chromatin material is evenly dispersed and that it is encased by a single circle of microtubules (*Mt*) that form the manchette. Vesicles (*ER*) are located beneath the plasma membrane. $\times 30,000$.

FIGURE 6 The nucleus, containing dense areas of chromatin (*Ch*), is surrounded by the manchette; a continuous profile of endoplasmic reticulum (*ER*) lies beneath the plasma membrane. $\times 48,000$.

10–13 *b*, and 15–21. Nuclear elongation commences with the appearance of a framework of microtubules next to the outer membrane of the nuclear envelope of the spermatid. The appearance of chromatin undergoes marked changes at successive stages in the course of spermatid differentiation. At an early stage of development, the nucleus, which contains a rather diffuse chromatin pattern, assumes a cylindrical form (Fig. 5). At a later stage of development, large chromatin masses are distributed throughout the nucleus (Figs. 6 and 8). The chromatin masses progressively enlarge and fuse to form a highly condensed uniform structure. The differences in the nuclear density in these micrographs is due primarily to the varied staining times used in this investigation. Most of the interchromosomal nucleoplasm is segregated into a nuclear vesicle which is bounded by the nuclear envelope (Fig. 7) and runs at one side of the nucleus for nearly its entire length (Fig. 13 *b*).

Following glutaraldehyde fixation, myelin figures are frequently observed within the nucleus and the nuclear vesicle of the developing spermatid (Figs. 7 and 8). As a result of elongation and elimination of nonchromatin nucleoplasm, the diameter of the nucleus decreases and the nuclear density increases markedly (Figs. 10–12). The system of microtubules is most extensive at the time when the nuclear volume is being reduced. The nuclear vesicle is separated from the nucleus by microtubules of the manchette (Figs. 13 *a–b*, 15, and 16) and later eliminated from the cell (Fig. 17). An irregularity in the circular profile of the nucleus, in cross-section, represents the point of separation of the nuclear vesicle (Figs. 16 and 21).

The elongating spermatids remain attached to the anucleate cytoplasmic mass by a constricted portion of the cytoplasm. An extraneous fuzzy coat composed of 20–30-A filaments extends from



FIGURE 7 A myelin figure and clear nucleoplasm are contained in the nuclear vesicle (NV) formed by the extrusion of the nuclear envelope (arrows). At a different level (figures at right), the nuclear vesicle is absent and the manchette microtubules completely encase the nucleus that is markedly reduced in diameter at this stage of development. $\times 32,000$.

the plasma membrane of the dense attachment region (Figs. 8 and 9). The anterior tips of the microtubules of the manchette terminate in this region.

Reduction of the Cytoplasm

Morphogenetic events during the final stages of spermiogenesis accomplish the reduction of cytoplasm and dissemblance of the system of microtubules that encase the acrosome, nucleus, and middle piece. The endoplasmic reticulum plays a significant role in this process. In the early stages of spermatid differentiation, smooth-surfaced vesicles appear next to the microtubules of the manchette (Figs. 6 and 11). In later stages of development, the vesicles coalesce to form a continuous membrane surrounding the manchette (Fig. 12 *b*). Further condensation of chromatin proceeds within the inner membrane of the nuclear envelope (Figs. 10–12 *a*). Subsequent distension of the perinuclear cistern and the cistern of the smooth

endoplasmic reticulum results in the relocation of the manchette to a thin cytoplasmic band (Figs. 18 and 20). The perinuclear cistern is sometimes continuous with the cistern of the endoplasmic reticulum (Figs. 18 and 19). Following disassociation of the manchette cytoskeleton, the nucleus and the perinuclear cistern are encased by two closely apposed membranes (Figs. 21 and 31).

Differentiation of the Middle Piece

Several mitochondria are present in the cytoplasm of the early spermatid. Just before formation of the manchette, six mitochondria aggregate in the cytoplasm between the centriole and the nucleus (Fig. 2). In transverse section, the mitochondria have circular profiles and surround a small dense zone of cytoplasm (Fig. 22). Only a few microtubules are associated with this complex during the early stages of middle-piece formation (Figs. 2 and 22). Fusion of the outer mitochondrial membranes results in the formation of a pinwheel

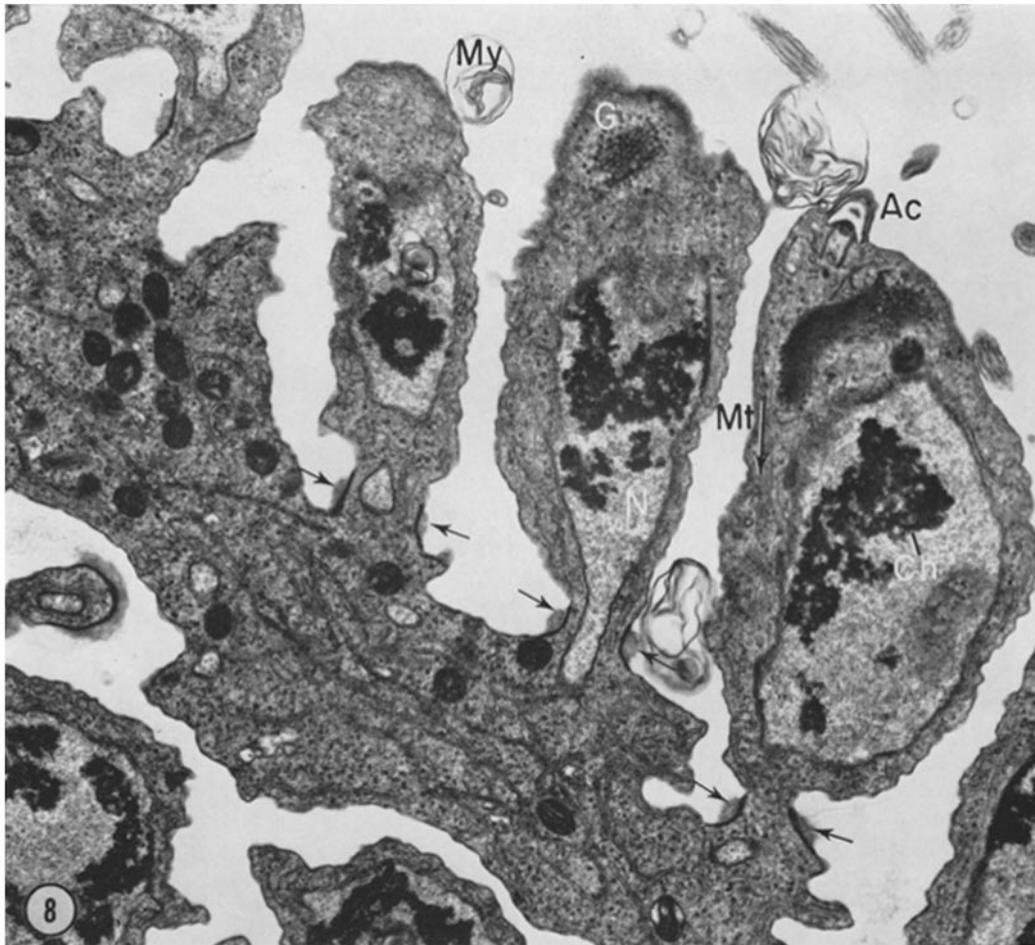


FIGURE 8 An oblique section through several spermatids in a stage of development similar to that of those shown in Figs. 6 and 7 demonstrates that nuclear condensation and elongation occur simultaneously. The spermatids are attached to a central nutrient mass by thin cytoplasmic bridges (short arrows) with an external "fuzzy coat." Numerous mitochondria and an elaborate endoplasmic reticulum are seen in the nutrient mass. *Ac*, acrosome; *Ch*, chromatin; *G*, Golgi complex; *Mt*, microtubules; *My*, myelin figures. $\times 11,500$.

framework that encases the dense intramitochondrial matrices (Figs. 23–28). As the microtubules of the manchette develop, each unit of the hexagonal complex is compressed into an elongate, triangular wedge; the cristae of the mitochondria are reduced in number and the central core of the mitochondria is reduced in diameter and density (Figs. 3, 4, 14 *a*, and 23–25). At the height of manchette development, the middle piece is encased by a thick sheath of microtubules (Fig. 24). Smooth membranes of the endoplasmic reticulum surround the manchette. Distension of the cisternae

accompanies reduction of the cytoplasm that surrounds the middle piece. Late in maturation, only a few microtubules surround the middle piece (Figs. 25–27 and 29 *a*), and finally only the plasma membrane encases the mitochondrial complex (Figs. 28 and 31).

The Basal Body and Flagellum

The centriole involved in manchette formation is also concerned with the development of the flagellum (Figs. 2, 3, and 14 *a*). At the distal region of the centriole, satellite bodies project

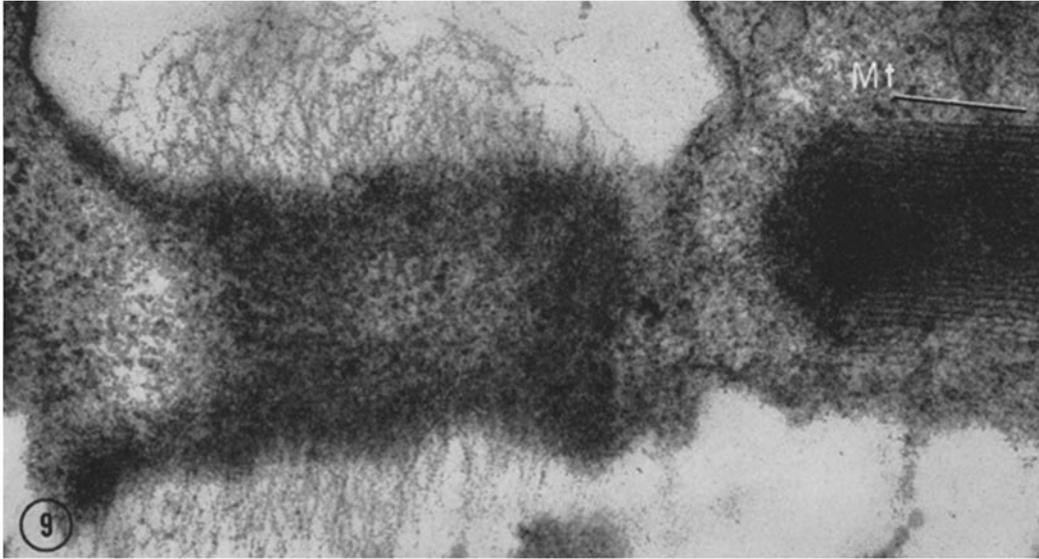


FIGURE 9 At high magnification, the zone of attachment between spermatid and nutrient mass appears as a dense cytoplasmic band from which 20-30-A filaments arise. The anterior tips of the manchette microtubules (*Mt*) terminate near the cytoplasmic bridge. $\times 54,000$.

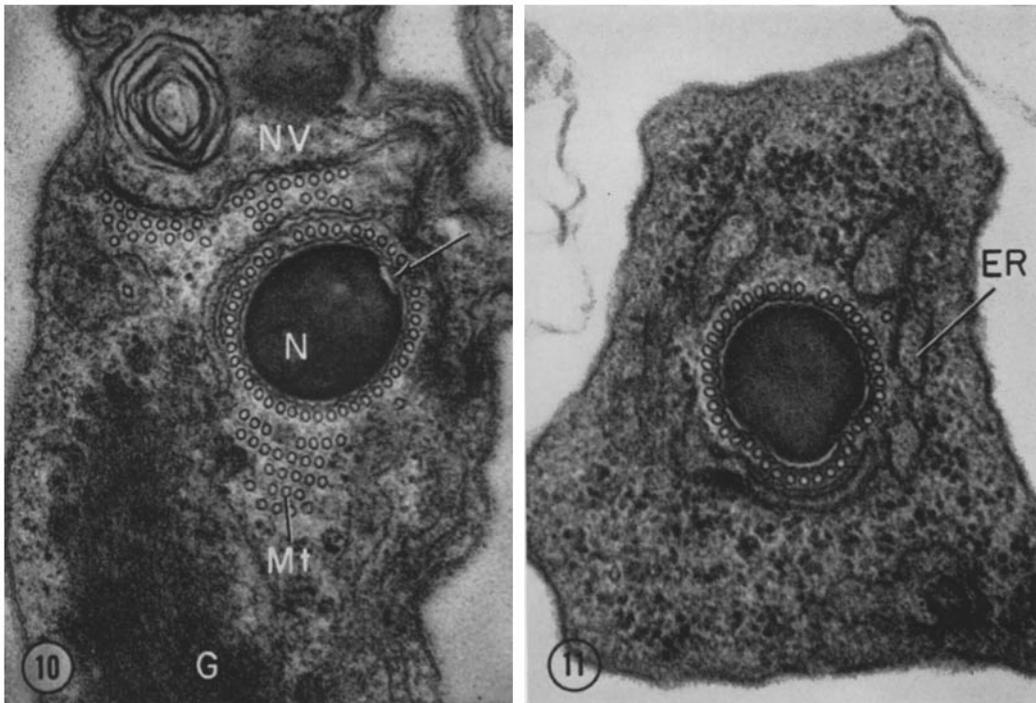


FIGURE 10 Numerous microtubules (*Mt*) surround a highly condensed nucleus (*N*) which exhibits a small irregularity (arrow) in which the extruded vesicle (*NV*) was severed from the nucleus. *G*, Golgi complex. $\times 52,000$.

FIGURE 11 Profiles of endoplasmic reticulum (*ER*) encircle the nuclear region of the spermatid at this stage of development. The cytoplasm is reduced in volume and contains numerous free ribosomes. $\times 54,000$.

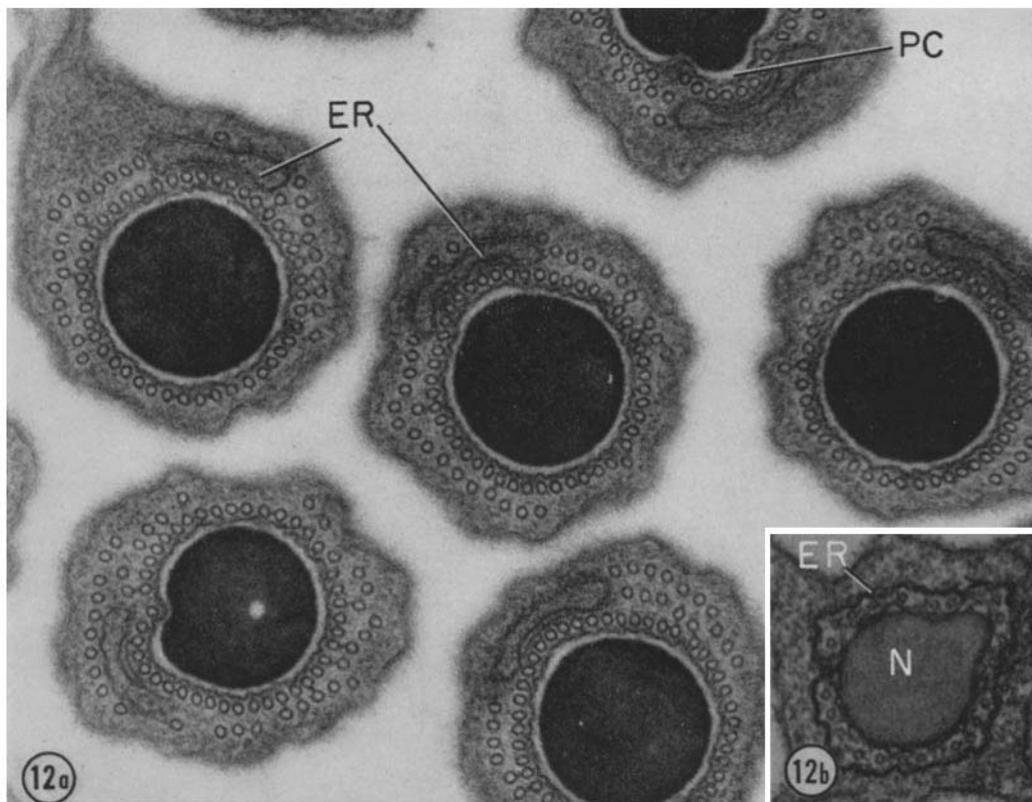


FIGURE 12 *a* At this stage of maturation a perinuclear cistern exists between the inner and outer membranes of the nuclear envelope (*PC*). Single profiles of endoplasmic reticulum (*ER*) are present around the inner band of microtubules, and the cytoplasmic volume is reduced further. $\times 64,000$.

FIGURE 12 *b* This cross-section shows the inner band of microtubules completely encircled by membranes of the endoplasmic reticulum. *N*, nucleus. $\times 50,000$.

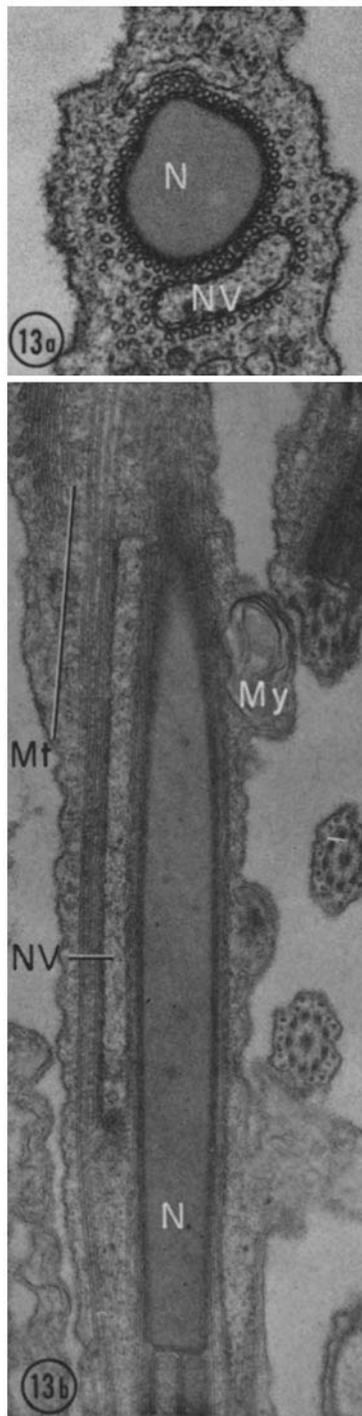
laterally into the cytoplasm (Fig. 14 *b*). Posterior to this, at the transitional zone between the centriole and the flagellum, a dense collar surrounds the peripheral microtubules of the centriole (Figs. 29 *a* and *b*). At the distal end of the centriole, a single central tubule, approximately 500 Å in diameter, gives rise to a central pair of flagellar tubules (Figs. 29 *a* and *b*). Dense granules that resemble glycogen are located between the peripheral tubules and flagellar membranes of the mature spermatozoa (Figs. 29–31). Following periodic acid–Schiff (PAS) staining of Epon-Araldite sections (Lane and Europa, 1965), the tails of the mature spermatozoa are intensely stained. However, after incubation of thick sections in saliva (Personne and André, 1964) the

PAS reactivity of the sperm tails is markedly reduced. These results indicate that the sperm tails contain a polysaccharide which is probably glycogen.

DISCUSSION

Role of the Centriole in Development of the Manchette and Flagellum

Microtubules play a significant role in the cytomorphogenetic events that lead to differentiation of the earthworm spermatid. The presence of microtubules in the cytoplasm of differentiating earthworm spermatids was demonstrated by Gatenby and Dalton (1959) and Bradke (1963 *a*). But the suggestion that the microtubules of the



manchette in spermatids of earthworm (Gatenby and Dalton, 1959) and python (Boisson and Mattei, 1965) are derived from the outer nuclear membrane is not supported by our observations. The consistent association of microtubules with the centriole and Golgi complex suggests that these organelles are involved in the production of microtubules. It is clear that the Golgi complex plays an important part in the segregation and concentration of structural proteins in developing spermatids (Fawcett, 1966). In the differentiating earthworm spermatid, the Golgi complex is involved in acrosome formation. However, the intimate association of the Golgi complex to the centriole and microtubules suggests a functional relationship. Perhaps it can be inferred that the Golgi complex sequesters precursor substances that may be utilized in the synthesis of microtubules. In early spermatid differentiation, cisternae and vesicles of the Golgi complex and microtubules are first randomly distributed around the centrioles near the nucleus. In a later stage, microtubules converge upon the centriole. Some of these centriole-associated microtubules extend anteriorly to form the manchette. Our observations, therefore, indicate that the centriole is involved in the organization of the macromolecules that form the microtubules. Such precursors are assembled into microtubules to form a scaffold that surrounds the acrosome, nucleus, and middle piece. This is supported by the fact that spindle microtubules arise from pericentriolar densities in dividing cells (Robbins and Gonatas, 1964; Anderson et al., 1966). The formation of microtubules in cilia and flagella by the basal body is correlative evidence that the development of microtubules may be induced or directed by a centriole; this has been demonstrated in a variety of cellular systems by Roth and Shigenaka (1964), Renaud and Swift (1964), and Roth et al. (1966). In addition, the distal centriole of the spermatid in the jellyfish may serve simultaneously as an attachment of spindle microtubules and as an inducer of flagellar microtubules (Szollosi, 1964). In *Lumbricus*, the centriole that serves as a spindle pole during the last meiotic division of the sperma-

FIGURE 13 *a* and *b* These micrographs reveal in cross- and longitudinal sections (respectively) how the manchette separates the nucleus (*N*) from the flattened extruded nuclear vesicle (*NV*). *My*, myelin figure. Fig. 13 *a*, $\times 55,000$. Fig. 13 *b*, $\times 36,000$.

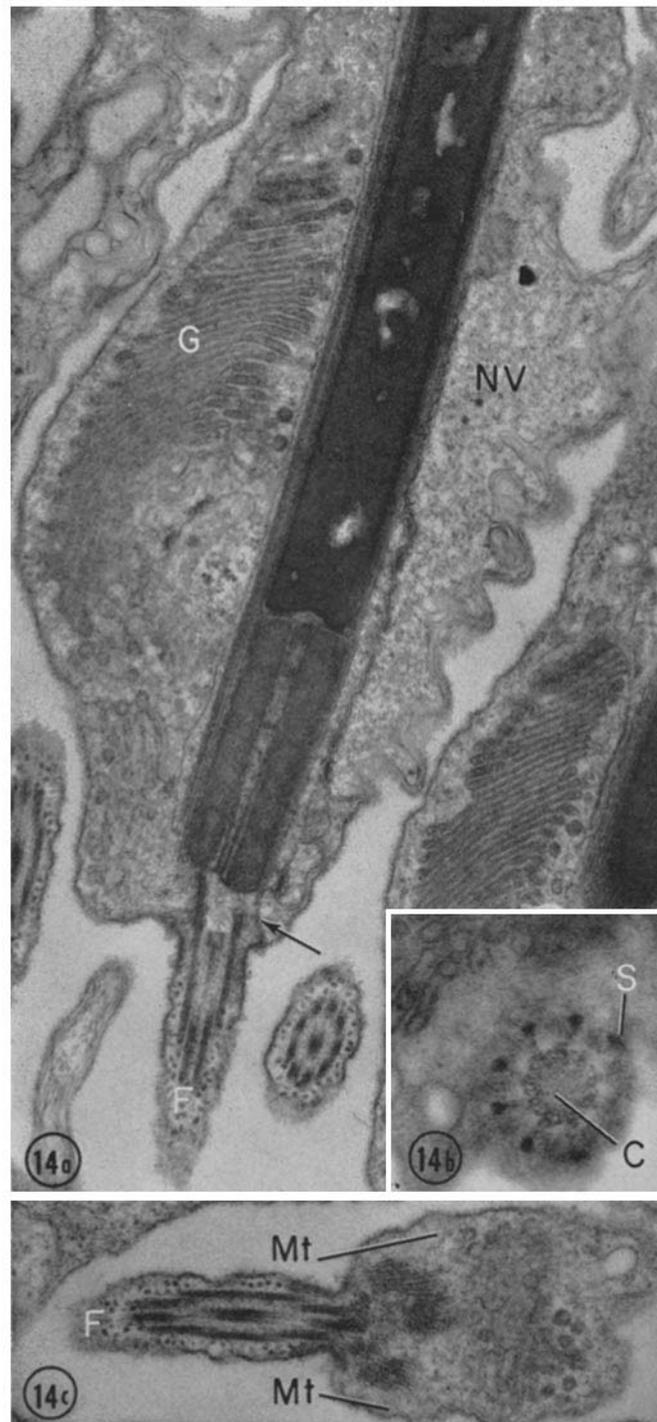


FIGURE 14 *a* In this longitudinal section through the basal portion of the spermatid, the manchette separates the elongated nucleus and middle piece from the cytoplasm, Golgi membranes (*G*), and extruded nuclear vesicle (*NV*). *F*, flagellum. $\times 40,000$. *b*, In this cross-section at the base of the flagellum (arrow, Fig. 14 *a*) dense satellites (*S*) radiate from the nine triplet tubules of the centriole (*C*). $\times 46,000$.

c, An oblique section at the same level as Fig. 14 *b* illustrates clearly the common origin of manchette and flagellar tubules. *Mt*, Microtubules; *F*, flagellum. $\times 57,500$.

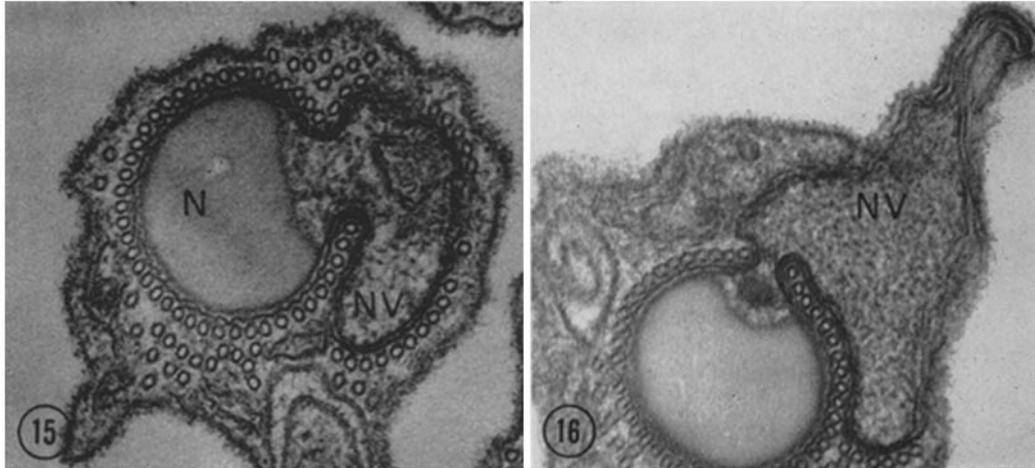


FIGURE 15 The sleeve of microtubules around the nucleus (*N*) seems to pinch off the nonchromatin nucleoplasm that is segregated into the nuclear vesicle (*NV*). $\times 96,000$.

FIGURE 16 In a stage subsequent to Fig. 15, the nearly extruded nuclear vesicle (*NV*) contains granular nonchromatin material. $\times 86,000$.

toocyte subsequently seems to induce the formation of both the manchette and flagellar microtubules.

The Role of the Manchette in Cytodifferentiation

Oriented microtubules have been demonstrated in a variety of differentiating cells (Burgos and Fawcett, 1955; Nagano, 1962; Bawa, 1964; Byers and Porter, 1964; Silveira and Porter, 1964; Anderson et al., 1966). The microtubules are regarded as resilient cytoskeletal elements that help to maintain cell shape by imparting stiffness to certain areas of the cell. The microtubules in these cells may also be involved in the internal movements of the cytoplasm and in alterations in cell shape. Our observations indicate that the system of microtubules not only determines the symmetry of the elongating spermatids but represents a specialized mechanism that maintains cell form and organization. The manchette is most extensive at the time when nuclear volume is being reduced. It may be inferred that the encircling microtubules are capable of synchronous centrifugal movement and may thus provide the motive force for extrusion of the nuclear vesicle. The directed redistribution of microtubules between the condensed nuclear material and nuclear vesicle leads to nuclear vesicle separation, segrega-

tion, and elimination. Compartmentalization and conservation may also be a function of the manchette since those elements that remain within the framework of microtubules are retained while all the cell constituents outside the scaffolding are discarded. The microtubules may also facilitate cytoplasmic reduction, but the endoplasmic reticulum seems to play a primary role in this process. Thus, the transient system of microtubules that constitutes the manchette plays a key role in the complex cytomorphogenetic processes of spermatid differentiation.

Endoplasmic Reticulum in Cytoplasmic Reduction

During the course of spermiogenesis, membranes of the endoplasmic reticulum appear in the cytoplasm outside the manchette. At the time when the manchette is highly organized, continuous membranes of the endoplasmic reticulum completely surround the system of microtubules. The flattened membranes of the endoplasmic reticulum that encircle the manchette are similar to the "mantle" described by André (1963) and Brokelmann (1961). As a result of the distension of the cistern, the peripheral thin layer of cytoplasm is separated from the central region of the spermatid which is encased by the manchette. Distension of

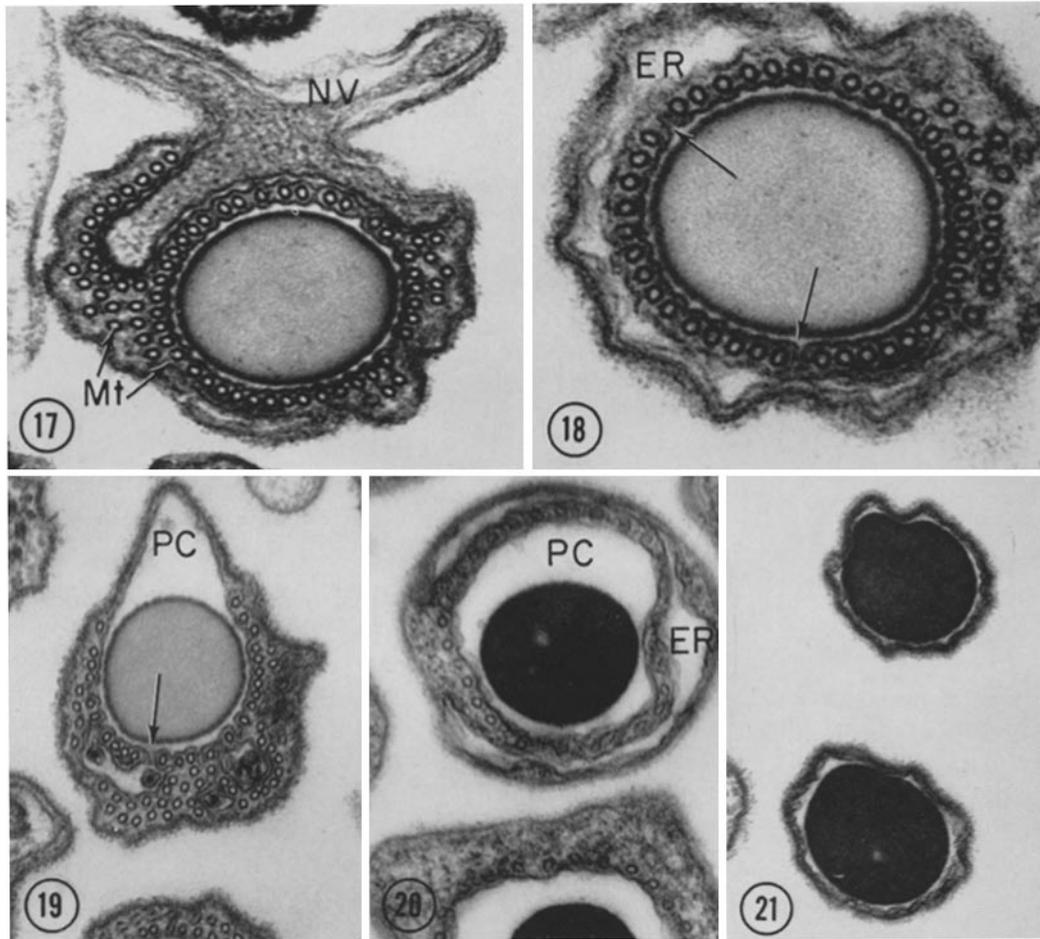


FIGURE 17 The extruded nuclear vesicle (*NV*) lies free in the cytoplasm. At this point, the cytoplasm is also greatly reduced in volume. *Mt*, Microtubules. $\times 81,000$.

FIGURES 18, 19, and 20 This series of micrographs illustrates that the perinuclear cistern (*PC*) between inner and outer membranes of the nuclear envelope becomes more distended and contacts the cisternae of the endoplasmic reticulum (arrows). Subsequent fusion of the cisternae leads to the separation of the manchette from the nucleus and the disassociation of the microtubular cytoskeleton.

Fig. 18, $\times 100,000$. Fig. 19, $\times 55,000$. Fig. 20, $\times 60,000$.

FIGURE 21 The nucleus of the mature spermatozoan is invested by a pair of closely apposed plasma membranes. The inner nuclear membrane encases the condensed chromatin. $\times 67,000$.

the cistern bounded by the outer membrane of the nuclear envelope occurs concurrently with dilation of the endoplasmic reticulum. In this manner, the manchette is separated from the nucleus and redistributed to a cytoplasmic band that is limited by the external membrane of the nuclear envelope

and the encircling endoplasmic reticulum. By subsequent fusion of these cisternae the microtubule system is disassociated, and the surrounding cytoplasm is sloughed off into the extracellular space. The endoplasmic reticulum is similarly involved in cytoplasmic reduction during spermatid differ-

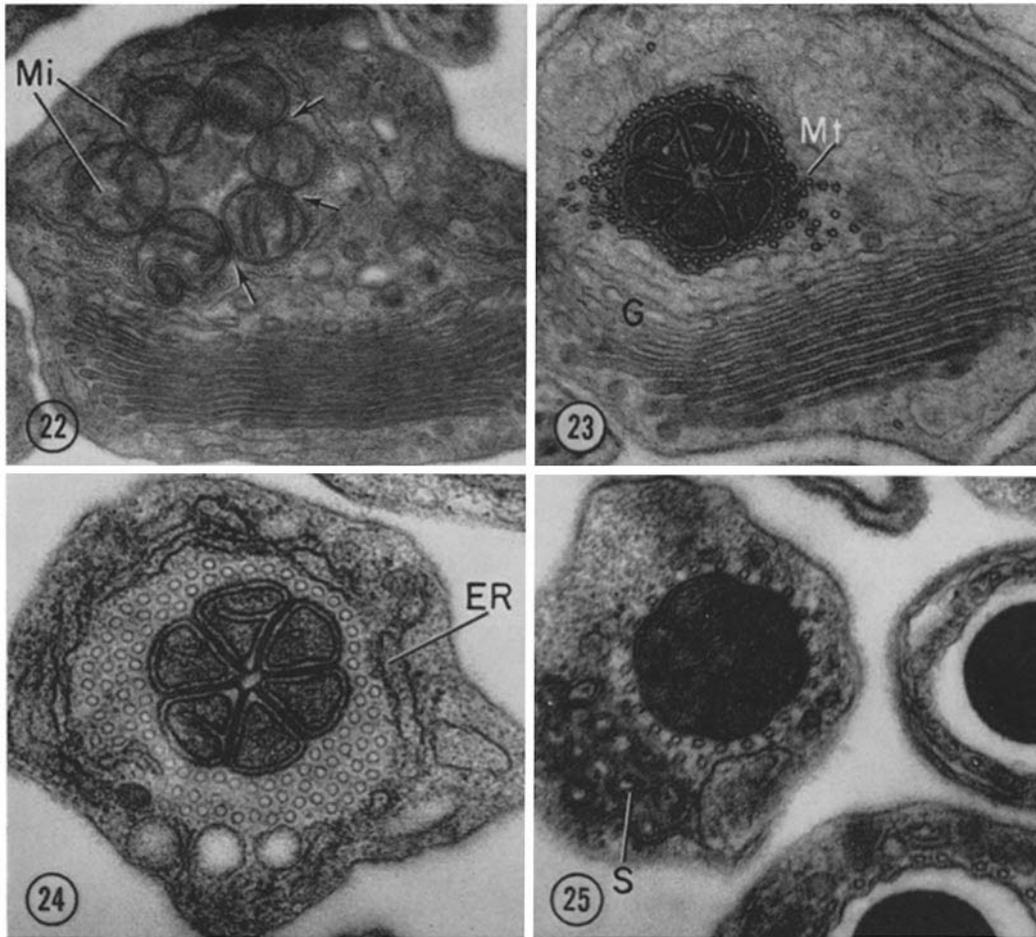


FIGURE 22 A circlet of six mitochondria (*Mi*) aggregate at an early stage in the formation of the mitochondrial middle piece. Lateral fusion of the outer membranes has occurred at several points (short arrows). The ring of mitochondria encloses a dense core of cytoplasm. The Golgi apparatus is extensive and the microtubules of the manchette partially surround the mitochondria. $\times 46,000$.

FIGURE 23 Further fusion of the outer membranes of adjacent mitochondria produces a perfect hexagonal sheath that encloses the dense mitochondrial matrices. *Mt*, microtubules. $\times 50,000$.

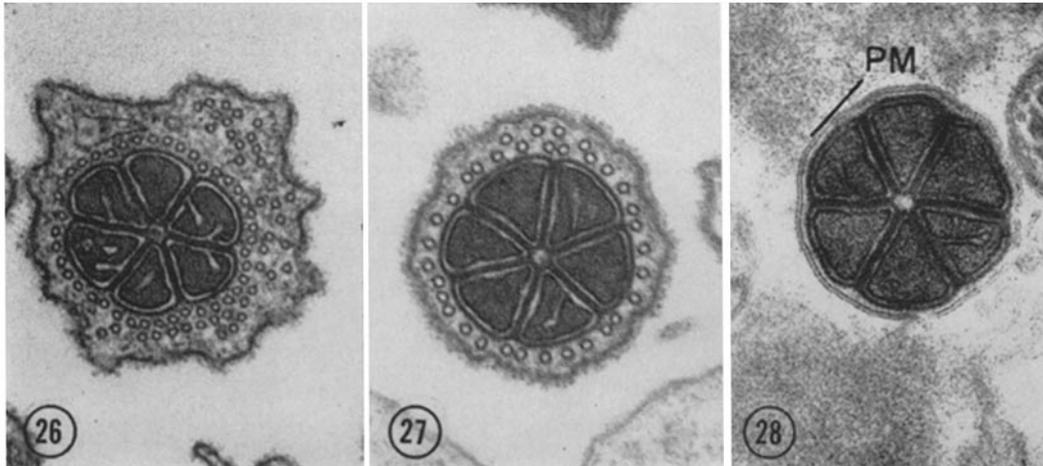
FIGURE 24 As the volume of the cytoplasm decreases around the middle piece, the endoplasmic reticulum becomes more prominent (*ER*). The fused mitochondria are surrounded by approximately 100 evenly spaced microtubules. $\times 55,000$.

FIGURE 25 The number of microtubules and the volume of cytoplasm become greatly reduced with further development of the spermatid. A highly convoluted membrane system (*S*) lies close to the manchette during spermatid differentiation. $\times 42,000$.

entiation in the silkworm (Yasuzumi and Oura, 1965). The cytoplasmic remnant is reabsorbed and remobilized by the anucleate nutrient mass, while the mature sperm are released into the lumen of the seminal vesicles.

Middle Piece Formation

Mitochondria in differentiating spermatids undergo extensive alteration in shape and substructure, and in intracellular distribution. Several



FIGURES 26, 27, and 28 This series of cross-sections of the middle piece illustrates the progressive loss of microtubules and cytoplasm, culminating in a mature middle piece surrounded solely by the plasma membrane (PM).

Fig. 26, $\times 50,000$. Fig. 27, $\times 55,000$. Fig. 28, $\times 73,000$.

mitochondria are present in the cytoplasm of the early earthworm spermatid, but only six mitochondria are mobilized to a region of cytoplasm between the nucleus and centriole. The mitochondria contain cristae that project into the matrix (Fig. 22). The mitochondrial substructure is unlike that of the atypical mitochondria in spermatids of other species where the cristae are folded or flattened against the limiting membranes (Fawcett, 1957). During the course of spermiogenesis, the spherical mitochondria are transformed into a middle piece consisting of six pyramidal subunits bound within a hexagonal pinwheel framework. The system of microtubules that encase the middle piece probably provides the motive force for lateral fusion and reorganization of the mitochondria. The compactness and position of the middle piece contribute to the integrity of this highly attenuated cell. In this capacity the middle

piece can serve effectively as an energy source for locomotion and fertilization.

Glycogen in the Flagellum

Dense granules between the peripheral tubules and the membrane of the flagellum are similar to the glycogen granules observed in spermatozoa by Personne and André (1964). The positive PAS reaction of the sperm tails demonstrates the presence of a polysaccharide in this region of the cell, and the decrease in staining intensity of the tails subsequent to incubation in saliva indicates that the polysaccharide is probably glycogen. Glycogen granules in the tail are presumably available as stores to be utilized in ATP production by the mitochondria.

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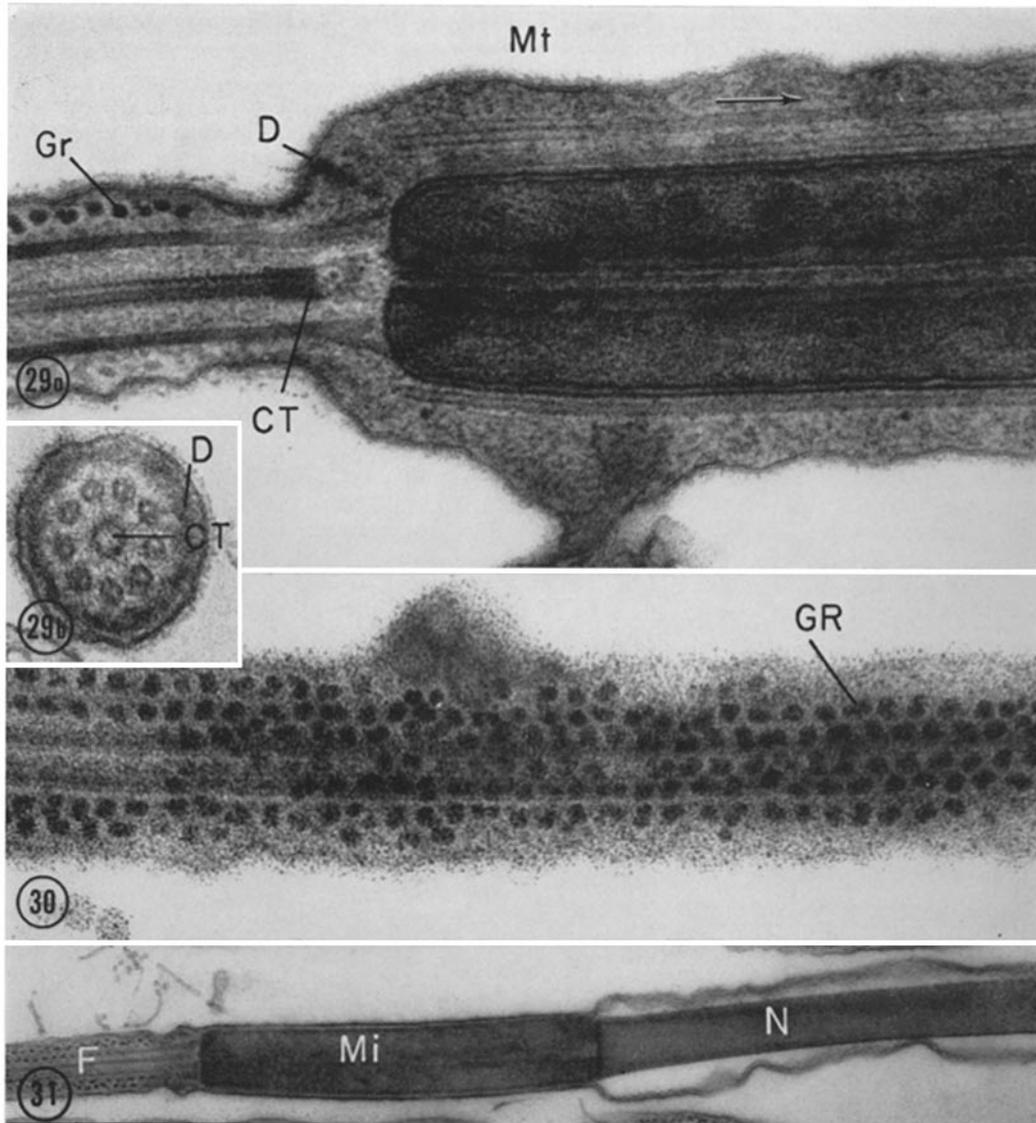


FIGURE 29 *a* In this longitudinal section through the middle piece and the base of the flagellum, the remaining microtubules of the manchette (*Mt*) converge toward a dense band (*D*) close to the base of the flagellum. The central pair of flagellar tubules fuse in this region to form a single basal tubule (*CT*) that has a diameter of approximately 500 Å. Dense granules (*Gr*) are arranged in a linear pattern between the peripheral tubules and the plasma membrane. $\times 75,000$. *b* Where the flagellum emerges from the neck piece of the spermatozoan, a continuous dense collar (*D*) surrounds the flagellar microtubules. At this level a single central tubule (*CT*) is indicated. $\times 75,000$.

FIGURE 30 A tangential section through the sperm tail reveals the regular arrangement of dense glycogen granules (*Gr*) around the peripheral flagellar tubules. $\times 75,000$.

FIGURE 31 A longitudinal section of a mature spermatozoan shows the flagellum (*F*) and middle piece (*Mi*) encased only by the plasma membrane, and the elongated nucleus (*N*) surrounded by the outer nuclear and plasma membranes. The inner nuclear membrane remains in contact with the condensed chromatin. $\times 12,000$.

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