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AN ULTRASTRUCTURAL STUDY OF PLANT SPERMATOGENESIS

Spermatogenesis in *Nitella*

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ABSTRACT

Spermatogenesis in the charophyte *Nitella* has been followed in antheridia prepared for light and electron microscopy. The antheridial filament cells contain paired centrioles which are similar in structure and behavior to the centrioles of animal cells. In the early spermatid, the centrioles undergo an initial elongation at their distal ends and become joined by a spindle-shaped fibrous connection. At the same time, their proximal ends are closely associated with the development of a layer of juxtaposed microtubules which will form the microtubular sheath. The architectural arrangement of these microtubules suggests that they constitute a cytoskeletal system, forming a framework along which the mitochondria and plastids become aligned and along which the nucleus undergoes extensive elongation and differentiation. The microtubular sheath persists in the mature sperm. During mid-spermatid stages, the centrioles give rise to the flagella and concomitantly undergo differentiation to become the basal bodies. The Golgi apparatus goes through a period of intensive activity during mid-spermatid stages, then decreases in organization until it can no longer be detected in the late spermatid. An attempt is made to compare similarities between plant and animal spermiogenesis.

INTRODUCTION

Although animal spermatogenesis has received a good deal of attention at the ultrastructural level in recent years, our knowledge of plant spermatogenesis is still quite limited. Flagellated plant sperm found in many of the nonflowering plants are functionally comparable to animal sperm, serving as carriers of genetic material for sexual reproduction. When observed at a gross level, these plant sperm appear to differ significantly from animal sperm in structure; however, at the ultrastructural level many similarities become apparent.

Among the cryptogams, the biflagellate sperm of the bryophytes and some species of algae most closely approximate the "typical" animal sperm in structure. These plant sperm possess a motility apparatus, condensed nucleus, and a localized aggregation of mitochondria, just as do many animal sperm; however, the acrosome, a conspicuous organelle of most animal sperm, has not been demonstrated in plants. Other organelles, such as plastids, are limited to plants.

A comparison of underlying developmental similarities present in the spermatogenesis of diverse types of organisms should lead to a better understanding of the integrated sequence of events which give rise to the mature sperm. Therefore, a study of spermatogenesis in the charophyte, *Nitella*, was undertaken for the purpose of comparing sperm formation in a representative plant with our

present knowledge of animal spermatogenesis. *Nitella* was chosen for this study because of its availability in different stages of development and the relative ease with which it may be handled.

Perhaps the most unusual aspect of spermatogenesis in plants, and one in which plant systems differ significantly from most animal systems, is the cyclic nature of the centrioles. In animals, the centriole can be considered a permanent cell organelle, replicating in phase with cytokinesis and passing from somatic to germ cell line in an unbroken sequence. Although centrioles have not been found in cells of the flowering plants, they are evident in a number of the cryptogams. In many instances, these centrioles are found only during certain portions of the plant's life cycle, notably during those stages leading up to the formation of motile gametes. In these organisms it appears as though centrioles per se are not permanent cell organelles and are not passed from vegetative to reproductive cell lines. Because of widespread interest in this organelle, much of this research concentrated upon the structure and behavior of the centriole in *Nitella* and a comparison of this centriole with its animal counterpart.

A number of investigators (de Harven and Bernhard, 1956; Amano, 1957; Bessis, 1957; Bernhard and de Harven, 1960; Gall, 1961; André and Bernhard, 1964; Fawcett, 1966) have reported on the fine structure of the typical animal centriole. The paired centrioles of most animal cells undergo replication at a specific time during division and apparently play a role in the architecture of the spindle (Amano, 1957; Inoué, 1964; Roth and Shigenaka, 1964; Krishan and Buck, 1965; Murray et al., 1965).

Several postulates concerning centriole replication have been advanced, based on electron microscopic observations. The most generalized mode of centriole replication is thought to be by a mechanism of procentriole formation (Gall, 1961), with the formation of one new centriole in association with each parent centriole prior to karyokinesis. A variation in this mode of replication, involving the formation of multiple centrioles in association with a single parent centriole, has also been described.

The question of the appearance or formation of multiple centrioles in the absence of a parent centriole has been examined by several investigators in recent years (Stockinger and Cireli, 1965; Dirksen and Crocker, 1965; Mizukami and Gall, 1966). Unfortunately, none of these investigations gives a definitive answer to the problem of the apparent de novo origin of centrioles. Dirksen and Crocker (1965) postulate that a nucleic acid template may be involved in the formation of multiple centrioles; however, the evidence for this is largely circumstantial.

Early electron microscopic studies confirmed a structural similarity between centrioles and the basal bodies of cilia (Fawcett and Porter, 1954; Burgos and Fawcett, 1956). This similarity has become more evident with the application of the newer preparative techniques (Gibbons and Grimstone, 1960; Fawcett, 1961; Reese, 1965; see also reviews by Sleigh, 1962, and Rivera, 1962). Studies of ciliogenesis in various metazoan cells have shown the participation of centrioles in the formation of cilia and flagella (Sotela and Trujillo-Cenóz, 1958 *a*, *b*; Bertaud and Gatenby, 1960; Gall, 1961; Sorokin, 1962; Szollosi, 1964).

A few cryptogam centrioles, observed by means of the electron microscope, have been described in the literature (Berkaloff, 1963; Berlin and Bowen, 1964; Renaud and Swift, 1964). In the stages in which they have been seen, they appear to be similar in most respects to the centrioles of animal cells.

The cyclic nature of the centrioles in *Nitella*, characterized by their appearance only in the reproductive cells, is a puzzling phenomenon. The present study is intended to provide information on centriole structure and behavior during spermatogenesis in *Nitella* and does not deal with the question of the ultimate origin of the centriole in this plant.

MATERIALS AND METHODS

Locally collected plants of the charophyte *Nitella missouriensis* Allen[1] were utilized. For the initial part of the study, standard light microscope techniques were employed. These include Feulgen- and aceto-carmine-stained squash preparations and hematoxylin-stained paraffin sections, as well as osmium tetroxide vapor-fixed squash preparations examined with phase-contrast optics.

For the ultrastructural studies, antheridia at various stages of development were fixed for 2 hr in 2.5% glutaraldehyde buffered to pH 7.2 with 0.05 M sodium cacodylate (Sabatini et al., 1963). After rinsing with the buffer, the antheridia were post-

[1] The author is indebted to Dr. Takashi Sawa for identification of the species of *Nitella* used in this study.

fixed in 1% osmium tetroxide buffered with sodium cacodylate (pH 7.2), stained overnight with 0.5% aqueous uranyl acetate, and embedded in a mixture of Epon and Araldite (mixture No. 1 of Mollenhauer, 1964). Thin sections on uncoated grids were stained with lead citrate (Reynolds, 1963) and examined with an RCA EMU 3D or 3F electron microscope. The diagrammatic reconstruction of a *Nitella* sperm (Fig. 30) was derived from the light and electron microscopic data.

LIGHT MICROSCOPE OBSERVATIONS

Spermatogenesis in *Nitella* takes place within specialized fruiting structures, the antheridia, which develop at the nodes of the branchlets (Fritsch, 1935). Within the antheridium, the reproductive cells are found in the form of numerous antheridial filaments associated with a number of accessory vegetative cells (Fig. 1). Each filament is derived from an antheridial initial cell which undergoes a succession of transverse mitotic divisions.

Stained squash preparations of the developing antheridia demonstrate that the individual filaments contain long segments which are synchronized with respect to a given division stage (Fig. 2). The cells resulting from the last antheridial division are the spermatids, the protoplasts of which differentiate within the cell walls to form the mature sperm. The young spermatids are characterized by a dense "shell" of Feulgen-positive material just within the nuclear envelope. This material is more clearly demonstrated by electron microscopy (see Figs. 12, 13).

As spermiogenesis progresses, the protoplast recedes from the cell wall. From this point on, each spermatid develops as a separate entity within its old cell wall. It is noteworthy that the synchrony described earlier for the division stages of the antheridial filament cells persists through the remainder of spermiogenesis despite the loss of intercellular connections.

The nuclei, which in the early spermatid assume a characteristic "shell" configuration, soon stain homogeneously and begin to differentiate, assuming a crescent shape when viewed in squash preparations (Fig. 3). During later spermatid stages, the nuclei elongate and begin to coil. Although not demonstrated in the figures presented here, this differentiation is accompanied by corresponding changes in the shape of the protoplast. The nucleus of the mature sperm appears as a slender, condensed rod, tapering at both ends and making about two and one-half turns within the antheridial cell wall (Fig. 4).

At maturity the antheridium breaks open, freeing the antheridial filaments. The sperm begin to move within the confines of their walls, and shortly afterward they are released through pores in the walls (Smith, 1955) (Fig. 5). The free-swimming sperm, after uncoiling, are about 60 μ long with two flagella of approximately the same length attached subterminally near the anterior end of the sperm. The flagella are directed posteriorly.

Three different regions can be distinguished within the body of the free sperm (Fig. 6). Progressing posteriorly from the anterior end of the sperm, these include (*1*) a linear array of mitochondria extending about one-fourth the length of the cell, (*2*) the elongate nucleus which occupies about one-half the cell, and (*3*) an aggregation of plastids in the posterior portion of the sperm. The contents of the sperm cell appear closely packed with little, if any, excess cytoplasm surrounding them.

ULTRASTRUCTURAL OBSERVATIONS

The ultrastructural studies were begun at a stage in which the antheridial filaments had already been initiated. The cells of the antheridial filaments, following a series of equational divisions, become spermatids, which differentiate to become the mature sperm. Emphasis has been placed on early and mid-spermatid stages, since it is during these stages that the major structural changes take place in the cell. Special consideration has been given to the centrioles because of their presence only in the reproductive cell line.

The early antheridial filament cells are cylindrical in shape with a length several times greater than their diameter. They are joined to their neighboring cells by numerous, well developed intercellular connections, and a pair of centrioles is found in close association with the nucleus of each cell. In the interphase cell, the centrioles are usually observed with their long axes parallel to each other and perpendicular to the surface of the nucleus (Fig. 7). They are separated from the nuclear envelope by a distance of about 60 mμ.

The centrioles follow a division cycle similar to that seen in animal cells. During early prophase stages, two pairs of centrioles are found on opposite sides of the nucleus (Fig. 8). The two centrioles of a pair occur at right angles to each other and retain this arrangement during subsequent division

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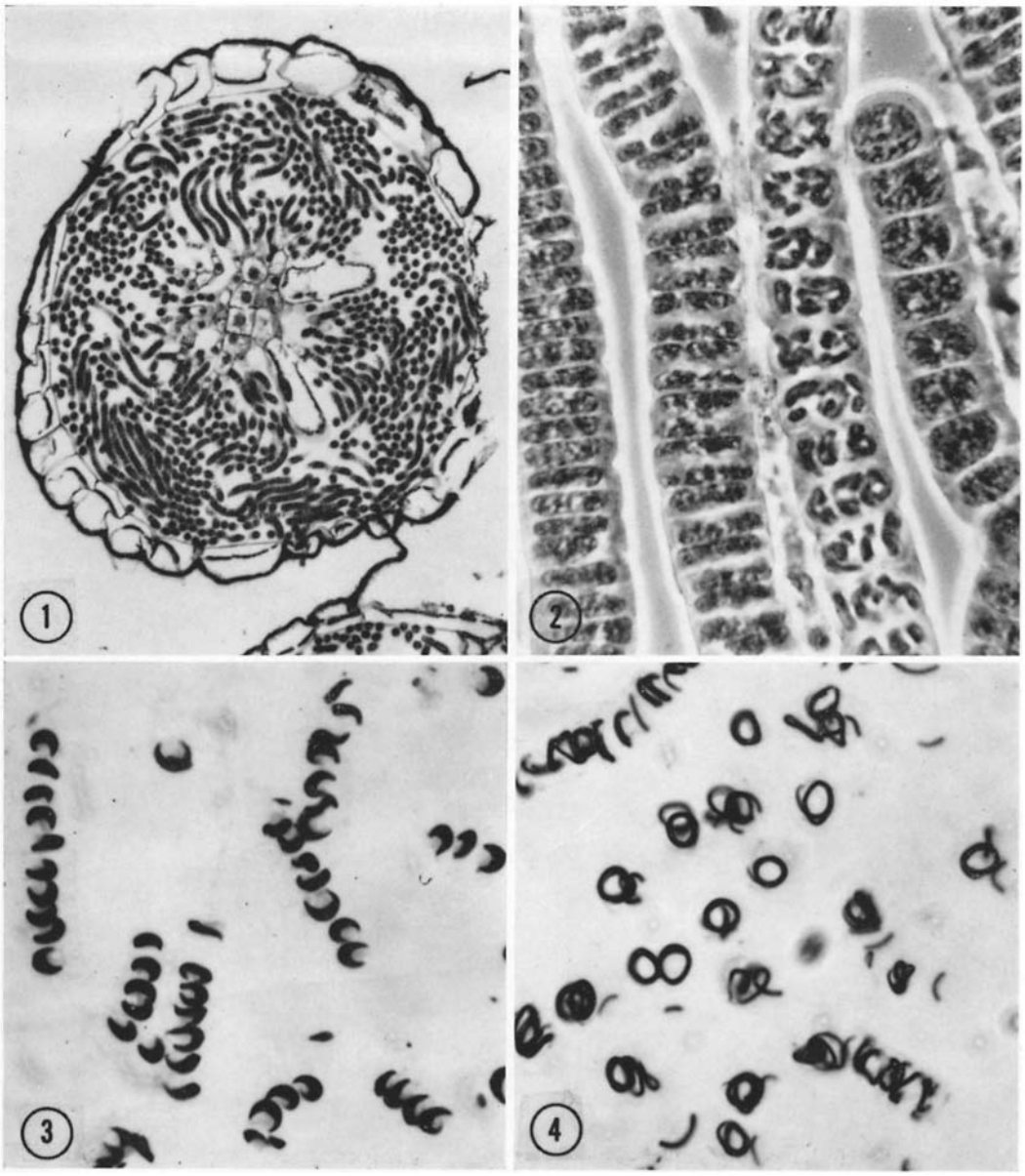

FIGURE 1 Light micrograph of a section through an antheridium showing the numerous antheridial filaments and associated vegetative cells. Hematoxylin-stained paraffin section. × 170.

FIGURE 2 Acetocarmine-stained squash preparation of a group of antheridial filaments showing long segments of the filaments in synchronous stages of division. × 2,300.

FIGURE 3 Feulgen-stained squash preparation of a mid-spermatid stage in which the nuclei have begun to elongate and coil. × 770.

FIGURE 4 Feulgen-stained squash preparation of a late spermatid stage showing the nuclei after the completion of elongation. × 1,150.

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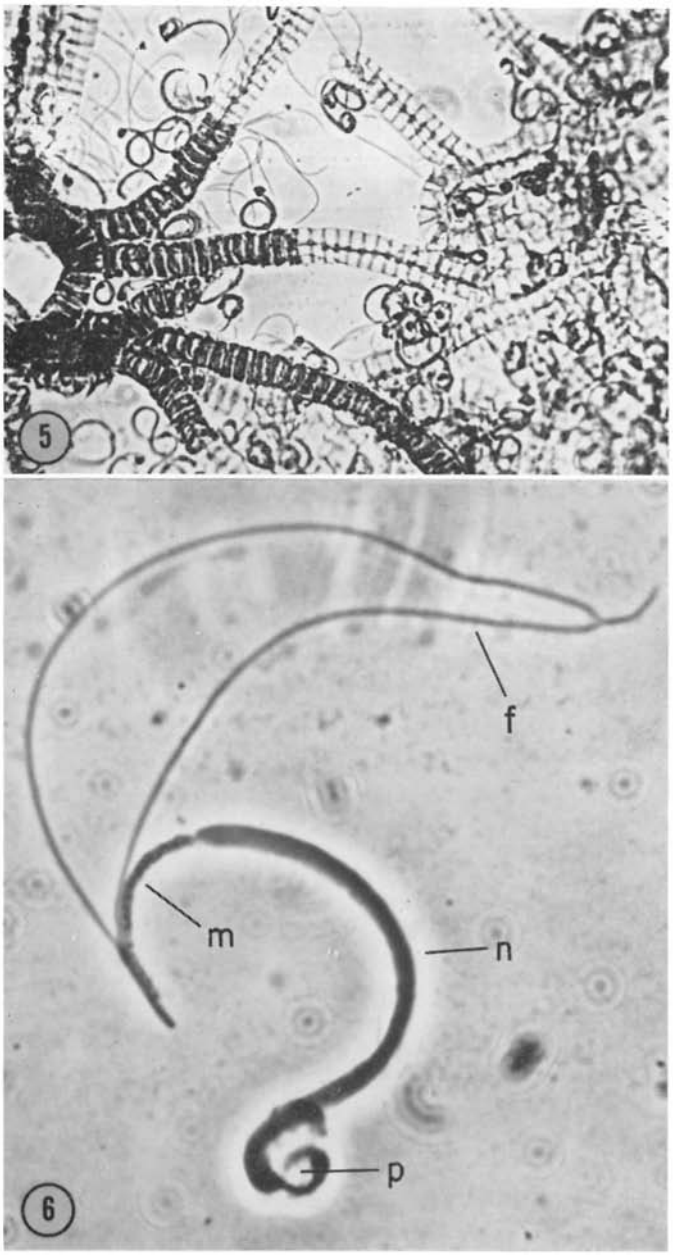

FIGURE 5 An OsO_4 vapor-fixed preparation of antheridial filaments at the time of sperm release. The darker portions of the filaments contain sperm, while the lighter portions are the empty cell walls from which the sperm have escaped. × 670.

FIGURE 6 Phase-contrast micrograph of an OsO_4 vapor-fixed sperm. *f*, flagella; *m*, mitochondria; *n*, nucleus; *p*, plastids. × 2,700.

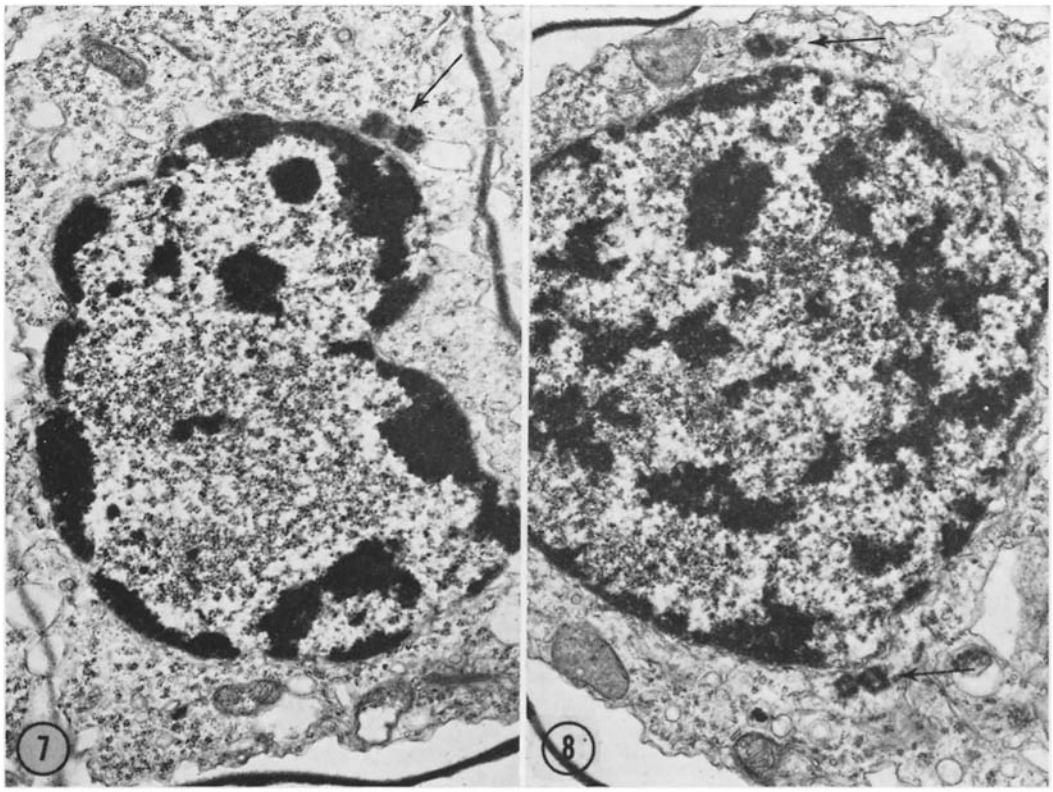

FIGURE 7 An interphase cell with two parallel centrioles (arrow) in the vicinity of the nuclear envelope. × 14,000.

FIGURE 8 An early prophase cell showing two pairs of centrioles (arrows) near the prospective spindle poles. × 14,000.

stages. One centriole of each pair may be shorter than the other during early prophase, but by late prophase they are usually the same length.

Centriole replication was not observed in this material; however, from the foregoing observations it is concluded that replication takes place during late interphase or very early prophase. During later prophase stages, the two pairs of centrioles move away from the nucleus and assume positions at, or near, the prospective spindle poles (Fig. 8). During this stage, a number of apparently randomly oriented microtubules are frequently seen in the vicinity of the centrioles (not illustrated).

During metaphase and anaphase, a well developed spindle is found, which appears to be made up of microtubules with a diameter of about 180 A (Figs. 9, 10). Kinetochores have been found on the chromosomes, providing points of attachment for some of the spindle fibers (Fig. 10). In a study of a substantial number of separate samples, no direct attachment of the spindle fibers to the centrioles was found, nor were any structures found which might be centriole derivatives to provide points of attachment for the spindle fibers at the poles. Consequently, it is not known whether the centrioles actually form the spindle apices or only occur in the general region of the spindle pole. During telophase, the centrioles are again found parallel to each other (Fig. 11).

For convenience, the spermatids of *Nitella* can be roughly classified as belonging to one of three developmental stages: early, mid, or late spermatid. These classifications are based on morphological appearances during successive stages of spermiogenesis and are intended to serve as markers when

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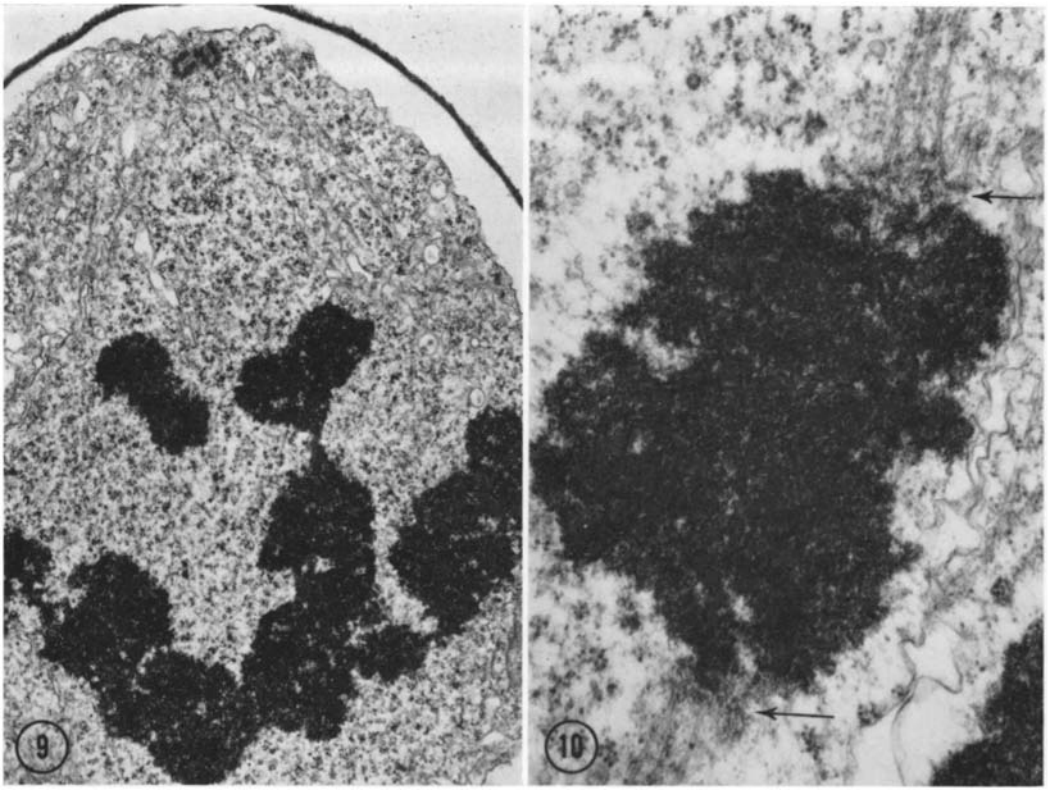

FIGURE 9 An early anaphase cell showing paired centrioles at one of the spindle poles. × 12,000.

FIGURE 10 A pair of metaphase chromosomes showing attachment of the spindle fibers at the kinetochore regions (arrows). × 36,000.

one is considering the events leading up to sperm maturation. Briefly stated, the three stages can be recognized by the following criteria: The early spermatid or shell stage occurs soon after the last antheridial filament cell division and is characterized by an accumulation of chromatin just within the nuclear envelope (Figs. 12, 13). During this stage, the protoplast becomes progressively separated from the cell wall, the centrioles elongate, and the microtubular sheath of the sperm is initiated. Increased activity of the Golgi apparatus is evidenced by a large increase in the size and complexity of this organelle and its associated vesicles. The Golgi apparatus does not appear to be in direct association with the centrioles, as is the case in many types of animal cells (Fawcett, 1966), but is located at the opposite side of the spermatid.

During the mid-spermatid stages, the chromatin undergoes a redistribution with a subsequent change in the shape of the nucleus (Figs. 14, 15). In addition, the mitochondria aggregate at one end of the spermatid and the plastids at the opposite end. The Golgi apparatus shows apparent atrophy, presumably associated with a decrease in its activity. The microtubular sheath continues to enlarge, and the flagella are elaborated near the anterior end of the spermatid.

Final maturation of the sperm takes place during late spermatid stages. In addition to a further condensation and coiling of the nucleus, there is a reduction of most of the matrix cytoplasm of the spermatid. The plastids in the posterior portion of the spermatid become filled with electron-transparent grains, and the motility apparatus is completed. Finally, the sperm becomes motile, and is released through a pore in the antheridial cell wall.

The separation of the protoplast from the an-

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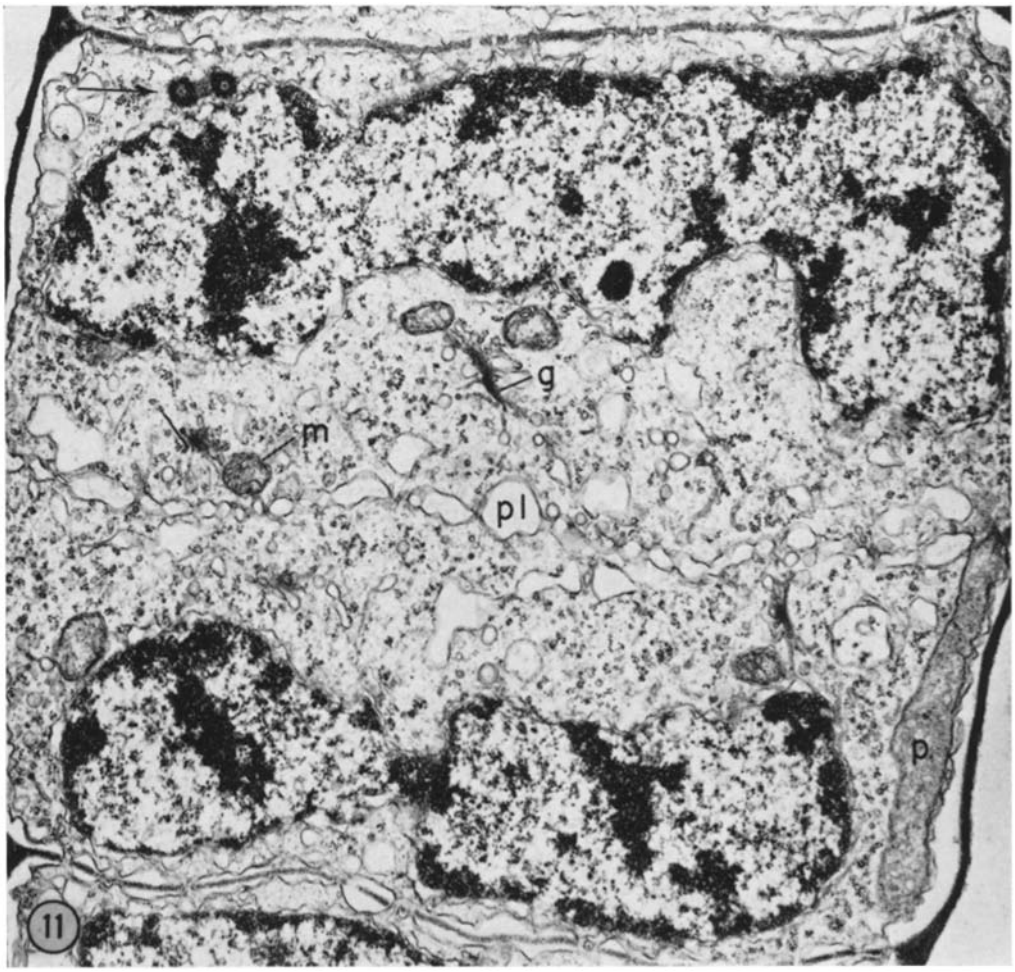

FIGURE 11 A cell in telophase illustrating the various organelles of the pre-spermatid antheridial filament cell. The cell plate is forming between the daughter nuclei. The centrioles of one daughter cell can be seen in a parallel arrangement (arrow). *pl*, cell plate; *g*, Golgi apparatus; *m*, mitochondrion; *p*, plastid. × 16,000.

theridial cell wall is a gradual process, beginning in the early protoplast. As the plasma membrane withdraws from the cell wall, the intercellular connections are temporarily retained, giving the protoplast a very irregular outline (Fig. 12). As the space between the spermatid and the wall increases, it becomes filled with a flocculent-appearing material of unknown origin. The separation continues until, except for a small region on the side, the plasma membrane is completely withdrawn from the wall. It is in this area that the pore develops through which the mature sperm will escape (Figs. 13, 14).

One of the earliest changes in the spermatid is the accumulation of electron-opaque material around the peripheral part of the nucleus, just within the nuclear envelope (Figs. 12, 13). The dense material of the shell is particulate in nature and of somewhat uneven distribution. The inner portion of the nucleus during this stage is loosely filled with granules which have a diameter of about 35 mμ.

An additional feature of the shell stage is a marked increase in the size of the Golgi apparatus, accompanied by an apparently large increase in activity, as evidenced by the profusion of Golgi-

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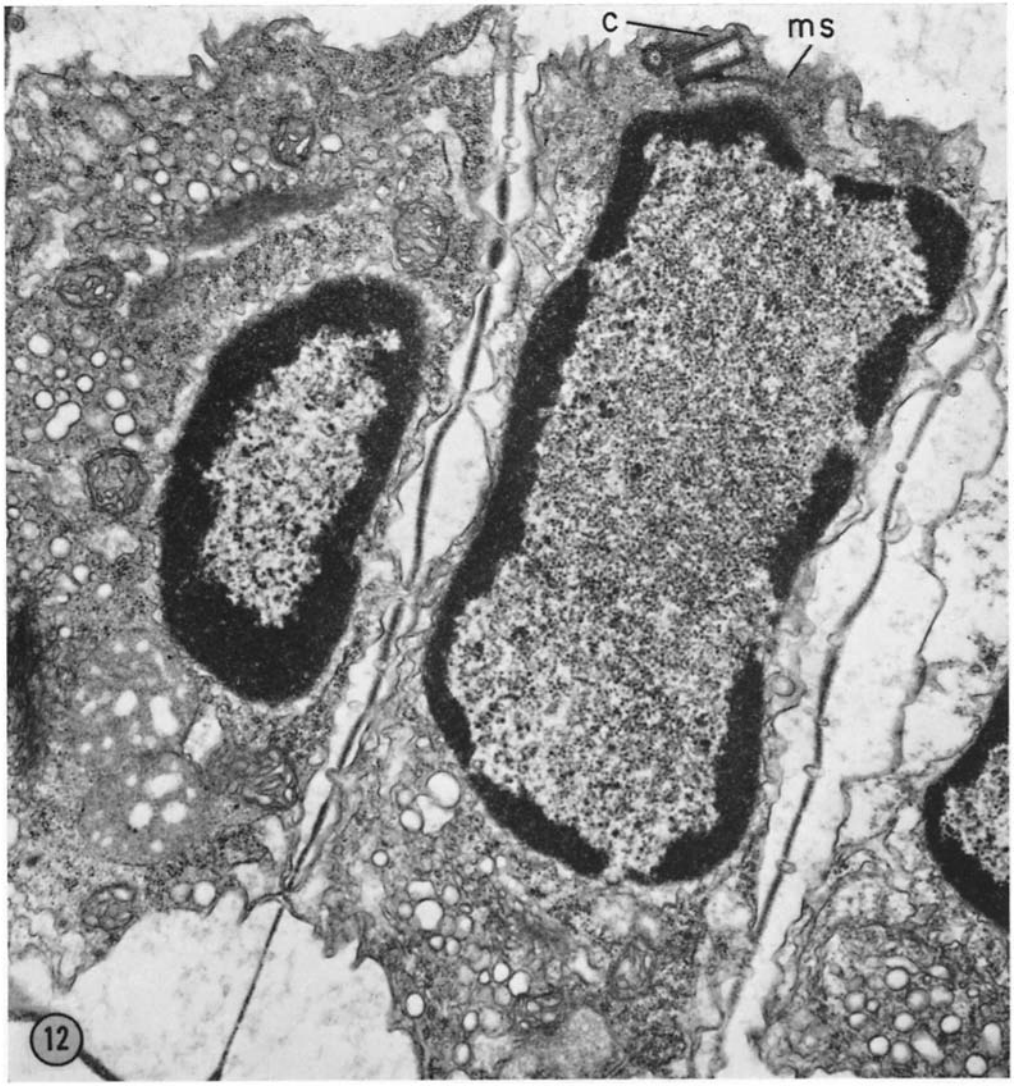

FIGURE 12 An early shell stage spermatid and portions of two adjacent cells. The centrioles (*c*) have undergone their initial elongation and portions of the microtubular sheath (*ms*) may be seen in their immediate vicinity. The protoplasts are beginning to separate from the cell wall, breaking some of the intercellular connections. × 19,000.

associated vesicles (Fig. 13). The Golgi apparatus remains active for a considerable period, regressing in late spermatid stages until it can no longer be detected in the mature sperm. This study gave no evidence of the direct shedding of the Golgi apparatus and residual cytoplasm characteristic of late spermatid stages in many animal species.

Nuclear changes following the shell stage involve a redistribution of the nuclear material and a gradual differentiation of this material into long filamentous profiles or fibrils which run roughly parallel to the long axis of the elongating nucleus (Fig. 15). These fibrils are composed of a number of subfibrils wound about one another to form a long multistranded helix. Neither the number of subfibrils making up a helix nor the total length of a helix was determined in this study. There is no obvious correlation between the number of fibrils,

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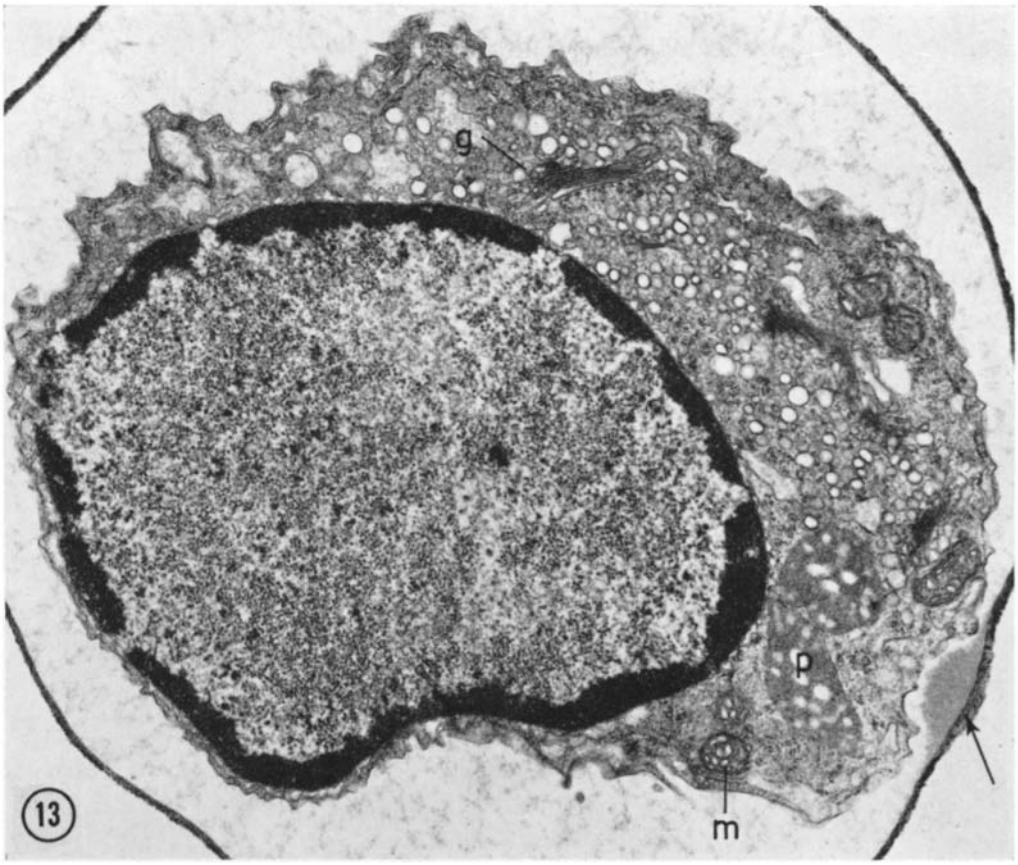

FIGURE 13 An early shell stage spermatid sectioned in a plane at approximately right angles to the cell in Fig. 12. An early stage of pore formation (arrow) can be seen in the vicinity of the plastids. *g*, Golgi apparatus; *m*, mitochondrion; *p*, plastid. × 16,000.

which appears to be very large, and the chromosome number, which for this species of *Nitella* is only six. Following this stage, the nuclear contents go through a number of complex changes before the sperm reaches maturity. The nucleus of the mature sperm appears very electron opaque and homogeneous (Fig. 28 *g*).

The earliest evidence of pore formation is seen during the shell stage when a small amount of granular material appears between the spermatid membrane and the cell wall (Fig. 13). As spermiogenesis progresses, this material increases in amount, and during late stages the spermatid becomes completely separated from the cell wall. The wall breaks down where it is in contact with the deposited material, and a pore is formed, which immediately becomes plugged with the material (Fig. 14). At the time of sperm release, the material forming the plug is broken down, permitting free passage of the sperm cell through the wall.

The centriole of *Nitella* is a cylindrical structure with a diameter of about 200 mμ and a length of 300 mμ (Figs. 16–18). The wall of the cylinder is composed of nine triplet fibers running the length of the centriole, embedded in an electron-opaque matrix. The subfibers of each triplet have a diameter of about 180 A and are composed of a wall approximately 50 A thick surrounding a lumen in which no structure has been demonstrated.

Transverse sections through the proximal end of the centriole show a well defined cartwheel structure in the center of the lumen (Fig. 17). This structure consists of a central "hub" with "spokes" radiating out toward the nine triplet fibers. The triplet fibers are inclined at an angle to the edge of the centriole, and the "spokes" appear to connect

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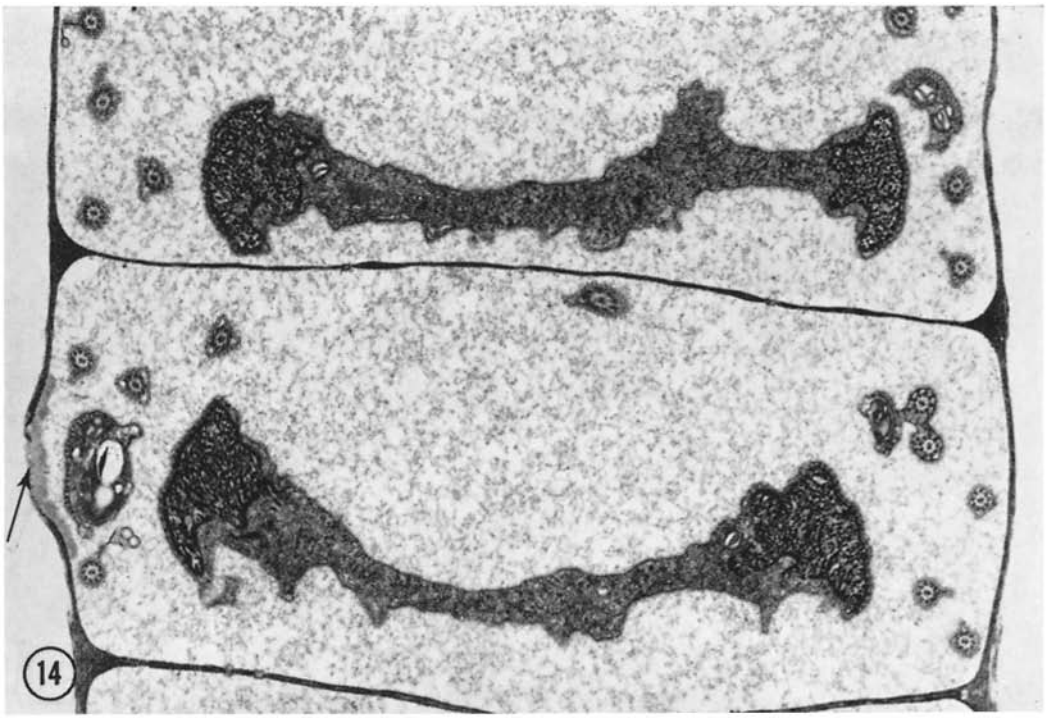

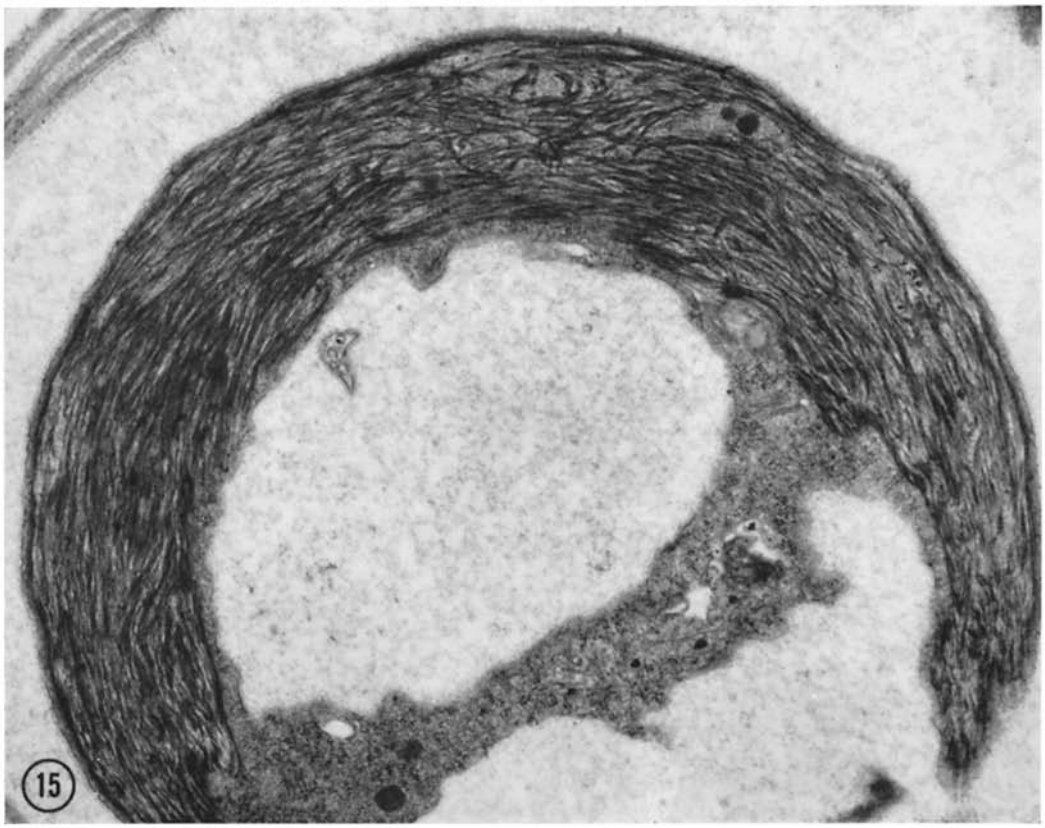

FIGURE 14 Transverse sections through two mid-spermatids intersecting the coiled nuclei as well as the mitochondrial and plastid regions and the flagella of the cells. In the cell wall near the plastid can be seen the developing pore through which the mature sperm will escape (arrow). × 15,000.

FIGURE 15 A longitudinal section through a portion of the differentiating nucleus of a mid-spermatid. × 23,000.

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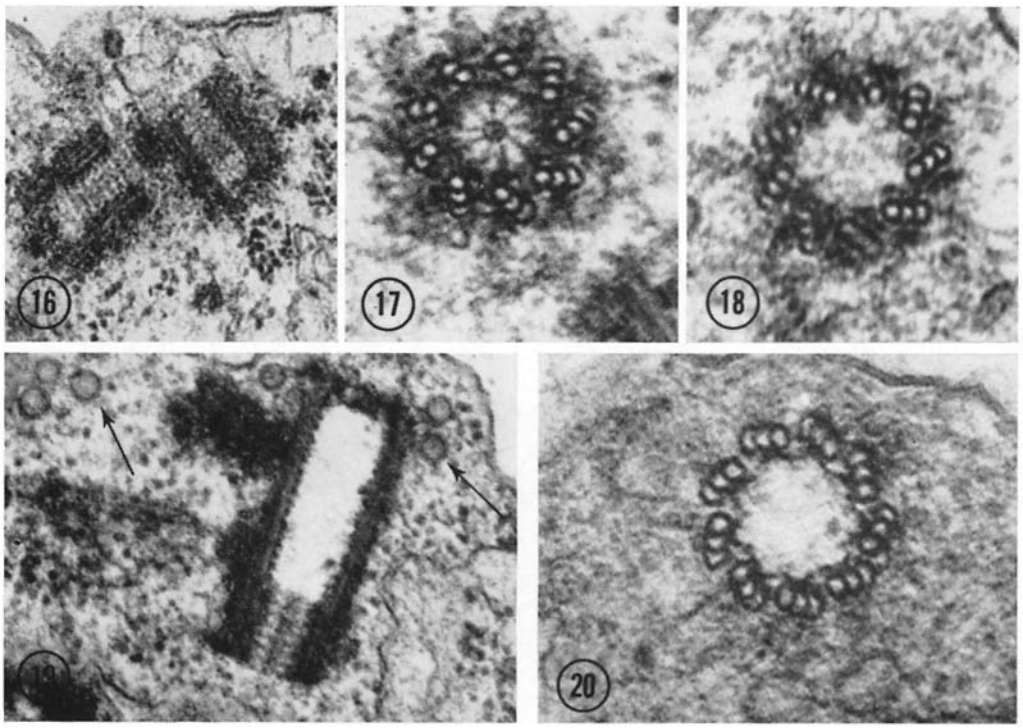

FIGURE 16 An enlargement of the pair of centrioles seen in Fig. 9. Both centrioles have been sectioned longitudinally. × 62,000.

FIGURE 17 A transverse section through the proximal end of a centriole showing the cartwheel structure in the lumen. × 118,000.

FIGURE 18 A transverse section through the distal end of the centriole. × 116,000.

FIGURE 19 A longitudinal section through the elongated centriole of an early spermatid. Several centriole-associated vesicles (arrows) can be seen near the distal end of the centriole. × 84,000.

FIGURE 20 A transverse section through the distal end of an elongated early spermatid centriole showing connections between adjacent triplet fibers. × 124,000.

with the innermost subfiber of each triplet. In addition, there is sometimes an indication of a connection between this same subfiber and the corresponding subfiber of each adjacent triplet. In most instances, the lumen of the "hub" contains what appears to be a granule, or rod, 70 A in diameter.

Longitudinal sections of the centriole indicate that the cartwheel structure occupies about ⅗ of the total length of the centriole (Fig. 16). The "hub" appears to be a continuous cylinder, while the "spokes" present a ladder-like appearance, suggesting that each "spoke" is actually a rod and not a continuous plate running the length of the "hub." The micrographs indicate that the triplets run essentially parallel to the long axis of the centriole.

During the shell stage, the pair of centrioles is situated at one side of the spermatid at a point at which the nucleus is fairly close to the cell membrane (Fig. 12). During this stage, both centrioles elongate at their distal ends until they reach a length of about 600 mμ (Fig. 19). They remain at this length until the flagella are elaborated. Transverse sections of this new portion of the centriole indicate that the adjacent triplet fibers are connected by lines of electron-opaque material (Fig. 20).

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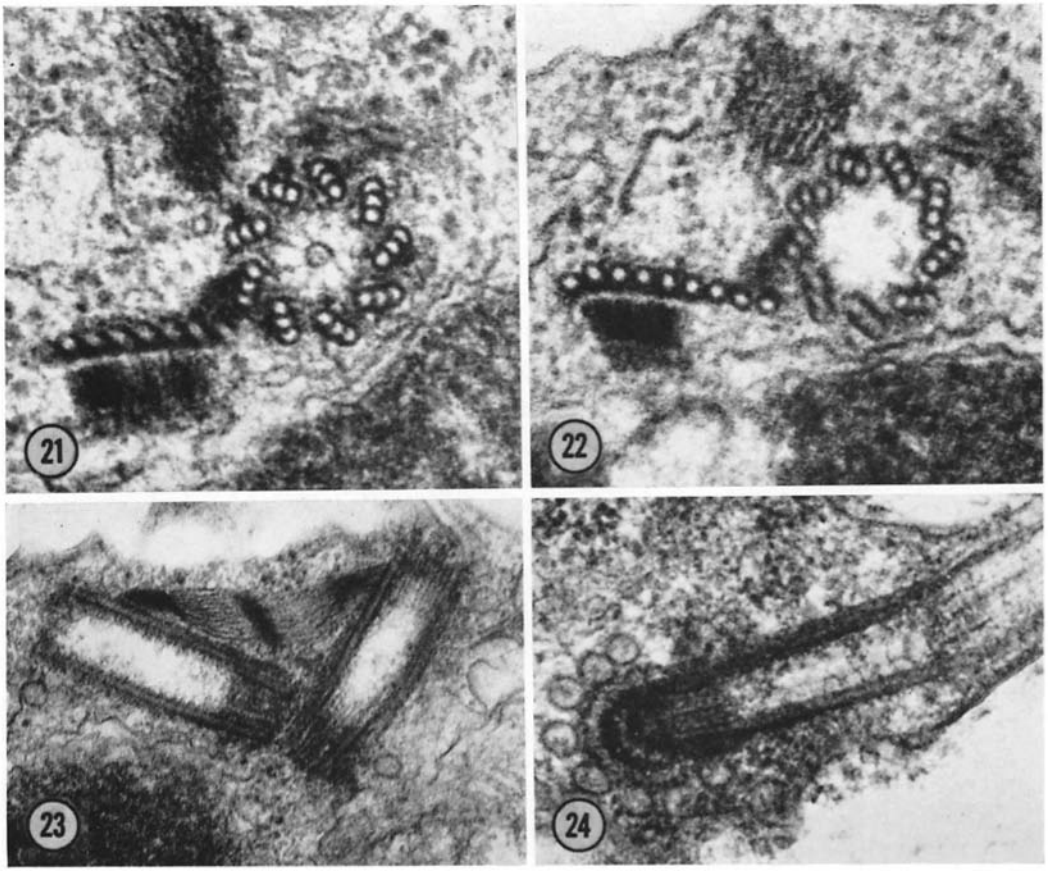

FIGURE 21 A transverse section through a centriole of an early spermatid showing its relation to the tubules of the developing microtubular sheath. × 124,000.

FIGURE 22 Similar to Fig. 21, except that the centriole has been sectioned through the distal end. × 125,000.

FIGURE 23 A pair of elongated centrioles from an early spermatid. The centrioles are joined by a fibrous connection. × 55,000.

FIGURE 24 A longitudinal section through a mid-spermatid centriole showing the developing headpiece at its proximal end. A portion of the flagellum can be seen extending out from the distal end. × 72,000.

The centrioles lose their parallel arrangement during the shell stage, but do not become separated. Instead, a spindle-shaped fibrous connection appears, linking the two centrioles together (Fig. 23). This connection appears to be composed of a number of parallel fibers with an electron-opaque band running across the middle of the connection. The tapering ends of the connection are attached to the centrioles at points approximately 140 mμ from their distal ends. The connection persists through most of spermiogenesis, and remnants of it occasionally may be identified in the late spermatid.

Following the elongation of the centrioles, there appears in the same region of the cell a number of microtubules having diameters similar to those of the centriolar subfibers (Figs. 21, 22). These tubules first appear in the immediate vicinity of the centrioles, arranged next to each other in a single layer. The tubules increase in length, following a

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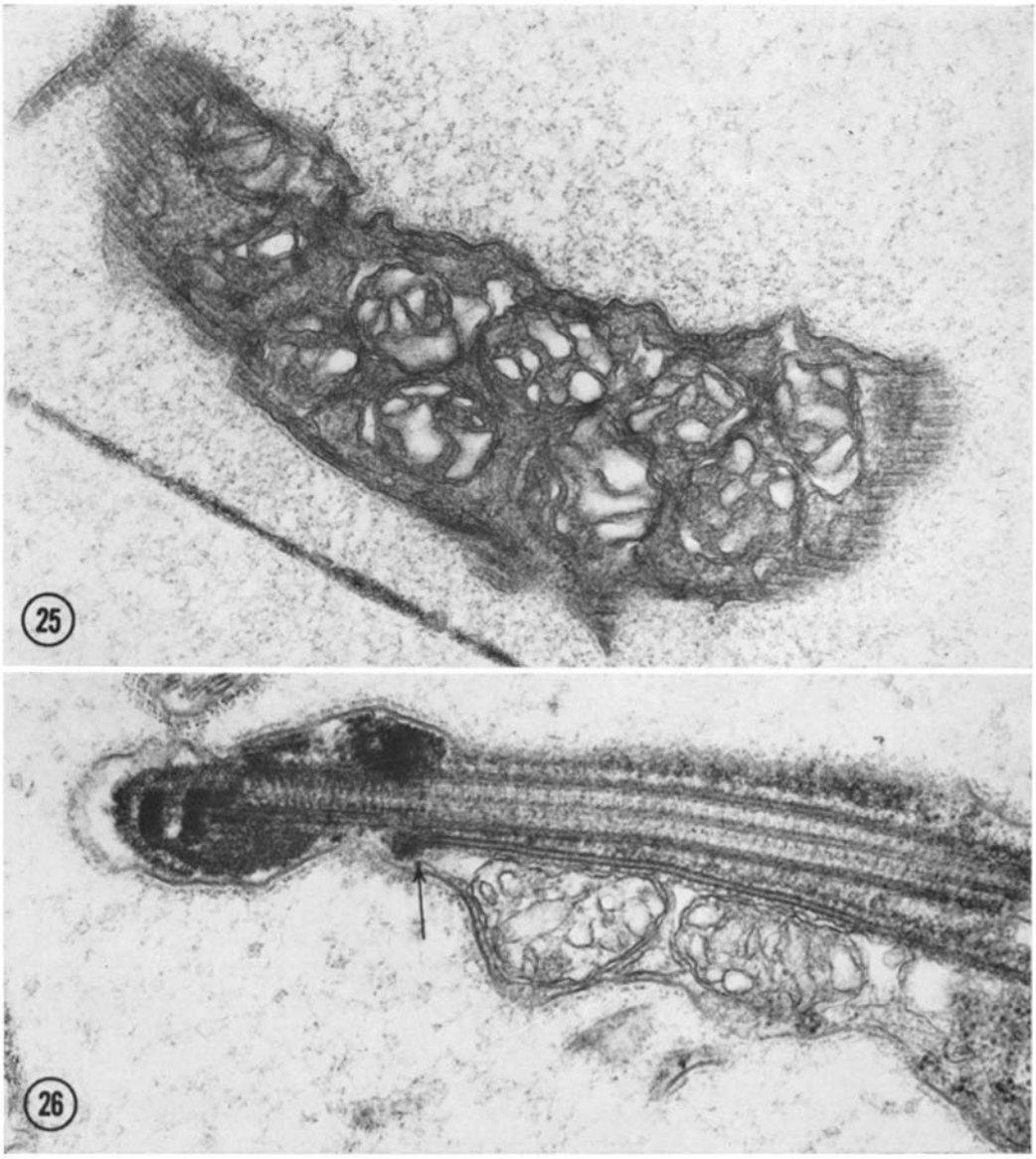

FIGURE 25 An oblique section through the mitochondrial region of a mid-spermatid illustrating the closely packed mitochondria and the parallel, ordered microtubules of the microtubular sheath. × 35,000.

FIGURE 26 A longitudinal section through the anterior end of a mid-spermatid showing the anterior end of the microtubular sheath (arrow) and its relation to one of the flagella. The basal body is capped by the headpiece. × 46,000.

course along the surface of the nucleus. Eventually, they extend beyond the nucleus into the cytoplasm and appear to provide a cytoskeletal framework along which the nucleus elongates and the other organelles of the mature sperm become arranged (Fig. 25). In the mature sperm, the microtubules form a sheath along one side of the full length of the sperm cell (Fig. 28). Transverse sections

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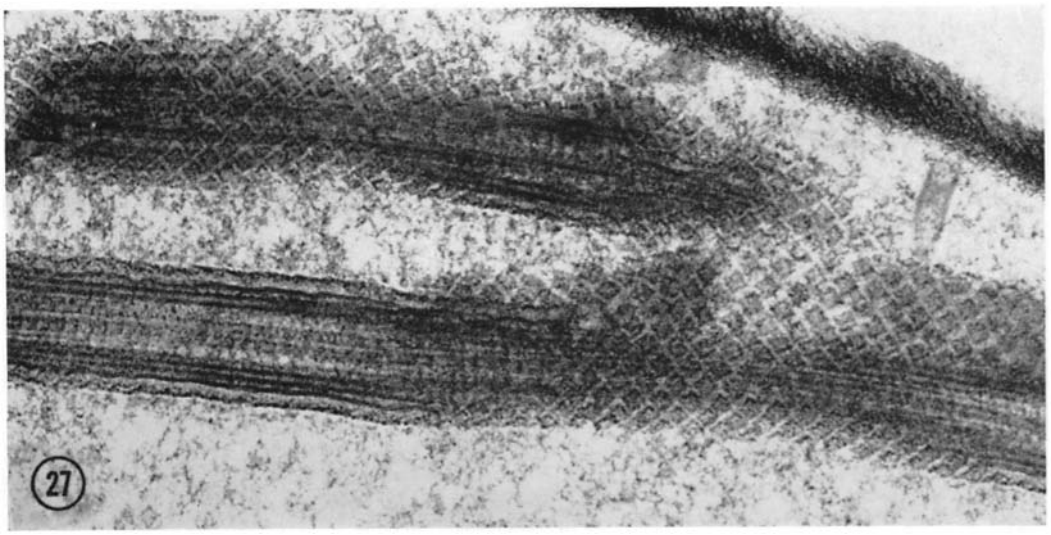

FIGURE 27 An oblique section through portions of two flagella. The thick outer covering and the scales can be seen in several aspects. × 57,000.

through different sperm show some variation in the number of microtubules comprising the sheath.

The microtubular sheath and at least one of the two centrioles of each spermatid are closely associated by means of a band of electron-opaque material which runs between one of the triplet fibers and one or more of the microtubules of the sheath. It has not been determined with certainty whether this association exists for just one or for both of the centrioles, but because of the angle at which the centrioles are oriented with respect to each other, it is difficult to imagine how both could be connected in such a manner.

The early stages of flagella formation were not observed in this material. The centrioles, which are originally at right angles to each other, shift in position until they are nearly parallel to each other and fairly close together. This shift in position results in a displacement of the fibrous connection to one side. The flagella extend from the distal ends of the centrioles.

At about the same time that the flagella are forming, a new structure appears at the proximal end of one of the centrioles, which in longitudinal sections of the centriole appears as an electron-opaque cap composed of several layers (Fig. 24). This structure, which will be referred to as the "headpiece" because of its eventual disposition in the mature sperm, is closely associated with a number of membrane-bounded vesicles, each having a diameter of about 65 mμ. The origin of these vesicles is unknown; however, they can be found in the cytoplasm surrounding the centrioles in younger spermatids (Fig. 19). The headpiece, as its name implies, forms the anteriormost part of the mature sperm, directly in front of the basal bodies (Fig. 26). Minor structural modifications of the headpiece during later stages of spermiogenesis were not studied in detail.

The motility apparatus of the mature sperm consists of the two anteriorly attached flagella and their associated basal bodies. Transverse sections of the flagella show the typical "9+2" configuration common to most motile cilia and flagella (Fig. 29 *b*). The microtubules comprising the fibers of the flagella are of the same diameter as the subfibers of the centrioles. In this study, the nine peripheral fibers did not show well defined arms such as those which have been described for a number of animal species (Afzelius, 1961). This apparent lack of arms may be due to inadequate preservation under the particular conditions of fixation employed.

The flagella are covered by a layer of amorphous material approximately 325 A thick. The outer surface of this layer is more dense than the remaining portion and, in grazing sections parallel to the surface, is seen to be composed of a regular array of square plates or scales (Fig. 27). These scales measure approximately 390 A on a side and are

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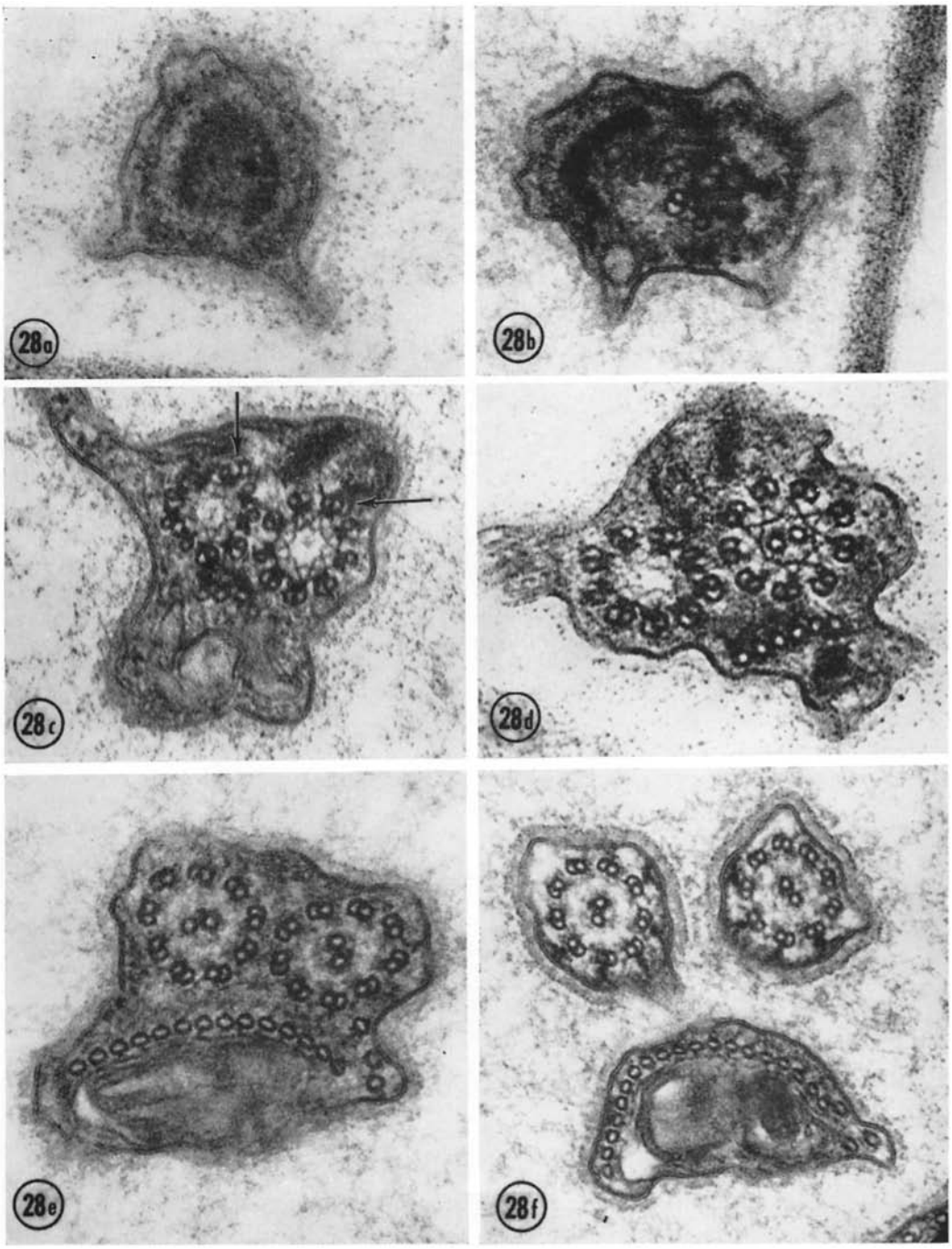
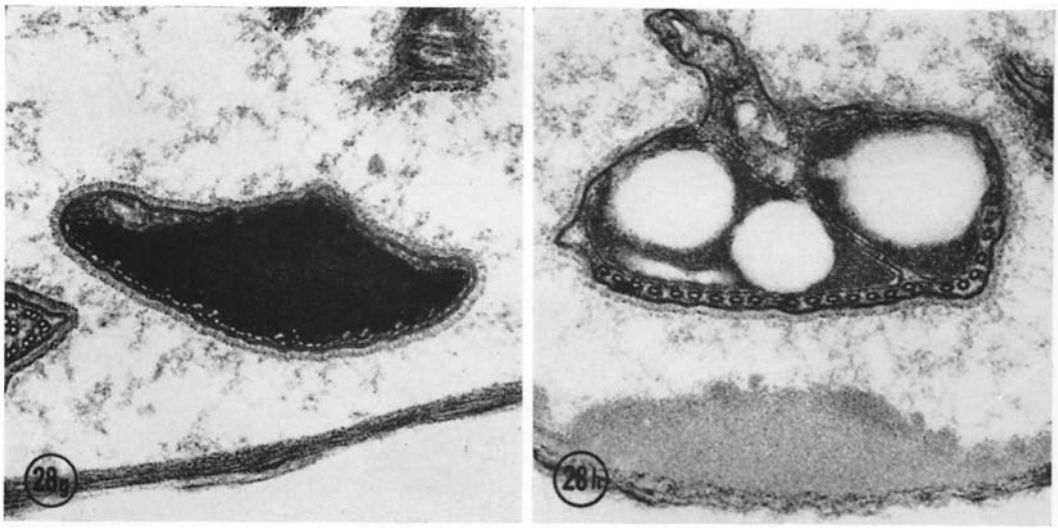


FIGURE 28 Representative transverse sections through different regions of late spermatids; (*a*) headpiece, × 109,000; (*b*) proximal end of the anterior basal body, × 113,000; (*c*) central region of the two basal bodies showing the occasional triplet fibers (arrows) found in the vicinity of the remnant of the fibrous connection, × 85,000; (*d*) the two basal bodies, one of which is sectioned in the transition region between basal body and flagellum, × 104,000; (*e*) and (*f*) vicinity of the junction between the flagella and the mitochondrial portion of the sperm cell. × 97,000 and × 86,000, respectively. (*g*) nucleus, × 49,000; (*h*) plastid region at posterior end of sperm, × 53,000.



For legend, see Fig. 28a-f

separated from each other by about 325 A. The scale layer is first noted at the time the flagella are forming.

Transverse sections of the basal bodies of late spermatids differ from those of the centriole in several respects. The peripheral fibers of the basal body are not triplet structures as are those of the centriole, but rather are doublets like the fibers of the flagellum (Fig. 28 *b–d*). An examination of a large number of sections of late spermatids has failed to reveal the presence of triplet fibers in the major portion of the basal body. Occasionally, one of the fibers may be a triplet, and in these instances it is usually the one nearest the remnant of the fibrous connection (Fig. 28 *c*). The cartwheel structure present in the proximal end of the centriole has not been identified in the basal body.

The transition zone between the flagellum and the basal body contains a specialized structure consisting of a series of electron-opaque lines connecting alternate peripheral fibers. In transverse sections of the basal body, this structure assumes the shape of a star (Fig. 28 *c*, *d*). The two central fibers of the flagellum are continuous through at least a portion of this region before they terminate. No other specialized structures have been observed in the region where the central fibers end.

The two basal bodies do not occur at the same level in the sperm, but are staggered, the anteriormost being the basal body associated with the headpiece. Longitudinal sections through the anterior end of the sperm show some electron-opaque material which appears to anchor one of the basal bodies to the microtubular sheath (Fig. 26). This material may represent a remnant of the band of material noted earlier between a centriole and the sheath during the shell stage.

Micrographs of lightly stained transverse sections of the centriole, flagella, and microtubular sheath indicate a similar ultrastructural pattern within the walls of all of their component microtubular elements (Fig. 29). This staining pattern may correspond to the arrangement of the subunits comprising the walls of these microtubules. Similar staining patterns have been described in other types of flagella (Ringo, 1967) where they have been examined in greater detail.

DISCUSSION

The complete sequence of stages in spermatogenesis is not entirely comparable in the animal kingdom and in those plants characterized by flagellated sperm. In the plants producing flagellated sperm, meiosis takes place during a different portion of the plant's life cycle and is not directly associated with gametogenesis (Wilson, 1925). The higher plants have evolved quite different mechanisms of sexual reproduction which do not depend on motile gametes. It is in the cryptogams which produce biflagellate sperm, notably the bryophytes and some of the algae, that we find a situation

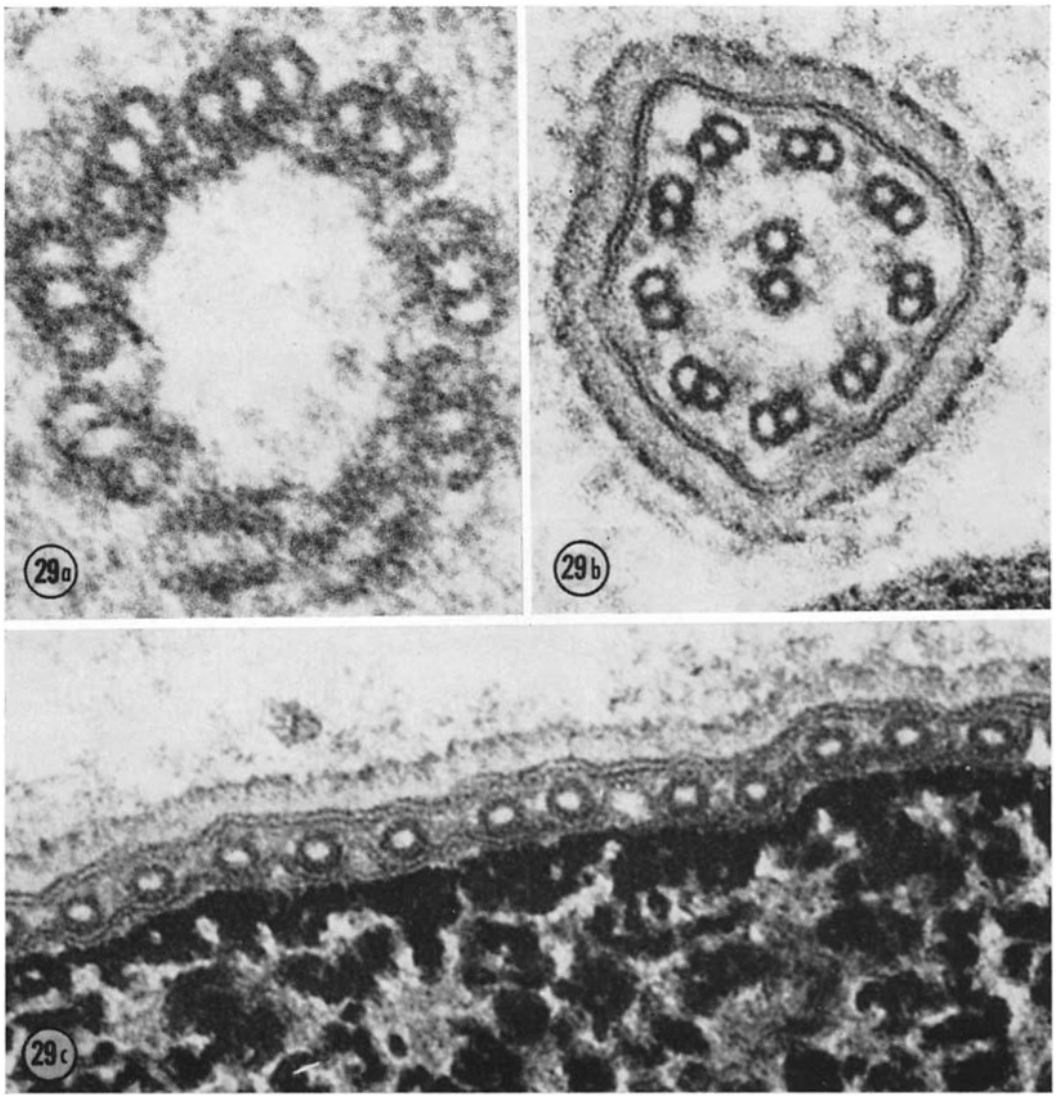


FIGURE 29 Enlargements of the centriole (*a*); flagellum (*b*); and a portion of the microtubular sheath (*c*); showing evidence of a similar substructure in the microtubules of all three organelles. Fig. 29 *a*, × 304,000; Fig. 29 *b*, × 194,000; Fig. 29 *c*, × 199,000.

which most closely approximates animal spermiogenesis.

Spermiogenesis in the charophytes is, in many ways, similar to animal spermiogenesis, as this study of *Nitella* has demonstrated. These similarities include nuclear condensation, localized aggregation of organelles such as the mitochondria, and a general loss of residual cytoplasm. Centriole behavior and the development of the motility apparatus are also similar to those in animal spermiogenesis, although it may be noted that, whereas in *Nitella* both centrioles form flagella, in most animal sperm only one of the two centrioles gives rise to a flagellum. No structure in plant sperm appears to be analogous to the acrosome of animal sperm.

The maturation of the spermatid nucleus of *Nitella* results in a dense, apparently homogeneous nucleus in the mature sperm. Similar examples of nuclear condensation have been described in

various animal species, both invertebrate (Dass and Ris, 1957; Gibbons and Bradfield, 1957; Rebhun, 1957; Yasuzumi and Ishida, 1957; Gall and Bjork, 1958) and vertebrate (Burgos and Fawcett, 1955, 1956; Nagano, 1962). The detectable structural changes which the nuclear material goes through appear to vary considerably from one species to another. In some, it goes through a series of fibrillar stages, while, in others, it goes through a series of granular stages, before the final condensed state is achieved. The fibrillar type of condensation similar to that seen in *Nitella* is found predominantly among the invertebrates. There is at present little information available relating these ultrastructural changes in the nucleus to underlying biochemical changes.

The mitochondria of the late spermatid of *Nitella* do not differ significantly in morphology from those of the pre-spermatid stages. They become arranged in a linear fashion in the anterior end of the spermatid, but do not appear to undergo any fusion or unusual associations. Whereas the mitochondria of most animal sperm are, in some manner, arranged in close association with at least a portion of the flagellum, no such direct association exists in *Nitella*, in which the mitochondria are some distance removed from the flagella (Fig. 30).

The antheridial filament cells of *Nitella* contain several plastids, which, in most instances, appear to be oriented parallel to the long axis of the filament (Fig. 11). Many dividing antheridial filament cells have been observed in which the plastids appear to be pinched in two by the forming cell plate. The longitudinal orientation of the plastids assures that they are intersected by the plate, providing a fairly constant number of plastids for each antheridial filament cell.

During mid-spermatid stages, material of undetermined composition begins to form and gradually accumulates within the plastids (Fig. 28 *h*). It may be speculated that this material is stored during spermiogenesis to be utilized as an energy source for the movement of the mature sperm. If this should prove to be the case, it is noteworthy that this material is rather distant from the motility apparatus, as the plastids are localized at the opposite end of the sperm cell.

During spermiogenesis in many animals, the Golgi apparatus of the spermatid has been shown to contribute to the formation of the acrosome (Burgos and Fawcett, 1955, 1956; Beams et al., 1956; Kaye, 1962; Hopsu and Arstila, 1965). Following acrosome formation, the Golgi apparatus, as well as other cellular components not incorporated into the mature sperm, are sloughed off from the spermatid as the residual body (André, 1963). The present studies of spermiogenesis in *Nitella* did not reveal any organelles resembling the acrosomes of animal sperm. During early spermatid stages, the Golgi apparatus appears to become very active, increasing extensively in size and becoming surrounded by a "cloud" of vesicles, presumably derived from the Golgi cisternae.

Scales which eventually cover the flagella first appear as the flagella are being elaborated. The formation of some types of algal scales has been investigated (Manton and Parke, 1962; Manton et al., 1965; Manton, 1966), and the origin of these scales in the Golgi apparatus has been demonstrated. In *Nitella*, a modification of the fixation procedure used in these experiments (Turner, data in preparation) has revealed the presence of scales in the vesicles and cisternae of the Golgi apparatus. The mechanism by which these scales become arranged on the surface of the flagella is not known.

In later spermatid stages, the Golgi apparatus decreases in size, and in very late spermatids it is no longer demonstrable. It is noteworthy that in this organism almost all of the ground cytoplasm, as well as Golgi apparatus and endoplasmic reticulum, is lost during late spermatid stages. They are not sloughed off as is the case in animal spermatids, and, since traces of them are not found within the antheridial cell wall, it is believed that they may be broken down and their components utilized in the formation of other structures.

The centrioles found in the antheridial filament cells of *Nitella* are similar in size and structure to the animal centrioles described in the literature (de Harven and Bernhard, 1956; Amano, 1957; Bessis et al., 1957, 1958; Bernhard and de Harven, 1960; Gall, 1961; André, 1964; Fawcett, 1966). The basic configuration of a cylinder, the wall of which consists of nine triplet microtubular elements, appears to be a uniform feature of most centrioles. The cartwheel structure which occupies the greater portion of the lumen of the centriole of *Nitella* has been demonstrated in animal centrioles (Gall, 1961) where it is confined to a small region near the proximal end. This structural formation has been noted in several other plant centrioles (Berkaloff, 1963; Berlin and Bowen, 1964; Renaud and Swift, 1964), and possibly it also

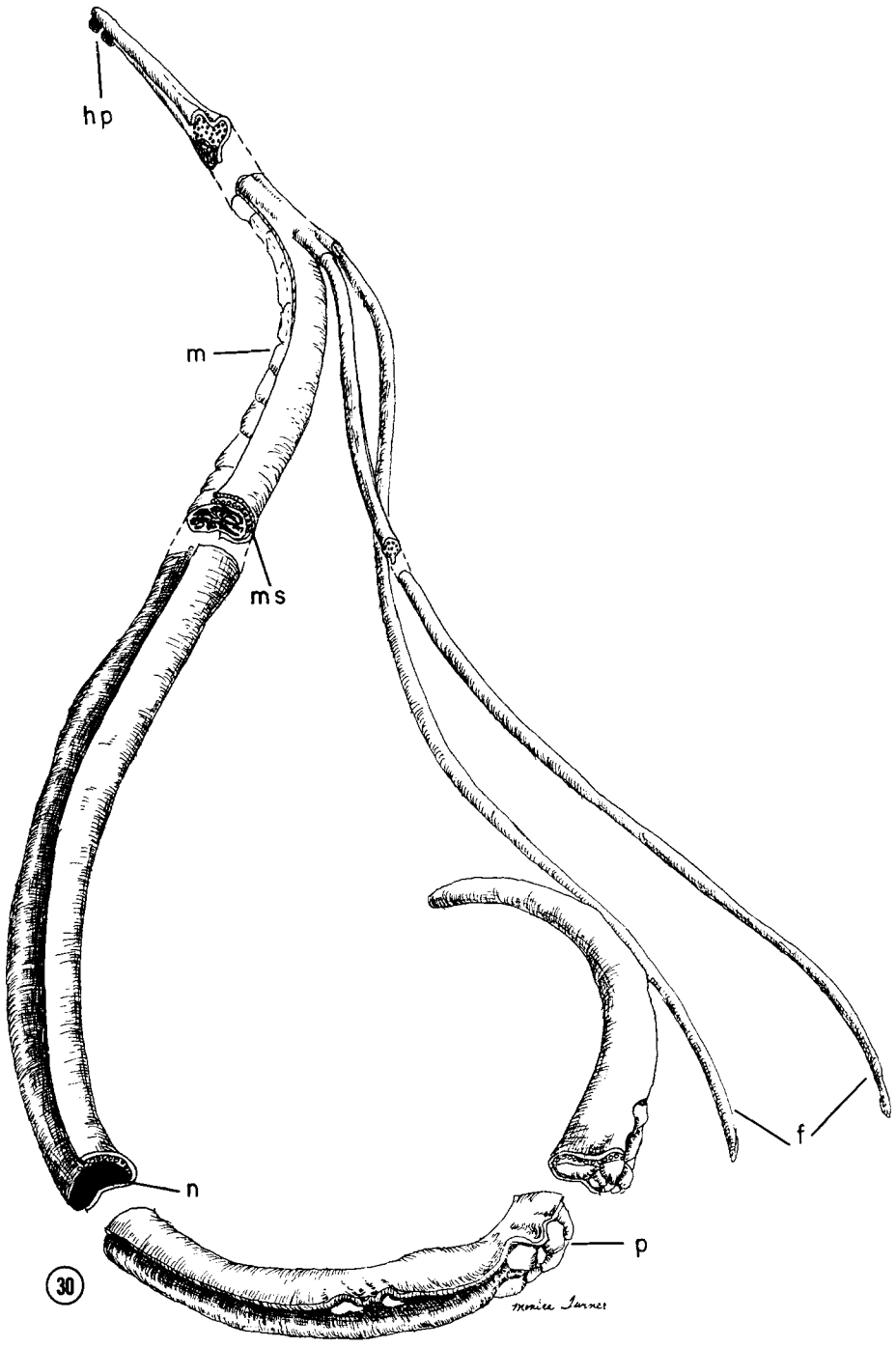


FIGURE 30 A diagrammatic reconstruction of a *Nitella* sperm derived from light and electron microscopic evidence; *hp*, headpiece; *f*, flagella; *m*, mitochondria; *ms*, microtubular sheath; *n*, nucleus; *p*, plastid.

may prove to be a consistent feature of most centrioles.

An extensive study of a large number of sections of the centrioles of *Nitella* failed to reveal the presence of any of the accessory structures which have been described as occurring in association with animal centrioles. These structures, which include satellites (Bernhard and de Harven, 1960), clavate extensions (Gonatas and Robbins, 1964) and, pericentriolar filamentous bodies (Sakaguchi, 1965), appear to represent points of origin or of attachment of microtubular elements such as spindle fibers and neurotubules in the animal cells which have been studied.

The mechanism of centriole replication and its temporal relation to the mitotic cycle in *Nitella* antheridial filament cells appear to follow the same patterns as those which have been postulated for animal cells (Gall, 1961; Robbins and Gonatas, 1964; Murray et al., 1965). During interphase, there are consistently only two centrioles in each tell, usually found parallel to each other and perpendicular to the nuclear envelope. This arrangement is not the one usually found in animal cells, in which the centrioles are normally perpendicular to each other, but has been encountered in several other cryptogams such as *Hemanthalia* (Berkaloff, 1963) and *Allomyces* (Renaud and Swift, 1964).

By early prophase, before breakdown of the nuclear envelope, two pairs of centrioles are present in each antheridial filament cell, located on opposite sides of the nucleus. The observation that, at this stage, the centrioles of a pair are at right angles to each other and one is frequently shorter than the other agrees with a scheme of replication by procentriole formation. That this mechanism is similar to the one described by Gall (1961) is further substantiated by the fact that the shorter centriole (presumably the daughter) is always closest to the proximal end of the parent centriole. Murray et al. (1965) described a gradual maturation of the daughter centriole during later stages of division of rat thymic lymphocytes. From the present study, it is not possible to tell at what stage the daugher centriole is mature, i.e., able to replicate itself.

The centrioles of dividing antheridial filament cells are located in the region of the spindle poles; however, as noted earlier, no evidence has been found for a connection between the spindle fibers and the centrioles. Sufficient data are not available to determine whether either or both of the centrioles of a pair bear any specific relationship to the axis of the spindle.

The apparent lack of centrioles in cells other than those of the antheridium would seem to indicate that centrioles per se are not transmitted through the vegetative cell line, a finding which leaves their origin in the antheridium uncertain. The possibility still exists that a centriole progenitor, such as a procentriole, is carried through the vegetative cell line as a self-replicating unit, differentiating only during spermatogenesis to form a morphologically discernible centriole. There is evidence in the fungus *Allomyces* (Renaud and Swift, 1964) that a self-replicating centriole of limited length exists in association with the vegetative nuclei, and that it differentiates into a full-sized centriole only during gametangial differentiation.

Electron microscopic evidence for the formation of centrioles in the apparent absence of preexisting centrioles in animal cells is very limited. However, in a study of artificially activated sea urchin eggs, Dirksen (1961) demonstrated the presence of a centriole at the center of the aster resulting from this activation. The centriole did not appear to be derived from a preexisting centriole in the egg, and it was noted that several asters may be present after activation, each presumably possessing a centriole at its center. Also, in a study of embryonic and very young rat tissue, Stockinger and Cireli (1965) have shown the formation of large numbers of centrioles in cells destined to become ciliated. These centrioles differentiate from granular precursors and apparently are not derived from preexisting centrioles.

Dirksen and Crocker (1966) also examined the formation of multiple centrioles in ciliated epithelial cells. These investigators hypothesized that the formation of the extra centrioles is mediated by a nucleic acid template originating in association with a mature centriole.

A contrasting situation is encountered in the formation of atypical sperm in the snail *Viviparus* (Gall, 1961). In this organism, the atypical sperm contain degenerate nuclei and are multiflagellate. It was demonstrated that the extra centrioles which produce the flagella are derived from procentrioles. The extra procentrioles arise simultaneously in association with the proximal end of a normal-appearing centriole. This is a variation of the normal scheme of centriole replication in which

only one procentriole is formed by each parent centriole.

Closely associated with the developing motility apparatus in the spermatids of *Nitella* is a system of microtubular elements representing the beginnings of the microtubular sheath. The microtubular sheath appears to originate in the immediate vicinity of the centrioles and is comprised of microtubules which are similar in appearance to the microtubular components of the centriole and the flagella. The development of the microtubular sheath precedes the elongation and coiling of the nucleus. It appears to form a structural framework or cytoskeleton along which the nucleus differentiates.

Examples of microtubular "skeletons" are found in a wide variety of sperm, both plant and animal. They appear in almost all descriptions of sperm which have been fixed in glutaraldehyde. It is probable that the structures which Manton refers to as fibrous bands correspond to microtubular sheaths (Manton, 1957, 1959). In animal spermatids, the manchette or caudal sheath, a complex of straight microtubules forming a cylindrical sheath around the developing sperm tail, may develop in a manner similar to the microtubular sheath of *Nitella* (Burgos and Fawcett, 1955; Bradke, 1963).

The sperm of the triclad flatworms are remarkably similar in structure to those of *Nitella* (Silveira and Porter, 1964). The body of the sperm is described as having an undulatory motion which is independent of the flagellar motion. According to these authors, the layer of microtubules which forms a sheath along the length of the flatworm sperm is implicated in this motion because of its disposition and because of the similarity of the microtubules to the fibers of the flagella. Preliminary observations on living *Nitella* sperm have not revealed an analogous, independent motion in the body of the sperm cell. The microtubular sheath in *Nitella* appears to function primarily as a cytoskeleton.

The homology of the centriole with the basal body was first proposed independently by Henneguy and Lenhossek in 1898. In recent years similarities in fine structure between these organelles have been confirmed by use of the electron microscope (Gibbons and Grimstone, 1960; Reese, 1965).

It has been shown in *Nitella* that the centrioles elongate considerably before ciliogenesis is initiated. This phenomenon of centriole elongation has been described in the literature for both plants and animals, e.g., in the fungus *Allomyces* (Renaud and Swift, 1964) and in the chicken spermatid (Nagano, 1962). This initial elongation, although widespread in occurrence, probably is not essential for ciliogenesis as there are some instances in which it is not encountered (Sorokin, 1962).

Concomitant with the process of ciliogenesis, the centriole also undergoes morphological differentiation as it becomes the basal body. The most significant change is the appearance of a stellate structure within the lumen of the distal end of the centriole, in the region which will become the transition zone between basal body and flagellum. It appears to be a common feature in most, if not all, of the basal bodies of plant flagella which have been studied (Lang, 1964; Manton, 1964), but has not been described in animal cells. The cartwheel structure which is such a prominent feature of the plant centriole appears to be lost in the transition from centriole to basal body.

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