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Spermatogenesis in Males of the Free-Living Nematode, *Caenorhabditis elegans*

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We have studied the morphology of spermatogenesis in the free-living nematode *Caenorhabditis elegans* with the light and electron microscopes. The gonad of the adult male is a single, reflexed cylindrical structure containing all stages of spermatogenesis arranged in a single wave of development. The primary spermatocytes are at the end of the gonad most distal from its opening into the cloaca. In this region, the cells are syncytial, and there is a central core containing cytoplasm common to all the neighboring cells, an organization reminiscent of the ovary in the hermaphrodite. Moving toward the cloaca one encounters the stages of meiotic prophase. At diplotene and diakinesis the cells contain many Golgi complexes. Some of these Golgi complexes are associated with an urn-shaped vesicle with a dark amorphous collar about its neck. Other Golgi complexes are seen next to aggregates of microfilaments. These "fibrous bodies" become enveloped with a flattened vesicle that forms a boundary two membranes thick. After these two structures have grown in size, their membranes fuse to form a composite structure. The membranes at the site of fusion then develop dark-staining thickenings as they fold into convoluted sacs and tubes. At about this time, the cells go through the two meiotic divisions. At telophase II the composite structures and fibrous bodies cluster at the spindle poles and cleavage furrows not only separate the daughter cells, but they also slough off a substantial volume of cytoplasm. In the resulting spermatid the nucleus condenses to form the small mass of dark-staining chromatin characteristic of sperm. The microfilaments of the fibrous bodies now disappear while the membranes of the composite structures continue to fold. The texture of the cytoplasm becomes more dense and now contains numerous slender, wavy tubular elements. A part of each composite structure now fuses with the plasma membrane of the sperm to make an almost spherical invagination of extracellular space partially filled by the tortuous bits of membrane-bound cytoplasm formed by the foldings of the membranes of the composite structure.

Caenorhabditis elegans is now established as an experimental genetic system (*3*). Work has begun using both the anatomical simplicity and genetical manipulability of *C. elegans* to study animal development. Among the existing developmental mutants there is a set defective in spermatogenesis (*12, 13*). Such mutants offer a means to probe individual steps of spermatogenesis. The value of such an analysis resides ultimately in the possibility of developing a detailed understanding of a relatively simple pathway of cell differentiation. Before aberrations of the normal pathway caused by mutations can be analyzed, however, it is important to have a thorough knowledge of spermatogenesis in the wild-type individual. We report here an electron microscopic analysis of the structure of spermatogenesis in adult males of a wild-type strain of *Caenorhabditis elegans*.

MATERIALS AND METHODS

Nematode strains. The *C. elegans* used in these studies were from the University of Colorado, Boulder, stock originally derived from the strain described by Brenner (*3*). The wild-type strain is designated N2.

Specimen preparations. Males were the offspring of crosses between N2 hermaphrodites and N2 males and were collected after 5 days of growth at 20°C. Aldehyde fixations are poor at penetrating an intact worm, so the nematodes were dissected in 2% glutaraldehyde in 0.08 *M* sodium phosphate buffer, pH 7.4. Because of the normal pressure within the body of the worm, the reproductive organs are expelled upon cutting the worm. Worms were cut beneath the pharynx to obtain well-fixed gonad from the anterior half of the worm; cuts were made midway along the worm to obtain good fixation of the posterior portions of the

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gonad. The cut worms were fixed in glutaraldehyde overnight at room temperature, then postfixed in osmium tetroxide for 1 hr, stained in uranyl acetate, dehydrated in ethanol, and embedded in Epon. Microtomy was performed on an MT-2 Porter-Blum ultramicrotome. Sections were stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

Thin sections were collected every 20 μm throughout the first 175 μm from the anterior extremity of the gonad and every 10 μm for the next 100 μm, and serial sections were collected from there until mature sperm were found.

RESULTS

The male gonad of *C. elegans* is a single, reflexed cylindrical structure that is located within the pseudocoelom of the worm. The different morphological regions of the male reproductive system as viewed by light microscopy are named as shown in Fig. 1. The terms distal and proximal are used with reference to the point at which mature gametes emerge, the cloaca in the tail. The distal arm of the gonad is thus the part more distal than the anterior loop and is found on the ventral side of the worm. It has been designated "the testis" by several authors (*1, 5*). The proximal arm is the part posterior to the loop on the dorsal side of the animal. We use this terminology to draw attention to morphological similarities between the gonad of the male and the hermaphrodite (*12*). The seminal vesicle is the posterior region of the proximal arm; the vas deferens connects the seminal vesicle to the cloaca.

Since the anterior edge of the loop of the gonad is the most anterior point of the gonad, we use it as the frame of reference for describing our cross sections. The positions of all subsequent cross sections are measured in micrometers from this point. To distinguish images of the proximal arm from those of the distal arm, we use positive numbers for the former and negative numbers for the latter (Fig. 1).

Pachytene nuclei are seen in the loop and the anterior portions of the proximal and distal arms by using Feulgen staining (*15*). Posterior to this region of the proximal arm are nuclei in diakinesis. Further posterior one observes nuclear divisions (*15*). Sperm are found in the extreme posterior part of the proximal arm (Fig. 1). Therefore, there appears to be a gradient in the stages of spermatogenesis progressing from the distal arm through the loop to the posterior part of the proximal arm. In this study, we corroborate this idea at the fine structural level and show that the various processes of spermiogenesis, such as nuclear condensation and membrane specialization, reveal a smooth sequence of development from the distal arm through the loop region and down the proximal arm of the gonad to the region of appearance of mature sperm.

The distal arm. The distal arm consists of cells arranged around a cytoplasmic core, referred to as a rachis by several authors (*16, 17*). There are frequent gaps in the cell membranes so that the cytoplasm of the peripheral cells is continuous with the core cytoplasm (Fig. 2). The fine structure of cytoplasm in the cells surrounding the core is identical in appearance to that of the core. It consists of many free ribosomes, mitochondria, and a few Golgi complexes. Occasionally one observes endoplasmic reticulum. This morphological relationship between the peripheral cells and the core is similar to that seen in the distal arm in the hermaphrodite gonad of *C. elegans* (*12*). There is, however, a difference between the distal arms of the two sexes. A cellular sheath and basal lamina surround the hermaphrodite gonad, whereas no cellular sheath is found in the male (Fig. 2).

The Loop. Cross sections through the loop reveal that the core continues from the distal arm through the loop. All of the nuclei in the loop are in pachytene (*15*), and consistent with this stage of meiosis, one can identify synaptonemal complexes.

The proximal arm. The cytoplasm of the anterior region of the proximal arm is essentially indistinguishable from that of the distal arm and the loop. The core continues from the loop to the proximal arm. The core of the proximal arm is also continuous

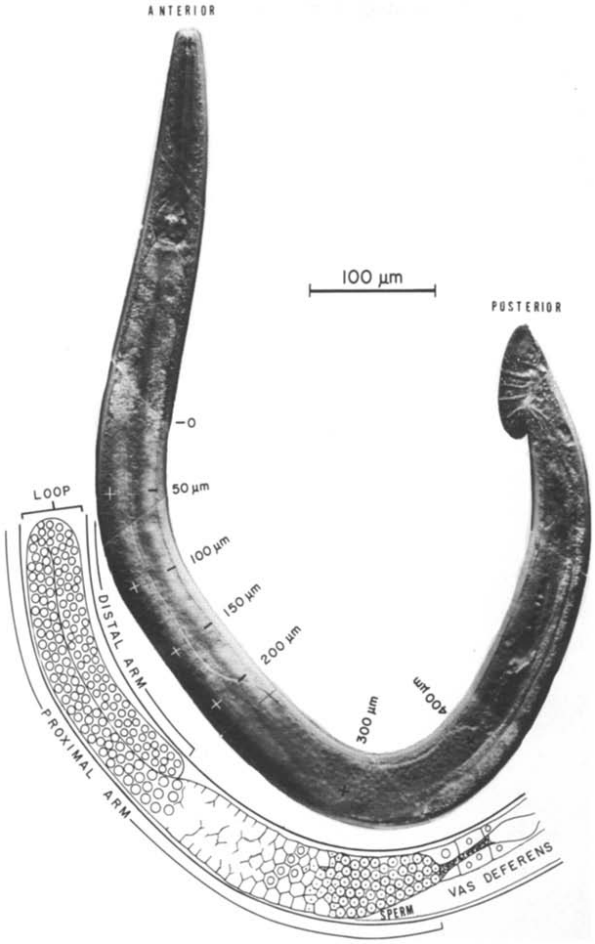


FIG. 1. Micrograph of an adult *Caenorhabditis elegans* male taken with Nomarski differential interference optics. The reproductive system is diagrammed. The terminology used in the text for the different portions of the reproductive system are illustrated. The positions of the cross sections for microscopy are relative to the anterior edge of the loop as the origin (o). Distances are measured from the origin with positive numbers referring to distances along the proximal arm and negative numbers referring to distances along the distal arm.

with the surrounding cells. Throughout the first 100 μm of the proximal arm the cytoplasm of the core and the cytoplasm of the surrounding cells remain very similar. There are, however, more Golgi complexes and endoplasmic reticulum in these first 100 μm of the proximal arm than in the distal arm.

At about +100 μm from the loop the first differences in appearance of the cytoplasm of the surrounding cells are noted. The peripheral cytoplasm contains many Golgi complexes, mitochondria, free ribosomes, and extensive endoplasmic reticulum, while the core cytoplasm shows only endoplasmic reticulum and ribosomes.

In the region +150 to +200 μm one first detects a special vesicle associated with one edge of many of the Golgi complexes. The special vesicle appears as a bulbous enlargement of a Golgi vesicle, surrounded on the cytoplasmic-facing side by a cup-shaped cisterna of endoplasmic reticulum (Fig. 3). The vesicles have constricted necks adjacent to the Golgi complexes, and electron-dense material is seen on the outside of these constrictions as if forming a collar. In the same area of the gonad another set of

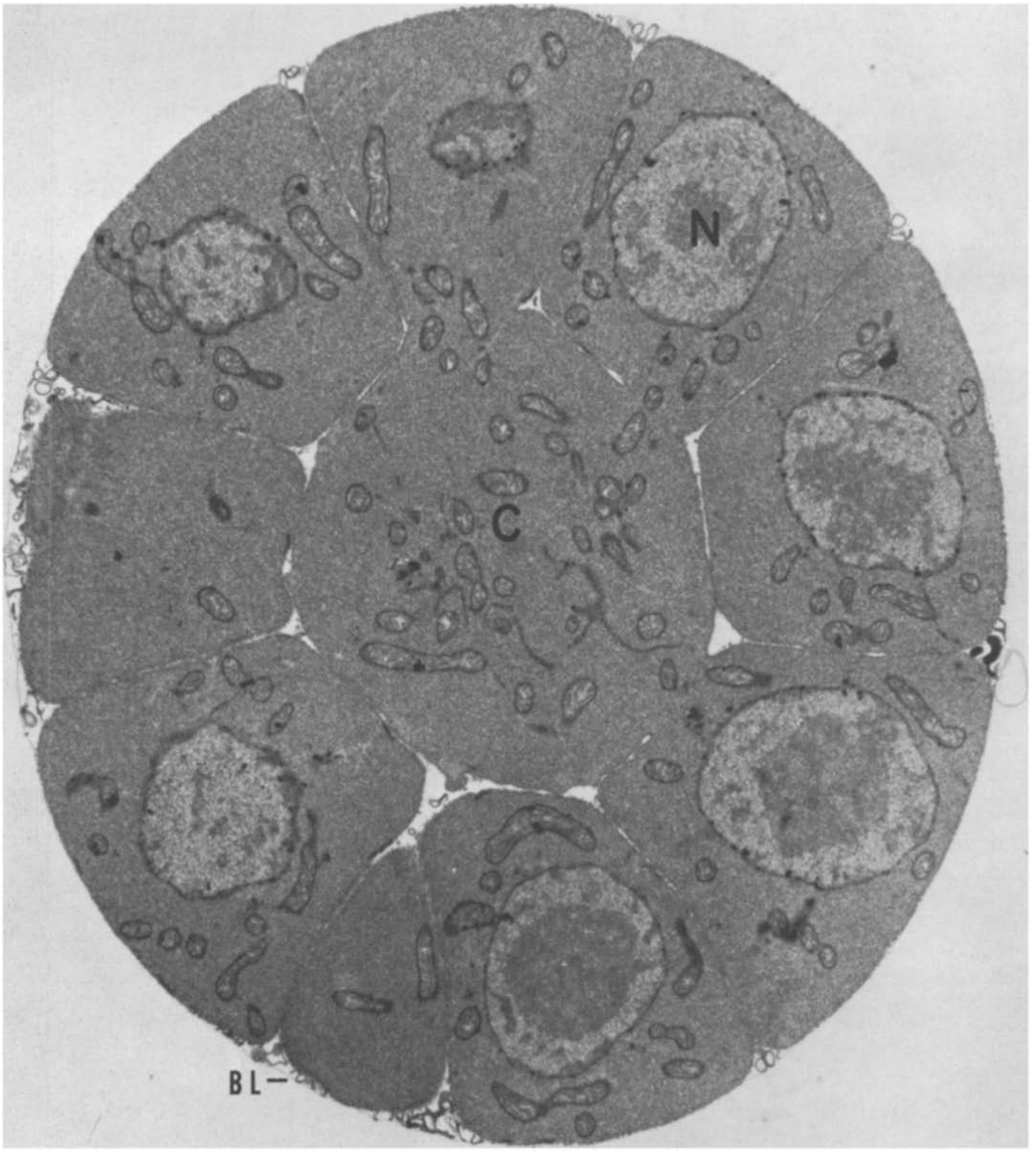
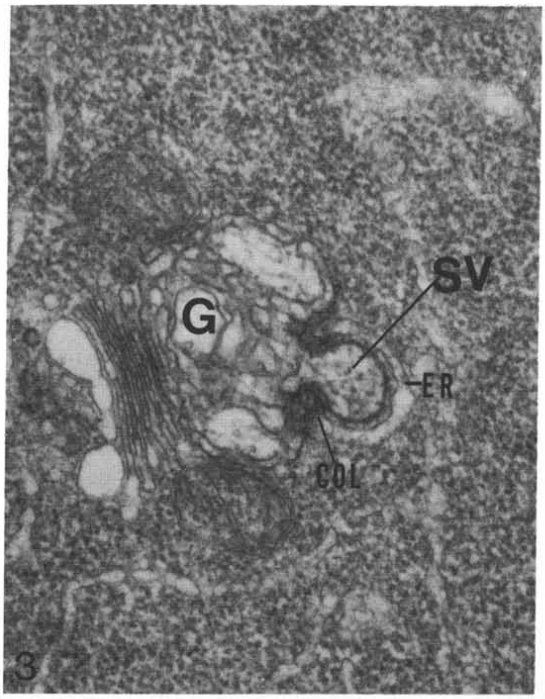


FIG. 2. Cross section of the distal arm. The gaps in the membranes are apparent showing the continuity of the peripheral cytoplasm with the core (C) cytoplasm. Nuclei (N) are located only in periphery. The male gonad is surrounded only by a basal lamina (BL). × 9700.

FIG. 3. Section at +200 μm from the anterior edge of the gonad showing the formation of the special vesicle (SV) in association with the Golgi complex (G). The special vesicle appears as a bulbous structure surrounded by endoplasmic reticulum (ER) and a dark collar (COL). × 44 000.

FIG. 4. Two fibrous bodies (FB) that first appear as dark striated figures. Section at +200 μm. × 44 000.

FIG. 5. Section at +220 μm at lower magnification showing that special vesicles (SV) and fibrous bodies (FB) are found only in the peripheral cytoplasm (P) of the gonad and not in the core cytoplasm (C). The core cytoplasm contains ribosomes and endoplasmic reticulum. × 12 000.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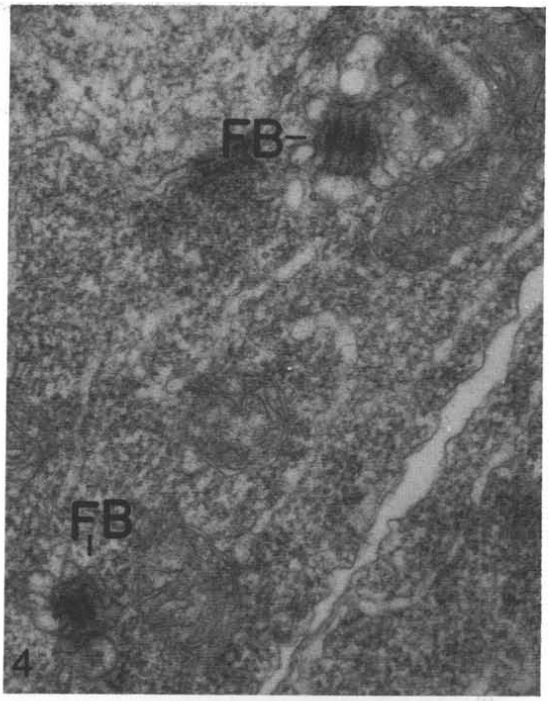
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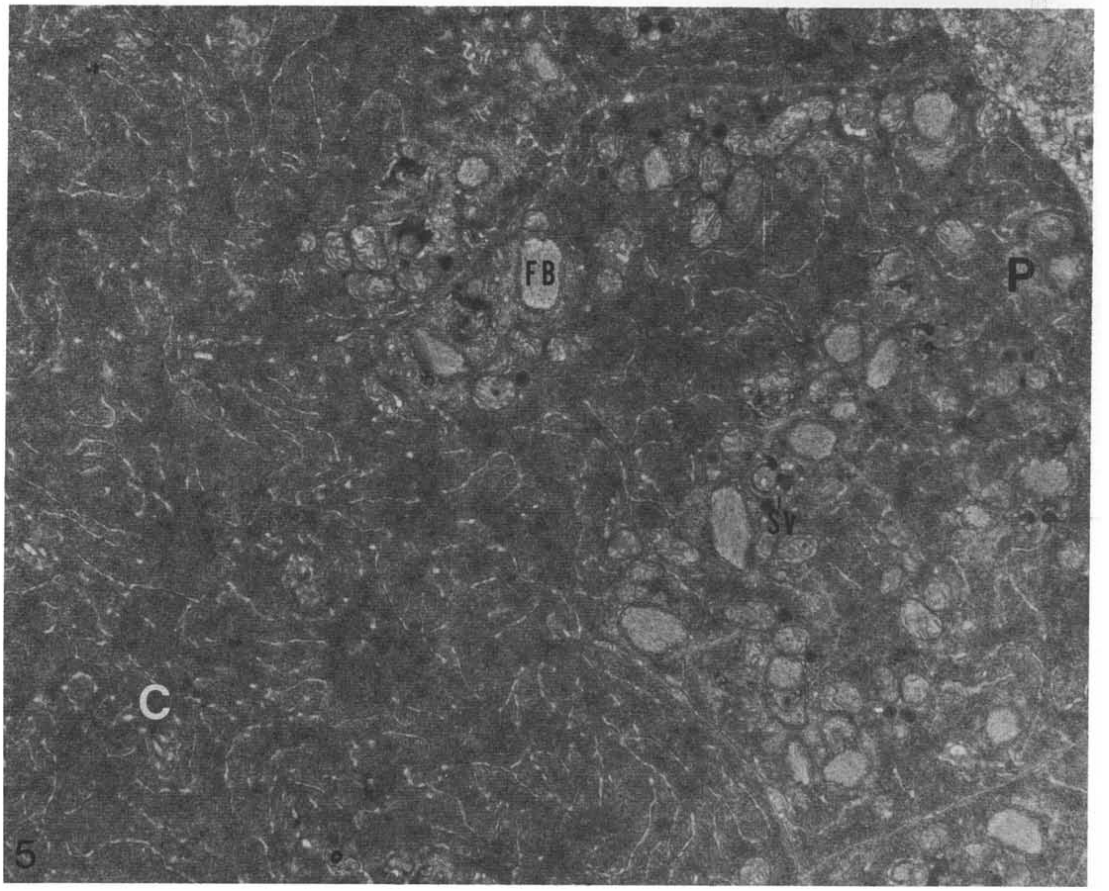
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novel structures is visible, referred to as fibrous bodies (Fig. 4). The fibrous bodies are adjacent to Golgi complexes, but it is not clear whether the fibrous bodies are formed from Golgi complexes or endoplasmic reticulum. The fibrous bodies become larger as one progresses toward the posterior part of the proximal arm. The formation of the special vesicles and fibrous bodies occurs in the peripheral area of the gonad, whereas the core contains only endoplasmic reticulum and ribosomes (Fig. 5).

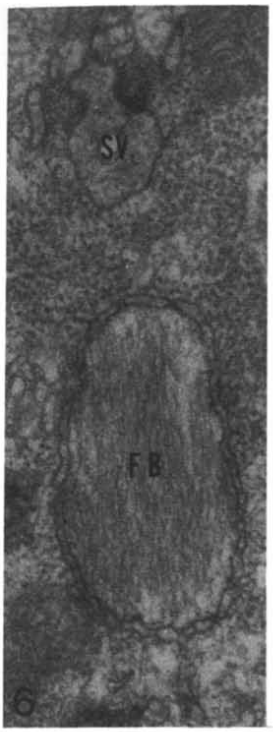
At about +275 μm from the loop the distinct core is absent. There are nuclei throughout the gonad cross section. In this area of the gonad the fibrous bodies reach their maximum size and are surrounded by two distinct membranes that may form a continuous layer around the fibers (Fig. 6). The fibers are 48 ± 11 Å wide. At about +280 μm in the gonad some of the special vesicles are near the fibrous bodies, and some are associated with the fibrous bodies (Figs. 6–8). The special vesicles at this stage show a new assymetry. The part on one side of the constriction, or collar, is lobular, but the part on the other side of the neck has finger-like projections. These projections seem to fuse with the membranes that surround the fibrous bodies to form a composite structure. Simultaneously, the double membranes surrounding the fibrous bodies become fenestrated, thus providing connections between the fiber-containing space and the rest of the cytoplasm.

In the subsequent sections, the composite structures formed by the fusion of the special vesicles with the fibrous body membranes form an additional specialization. The membranes in the region of the fusion of the two organelles are highly folded. Also an electron-dense material appears on the membranes (Figs. 9–11). The material appears as a depòsition of osmiophilic substance on the cytoplasmic surface of the membrane. As the folding becomes more pronounced, the channels connecting the cytoplasm with the fibrous body become larger. The infolding ultimately becomes so pronounced that in thin section one sees stacks of flattened vesicles with the electron-dense material on the concave surface. During the removal of the double membrane from the fibrous bodies, the fibers disappear and the cytoplasm contains a high density of small vesicular structures. These structures have been referred to as "tubular elements" by Beams and Sekhon (*2*) and are visible in Fig. 9.

From +280 μm onward the gonad contains nuclei undergoing meiotic divisions from studies done on Feulgen-stained gonads and from observations on living specimens using Nomarski differential interference contrast microscopy (*15*). It is apparent from light microscopy of living material that each cell that enters this region of the proximal arm undergoes two divisions (Fig. 12). At the end of the second meiotic division an anucleate mass of cytoplasm is sloughed off. These anucleate cytoplasmic bodies were first observed by Delavault (*7*) who referred to them as "cytoplasts." Electron microscopy of cells in their final meiotic division shows that the cells are elongated with a nucleus at each end (Fig. 13). All of the organelles, in particular the fibrous bodies and special vesicles, are located near the poles of the elongated cell. The cytoplasm in the middle of the dividing

FIGS. 6–8. Micrographs of sections taken at +280 μm showing the relationship of the special vesicles (SV) to the fibrous bodies (FB). In Fig. 6, a special vesicle is near a fully formed fibrous body. Note that the fibrous body is surrounded by two membranes. In Fig. 7, finger-like projections of the special vesicle are adjacent to the fibrous body. In Fig. 8, the projections are fused with the membranes on the surface of the fibrous body, and these membranes are absent from other areas on the surface of the fibrous body. × 42 000.

FIGS. 9–11. Micrographs showing the folding of the fused membranes of the special vesicles (SV) and the fibrous bodies (FB), and the electron-dense material on those membranes. Simultaneously "tubular elements" (TE) appear in the cytoplasm. × 71 000.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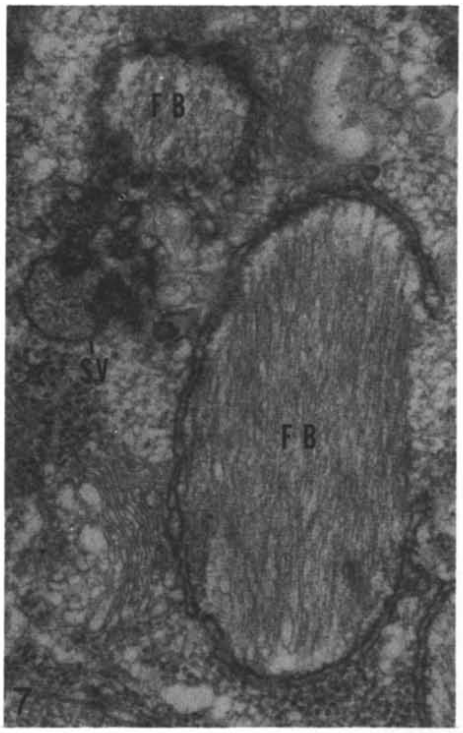
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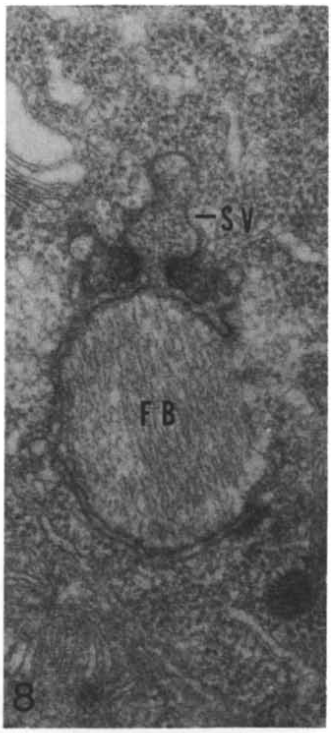
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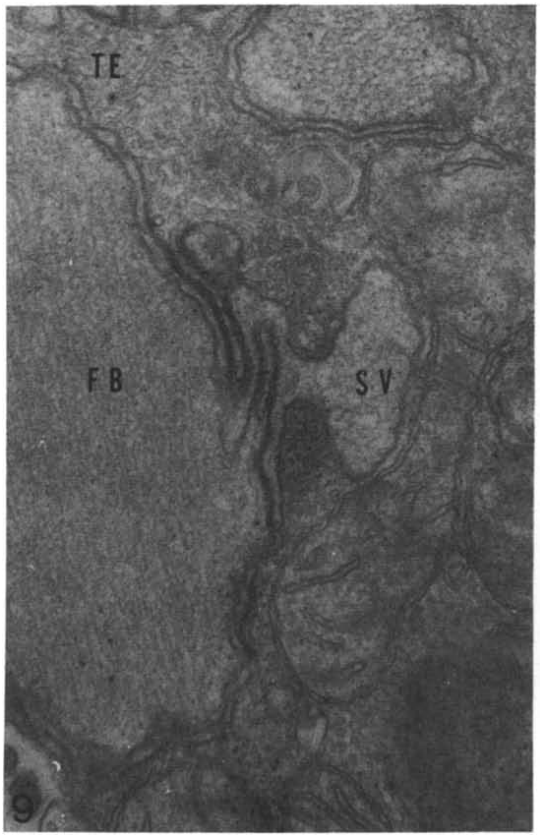
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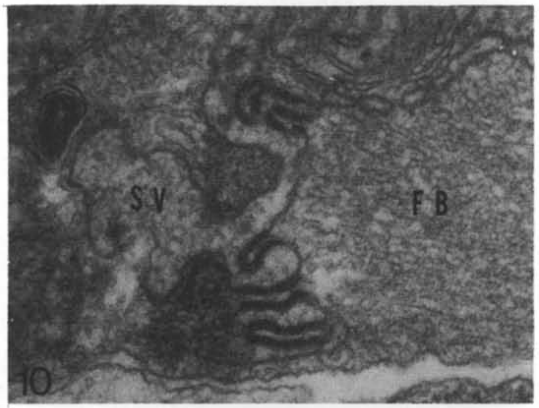
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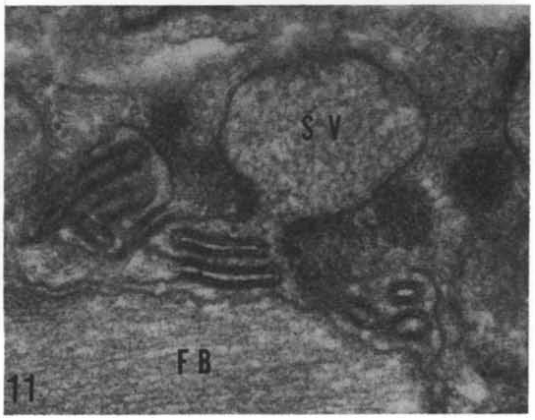
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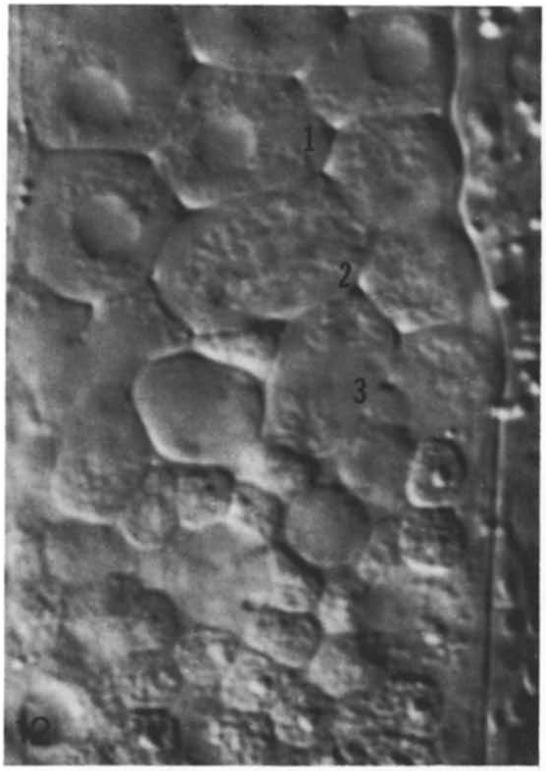
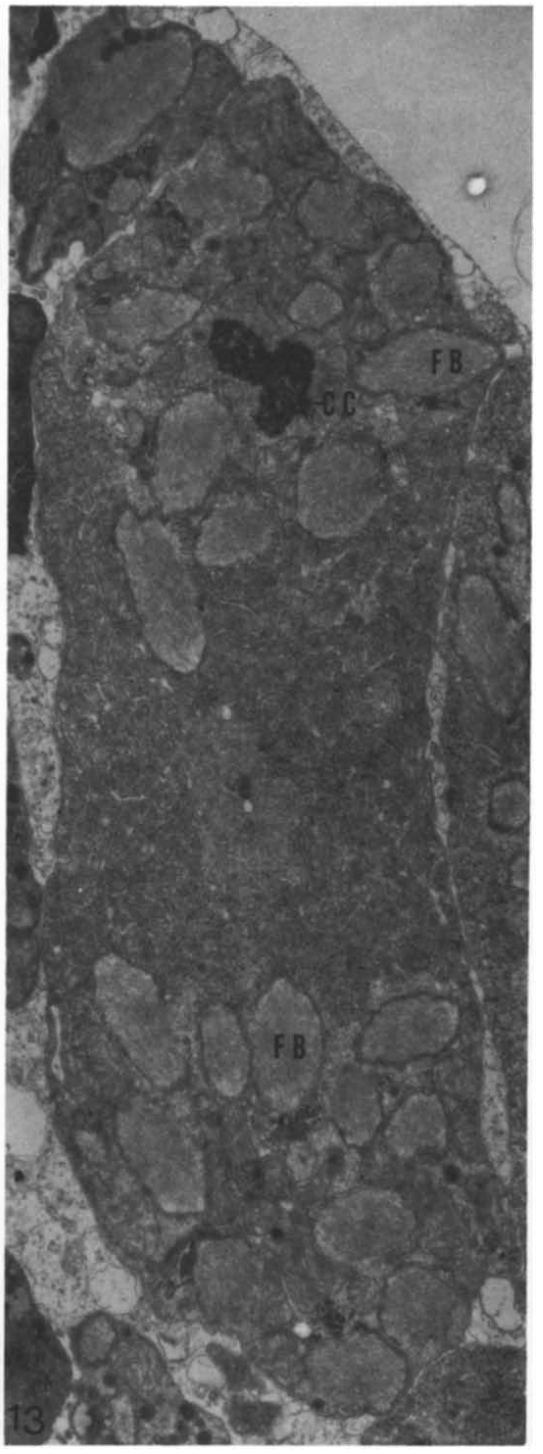
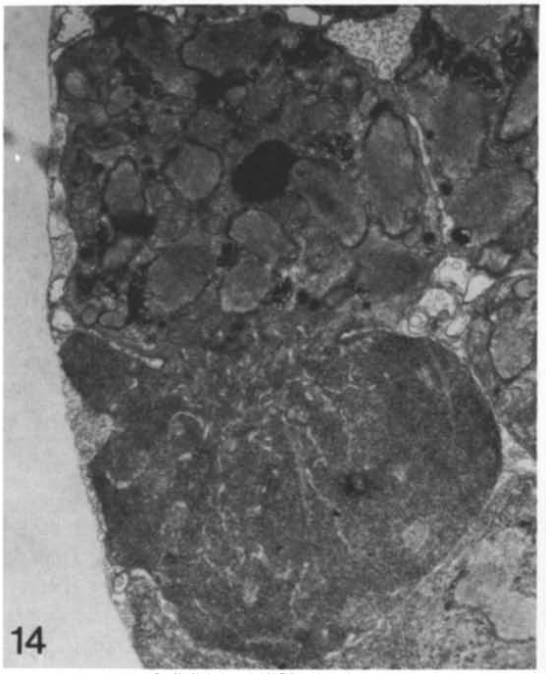






FIGS. 12–14. Meiosis.

cell contains largely ribosomes and endoplasmic reticulum, and it is this midregion that is pinched off during cytokinesis (Fig. 14). Thus, as a result of this division, presumably meiosis II, one observes both spermatids and anucleate cytoplasmic bodies in the gonad.

The spermatid. The spermatid has a condensed nucleus with adjacent structures we identify as centrioles (Fig. 15). Mitochondria are visible in the spermatid, but the cytoplasm consists mainly of the fibrous bodies with their attached vesicles. Much of the periphery of each fibrous body is no longer bounded by membranes.

A late spermatid is shown in Fig. 16. The fibrous bodies of the spermatid have disappeared. The special vesicles have completed enfolding the membranes and have become distinct structures which we have termed special membrane structures. The special membrane structures are highly asymmetrical; on one side of the collar the vesicle is round, on the other side it is folded. All of the special membrane structures are found near the periphery of the cell. The round side of a special membrane structure usually faces the plasmalemma. The most prominent cytoplasmic organelles of the late spermatid are the special membrane structures and the mitochondria. The nucleus of the late spermatid is fully condensed. The dark halo that surrounds the nucleus makes it difficult to discern a nuclear envelope if one is present. The centrioles are located within the dark halo (Fig. 16).

Sperm. There are two regions to the cytoplasm of a mature sperm (Fig. 17). One region contains no visible organelles, and here the cytoplasm appears amorphous. The other region contains the nucleus and all the cytoplasmic organelles. As in the late spermatid, the most prominent organelles are the mitochondria and the special membrane structures. In the sperm, however, the membranes of the special membrane structures are now probably continuous with the plasmalemma. This is likely because the electron density within most of the special membrane structure is about the same as the extracellular space, because there appears to be a continuity through the region delimited by the collar, and because we have found images that appear to represent intermediates in the presumed fusion process.

The material that used to define the collar of the special vesicle now appears as a ring just below the plasma membrane delimiting a pore that opens into the cup of the special membrane structure. The cup contains numerous images of folded membranes. Presumably these are the folded membranes of the composite structure. The concave surfaces of the folded membranes still show evidence of the electron-dense deposition.

DISCUSSION

Spermatogenesis in *C. elegans* follows the general pattern observed in other nematodes with a telogonic germ cell production in which the stages of spermatogenesis progress along the long axis of the gonad (*1, 8*). Spermatogenesis in the free-living nematode *C. elegans* is very similar to that in the parasitic nematode *Rhabditis pellio* (*2*). The anucleate core of the distal portion of the gonad described here is similar to

FIG. 12. Light micrograph of the zone of meiotic divisions. A primary spermatocyte (1) is shown. Cells undergoing a first meiotic division (2) and a second meiotic division (3) are also apparent. The smaller cells with prominent round nuclei in the lower part of the photograph are sperm. Nomarski differential interference contrast optics. × 4100.

FIG. 13. Electron micrograph of a cell undergoing the second meiotic division. The stage of division corresponds to the cell 3 in Fig. 12. The organelles, in particular all of the fibrous bodies (FB), are clustered at each pole of the dividing cell. Nuclei at this stage consist of highly condensed chromatin (CC). × 14 000.

FIG. 14. Electron micrograph of a cell at a late stage of the second meiotic division when the anucleate cytoplasm, devoid of organelles, is sloughed off. × 11 000.

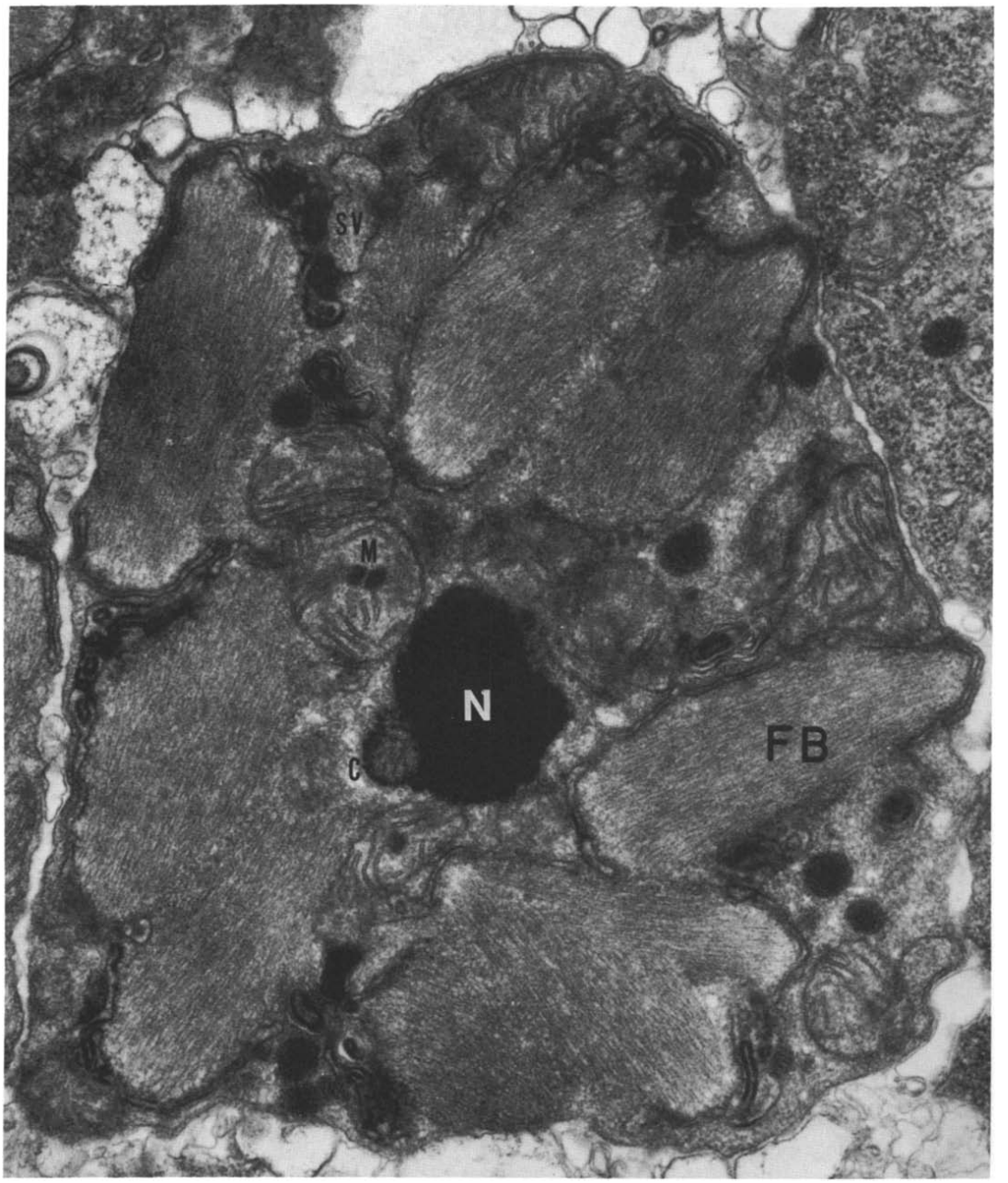


FIG. 15. Spermatid, showing the dense nucleus (N) without an apparent nuclear envelope. Centrioles (C) are adjacent to the nucleus (see also Fig. 16). The fibrous bodies (FB) are apparent, and their membranes have become reticulated. Special vesicles are associated with the edges of the fibrous bodies. The dense folded membranes are apparent where the special vesicles have joined the fibrous body membranes. The cytoplasm also contains many mitochondria (M). × 35 000.

that found in the male gonad of the distantly related nematodes *Heterakis gallinarum* (*16*) and *Dipetalonema* (*17*). On the other hand, there are male nematodes that do not have a core within their gonads such as *Aphelenchoides blastophorus* which is also only distantly related to *C. elegans* (*21*). In the gonad of *C. elegans* males, the

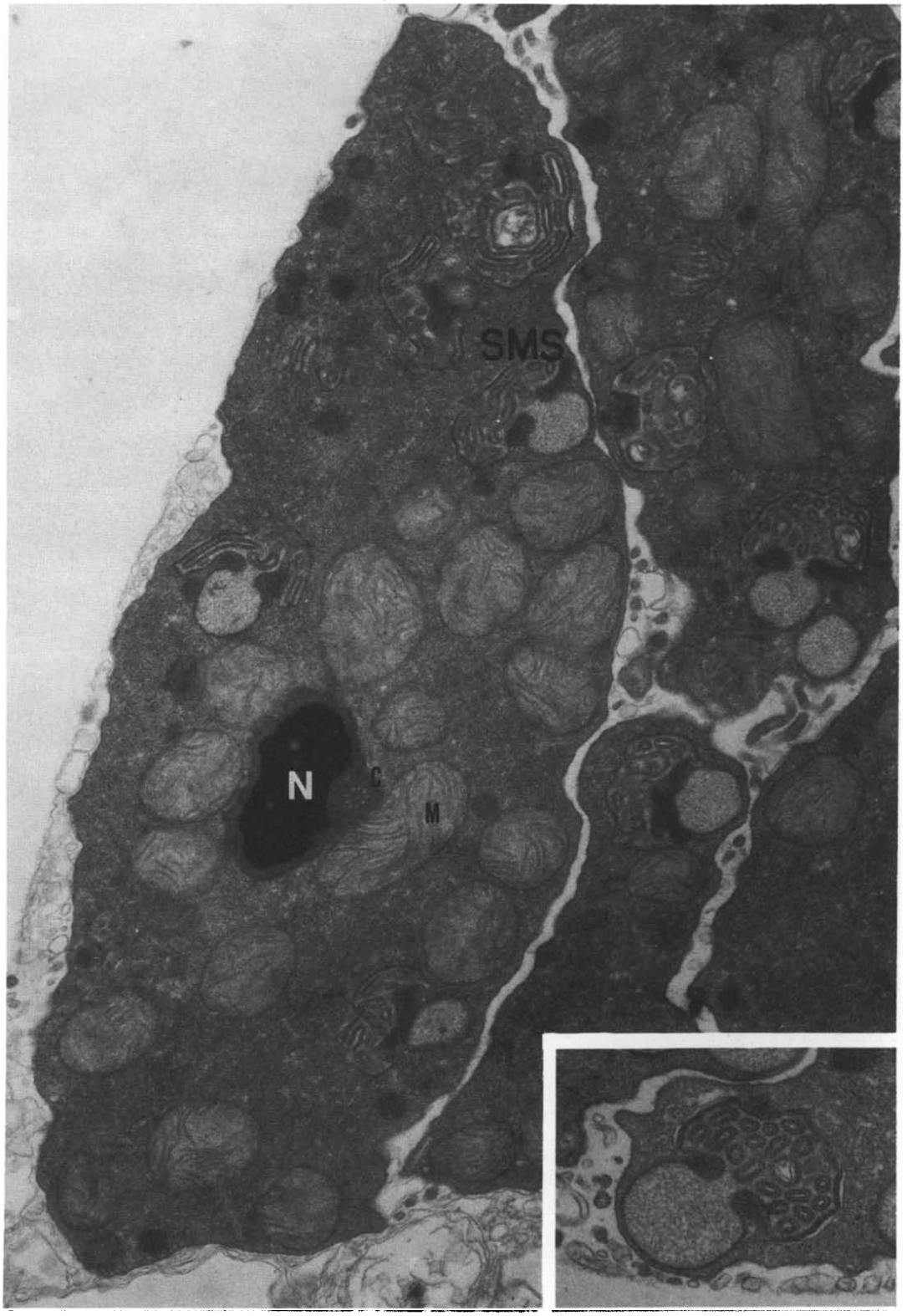
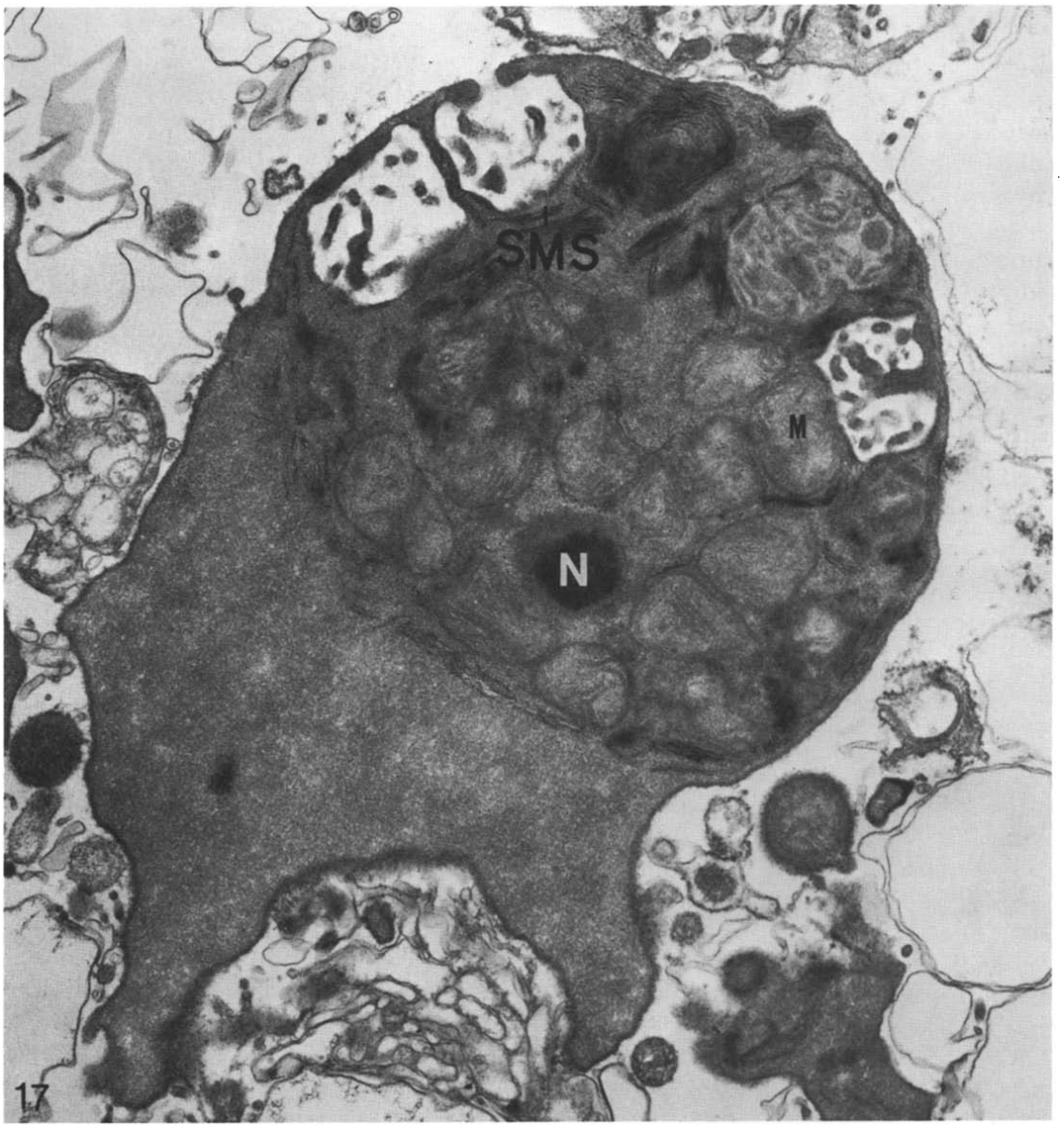


FIG. 16. Late spermatid. The special membrane structures (SMS) are apparent. They are derived from the association of the special vesicles with the fibrous bodies. The special membrane structures (SMS) lie close to the cell periphery. One is shown at a higher magnification in the insert. The nucleus (N) is highly condensed and is surrounded by an opaque halo. The centrioles (C) lie within the halo. × 34 000; inset, × 48 000.



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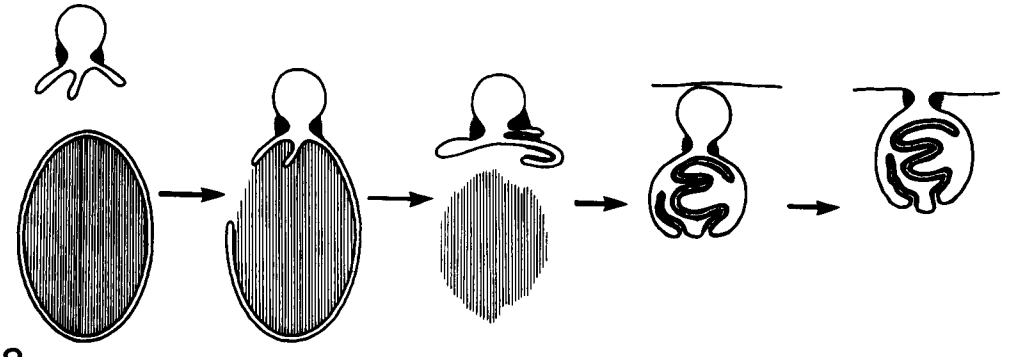

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core extends throughout the length of the distal arm, the loop region, and the anterior part of the proximal arm, up to the point where cytodifferentiation begins. This morphology is similar to the rachis described by Lee in *Heterakis gallinarum* (*16*). The core cytoplasm in *C. elegans* males is continuous with the cytoplasm of the surrounding cells of the gonad. Throughout most of the gonad the appearance of the core cytoplasm is the same as that observed in the cells surrounding it. Only in the last part of the gonad is the core cytoplasm distinctly different from the cytoplasm of the germ cells around it. In this region, the core contains an abundance of endoplasmic reticulum and free ribosomes but few other organelles, whereas at the same level of the gonad the cells are rich in Golgi complexes and mitochondria and contain less endoplasmic reticulum.

The most distal portions of the male and hermaphrodite gonads are strikingly similar in fine structure: Both are constructed with a core. The similarity is perhaps surprising since one gonad is making sperm, the other eggs. In oogenesis the core cytoplasm seems to serve a function: It appears to represent the pathway by which multiple cells may contribute cytoplasm to a single nucleus. In spermatogenesis, this function is obviated, and it is not clear what role the core might play. Possibly it is a vestigial manifestation of the close similarity between male and hermaphrodite, although the pathways of organogenesis of the two gonads are distinguishable at an early stage (J. Kimble, personal communication). This problem and the role of the sheath cells that surround the hermaphrodite gonad but not the male gonad will be interesting to study in the temperature-sensitive transformer mutant that has been described (*15*).

We have followed in detail the formation of the special membrane structures during spermatogenesis. These structures are present in the sperm of most nematodes and have been given different names by several authors as has been discussed by McLaren (*17*). Most investigators agree that the special membrane structures are derived from the Golgi complexes and the endoplasmic reticulum (*2, 6, 16, 17, 19*). The details of their formation have until now been unclear. We conclude from our studies that the special membrane structures are formed by fusion of two organelles, the special vesicles and the fibrous bodies. The membranes on one side of a special vesicle fuse with the membranes surrounding a fibrous body. Concomitant with this membrane fusion, the fibrous body becomes denuded of its own surrounding membranes and invaginated membranes appear in the region of the original fusion between the two organelles. It appears as if the special vesicle pulls the surface membranes from the fibrous body and packages the membranes by invagination. At the time that this invagination is occurring, there is deposition of electron-dense material on the membrane surfaces. Perhaps the dense material is mechanically important for the invagination process. After the formation of the special membrane structures, the fibrous bodies no longer have limiting membranes, and soon thereafter the fibers are no longer visible. The molecular identity and function of the fibers remain unknown and must await the completion of chemical studies on the sperm. The abundance of fibers is striking. If the fibrous material is depolymerized and remains undegraded in the mature sperm, it is likely it will appear as the major molecular component. Clearly the fibers change state as the membranes

FIG. 17. Mature sperm showing the two areas of cytoplasm, the pseudopodial end and the perinuclear area containing the organelles. There are several mitochondria (M). The special membrane structures (SMS) have fused with the plasmalemma and their insides are now continuous with extracellular space. × 34 000.

FIG. 18. Diagrammatic representation of the association of a special vesicle with a fibrous body to form a special membrane structure which then fuses with the plasma membrane.

fold up during formation of the special membrane structures, but whether the fibers depolymerize and become the amorphous cytoplasm of the pseudopod, the tubular elements of the cytoplasm, or some unidentified structure is not understood. The tubular elements of the sperm cytoplasm have been referred to as "microtubular elements" by Shepherd *et al.* (*20*) and as smooth endoplasmic reticulum by Foor (*11*) and Burghardt and Foor (*4*), but at this time their composition and function also remain unknown.

Pasternak and Samoiloff (*19*) have observed the formation and the disappearance of fibrous bodies during spermatogenesis in the free-living nematode *Panagrellus silusia.* These authors raised the question of the function of the fibrous bodies particularly in view of their transient appearance. One possibility is that the fibrous bodies provide a mechanism for packing the fibrous material so that it remains in the sperm and is not discarded with the spermatocyte cytoplasm that is sloughed off at the second meiotic division.

The centrioles observed in the spermatids and sperm consist of a ring surrounded by nine single microtubules. The same type of centrioles have been observed in other nematode sperm (*2, 8–11, 16*).

We have observed what appear to be mature sperm in the *C. elegans* male. Pasternak and Samoiloff (*19*) have also observed mature sperm in the male of the free-living nematode *Panagrellus silusiae,* but in several other nemtodes final maturation of the sperm takes place after transfer to the uterus (*2, 8–11, 16*). We have also examined virgin males and found their sperm identical to those sperm we have described here. Also sperm in unmated hermaphrodites of *C. elegans* appear to have the same fine structure as the male sperm described here.

In the mature sperm of *C. elegans,* the special membrane structures appear to have fused with the plasmalemma and emptied part of their contents to the exterior. Our understanding of the stages of this process is diagrammed in Fig. 18. The function of these special membrane structures is presently unknown and must await further studies.

This fine structure study has characterized stages in the morphogenesis of the sperm of *C. elegans.* In future work we hope to use mutants defective in spermatogenesis to identify the functions of the structures described here and ultimately to dissect the various steps in this morphogenetic pathway with the goal of improving our understanding of the molecular biology of cytomorphogenesis.

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REFERENCES

1. ANYA, A. O., *Advan. Parasitol.* **14,** 267 (1976).
2. BEAMS, H. W., AND SEKHON, S. S., *J. Ultrastruct. Res.* **38,** 511 (1972).
3. BRENNER, S., *Genetics* **77,** 71 (1974).
4. BURGHARDT, R. C., AND FOOR, W. E., *J. Parasitol.* **61,** 343 (1975).
5. CHITWOOD, B. G., CHITWOOD, M. B., Introduction to Nematology. University Park Press, Baltimore, 1974.
6. CLARK, W. H., THOMSON, W. W., AND MORETTI, R. L., *J. Cell Biol.* **39,** 25a (1968).
7. DELAVAULT, R., *Arch. Anat. Microsc. Morphol. Exp.* **41,** 41 (1952).
8. FOOR, W. E., *Biol. Reprod. Suppl.* **2,** 177 (1970).
9. FOOR, W. E., JOHNSON, M. H., AND BEAVER, P. C., *J. Parasitol.* **57,** 1163 (1971).
10. FOOR, W. E., AND MCMAHON, J. T., *J. Parasitol.* **59,** 753 (1973).
11. FOOR, W. E., *J. Parasitol.* **60,** 125 (1974).
12. HIRSH, D., OPPENHEIM, D., AND KLASS M., *Develop. Biol.* **49,** 200 (1976).
13. HIRSH, D., AND VANDERSLICE, R., *Develop. Biol.* **49,** 220 (1976).
14. JAMUAR, M. P., *J. Cell Biol.* **31,** 381 (1966).
15. KLASS, M., WOLF, N., AND HIRSH, D., *Develop. Biol.* **52,** 1 (1976).

16. LEE, D. L., *J. Zool.* **164,** 181 (1971).
17. MCLAREN, D. J., *Parasitology* **66,** 447 (1973).
18. NEILL, B. W., AND WRIGHT, K. A., *J. Ultrastruct. Res.* **44,** 210 (1973).
19. PASTERNAK, J., AND SAMOILOFF, M. R., *Canad. J. Zool.* **50,** 147 (1972).
20. SHEPHERD, A. M., CLARK, S. A., AND KEMPTON, A., *Nematologica* **19,** 551 (1974).
21. SHEPHERD, A. M., AND CLARK, S. A., *Nematologica* **22,** 1 (1976).