Spermatogenesis in the insect-parasitic nematode, *Heterorhabditis bacteriophora* Poinar (Heterorhabditidae : Rhabditida)

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SUMMARY

Spermatogenesis occurs in the ovotestis of the first generation hermaphroditic females and the testes of the second generation males of *Heterorhabditis bacteriophora* Poinar, 1976. The development and morphology of the various stages appear to be the same in both forms. Spermatogonia undergo mitosis and mature into primary spermatocytes. Each primary spermatocyte divides into two secondary spermatocytes and these in turn divide into two spermatids. The spermatids undergo several stages of maturation involving the loss of their residual body and reorganization of their fibrous bodies. They develop into spermatozoa, characterized by a small condensed nucleus and a distinct pseudopod. Mature spermatozoa do not occur in the testis but develop in the amphimictic female after mating. The mature spermatozoa are amoeboid and basically similar to most nematode sperm. The appearance of fibrous bodies in the primary spermatocyte and their development and eventual dissolution in the spermatozoon, along with the formation of membranous specializations have been studied at the ultrastructural level.

RÉSUMÉ

La spermatogenèse chez le nématode parasite d'insecte Heterorhabditis bacteriophora *Poinar (Heterorhabditidae : Rhabditida)*

Chez *Heterorhabditis bacteriophora* Poinar, 1976, la spermatogenèse a lieu dans les ovotestis des femelles de première génération et dans les testicules des mâles de seconde génération. Le développement et la morphologie des différents stades apparaissent identiques dans l'un et l'autre cas. Les spermatogonies subissent une mitose et se transforment en spermatocytes primaires. Chaque spermatocyte primaire se divise en deux spermatocytes secondaires qui à leur tour se divisent en deux spermatides. Ces spermatides passent par différents stades de maturation au cours desquels leur corps résiduel est perdu et leurs corps fibreux réorganisés. Ils évoluent en spermatozoïdes, caractérisés par un noyau petit et condensé et un pseudopode distinct. Les spermatozoïdes matures ne s'observent pas dans le testicule : ils se développent en effet dans les femelles amphimictiques après fécondation. Les spermatozoïdes mûrs sont amiboïdes et pour l'essentiel similaires à ceux de la plupart des nématodes. La structure et le développement des corps fibreux chez le spermatocyte primaire ainsi que leur éventuelle dissolution chez le spermatozoïde, de même que la formation de différenciations membraneuses ont été étudiés au niveau ultra-structural.

Heterorhabditis bacteriophora Poinar, 1976 and other members of this entomogenous genus are capable of developing in a wide range of insect hosts (Poinar, 1979). They are unique in exhibiting heterogamy during development within a single host. Infection is initiated by a third stage infective or « dauer » juvenile which is the only free-living stage in the live history of this nematode. After penetrating into the body cavity of an insect, the infective stage develops into a first generation, hermaphroditic female which produces progeny that develop into a second generation amphimictic population. The progeny of this latter generation normally develop into infective stage juveniles which leave the host cadaver and search for new insect hosts.

Spermatogenesis actually occurs twice in the normal life history of *H. bacteriophora*. The first time is in the ovotestis of the first generation protandric hermaphrodite. The second time is in the testis of the second generation male. The purpose of this paper is to examine spermatogenesis in these two sites. Since some confusion exists as to the terminology of the various stages encountered in spermatogenesis, we have followed the outline of Shepherd (1981).

Materials and methods

The nematode *H. bacteriophora* was originally described from a lepidopterous larva in Australia (Poinar, 1976) however it has a wide host range and for the present studies, was reared on larvae of the wax moth, *Galleria mellonella L.* Infected insects were dissected daily over a period of two weeks to follow the development of the nematode. For light microscopic studies, the hermaphroditic females and males were placed in a drop

of saline on a microscope slide and their bodies ruptured with a fine needle to allow extrusion of the reproductive tissue. They were then examined under bright field and Differential Interference Contrast with a Nikon Optiphot microscope.

For electron microscope studies, nematodes were ruptured as described above and the reproductive tissue was immersed in 2.5 % phosphate-buffered (0.1 M, pH 7.2) glutaraldehyde for one hour. Specimens were postfixed in 1 % osmium tetroxide in phosphate buffer for one hour, dehydrated and embedded in Araldite 6005. Sections were stained with saturated aqueous uranyl acetate followed by lead citrate and examined in a Philips EM 300 electron microscope. The following stages were examined for sperm development; pre-adult and adult hermaphroditic females, males, and mated amphimictic females.

Results

Light microscope observations

First generation hermaphroditic female : Spermatogenesis occurred in the ovotestis of the fourth stage or pre-adult female. At this time the ovotestis had reached its final shape with the distal tip reflexed and the proximal portion attached to the oviduct and uterus. The vulval opening and portions of the vagina were formed only after the final molt to the adult stage. The spermatogonia were found in the distal region of the non-reflexed portion of the ovotestis. Production of the primary and secondary spermatocytes and spermatids took place over a 48 h period just before the final molt, maturation of the spermatids into mature spermatozoa took longer. The regions of development that occurred in the testis (germinal, maturation and meiotic zones) could not be distinguished in the ovotestis. At the final molt, spermatids shedding their residual bodies could be found in the proximal portion of the ovotestis at the junction with the oviduct. The residual bodies were clear and uniform under the light microscope in contrast to the spermatid body which showed various surface irregularities, especially elongate bacilliform structures which represented the fibrous bodies. Only later after the eggs had formed and were moving down toward the oviduct were mature sperm seen in the ovotestis.

Second generation male : Spermatogenesis in the testis occurred in the adult male. Spermatogonia were located in the distal reflexed portion of the testis arranged around the rachis (Figs. 1a, 2a). The cells were small, but contained distinct nuclei with nucleoli. The primary spermatocytes appeared in the maturation zone at the distal portion of the rachis (Figs. 1b, 2b). These cells were large and contained prominent nuclei and nucleoli. Small fibrous bodies were distributed throughout the cytoplasm. Initially the primary spermatocytes were attached to the rachis. Later, they separated and divided into secondary spermatocytes (Figs. 1c, 1d, 1e, 2c). The secondary spermatocyte was characterized by nuclear dissolution. The nuclear area was represented by a wide cavity in the center of the cell (Figs. 1c, 1d) and the fibrous bodies collected on the periphery of this cavity (Fig. 2c). After separating, the secondary spermatocyte divided into two spermatids (Figs. 1e, 1f, 2d). These immature spermatids exhibited nuclear dissolution and larger fibrous bodies. They developed into mature spermatids which possessed a radial arrangement of the fibrous bodies and a more concentrated nucleus (Figs. 1g, 2e). At this point, the spermatids shed their residual bodies. Each spermatid was observed to shed a single residual body and afterwards entered a circular resting stage during which time the nucleus became prominent (Figs 1h, 2f). This stage was considered the immature spermatozoon and as the pseudopod formed, the nucleus condensed until a stage we described as the mature spermatozoon was formed (Figs. 1i, 2g). The final developmental stage in the testis was the immature spermatozoon. The mature spermatozoa were found only in the amphimictic female. The female also occasionally contained secondary spermatocytes and spermatids since if these stages were located in the lower portion of the gonad, they would be transferred into the uterus during mating.

Electron microscope observations

Sperm development in the hermaphroditic female and in the male appeared to be similar. The steps of spermatogenesis described here were observed in the male gonad.

Spermatogonia in the distal germinal zone of the gonad had very little cytoplasmic differentiation (Fig. 3a). They were packed with free ribosomes and contained a few small mitochondria. Golgi and endoplasmic reticulum were not obvious. The nuclei were large with prominent nucleoli. Chromatin was diffuse throughout the nucleoplasm.

Cells located in the distal, germinal zone of the gonad appeared to be syncytial with the nucleus-containing cell bodies interconnected by a central cytoplasmic core or rachis. The more proximal spermatogonia contained several small vacuoles. These vacuoles may have a patch of amorphous electron-dense material applied to the inner surface.

The cytoplasm of spermatocytes located in the maturation zone of the gonad was more highly differentiated than that of the spermatogonia and contained extensive rough endoplasmic reticulum as well as Golgi. The nuclei were observed to have more dense chromatin (Fig. 3b). Primary spermatocytes were interconnected by a cytoplasmic bridge, the rachis (Figs. 3b, 3c). The rachis was surrounded by a prominent plasma membrane which was separated from the cytoplasm by an apparent

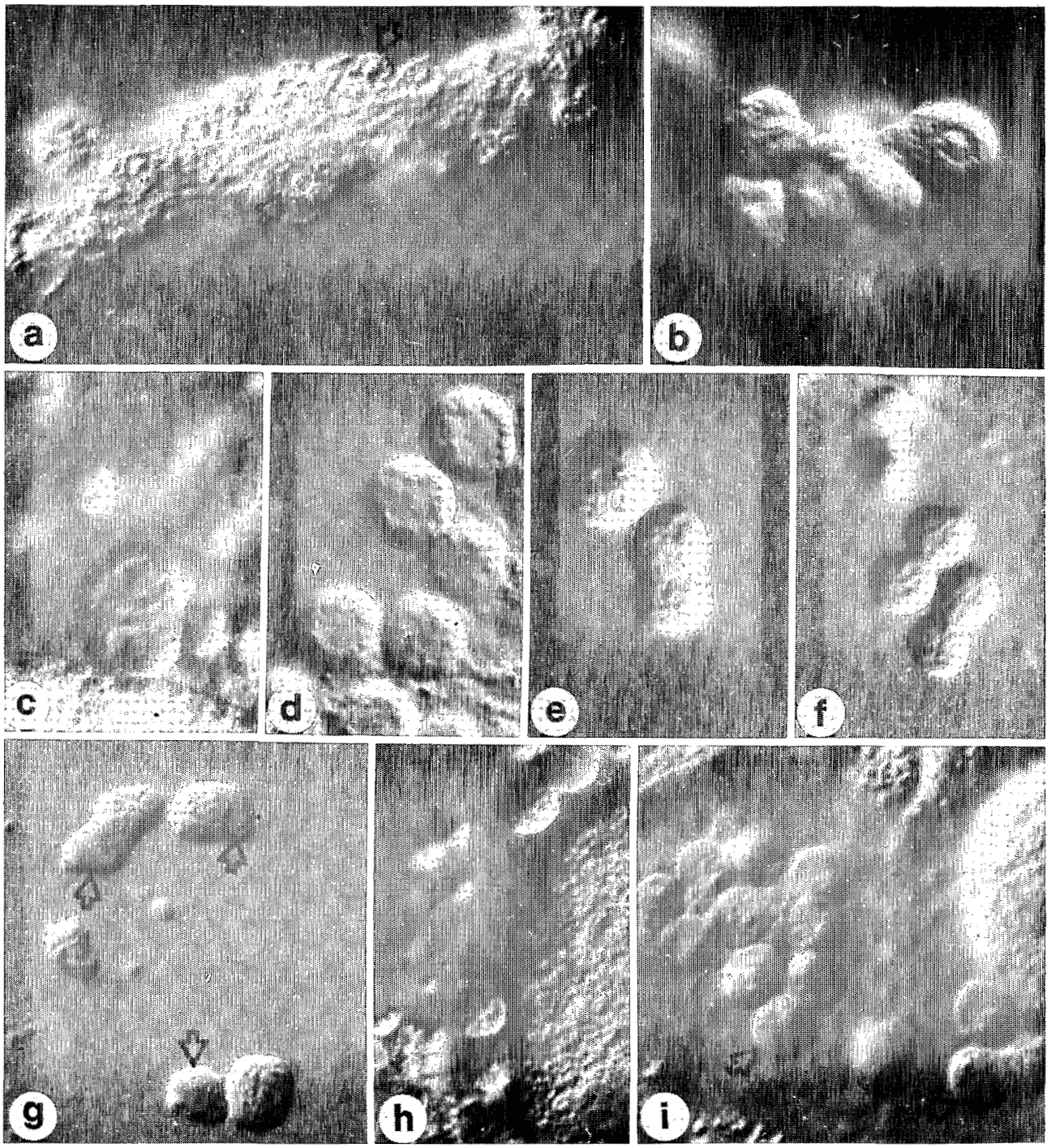


Fig. 1. Stages encountered during spermatogenesis in *H. bacteriophora*. All photos were taken at the same magnification (X 100). a. Spermatogonia (arrows) attached to rachis; note small size and prominent nuclei. b. Primary spermatocytes still attached to rachis. c. Detached primary spermatocyte dividing into two secondary spermatocytes. d. Secondary spermatocytes. e. Two secondary spermatocytes, one starting to divide into two spermatids. f. Two secondary spermatocytes completing their division into immature spermatids. g. Mature spermatids at various stages of losing their residual bodies (arrows). h. Immature spermatozoa; note prominent nuclei and absence of distinct pseudopod. i. Mature spermatozoa with pseudopods (arrows) and distinct nuclei.

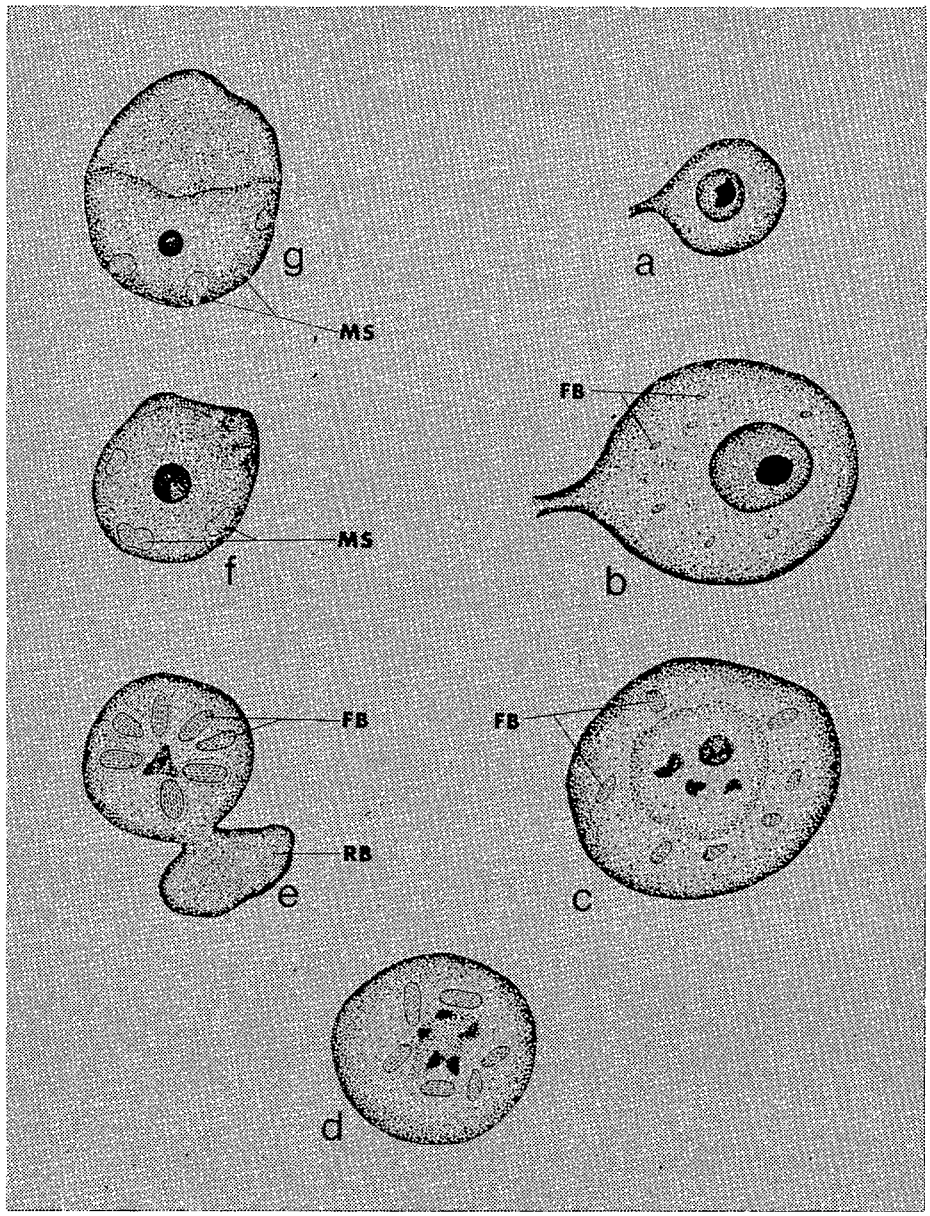


Fig. 2. Schematic illustration of the stages encountered during spermatogenesis in *H. bacteriophora.* The spermatogonium (a) is characterized by its small size, prominent nucleus and nucleolus and attachment to the rachis. This stage matures into the primary spermatocyte (b), characterized by its larger size, prominent nucleus and nucleolus, small fibrous bodies and attachment to the rachis. This stage detaches from the rachis and divides into two secondary spermatocytes. The secondary spermatocyte (c) exhibits nuclear dissolution, and an increase in fiber body size. The secondary spermatocyte divides to produce two spermatids. The immature spermatid (d) is characterized by nuclear reorganization and an increase in size of the fibrous bodies. This stage develops into a mature spermatid which sheds the residual body (c). The mature spermatid is characterized by a partially reorganized nucleus, with fibrous bodies arranged in a radial pattern and surrounded by loose membranes (precursor of the membranous specializations). The mature spermatid develop into an immature spermatozoon (f) characterized by a small pseudopod (not always apparent), membranous specializations in the cytoplasm, a distinct nucleus and dissolution of the fibrous bodies. This stage develops into the mature spermatozoon (g) characterized by a well developed pseudopod, a small condensed nucleus and membranous specializations which now open to the exterior at the border of the cell. All figures were made at the same scale. FB = fibrous bodies, MS = membranous specializations, RB = residual body.

clear zone about 100 nm wide. The cytoplasm was filled with masses of dense tubules 35 nm in diameter each of which was also surrounded by a clear zone. Occasional mitochondria were observed within the rachis as well as microtubules, small electron-dense granules surrounded by multiple membranes, and vacuoles. No other cell organelles were observed in this region. Spermatocytes undergoing meiosis separated from the rachis.

As the spermatocytes differentiated, a distinctive structural complex known as the fibrous bodies (FB) was formed throughout the cell. Vacuoles similar to those observed in the spermatogonia now had an electron-dense bulbous deposit associated with one end. This deposit was observed to protrude into the lumen of the vacuole (Figs. 3c, 3d) or be situated within the cytoplasm (Fig. 3c). The vacuoles with deposits were frequently in contact with mitochondria. Ribosomes were observed close to the vacuole membrane. The deposits in older primary spermatocytes were larger and now displayed a fibrous structure (Fig. 3e). The fibers were approximately 7 nm in diameter. A definite association of the vacuoles with other organelles was not observed. As the spermatocytes continued to mature, the fibrous deposits enlarged and in the larger deposits, a close association with the Golgi complex was observed. This association appeared on the vacuole side of the fibrous deposit (Fig. 4a). Two unit membranes appeared partially surrounding larger fibrous deposits (Fig. 4b). These membranes seemed to be continuous Golgi saccules which proliferated outward and around the deposits (Fig. 4c). In more mature spermatocytes, the fibrous deposits were completely surrounded by the two membranes and the structure formed was the fibrous body (FB). At on edge of the fibrous body, a vacuole was observed with electron-dense accumulations on either side (Fig. 4c). This vacuole was assumed to be the original vacuole associated with the fibrous deposits. These accumulations condensed and formed a collar which later appeared to constrict the vacuole (Fig. 4d). The condensed accumulations lay pressed against the vacuole membrane which appeared thickened in this region. A fine fibrillar coat, possibly glycoprotein, was observed on the inner vacuole membrane in the area of contact with the collar. Fibrous bodies continued to enlarge and in the spermatid, they were elongate, having a bacilliform shape in longitudinal section and rested preferentially in a radial arrangement around the nucleus (Fig. 4f).

In spermatids, the cytoplasm became polarized with the nucleus, fibrous bodies and mitochondria in one end and the other cell organelles localized in another. This polarization was first observed in secondary spermatocytes where the FB, mitochondria and nucleus were centrally located and the ribosomes peripherally located (Fig. 4e). In successive stages in the spermatid the ribosome-containing portion of the cell became more distally located and as the cell elongated the nuclear membrane disappeared and the chromatin condensed (Fig. 4f). Occasional sections showed a dumbbell-shaped form with a nucleus and associated organelles at each end and the central area filled with ribosomes. The ribosome-containing area budded off as cytokinesis occurred and became the residual body (Fig. 4g). The remaining nuclear-containing cells were spherical and represented immature spermatozoa (Fig. 5a). The nucleus appeared centrally located with the fibrous bodies radiating outward. Occasional residual bodies were observed which also contained dense nuclei.

During the formation period of the fibrous bodies, nuclei were observed in the spermatocytes in which the nuclear membrane was absent. Various stages in the condensation of the nuclear material were observed. The condensing nuclear material was first seen as diffuse electron-dense fibers in a clear area of the cytoplasm. In a more condensed stage in the spermatid, the fibrous strands appeared thickened and arranged in spherical configurations (Fig. 4a). In the final stages of condensation, the nucleus was electron-dense and homogeneous. The outline in section was bilobular with occasional channels possibly representing indentations (Fig. 5a). Surrounding the dense nucleus was a halo of interconnected tubular elements 22 nm in diameter (Figs. 5c, 5d, 5i). The tubular elements were filled with a dense amorphous substance. These tubular elements lay in a clear zone devoid of organelles, however one side of the halo was extended towards the cell membrane and within this extension the centrioles could be seen.

The fibrous bodies in the spermatids began to lose their associated double membranes (Fig. 4g). This process continued as the membranes became infolded, perhaps even vesiculated and detached from the fibrous bodies presumably by a contractive mechanism of the Golgi complex. This appeared to pull the membranes back to the area where the vacuoles with their electron-dense collars were originally formed (Fig. 5b). This resulted in the formation of membranous specializations (MS) which appeared as large vacuoles containing small microvilli-like internal protections capped by the vacuole with its electron-dense collar (Figs. 5c, 5d). A rearrangement in the position of the fibrous bodies and the membranous specializations occurred with the MS moving to the exterior of the cell and FB becoming more internal (Fig. 5c). As the spermatozoon continued to mature, the cell became elongate with the FB moving to the pseudopodial area of the cell and the MS moving to a position beneath the plasma membrane (Fig. 5d). The MS were aligned adjacent to the plasma membrane with the dense collar oriented in a position directly beneath the plasma membrane (Fig. 5e). Progressive stages suggested that a fusion of the plasma membrane with the membrane of the MS occurred at the electron-dense collar in the mature spermatozoon. This area of fusion appeared plugged with an amorphous deposit which was subsequently released into the oviduct as the MS opened to the exterior (Fig. 5f). Morphological evidence showed

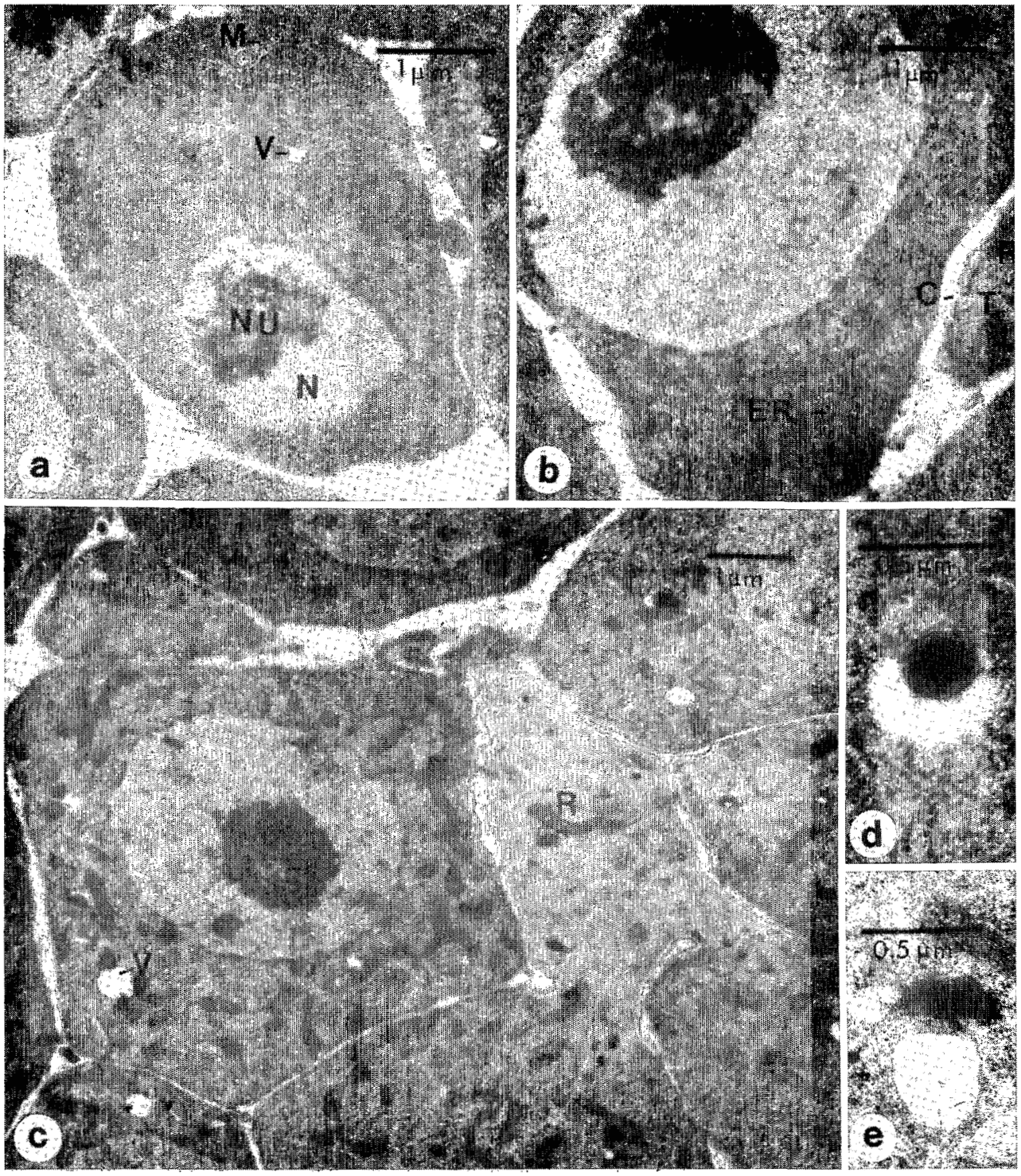


Fig. 3. Spermatogenesis in *H. bacteriophora*. All micrographs taken from males. a. Spermatogonia with cytoplasm filled with free ribosomes and a few small mitochondria (M) but otherwise seemingly devoid of other organelles. An empty vacuole (V) is observed within these cells. The nucleus (N) has a prominent nucleolus (NU) and diffuse chromatin. b. Primary spermatocyte showing an increase in the organelles distributed in the cytoplasm and the beginning appearance of rough endoplasmic reticulum (ER). The rachis (R) has a prominent plasma membrane separated from the cytoplasm by a clear zone (C). Within the rachis are electron-dense tubules (T) also surrounded by a clear zone. c. Primary spermatocytes showing interconnection by a cytoplasmic bridge, the rachis (R). Present in the spermatocytes are vacuoles with electron-dense accumulations (V). d. A vacuole from a spermatocyte showing an electron-dense accumulation as a bulbous protrusion. e. Vacuole with a dense accumulation showing fibrillar structure.

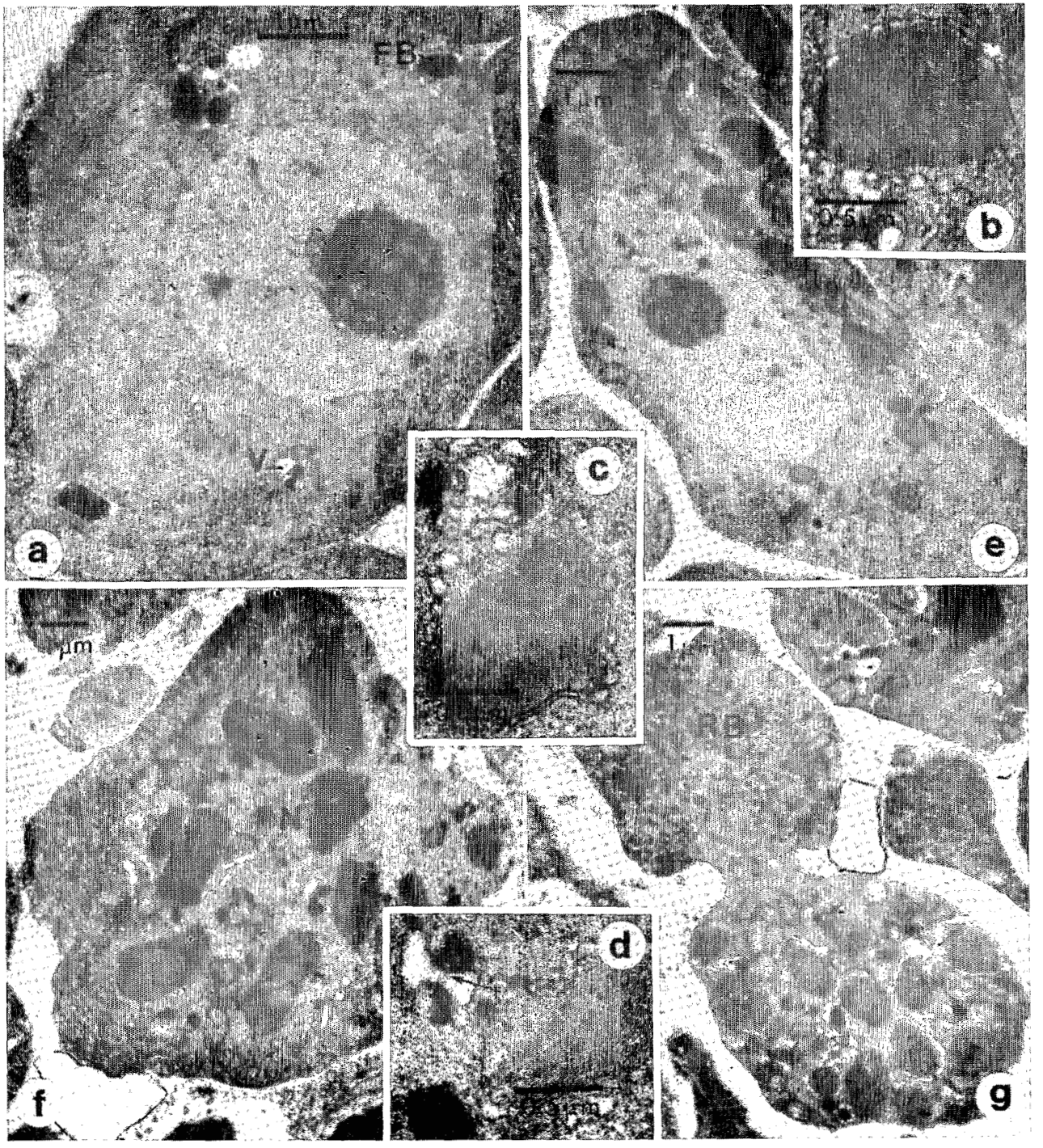


Fig. 4. Spermatogenesis in *H. bacteriophora*. All micrographs taken from males. a. Spermatocyte with fibrous bodies in various stages of formation from vacuoles with accumulations (V) to fibrous deposits surrounded by Golgy membranes (FB). b. Fibrous body illustrating envelopment by the Golgi membranes. Arrow indicates leading edge of the Golgi saccule. c. Fibrous body with associated vacuole and Golgi membranes. Electron-dense accumulations (A) are seen on either side of the vacuole. d. A further step in the formation of a vacuole is seen with the electron-dense accumulation (A) constricting either side. A fibrillar deposit, possibly glycoprotein is seen on the inner vacuole membrane (arrow). e. A secondary spermatocyte is shown in which the fibrous bodies are all surrounded by membranes and the cytoplasm is beginning to show polarity. f. A spermatid is shown where the fibrous bodies have reached the maximum size. The characteristic bacilliform structure of the fibrous bodies as well as their seemingly preferential radial arrangement around the nucleus is apparent. The nucleus (N) is in an intermediate stage of nuclear condensation. g. A spermatid in the process of budding off its residual body (RB).

an apparent decrease in visible MS in older spermatozoa as compared with immature forms and the presence of tubules closely associated with the spermatozoa of the same size and morphology as those observed within the unfused MS. The mature spermatozoon is characterized by fusion of the MS with the plasma membrane and the presence of pseudopodia (Fig. 5i).

The immature spermatozoa in the seminal vesicle were roughly spherical and contained an electron-dense nucleus (Fig. 5a). The fibrous bodies were arranged radially. Associated with the FB were vacuoles with electron-dense collars surrounded by Golgi membranes. Mitochondria were interspersed with the fibrous bodies. The cytoplasm was dense and devoid of any other organelles. In more mature spermatozoa the fibrous bodies have separated from the membranous specializations as described above. The organelles underwent a rearrangement within the cell with the MS first moving to the periphery of the cell. The fibers within fibrous bodies still retained their close association and remained around the nucleus (Fig. 5c). The fibers moved as a unit to one end of the cell as the spermatozoa became more elongate. This divided the cell into an organelle containing end with a dense nucleus surrounded by mitochondria, peripherally located membranous specialization (Fig. 5d), and a fiber containing end.

Spermatozoa were observed in the amphimictic female after mating. In the mature spermatozoon the membranous specializations had fused with the plasma membrane, releasing their contents (Fig. 5i). The end which contained the fibers became the pseudopodia and was now relatively homogeneous in appearance due to the disappearance of the protein fibers. Small electron-dense granules 4 nm in diameter were seen in the pseudopodia. The spermatozoa were amoeboid and varied in form from elongate to roughly spherical depending on whether the pseudopodia were extended in a single direction or fragmented into several directions. There appeared to be fewer membranous specializations in mature spermatozoa than in the immature spermatozoa after MS fusion with the plasma membrane. In some sections spermatozoa could be seen with only one or two MS in the plane of section. At the surface of the spermatozoa were tubular elements similar to those found in MS that were associated with depressions in the cell (Fig. 5g). Tubular elements of the same dimensions were also observed closely associated with the smoother outer surface of the spermatozoa (Fig. 5h) indicating a complete opening of these membranous specializations thereby exposing the microvilli to the oviduct lumen.

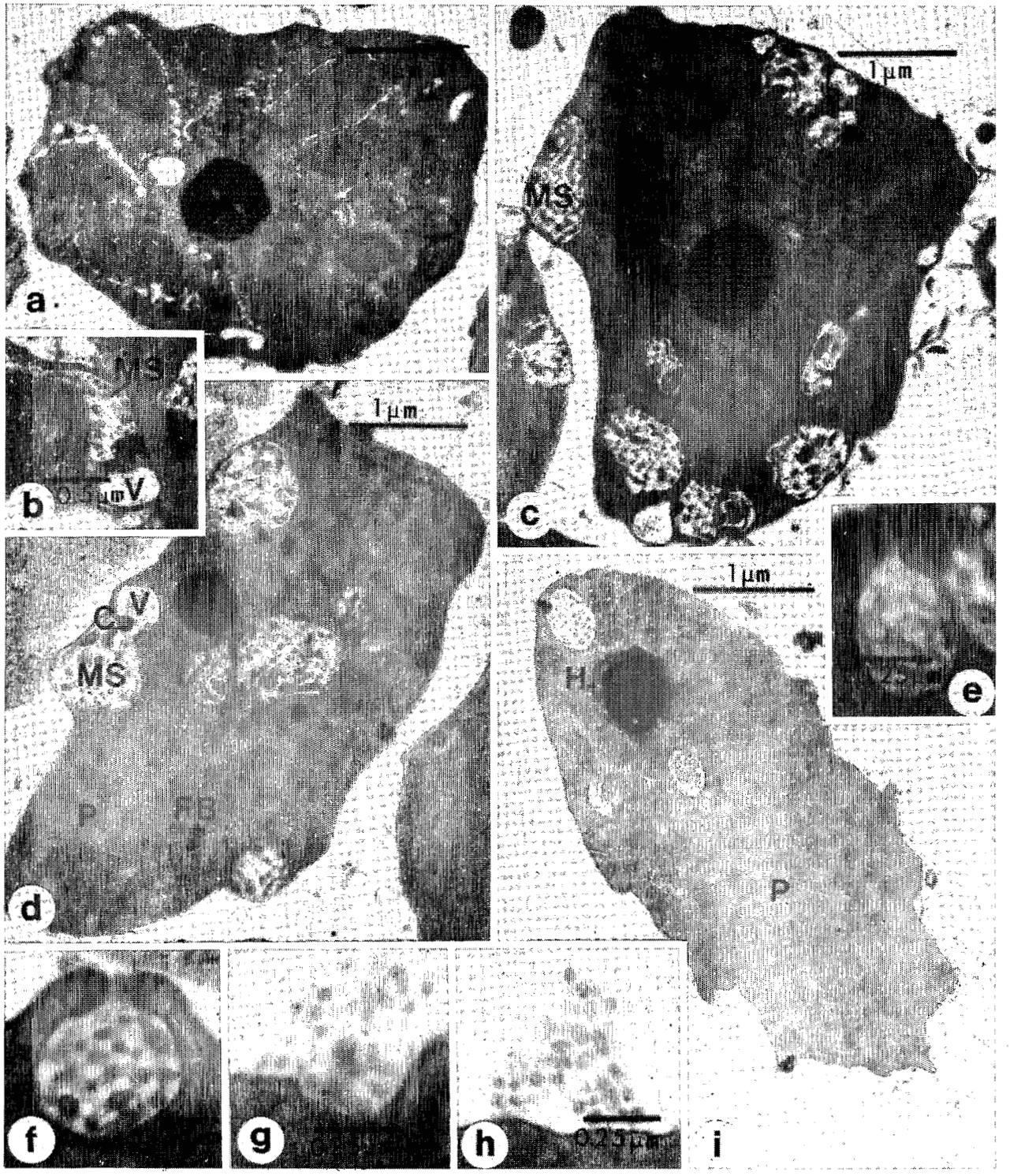
Sperm development in hermaphroditic females and males appeared the same ultrastructurally. The earliest point at which a sperm could be identified in the pre-adult hermaphroditic female was when the fibrous bodies first began to form in the primary spermatocytes.

Discussion

Heterogamy or the alternation of amphimictic and autotokous generations in nematodes is associated with animal parasitism involving both vertebrates and invertebrates (Poinar & Hansen, 1983). Of these cases the majority involves parthenogenesis as the autotokous mode of reproduction. Heterogamy involving hermaphroditism occurs only in the genera *Heterorhabditis* and *Rhabdias*. The latter is a parasite of amphibians and exhibits also heterogony. The parasitic female is a protandrous hermaphrodite and the free-living forms are amphimictic (Runey, Runey & Lauter, 1978). Thus *Heterorhabditis* is unique in being the only known hermaphroditic heterogamic nematode possessing homogony. Developmentally, the first generation hermaphrodite of *H. bacteriophora* resembles the free-living hermaphrodite of *Caenorhabditis elegans* and *C. briggsae*.

In *H. bacteriophora*, spermatogenesis occurs in both the first generation hermaphrodite and the second generation male. Spermatogenesis is initiated at the beginning of the fourth stage juvenile and terminates at the adult molt which occurs approximately 48 h later at 20°. Similar patterns of spermatogenesis in fourth stage

Fig. 5. Spermatogenesis in *H. bacteriophora*. All micrographs taken from males except 5 i. a. An immature spermatozoon with a fully condensed nucleus. The radially arranged fibrous bodies show a vesiculated membrane indicative of the first stage of retraction. b. This fibrous body is no longer surrounded by a Golgi membrane which has retracted, folded inward and formed a membranous specialization (MS). An electron-dense accumulation (A) is seen at the base of the vacuole (V). c. An immature spermatozoon in which the associated membranes of all the fibrous bodies have now formed membranous specializations. These membranous specializations (MS) have moved to a more peripheral position in the cell lying in most cases just beneath the plasma membrane. The perinuclear halo (H) is evident. d. In this immature spermatozoon the fibrous bodies (FB) are moving into the area of the cell which will become the pseudopodia (P). Note the electron-dennse collar (C) at the junction of the vacuole (V) and membranous specialization (MS). e. A membranous specialization which has positioned itself just below the plasma membrane and has apparently fused with the plasma membrane at or near the dense deposits. The channel to the outside is still plugged with an amorphous substance. f. The opening of this membranous specialization to the outside has formed but the passage still contains some amorphous material. g. A cup-shaped indentation on the surface of the sperm has associated vesicular profiles similar in size and structure to the microvilli-like projections in the membranous specialization so this probably represents a further stage in the



opening of these organelles. h. The same elongate vesicular material is seen associated with a flat surface of the sperm suggesting a complete unfolding of a membranous specialization. i. A mature spermatozoon is shown from a mated amphimictic female. The membranous specializations have fused with the cell surface. The electron-dense nucleus surrounded by a perinuclear halo (H) is located in one end together with the mitochondria. The extension of the pseudopodial (P) end produces an elongate from. Fibrous bodies are not visible at this stage.

juvenile hermaphrodites were noted by Wessing (1961) for *Rhabditis anomala* and Hirsh, Oppenheim and Klass (1976) for *Caenorhabditis elegans*. In these and *Heterorhabditis*, sperm production is completed by the end of the fourth stage juvenile although sperm maturation continues into the adult stage. This is in contrast to hermaphrodites like *Rhabdias ranae* (Runey, Runey & Lauter, 1978), *Rhabditis dolichura* (Maupas, 1900) and *Rhabditis sechellensis* (Potts, 1910) where spermatozoa and ova appear to be produced throughout the adult stage.

Another ununsual feature of *H. bacteriophora* is that the progeny of the hermaphrodites normally develop into males and females with an equal sex ratio. The males are relatively short lived (most perish within 48 h at 20°) but actively mate with the females. The females of the second generation are not hermaphrodites since sperm have never been observed in individuals held in isolation and unless males are present, the eggs will not develop.

However, if the progeny of the *Heterorhabditis* hermaphrodites are forced into « dauer » juveniles, then the dauer will always develop into another hermaphrodite. Thus, by restricting nourishment, a continuous population of hermaphrodites will be formed, similar to the natural condition found in *C. elegans*. Such a developmental pattern should provide an interesting cytogenetic study.

The process of spermatogenesis in the hermaphroditic female and in the male are similar. No ultrastructural distinctions can be made between the spermatocytes, spermatids or immature spermatozoa of either. Within the mated female mature spermatozoa also appear identical in structure to those observed within the hermaphroditic female.

The electron microscope observations reported here of spermatogenesis in *H. bacteriophora* are similar to those reported in *Caenorhabditis elegans* (Wolf, Hirsh & McIntosh, 1978; Ward, Argon & Nelson, 1981), *Panagrellus silusiae* (Pasternak & Samoiloff, 1972) and *Rhabditis pellio* (Beams & Sekhon, 1972). Features in common appear to be the formation of the fibrous bodies, the membranous specializations which subsequently fuse with the surface of the spermatozoon and a non-membrane bound nucleus. They differ from sperm of the « ascaroid » type (Foor, 1970) in that they lack a large refringent body. The fibrous bodies described in *C. elegans* have been found to contain the major sperm protein (Ward & Klass, 1982) which is subsequently located in the pseudopodia of the spermatozoa. Since the fibrous bodies were seen to migrate into the pseudopodia and underwent disassociation there it would seem that the same is also true for *H. bacteriophora*. Ward and Klass (1982) have suggested that the major sperm protein may participate in amoeboid motility although the presence alone of this protein is not sufficient for movement since non-motile mutant spermatozoa were also found to contain it. Lee (1971) in addition to considering the contractile function of the fibrous elements, suggested they may be a storage protein. Large deposits of fibrous material which may ou may not be associated with membranous specializations or organelles seem to be widespread in nematode sperm although, for example, fibrous bodies or deposits were not observed in the nematodes *Capillaria hepatica* (Neill & Wright, 1973). The retention of ultrastructurally recognizable fibrillar (fibrous) bodies in the spermatozoon is variable. Shepherd and Clark (1983) for example, have noted that the structure of the fibrillar material can be retained in the testicular spermatozoa of *Heterodera* spp., *Meloidogyne incognita incognita, M. acronea, M. arenaria, M. hapla* and *M. incognita wartelli* but not in *M. graminicola, M. oryzae* or *Globodera* spp.

Membrane specializations also appear to be characteristic organelles of many nematode sperm, although several types occur. Many membrane specializations, as in *H. bacteriophora* arise closely associated with fibrous bodies. In some the specialized membranes appear to arise from the Golgi as in *H. bacteriophora* whereas in others such as *Ascaris lumbricoides* (Foor, 1970) an association with mitochondria is suggested. The membrane specializations of *H. bacteriophora* after fusion with the cell membrane, appear to continue opening thus exposing the microvilli-like tubules. A similar situation is described in the inter-uterine spermatozoa of *Panagrellus silusiae* (Pasternak & Samoiloff, 1972). In both cases the released tubules suggest an analogy with the filopodia type of membrane specialization of the Heteroderoidae described by Shepherd and Clark (1983). Foor (1970) noted the absence of the typical membrane specializations in *Dioctophyma renale*. In the *in utero* spermatozoon, membranous elements were associated with tortuous channels which open to the exterior of the cell. In *Capillaria hepatica* Neill and Wright (1973) described membrane specializations just below the cell membrane in the tail of the mature sperm. These are formed by tubules which collapse upon themselves resulting in concave vesicles. They then appear to close upon themselves and fuse with adjacent units to give double membrane loops enclosing a portion of cytoplasm.

The function of the membrane specializations has been speculated upon. They appear to release a deposit into the uterus. Ward *et al.* (1982) suggest that in *Caenorhabditis elegans* this material may prevent cell-body adhesion to substrate. Clark, Moretti and Thomson (1967) and others suggested that they may have an acrosomal-like function in *Ascaris lumbricoides*. However this has been questioned since the sperm of *Ascaris* appears to enter the egg at the pseudopodial end according to the studies of Favard (1961) and Foor (1970). In *H. bacteriophora* the end of the sperm containing the membranous specializations appears to enter the egg first (manuscript in preparation). Shepherd, Clark and

Kempton (1973) suggest that the filopodia of *Heterodera* may have an acrosomal function. They report a close adherence of the filopodia to the oocyte membrane with the apparent discontinuity of the membrane in the region of contact.

In conclusion, the general pattern of spermatogenesis in *H. bacteriophora* follows that reported for other nematodes, especially members of the Rhabditida. The mature spermatozoon is distinctly amoeboid and resembles that of *C. elegans*. In *H. bacteriophora*, however, all dividing stages separate completely from the parent cell before undergoing further division.

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