# Ultrastructure of the genital ducts and sperm behavior in the insect parasitic nematode, *Heterorhabditis bacteriophora* Poinar (Heterorhabditidae : Rhabditida)

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SUMMARY

The testis wall of *Heterorhabditis bacteriophora* Poinar, 1976 was observed to consist of a thin attenuated epithelial sheath. The cells of the seminal vesicle were large and phagocytized residual bodies and defective sperm were observed within the cytoplasm. The walls of the *vas deferens*, ovary and ovotestis were composed of glandular cells. The oviduct cells were thicker and characterized by distinctive cellular junctions. The uterine walls were thin cytoplasmic sheaths. In mated amphimictic females, spermatozoa accumulated in the seminal receptacle between the uterus and oviduct. In the hermaphroditic females, spermatozoa congregated at the junction of the ovotestis and oviduct. Three distinct types of associations of the spermatozoa with the walls of the reproductive tracts were observed. These structural associations were suggestive of spermatozoan movement, attachment and engulfment.

RÉSUMÉ

*Ultrastructure des conduits génitaux et comportement des spermatozoïdes chez le nématode parasite d'insectes* Heterorhabditis bacteriophora *Poinar (Heterorhabditidae : Rhabditida)*

Les observations ont montré que la paroi du testicule de *Heterorhabditis bacteriophora* Poinar, 1976 consiste en une gaine épithéliale fine et amincie. Les cellules de la vésicule séminale sont volumineuses et des corps résiduels phagocytés ainsi que des spermatozoïdes déficients ont été observés dans leur cytoplasme. Les parois du *vas deferens*, de l'ovaire et de l'ovotestis sont composées de cellules glandulaires. Les cellules de l'oviducte sont plus épaisses et se caractérisent par des jonctions cellulaires distinctes. La paroi de l'utérus est une gaine cytoplasmique fine. Chez les femelles amphimictiques fécondées, les spermatozoïdes s'accumulent dans le réceptacle séminal, situé entre l'utérus et l'oviducte. Chez les individus hermaphrodites femelles, les spermatozoïdes s'agglomèrent à la jonction de l'ovotestis et de l'oviducte. Il a été observé trois types d'association entre spermatozoïdes et parois du tractus génital. Ces associations structurales suggèrent mouvement, fixation et « engloutissement » des spermatozoïdes.

The insect-parasitic nematode, *Heterorhabditis bacteriophora* Poinar, 1976, is unique in being the only known hermaphroditic heterogamic nematode possessing homogony (Poinar & Hess, 1986). 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 the second generation adults normally develop into infective stage juveniles which leave the host cadaver and search for insects.

Spermatogenesis occurs twice in the normal life history of *H. bacteriophora*, once in the ovotestis of the first generation protandric hermaphrodite and once in the testis of the second generation male (Poinar & Hess, 1986). Sperm development in hermaphroditic females and males was the same ultrastructurally and the spermatozoa observed within the mated amphimictic female appeared identical in structure to those observed within the hermaphrodictic female.

As described by Poinar and Hess (1986) the mature spermatozoa had two morphologically distinct regions, the main cell body and a pseudopodial area. The main cell body contained the condensed nucleus, mitochondria and membrane specializations. These membrane specializations were villi-lined vacuoles, the lumen of which opened to the exterior through an electron-dense collared orifice. The cell organelles were restricted to the main cell body which varied in shape from rounded to flattened. The pseudopodial region of the spermatozoa was pleiomorphic and appeared capable of movement around the main cell body so that the spermatozoa varied in shape from fusiform to round.

The present paper describes aspects of the behavior

of the developing and mature sperm in the male and hermaphroditic and amphimictic females of *H. bacteriophora*. The ultrastructure of the walls of the reproductive tract in the male, amphimictic female and hermaphroditic female is presented.

## Materials and methods

The nematode, *H. bacteriophora*, was originally described attacking a lepidopterous larva in Australia (Poinar, 1976); however it has a wide host range and for the present studies, it was reared on larvae of the wax moth, *Galleria mellonella*. Infected insects were dissected daily over a period of two weeks to follow the development of the nematode. For light microscopy, the hermaphroditic females and the 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 behavior; pre-adult and adult hermaphroditic females, males, and mated amphimictic females.

## Results

### Light microscope observations

The reproductive system of the male *H. bacteriophora* consists of a single (monorchic) reflexed testis composed of a distal germinal zone followed by a growth zone. Adjacent posteriorly to the latter is the seminal vesicle where developing sperm collect. Proximal to the seminal vesicle is a fairly long *vas deferens* containing thick, glandular walls.

The females of *H. bacteriophora* have a didelphic reproductive system with well developed ovaries connected to a constricted, thick walled oviduct which leads into a thin walled uterus. In the hermaphrodite, sperm produced in the ovotestes of the fourth stage juvenile collect in the distal portion of the oviduct (Fig. 1 a). In the amphimictic female, sperm from the male migrate to the distal portion of the uterus and collect in a specialized area we call the seminal receptacle (Fig. 1 b). We prefer to use the term spermatheca (often used synonymously with seminal receptacle) when the region is more specialized and set off from the remainder of the uterus.

In both hermaphrodites and males, the mature spermatozoa are similar in morphology and are characterized by a condensed nucleus, granular appearing bodies and a well developed pseudopod (Fig. 1 c).

In several cases, penetration of a spermatozoon into an egg was observed in mated amphimictic females. After initial contact, the egg membrane appeared to loosen and expand somewhat. There was fusion of the egg and spermatozoan membrane and for several seconds, the sperm was enveloped in a clear membrane which appeared to be an extension of the egg membrane. This membrane was soon retracted and pulled the spermatozoon into the body of the egg (Fig. 1 d). By this time, the sperm nucleus had enlarged from its original size in the spermatozoon.

Mature spermatozoa of *H. bacteriophora* are amoeboid in shape and movement. However, only a few sperm demonstrated full amoeboid movement. Most of the spermatozoa were in a « resting » position and at most, glided back and forth, bumping into and occasionally adhering to each other and moving along the egg surface.

### Electron microscope observations

#### *Developing sperm and the male gonad*

The wall of the testis in the areas where spermatogonia and primary spermatocytes were located was observed to be composed of a thinly attenuated epithelial sheath containing ribosomes and some rough endoplasmic reticulum (RER) (Fig. 2 a). The exterior of this sheath was covered with a basal lamina. Nuclei in the main cell body were flattened and elongate. Also present within the main cell body were lipid droplets and electron-dense granules. Within these areas of the testis the developing sperm cells were interconnected by a cytoplasmic bridge, the rachis. The germ cells were tightly packed and the wall of the testis was closely applied to the surface of the enclosed cells. In the spaces between the germ cells, membranous profiles, small cytoplasmic vesicles and what appeared to be empty vacuoles were observed (Fig. 2 a).

When the germ cells no longer were associated with the rachis the wall of the testis was no longer closely appressed to the surface of the germ cells. The germ cells in this region had disassociated and were not tightly packed. Interspersed between the developing spermatocytes were many circular membranous profiles (Fig. 2 b). Some of these profiles had remnants of cytoplasm associated with them. These profiles increased in number and relative size further down the testis where sperm maturation continued.

Just before the *vas deferens* in the area which could be designated as the seminal vesicle, the lumen of the testis

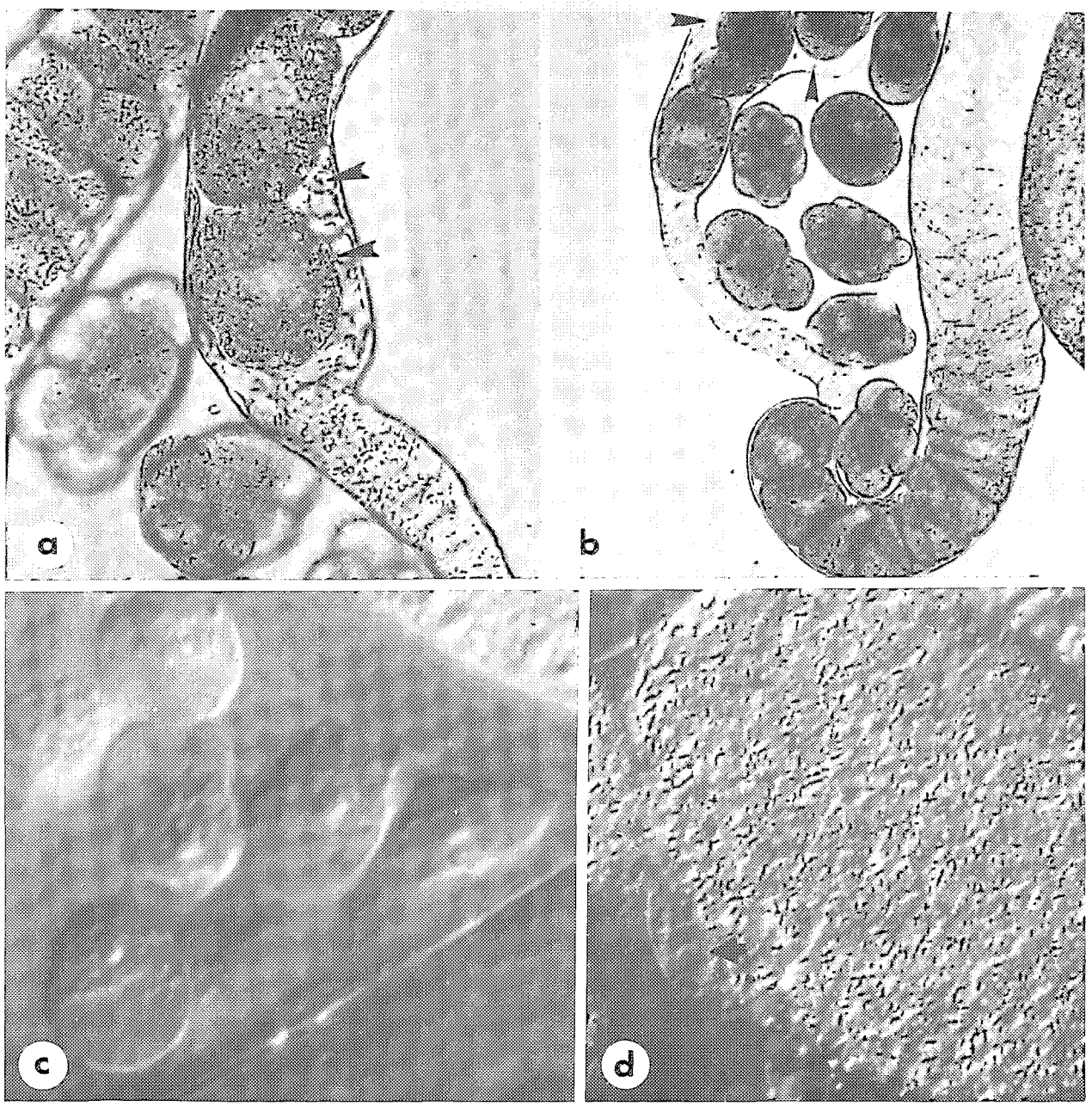


Fig. 1. Sperm behavior in *H. bacteriophora.* a) Sperm (arrows) collected in the proximal portion of the ovotestis of a hermaphroditic female (× 500). b) Sperm (arrows) collected in the seminal receptacle of a mated amphimictic female (× 250). c) Mature spermatozoa in a mated amphimictic female (× 3 000). d) Spermatozoon (arrow) inside egg membrane of a mated amphimictic female (× 1 000).

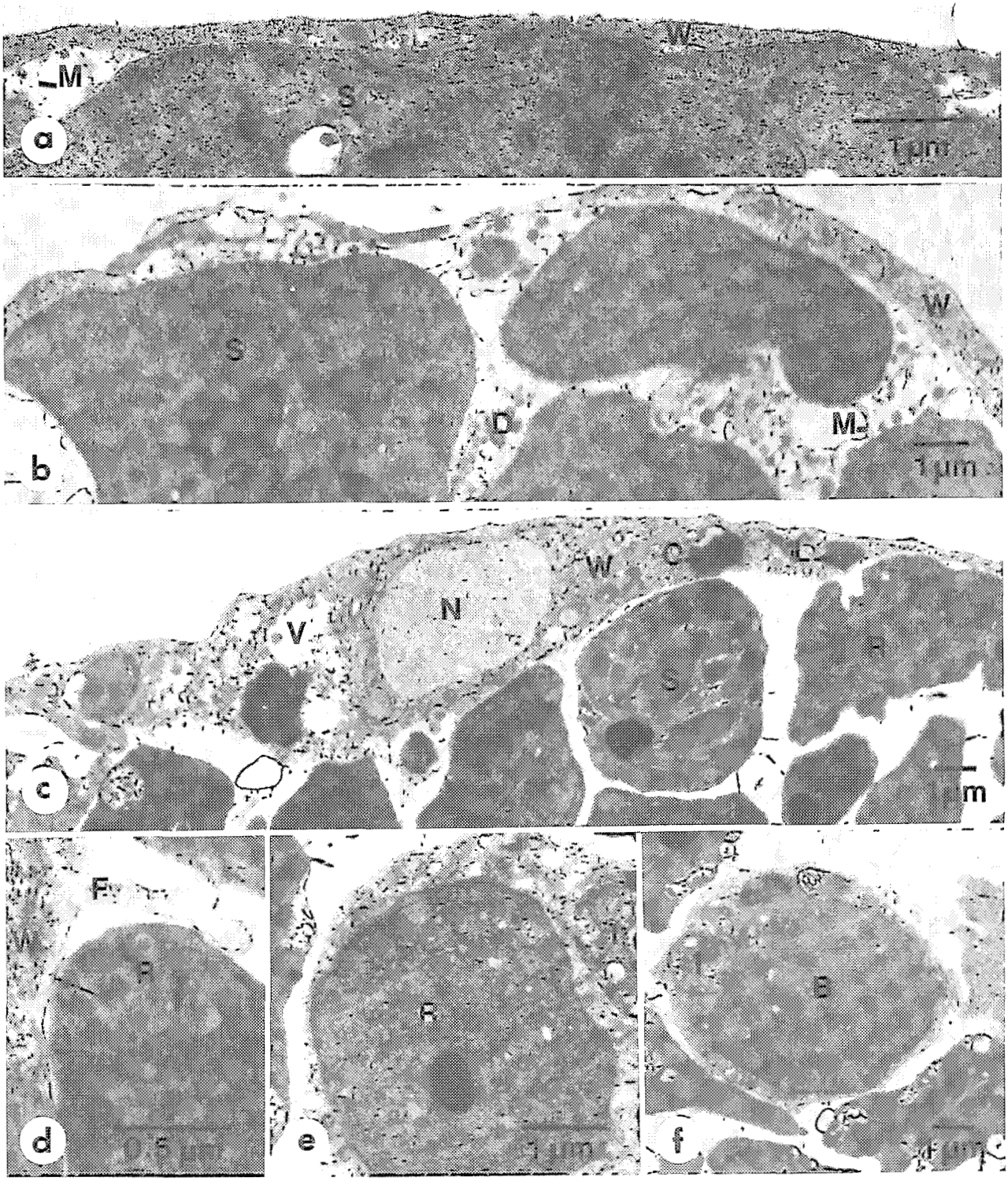


Fig. 2. a) The wall (W) in the growth region of the testis is a thinly attenuated epithelial sheath. *M : membranous profiles; S :* primary spermatocyte. b) The wall (W) of the testis where the germ cells have detached from the rachis is more separated from the developing germ cells and the lumen of the testis is filled with membranous profiles (M) and cellular débris (D). S : secondary spermatocyte. c) The wall (W) of the testis in the area where immature sperm accumulate (seminal vesicle) is thicker and contains lysosomes (L), vacuoles (V) and phagocytized débris. C : concentric membrane swirls; N : nucleus; R : residual body; S : immature spermatozoa. d) A finger-like projection (F) is shown extending from the testis wall (W) around a residual body (R). Arrows indicate attachment sites. e) A defective residual body (R) in which a nucleus has been sequestered has been completely engulfed by the testis wall. Also present in the cytoplasm is an inclusion body (I) containing remnants of endoplasmic reticulum.f) A phagocytized residual body has lost its distinctive structure becoming a homogeneous intermediate body (B) in the digestive process. I : inclusion body.

contained cast off residual bodies. The epithelial cells of the testis wall were thicker in this region (Fig. 2 c). The cytoplasm of the cells in the seminal vesicle contained more rounded nuclei with relatively clear nucleoplasm and prominent nucleoli. Rough endoplasmic reticulum was prominent and there were frequent small mitochondria. Present within the cytoplasm were electron-dense membrane bound granules which were presumed to be lysosomes. Also present in relatively high numbers were whorls of concentric membranes, some more compressed and electron-dense than others. Occasional lipid droplets and vacuolated areas were present. From the seminal vesicle wall, finger-like projections extended into the lumen of the testis. Some of these projections made contact with the discarded residual bodies. Intimate contact between the membranes of the testis wall and those of the residual bodies was observed suggesting attachment sites (Fig. 2 d). These projections were observed to extend for varying degrees around the residual bodies. Portions of residual bodies were surrounded by an engulfing rim of cytoplasm while still in the testis lumen. These phagocytic projections also contained various inclusion bodies (Fig. 2 e). Defective germ cells were endocytized. Homogenous intermediate bodies, which may represent residual bodies in a later stage of phagocytosis, were observed (Fig. 2 f).

The wall of the *vas deferens* was the thickest in the male reproductive system (Fig. 3 a). Nuclei were rounded with lobulate edges and the nucleoplasm was clear with prominent nucleoli. Small electron-dense granules and vacuoles were frequent in the cytoplasm and at the surface of the cells. Deposits of electron-dense material were observed within the vacuoles, some of which were open to the lumen of the testis.

*Spermatozoa and the reproductive tract in the amphimictic female*

Spermatozoa deposited within the amphimictic female accumulated in the seminal receptacle between the uterus and oviduct. The uterine wall was thin with attenuated cytoplasm rich in rough endoplasmic reticulum and contained large vacuoles (Fig. 3 b). The walls of the seminal receptacle were characterized by an increase in cell size. The wall was one cell thick and composed of epithelial cells with elongated interdigated processes rich in lipid droplets and RER (Fig. 3 b). The main cell body contained a nucleus with a prominent nucleolus and patches of electron-dense chromatin. Glycogen deposits were occasionally observed. Mitochondria were infrequent. The basal region of the cell was infolded in the area where adjacent cells met.

The wall of the oviduct contained the thickest cells of the female reproductive tract and was characterized by the complex cellular junctions. These junctions were also observed in the oviduct of the hermaphroditic female and will be described in that section. The cells were ultrastructurally very similar to those in the seminal receptacle. However, they also contained electron-dense paracrystalline deposits (Fig. 3 c), varying in section from rectangular to multifaceted. They appeared to be composed of spherical, 8 nm subunits which were resolved at the periphery of the body. The paracrystalline deposits were also observed associated with large electron-dense inclusion bodies (Fig. 3 c) containing membranes and mitochondria. Within the oviduct wall spermatozoa were frequently observed. These were of the same relative electron density as the inclusion bodies, but were abnormal in other structural aspects and appeared to be undergoing breakdown (Figs 3 d, 4 c, 4 f). In some, the pseudopodial end, which is organelle free, showed a loose substructure with the ground substance organized in coils interspersed with clear areas (Fig. 4 d). Also infrequently embedded in the oviduct wall were bacterial cells, sometimes in clusters of two and more.

Three distinct types of associations of the spermatozoa with the wall of the reproductive tract were observed in the amphimictic female. These structural associations were suggestive of spermatozoa movement, attachment and engulfment.

Spermatozoa which exhibited morphology suggestive of movement along the cytoplasmic surface of the uterus and oviduct were elongate in form. The pseudopodial end of the cell contained large projections or lamellipodia that made contact with the wall surface. Usually two or three such projections were observed in the plane of sectioning. In addition to the large projections from the pseudopodial end, several smaller projections or microspikes occurred, varying from slight bumps to finger-like extensions. The lamellipodia made contact intermittently with the surface of the uterus and oviduct. Frequently discontinuities in the plasma membrane appeared, however the integrity of the spermatozoan membrane was maintained (Fig. 3 e). In areas of close contact the plasma membranes were separated by a gap of 8 to 12 nm (Fig. 3 f). Within areas of contact, adhesion plaques were observed in which there was no discernible gap. Sections suggested a fusion of the plasma membrane of the wall with that of spermatozoa. In other instances packs of fibers 4-6 nm in diameter were observed traversing the gap. At the leading edge of the cells, the lamellipodia extended forward along the wall without contact. The more rigid main cell body (MB) always appeared to have a fine line of pseudopodia between it and the underlying wall surface. Adhesion plaques were observed in these areas also. The main cell body was flattened and elongate and appeared to float on this pseudopodial base. In some cases, portions of pseudopodia were on either side of the MB (Fig. 4 f). A slight difference in the electron density of the pseudopodial portions of the spermatozoa was observed. In areas of extension the ground substance was slightly less electron-dense than in areas of close contact with the reproductive tract wall.

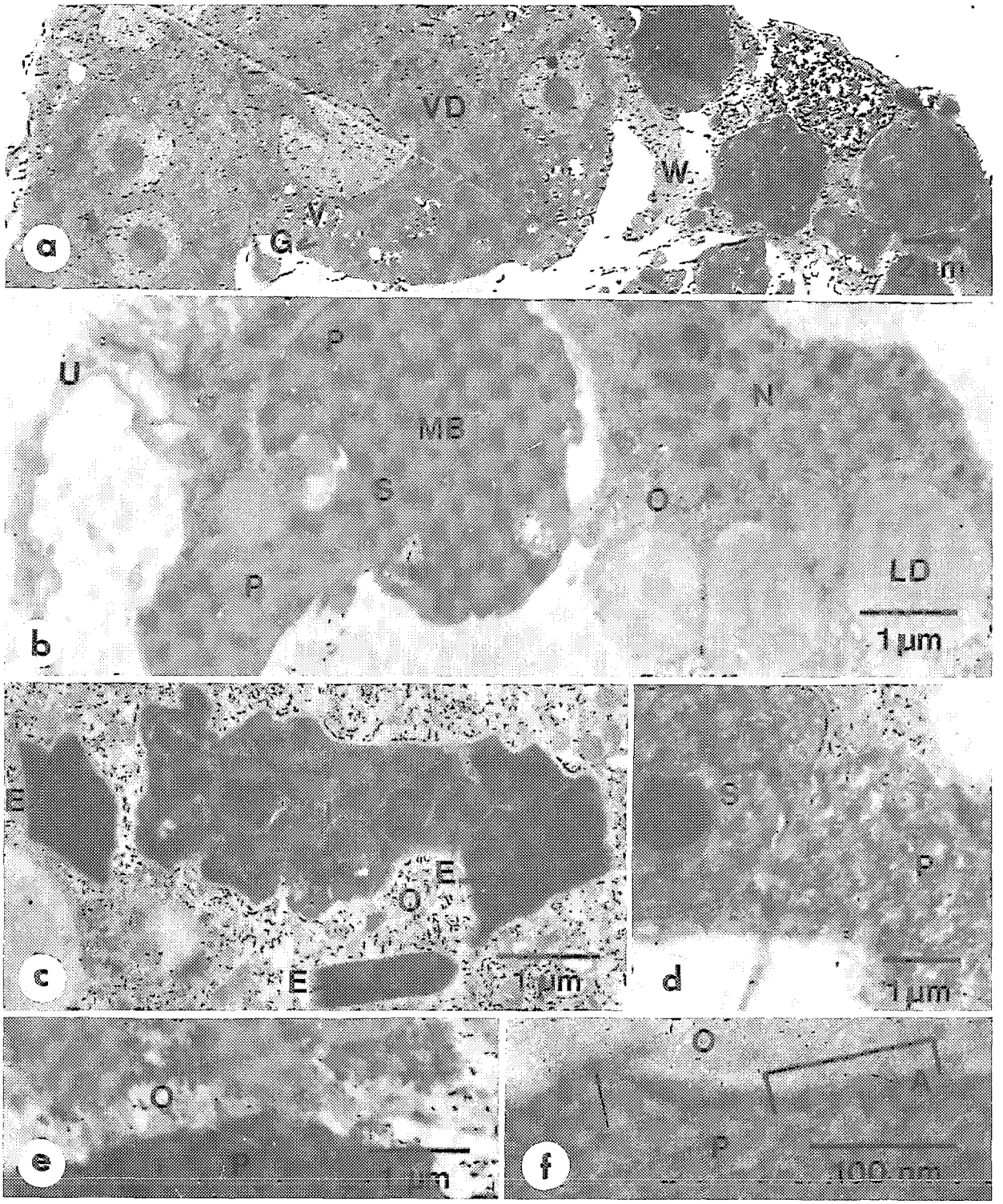


Fig. 3. a) Junction of the proximal portion of the testis wall (W) (seminal vesicle) with the glandular *vas deferens* (VD). The cytoplasm contains electron-dense granules (G) and vacuoles (V) of different densities which appear to fuse with the plasma membrane releasing their contents. b) The junction of the uterus (U) with the proximal oviduct (O) and seminal receptacle in the amphimictic female. LD : lipid droplets; MB : main cell body; N : nucleus; P : pseudopodia; S : mature spermatozoa passing from uterus to oviduct. c) Wall of the oviduct (O) in the amphimictic female showing electron-dense paracrystalline deposits (E) and their association with dense inclusion bodies (I). d) A spermatozoan (S) engulfed within the oviduct wall showed a less compact substructure in the pseudopodia end (P). e) The pseudopodia (P) of a spermatozoan is in close contact with the wall of the oviduct (O) where discontinuities in the plasma membrane were observed. f) The pseudopodia (P) of a spermatozoan in close contact with the wall of the oviduct (O) showing contact adhesion plaques (arrows) and places of contact (bracketed) where packs of fibers (A) traverse the gap between cells.

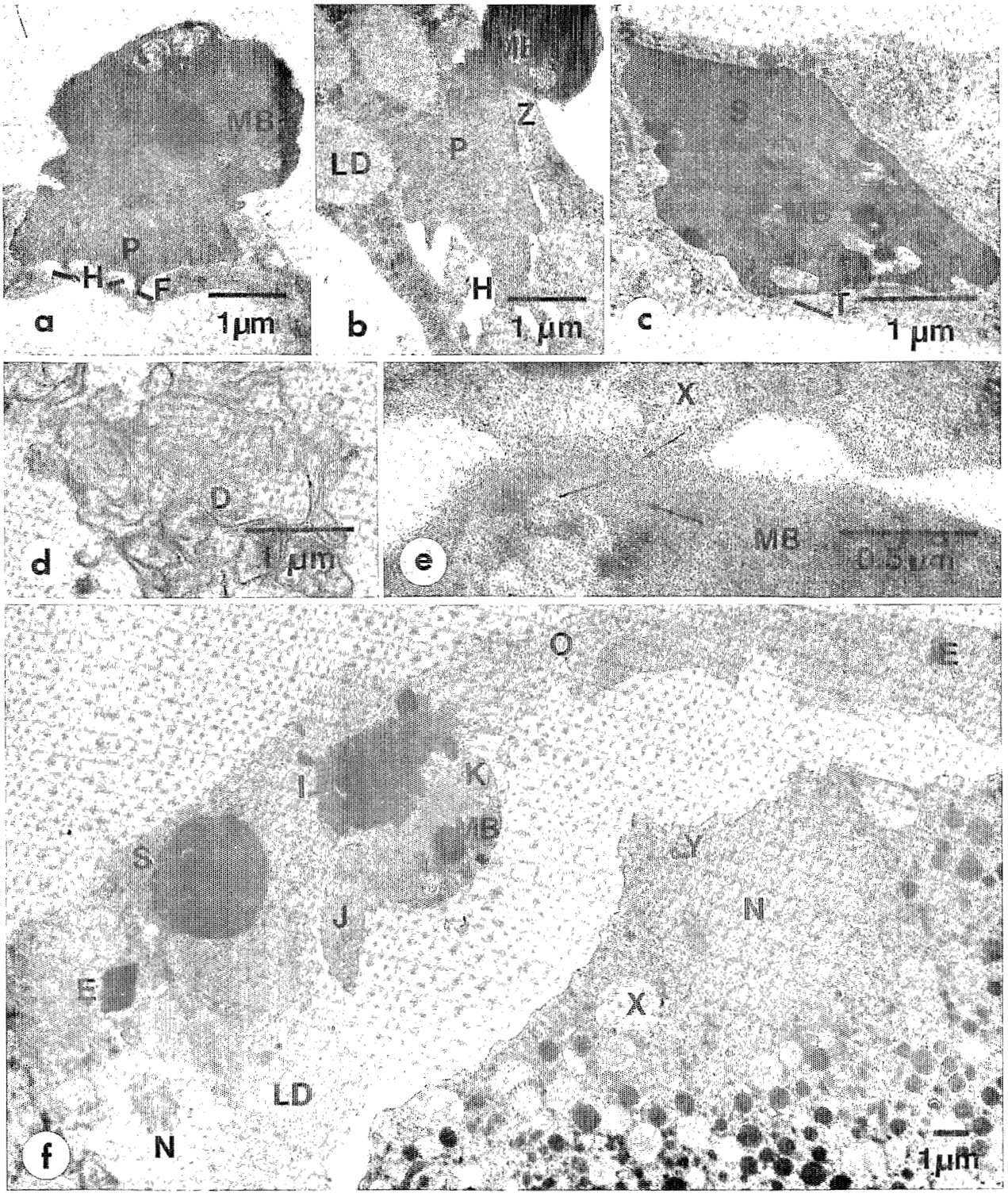


Fig. 4. a) In the amphimictic female, spermatozoa were observed anchored by their pseudopodia (P) within the wall of the oviduct. Gaps (H) occur where the oviduct cytoplasm does not make contact with the finger-like projections (F) of the pseudopodia. MB : main cell body. b) Anchored spermatozoa in the amphimictic female were surrounded by a cleared zone (Z) in the cortical area of the cell forming a collar. H : gaps; LD : lipid droplets; MB : main cell body; P : pseudopodia. c) This spermatozoon (S) completely engulfed by the wall of the oviduct of the amphimictic female had lost the rounded shape of main cell body (MB) and is in intimate contact with the cytoplasm of the oviduct around much of its surface. Fibrillar stands (T) are seen close to one edge. Rough endoplasmic reticulum is concentrated in this area. d) Packs of coiled membranes with associated cellular debris (D) were found in the oviduct and uterus of the amphimictic female. e) A point of contact between the oocyte (X) and the main cell body (MB) of the spermatozoan. Fine filaments extend from the place of contact into the cytoplasm of the spermatozoan (arrows). f) Proximal oviduct (O) of amphimictic female with oocyte in lumen (X). The oocyte nucleus (N) lacks a membrane and appears to be dividing. A small electron-dense body (Y) is seen next to the oocyte nucleus. Also in the lumen is an elongate spermatozoan in close contact with the surface of the oviduct in a manner suggestive of movement. There is an extended leading edge (J) of the pseudopodia before the main cell body (MB) and a trailing edge (K). Within the oviduct are an engulfed spermatozoon (S), electron-dense crystalline deposits (E), a dense inclusion body (I), a large number of lipid droplets (LD) and a characteristic nucleus (N).

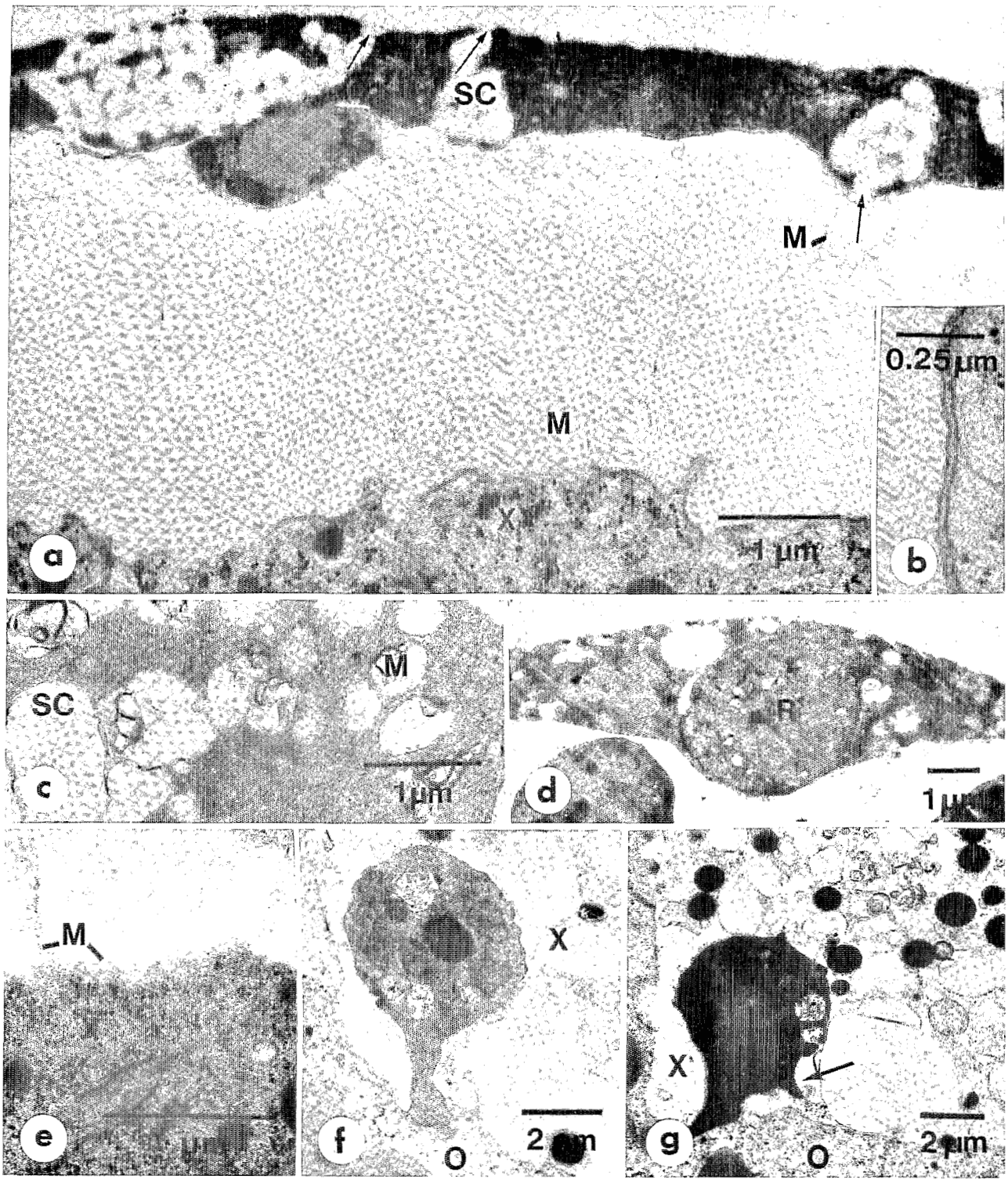


Fig. 5. a) The ovotestis of the hermaphroditic females shows the glandular nature of the cells. Arrows indicate opening of the multilobulate secretory cavities on the basal and lumen surface of the cells. There are membranous profiles (M) associated with the opening of the secretory chambers (SC) as well as the oocyte (X). b) Enlargement of surface of oocyte shows two unit membranes on the exterior. c) The lobulate secretory cavities (SC) of the ovary-testis contained a low-density flocculant material as well as electron-dense membranous profiles (M). d) The wall of the ovotestis contained large membrane-bound inclusions which resembled lysing residual bodies (R). e) The surface of the oocyte (X) in the ovary and more distal portions of the ovotestis were thrown into small villi-like processes. These processes were in contact with the membranous profiles (M) found in the lumen. f) Spermatozoa in the hermaphrodite were observed anchored on the oviduct wall (O) by a pedunculated pseudopodia as they penetrated into the passing oocyte (X) with the main cell body. g) A clear-zone in the oocyte (X) surrounds this spermatozoan still attached at one area to the oviduct wall (O) of the hermaphrodite. Another portion of the pseudopodia (P) is seen to be free within the oocyte at arrow.

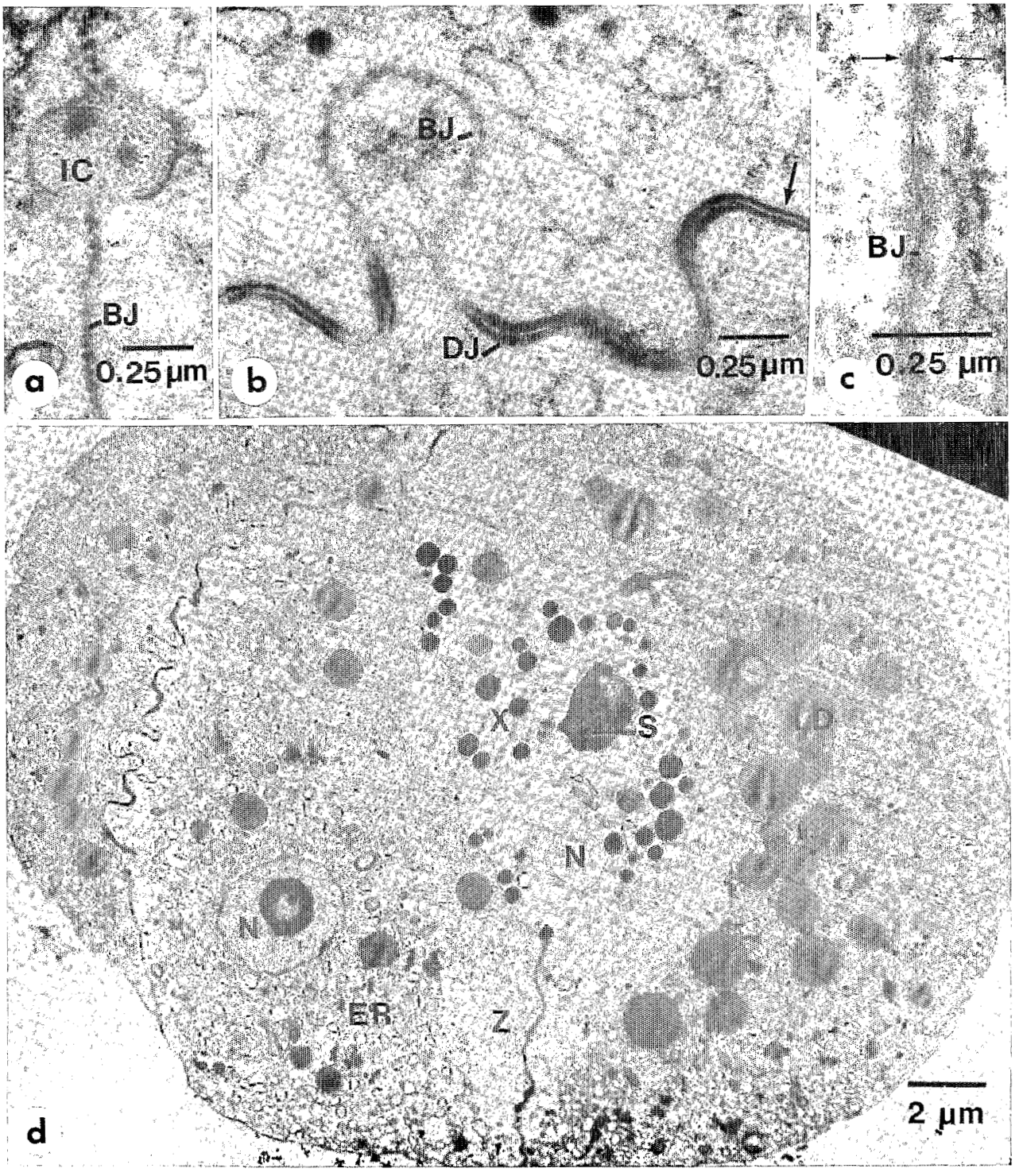


Fig. 6. a) The oviduct of *H. bacteriophora* contained distinct cellular junctions (B) with a beaded structure. These junctions opened periodically to produce a space between the membranes which contained electron-dense granules (IC). b) The beaded junctions (B) present at the basal and lumen side of the oviduct alternated with banded junctions (DJ) which possessed in some areas filaments bridging gaps (arrow). c) The beaded junctions (BJ) possessed opposite electron-dense granules (arrows) seemingly between the inner and outer electron-dense leaflet of the membrane. Where the beads occurred there frequently was no gap apparent between the membranes. d) The oviduct in the hermaphrodite here is the same in the female with a compressed lumen through which the oocyte (X) must pass. The oviduct cytoplasm contains extensive vesiculated endoplasmic reticulum (ER), lipid droplets (LD), nuclei (N) with circular nucleoli, a fairly clear cortex zone (Z) and distinctive cellular junctions. A, oocyte passing through is compressed and distorted. Within the oocyte is a spermatozoan (S) in the vicinity of the oocyte nucleus (N).

Spermatozoa were observed which appeared to be anchored or attached within the wall of the reproductive tract, particularly within the oviduct cells. These spermatozoa were for the most part slightly ovoid with the pseudopodial end largely concentrated beneath the rounded main cell body. The pseudopodial portion of the spermatozoa sometimes interdigitated within the cells, extending finger-like projections for varying distances into the cytoplasm (Figs 4 a, 4 b). This interdigitation was most striking in the thicker cells of the oviduct (Fig. 4 b). Some of the pseudopodia were surrounded by gaps where the cytoplasm of the wall did not make contact with the spermatozoa. In others varying degrees of contact were observed. These contacts were structurally similar to those described above for motile spermatozoa. Clear cortical zones in the cytoplasm at the surface of the reproductive wall were almost always observed associated with any points of contact with spermatozoa. This clear cortical zone occurred in a collar-like arrangement around the opening in the cell through which the spermatozoa projected (Fig. 3 b). Cleared zones were not present in contact regions deeper in the cytoplasm as seen in Figure 4 b.

As mentioned before spermatozoa appeared to be undergoing breakdown within the oviduct wall. Early stages in the digestive process were observed. Phagocytized spermatozoa were completely surrounded by the cytoplasm of the oviduct (Fig. 4 c). Close contact occurred almost completely around the spermatozoa with only occasional areas of an oviduct membrane visible. Cell organelles, particularly rough endoplasmic reticulum appeared closely associated with the spermatozoan surface. On occasion multiple layers of fibrillar strands were observed within the gaps between the oviduct cytoplasm and the spermatozoa.

Penetration of spermatozoa into the egg was not observed with the electron microscope in the amphimictic female. Eggs present in the oviduct where spermatozoa collected were covered with a double membrane. The outer membrane frequently was blebbed into pockets. These pockets were observed to be in contact with the main cell body of the spermatozoa held in the wall of the oviduct (Fig. 4 e). Associated with these contacts were filaments 6-8 nm in diameter in the cytoplasm of the spermatozoa. In some points of contact it was not possible to resolve the outer membrane. Some eggs were observed in which a nuclear region, not bounded by a membrane, was present close to the periphery of the egg. Chromosomes appeared within this region (Fig. 4 f). These eggs had a small electron-dense body located near the surface.

The lumen of the oviduct and uterus contained packs of coiled membranes which in some instances had small amounts of cellular debris associated with them (Fig. 4 d). Also present were villi-like processes that were observed close to the spermatozoa similar in size and structure to those within the membranous specializations of the spermatozoa. In many cases where these villi-like processes were found, the associated spermatozoa did not appear to have membranous specializations in the plane of sectioning.

*Spermatozoa in the hermaphroditic female*

In the region of sperm maturation, the wall of the ovotestis (OT) in the adult hermaphroditic female was characterized by the presence of secretory cells. This same type of cell was observed distally in the ovotestis where only eggs were developing but was not present in juvenile hermaphrodites where the wall appeared very similar in structure to the wall of the male testis in the germinal region.

The secretory cells contained multilobulate cavities that opened to the exterior of the cell both on the lumen side and on the exterior surface of the wall beneath the basal lamina (Fig. 5 a). The cavities, which were membrane bound, contained a low-density flocculent network. Frequently present within the cavities were electron-dense multilayered membranous profiles (Fig. 5 c). The cells were packed with rough endoplasmic reticulum and contained numerous mitochondria. The secretory cells were spindle-shaped with the main cell body protruding into the lumen. The nuclei were elongate and possessed diffuse chromatin and a prominent nucleolus. Single lipid droplets were observed in the main cell body. Also occurring within the main body of some of the cells were large membrane-bound inclusions (Fig. 5 d). These inclusions resembled residual bodies and contained endoplasmic reticulum, ribosomes and Golgi complexes. Stages suggestive of digestion were observed. A dense amorphous substance was deposited between the cell membrane and that of the phagocytized residual body. Some of the inclusion bodies were quite electron-dense.

In the adult hermaphroditic female, developing eggs and sperm were observed within the lumen of the ovotestis. In the distal ovotestis, the surface of the ova were bounded by a single plasma membrane and contained villi-like protrusions of various lengths (Fig. 5 e). Also within the lumen of the ovotestis were membranous profiles. These membranous profiles were in contact with the surface of the ova (Figs 5 a, 5 e) as well as the walls of the OT, particularly at the openings of the glandular cavities (Fig. 5 e). In the more proximal part of the OT, these membranous profiles were associated with both the ova and the sperm. The ova in the proximal ovotestis were covered by an additional membrane (Fig. 5 b).

Mature spermatozoa accumulated in the hermaphroditic female at the junction of the proximal OT and the oviduct. The oviduct was characterized by thick walls, a compressed lumen and distinct cellular junctions (Figs 6 a-d). The cytoplasm contained droplets of varying electron densities. There was extensive vesiculated

rough endoplasmic reticulum and electron-dense membrane bound granules 80 nm to 160 nm in diameter (Fig. 6 d). The nuclei were generally round with diffuse chromatin. The exterior surface of the wall was covered by a basal lamina attached to the plasma membrane by hemidesmosomes. The lumen surface was smooth. The cells were joined by two types of junctions, one characterized by dense bands and the other with a beaded membrane. In the first type of junction parallel electron-dense bands 20-25 nm thick were deposited on the membranes in patches along the entire length of the intracellular contact (Fig. 6 b). The intercellular space in these areas measured around 6-8 nm and regular septa were not observed although in some areas small granules were observed in the intercellular space as well as some filaments bridging the gap between the bands. At the luminal and basal sides of the intercellular spaces as well as between the electron-dense bands a beaded surface specialization of the membrane was observed (Figs 6 a-c). These beads were oval, approximately 10 × 20 nm and occurred opposite each other (Fig. 6 c). The intercellular space was compressed to 4-6 nm in this area and in somes cases a gap was not apparent. At infrequent points along the membrane between the beaded junctions, round to oval spaces between the membranes occurred (Fig. 6 a). The spaces were filled with electron-dense granules 20 nm in diameter. Some of the granules appeared to have an electron-lucent core. Cellular organelles were generally not associated with the cortical cytoplasm on either side of the intercellular space as well as the luminal side of the oviduct.

Within the hermaphroditic female the mature spermatozoa exhibited variable morphology suggestive of movement. Fusiform spermatozoa were observed. Forms suggestive of translocation by movement of the pseudopodia along the cellular surface were observed similar to those described in the amphimictic female. In some instances the pseudopodia were extended predominately in a putative leading direction. Spermatozoa were observed with most of the pseudopodia at the leading edge of the cell, with some under the main body, and some trailing behind. In many sections the pseudopodia were seen to have projections in multiple directions indicative of their extensive pliability.

The predominately occurring form of spermatozoa was rounded in the nuclear-containing end with the pseudopodia attached or anchored to the luminal surface of the wall. This contact involved an interdigitation of the pseudopodia with the cytoplasm in a manner apparently structurally identical to the mechanism of attachment described for spermatozoa in the amphimictic female. In the portion of the oviduct with a constricted lumen however spermatozoa were anchored more at the surface of the wall and the pseudopodia appeared pedicellate (Figs 5 f, 5 g). These spermatozoa had their main cell body embedded within the cytoplasm of the ova. Spermatozoa were observed within the ova indicating fertilization had occurred (Fig. 6 d). Some spermatozoa were found in close proximity to the ova nucleus.

## Discussion

Spermatozoa produced by the hermaphrodite of *H. bacteriophora* accumulate in the lower portion of the ovotestis adjacent to the distal portion of the oviduct and fertilize the eggs as they enter and pass through the oviduct. Spermatozoa inseminated in the amphimictic females migrate to the seminal receptacle at the distal portion of the uterus and fertilize the oocytes as they leave the oviduct or enter the uterus. In *Caenorhabditis elegans*, apparently both hermaphrodite and male produced spermatozoa accumulate in a spermatheca and fertilize the oocytes after they leave the oviduct (Ward & Carrel, 1979).

Ameboid sperm is a characteristic of nematodes in general (Poinar & Hansen, 1985) and pseudopod extension in relation to movement has been observed in *C. elegans* (Ward & Carrel, 1979, Ward *et al.*, 1982) and *Ascaris* (Abbas & Cain, 1979). In the present study we described a sequence of morphological stages suggestive of spermatozoan movement along the reproductive wall. The pseudopodial end of the cell was apparently responsible for movement with the more rigid main cell body « floating » on the pseudopodia base as it moved. It is suggested that pseudopodia extend lamellipodia along the leading edge of the cell and that these attach with specialized structures-adhesion plaques. Adhesion plaques are observed at points along the base of the cell suggesting that successive attachment-disattachment-reattachment occurs as the cell moves. Trailing ends of the pseudopodia are observed which are not attached. Differences in the density of the pseudopodial cytoplasm were observed with the extended edges being less dense than the more compacted base.

The cytoplasmic wall in the distal portion of the testis of *H. bacteriophora* is a thinly attenuated epithelial sheath closely applied to the developing germ cells. Where the germ cells have detached from the rachis this close association is no longer found. In many nematodes studied with the electron microscope the wall of the testis has been described as a thin cytoplasmic sheath similar to that seen in *H. bacteriophora* (Shepherd & Clark, 1976; Shepherd, Clark & Kempton, 1973). Exceptions are *C. elegans* where the male gonad was found to be surrounded only by a basal lamina (Wolf, Hirsch & McIntosh, 1978) and *Capillaria hepatica* which is surrounded by two basal lamina with small muscle cells between them (Neill & Wright, 1973). In *H. bacteriophora* the testis wall was observed to phagocytize cast-off residual bodies. This phenomenon was also observed in *Heterodera* spp. (Shepherd, Clark & Kempton, 1973) and *Ascaris megalocephala* (Favard, 1961). In the hologonic testis of *Capillaria hepatica* Neill and Wright (1973) described subtesticular cells which develop sper-

matogenic cells up to the early spermatid stage and engulf degenerated germ cells and residual bodies. They suggest these cells function in a threefold role — nutritional, regulatory and supportive. The *vas deferens* in *H. bacteriophora* was apparently glandular in that electron-dense granules and vacuole contents were released into the lumen. Shepherd and co-workers (1973, 1976) described secretory cells in the *vas deferens* of *Heterodera* spp. and *Aphelenchoides blastophthorus.*

The structure of the testis can be compared to that of the ovotestis in the hermaphroditic female. In young hermaphrodites the structure of the wall is very similar to that of the germinal and growth zones of the testis. However in the adults where all stages of developing sperm are observed the wall of the ovotestis is modified and consists of secretory cells. Residual bodies were observed within these cells suggesting that phagocytosis occurred also in the ovotestis. In the adult gonad of *Caenorhabditis elegans* Hirsh, Oppenheim and Klass (1976) reported the entire gonad to be surrounded by a tubular layer of extremely thin flat cells with endoplasmic reticulum and vesicles. Cells in the proximal arm were considered myoepithelial cells, containing arrays of longitudinally arranged thick and thin filaments. Such filaments were not observed in *H. bacteriophora.*

The structure of the walls of the reproductive tracts in the hermaphrodite and in the amphimictic female are very similar. Of course in the amphimictic female residual bodies are not present to be engulfed. On the other hand spermatozoa were observed being phagocytized by the oviduct wall in the amphimictic female. This was not observed in the hermaphrodite although it is possible that it was missed. In *Ascaris megalocephala* degenerated sperm in the lumen of the uterus were captured by the uterine wall by long microvilli. The sperm fused with the uterine cells and digestion ensued (Favard, 1961). In both the hermaphrodite and female nematodes spermatozoa were observed anchored in the reproductive tract wall by their pseudopodia end. The location in which anchoring occurred varied. In the female, spermatozoa were embedded in the proximal portion of oviduct and the expanded distal portion of the uterus. In the hermaphrodite, spermatozoa were anchored in the distal portion of the oviduct. Anchoring of spermatozoa by interdigitation of the pseudopodial end has been described in other electron microscope studies. In *Heterodera* spermatozoa were observed insinuated between the oocytes and wall of the oviduct and buried within the oviduct wall (Shepard, Clark & Kempton, 1973). Spermatozoa were anchored in the cells of the seminal receptacle of *Heterakis gallinarum* (Lee, 1971). Foor (1968)) described spermatozoa attached to the uterine wall near the oviduct/uterus junction by interdigitation of the pseudopodia into the cells in *Ascaris lumbricoides.* No evidence of engulfment of the spermatozoa by the oviduct was observed. In *Caenorhabditis elegans* spermatozoa were embedded by their pseudopodial end in the spermatheca (Ward & Carrel, 1979). Wright and Sommerville (1977) found the spermatozoa of *Nematospiroides dubius* anchored by their anterior end (pseudopodial end) to the wall of the uterus.

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