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Ultrastructural aspects of the oesophageal and reproductive systems of the equine parasite *Strongylus vulgaris*

M.S. Mobarak and M.F. Ryan*

Department of Zoology, University College Dublin, Belfield, Dublin 4, Ireland

Abstract

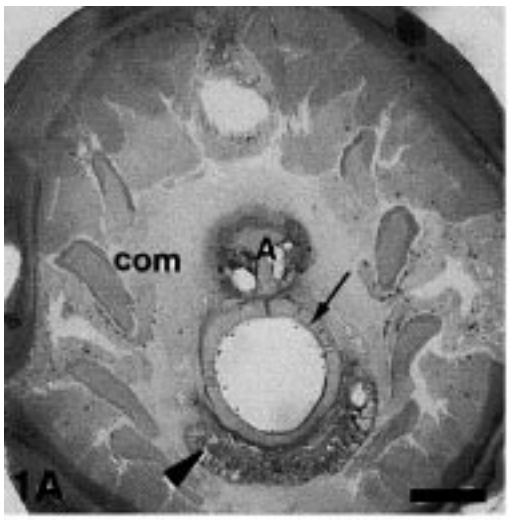
The ultrastructure of the dorsal oesophageal gland ampulla and its relationship with the oesophagus, oesophageal ultrastructure, and control mechanisms in oesophageal activity were studied. Terminal ducts of the subventral glands open through the oesophageal crown at the base of the buccal cavity. The terminal duct of the dorsal oesophageal gland running through the dorsal gutter opens to the exterior at the rim 'groove' of the buccal capsule. The posterior oesophageal region is clavate and the cuticle of the lumen folds to form outlet valves, 'valvulae'. An inconspicuous oesophago-intestinal valve (three lobes) connects oesophagus and intestine and is visualized in the open and shut position. In the female reproductive tract, with the exception of the uterus, the cells lie on a thick, irregular (convoluted) basal lamina. The apical plasma membrane of the uterus, and seminal receptacle, extend into the lumen by microvilli-like projections with which spermatozoa make intimate contact. The lumen of the uterus is filled with oocytes, fertilized and unfertilized. Testicular cells have two parts linked by a rachis. Spermatocytes are elongated with a large nucleus, distinct nuclear membrane, and many granules. The apical membrane of the rachis forms long microvilli-like projections with balloon-like tips. The amoeboid spermatozoa contain membrane specializations, a nucleus devoid of a membrane, and are enclosed by a pseudopodial-like extension.

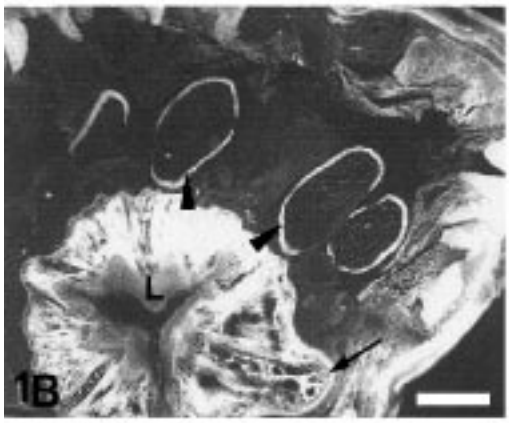
Introduction

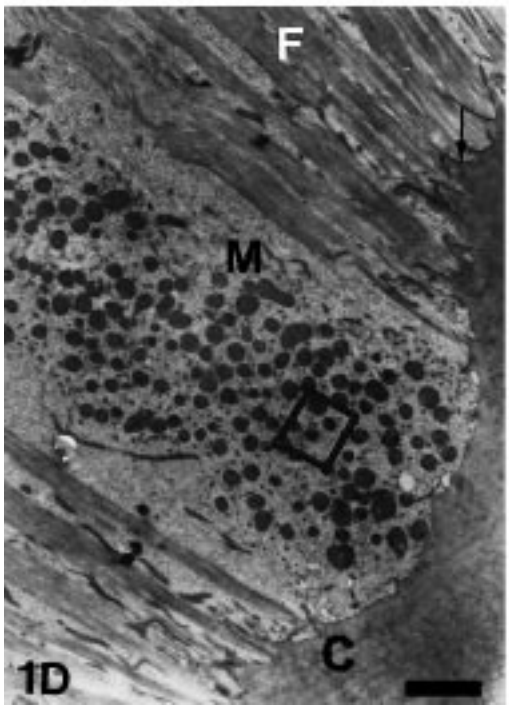
The structure of the alimentary tract and its associated glands has been examined in a wide range of nematodes (Bird, 1971; Chitwood & Chitwood, 1974; Mehlhorn, 1988; Bird & Bird, 1991). The oral opening is considerably modified in parasitic nematodes, and in Strongylida is surrounded by one or two leaf crowns. It leads to the buccal cavity, between mouth and oesophagus. In strongylids the buccal cavity lumen is wide and cuticularly lined. We have described in detail the ultrastrusture of the equine parasite *Strongylus vulgaris* including that of the buccal capsule, dorsal gutter, teeth (Mobarak, 1995; Mobarak & Ryan, 1998b, 1999) and aspects of feeding and the secretion–excretion system (Mobarak, 1995; Mobarak & Ryan, 1998b, 1999). Oesophageal glands open into the buccal cavity releasing enzymes for extracorporeal digestion, and facilitating attachment of the worm to the host's intestinal wall. The buccal cavity opens into the oesophagus which is clavate-shaped and 1.3–1.4 mm long, attaining its maximum width at the posterior third of its length (Popova, 1964).

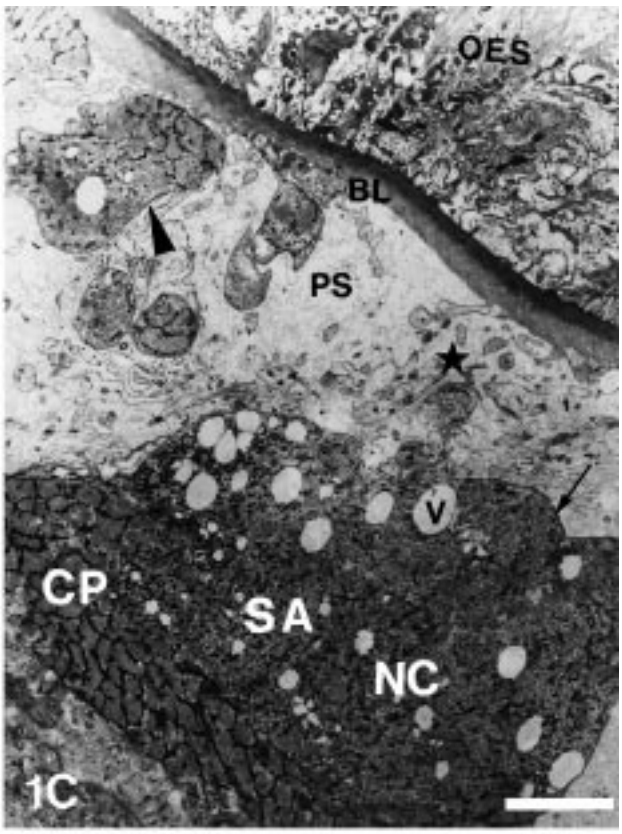
There are two kinds of muscle, somatic and specialized. Somatic muscles lie next to the hypodermis in the intercordal areas where they form a single layer. There are three types of muscle cell: flat (platymyarian), U-shaped (coelomyarian) in which the fibres extend up to the side of the cell and the sarcpolasm bulges into the pseudocoelom; and finally, circomyarian in which the fibres encircle the sarcoplasm (Bird, 1971; Mehlhorn, 1988; Bird & Bird, 1991). Specialized muscle cells occur in various locations and have various functions: the somato-oesophageal muscles extending from the body wall to the oesophagus; the depressor anal (H-shaped) and dilator anal muscles in

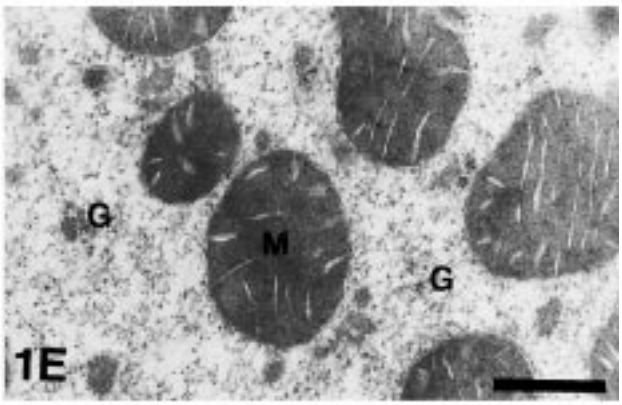
*Author for correspondence
Fax: 353 1 706 1152
E-mail: mfryan@ucd.ie

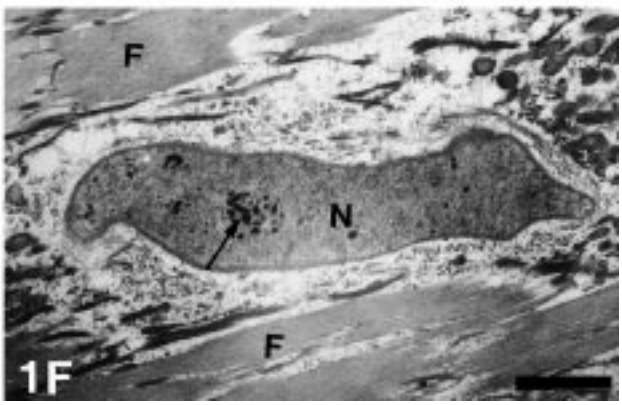












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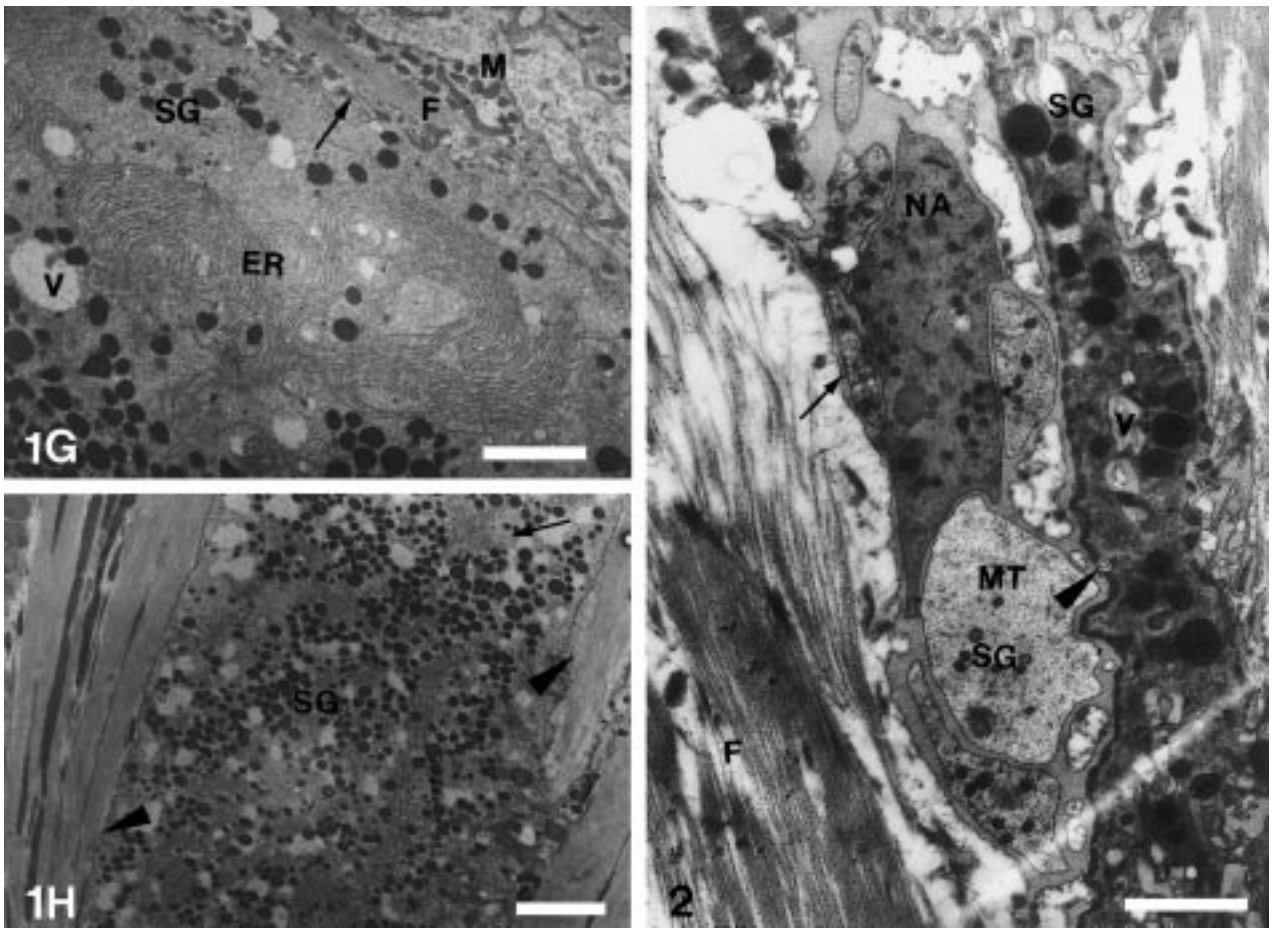

Fig. 1. A, Light micrograph (LM) of transverse sections (TS) through the oesophageal anterior region showing: round cuticular crown (arrowed) with lamellae surrounded by cephalo-oesophageal ligaments (arrowhead); eight lateral cephalo-oesophageal muscles (com); and dorsal oesophgeal gland ampulla (A), scale bar = 500 μm. B, LM of a TS below fig. 1A showing: tri-radiate cuticular lumen (L); basal lamina (arrowed); and four lateral cephalo-oesophageal muscles (arrowheads), scale bar = 500 μm. C, transmission electron micrograph (TEM) of a TS through a cephalo-oesophageal muscle cell showing: contractile portion (CP) with muscle fibres and non-contractile portion (NC); a dense sarcoplasm (SA); membrane-bound vesicles (V); sarcolemma (arrowed); small muscle cells (arrowhead) attached to the oesophagus (OES) basal lamina (BL); and pseudocoelom (PS) with pseudocoelomic membrane (*), scale bar = 5.0 μm. D, TEM of a TS through the apex of oesophageal muscle cell showing mitochondria (M); cuticle (C); and radial muscle fibres (F) attached to cuticle lumen by hemi-desmosomes (arrowed), scale bar = 2.0 μm. E, detail of boxed area in D showing: mitochondria with many cristae (M); alpha and beta glycogen deposits (G), scale bar = 0.5 μm. F, oesophageal muscle cell nucleus (N) with dense chromatin bodies (arrowed), scale bar = 2.0 μm. G, TEM of a TS through dorsal oesophageal gland ampulla showing: whorled rough endoplasmic reticulum (ER); electron-dense sercretory granules (SG); plasma membrane (arrowed); muscle cell with mitochondria (M); fibres (F); and vesicles (V), scale bar = 2 μm. H, TEM of a TS through dorsal oesophageal gland (posterior end) showing: electron-dense secretory granules (SG), rough endoplasmic reticulum with cisternae (arrowed); and plasma membrane separating gland cell from muscle cell (arrowheads), scale bar = 5.0 μm. Fig. 2. TS through dorsal oesophageal gland duct showing: thick, electron-dense lining (arrowhead); electron-dense secretory granules (SG); membrane-bound vesicles (V); nerve axons (NA) in intimate contact with the duct; microtubules (MT); and muscle fibres (F), scale bar = 5.0 μm.

the female; muscle associated with the repoductive system of the male nematode, e.g. copulatory, bursal, spicular, gubernacular muscles; and the vulvar muscle in the female (Bird, 1971; Levine, 1980; Bird & Bird, 1991).

Nematode sex organs are simple tubular structures, continuous with their ducts, allowing spermatozoa or eggs to be shed in the body cavity. This continuity of sex organs and ducts enables the reproductive system to function efficiently despite the high turgor pressure of the pseudocoelomic cavity (Bird, 1971; Mehlhorn, 1988). The present study describes the ultrastructure of the dorsal oesophageal gland ampulla (DOG.A), clarifies its relationship with the oesophagus, and describes oesophageal ultrastructure. It also provides orginal data on the ultrastructure of the reproductive systems of this species.

In the Strongylida, the male tail is modified to form a copulatory bursa that holds the female in position during copulation. Worms lie with anterior ends in opposite directions and the male clasps the female about the vulva

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with the bursa comprising two lateral lobes and a median dorsal lobe. Copulatory spicules are inserted together through the vulva and vagina into the ovijector to dilate the vaginal opening. Spermatozoa are passed into the reproductive tract of the female by muscular action of the male ejaculatory duct (Bird, 1971). During this process the male releases a colourless substance from a pair of copulatory glands that thicken and darken with age and are of unknown function (Bird, 1971; Lee & Atkinson, 1976).

Fertilization occurs when the oocyte enters the spermatheca. The pseudopodium of the sperm contacts the oolemma and the gamete membranes interdigitate and fuse. The whole sperm then enters the cell, which is followed by egg shell formation and by completion of the two meiotic divisions resulting in expulsion of two polar bodies (Mehlhorn, 1988). The eggshell consists of four layers: outermost (uterine), vitelline, chitinous, and inner (lipid) layer.

Materials and methods

Sampling and preparatory methods for *S. vulgaris* were as previously described (Caffrey & Ryan, 1994). Fixation, dehydration and embedding for light and electron microscopy (transmission and scanning) were as previously described (Mobarak & Ryan, 1998a,b).

Results

Oesophagus

The buccal cavity leads directly into the oesophagus (pharynx) connecting the buccal cavity and the intestine. The anterior portion of the oesophagus widens to form the oesophageal funnel with a cuticular, round lumen forming a ring (crown) with a pierced lamella of tissue connected to the buccal cavity: this cellular tissue represents cephalo-oesophageal ligaments (fig. 1A). The oesophagus has a clavate shape, 1.5–1.8 mm long, and comprises two regions: an anterior, narrow region, 0.5 mm long and a posterior, wide region, 1.0 mm long. At the end of the posterior region lies the oesophageal-intestinal valve consisting of three cuticle-lined lobes projecting into the intestinal lumen (fig. 3A).

Anteriorly, the nerve ring surrounds the oesophagus. Just below, is an excretory sinus with two transverse excretory canals attached laterally and joining the lateral excretory canal in the lateral cord. Despite the typically tri-radiate lumen of the oesophagus, the outer wall (basal lamina) is usually circular in section. It presents as a 0.9 μm thick, continuous sheet, acting as a sheath, and as an outer attachment for the radial muscle fibres and the cephalo-oesophageal muscles (fig. 1B,C).

The three rays subdivide the oesophagus into dorsal and two sub-ventral sectors (fig. 1B). The cuticle lining the lumen differs from that of the body cuticle by consisting of four layers: a thin, electron-dense membrane lining the lumen; followed by a less electron-dense layer; a third layer of fairly uniform density and fine fibres; and the fourth layer, to which the radial muscle fibres attach directly, is thick and very dense. Where the cuticle is thickened there may be transverse ridges or striation. This layer is considerably thicker at the extremity of each arch ray (fig. 1B), starting about 120 μm behind the anterior opening of the oesophagus (behind the funnel) and terminating about 70 μm from the oesophago-intestinal 'valvulae' (fig. 3B). Such thickening presumably facilitates attachment of the radial and apical fibres.

The tri-radiate oesophagus is a cellular structure, containing muscle cells, apical cells and oesophageal glands. The muscle series occurs along the oesophageal length, one to each sector. The gland cell of the sector divides each muscle cell into two parts that join at the apex. Apical cells separate between the muscle cells. Each apical cell is narrow and extends from the apex of the ray to the outer edge of the oesophagus (basal lamina), and contains many bundles of dense fibres.

In each sector, muscle cells are surrounded by a well-defined cell membrane. Each cell consists of radially-directed myofilaments attached to the oesophageal cuticular lumen at one end, and inserted in the oesophageal basal lamina at the other (fig. 1D). Thick and thin myofilaments in these radial muscles correspond to actin and myosin filaments respectively. The muscle cells contain many mitochondria, each with many cristae between the bundles of myofilaments or accumulated at the apex beneath the cuticle of the lumen (fig. 1D). Muscle cell cytoplasm contains alpha and beta glycogen deposits (fig. 1E), and the nucleus is large, elongated, with clumps of chromatin bodies and dense nucleoplasm lying between myofilaments (fig. 1F).

The three oesophageal glands extend as a duct anteriorly along the entire length of the oesophagus, terminating at the end of the posterior oesophageal region (bulb) (figs 1B, 3B). The terminal duct of the dorsal oesophageal gland (DOG), running through the dorsal gutter (DG) internal canal, opens at the rim of the buccal capsule 'ring canal'. The terminal ducts of the two sub-ventral oesophageal glands (SVOG) that open at the base of the buccal cavity, through the oesophageal crown, are lined with cuticle and have round orifices. A large, irregularly-shaped nucleus containing clumps of condensed chromatin is situated at the end of each gland.

Each of the three oesophageal glands occupies a sector and each gland is a separate cell. The DOG extends further than the SVOG to form an ampulla, is filled by various-sized, electron-dense secretory granules, and mitochondria with many cristae. The dense cytoplasm contains glycogen deposits and no nucleus was observed (fig. 1G). The ampullar cytoplasm is an extension of the DOG cytoplasm and consists of whorled, rough endoplasmic reticulum (RER). Large translucent vesicles are situated close to the RER, perhaps as a preliminary stage of granule formation. It is separated from the adjacent muscle fibres by a plasma membrane (fig. 1G). Posteriorly, the DOG contains many electron-dense secretory granules closely attached to the RER with many cisternae. There are few mitochondria and a well differentiated plasma membrane separates DOG and muscle cells (fig. 1H)

The SVOG cell resembles the DOG by containing many various-sized, electron-dense granules with extensive RER, lipid droplets, and glycogen, separated from muscle cells by a well defined plasma membrane. Each gland duct, running the entire length of the oesophagus, is lined

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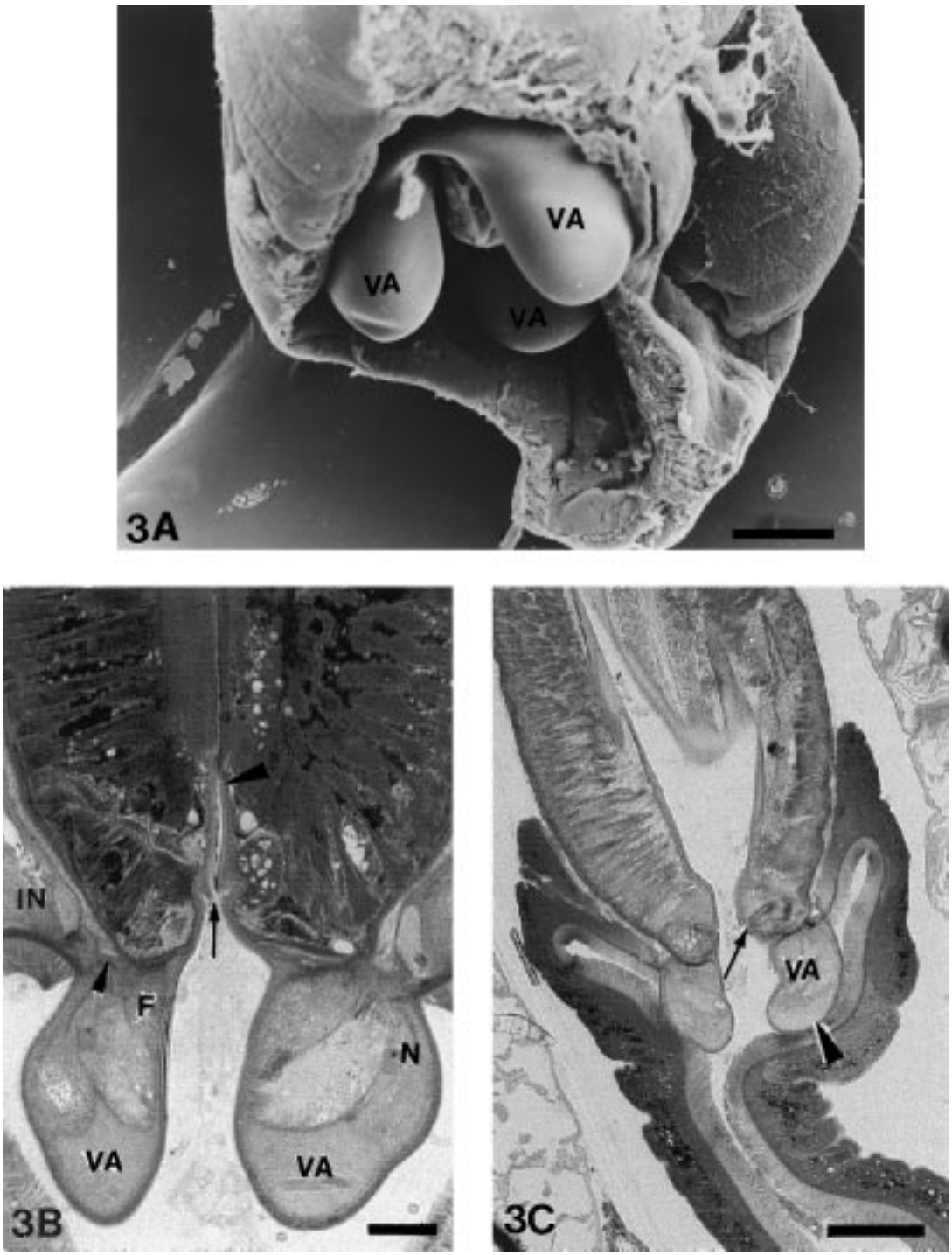

Fig. 3. A, Scanning electron micrograph of three-lobed oesophageal-intestinal valve (VA), scale bar = 100 μm. B, light micrograph of longitudinal section through posterior oesophageal end showing: end of cuticular thickening (large arrowhead); closed outlet 'valvulae' (arrowed); oesophageal-intestinal valve (VA) lined with cuticle, separated from oesophagus by basal lamina (arrowhead), containing nucleus (N), fibres (F), and intestine (IN), scale bar = 100 μm. C, outlet 'valvulae' opened (arrowed); oesophageal-intestinal valve (VA) pushed toward the intestinal microvilli (arrowhead), scale bar = 500 μm.

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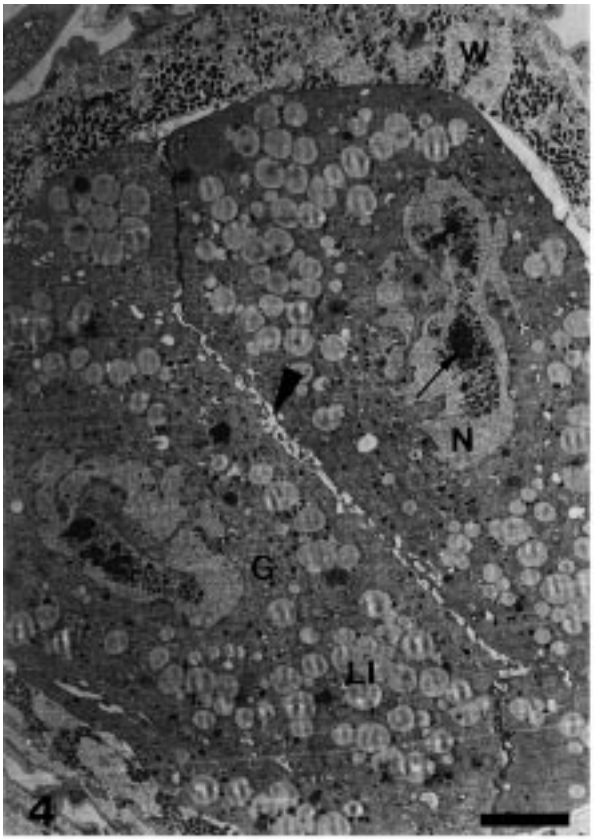

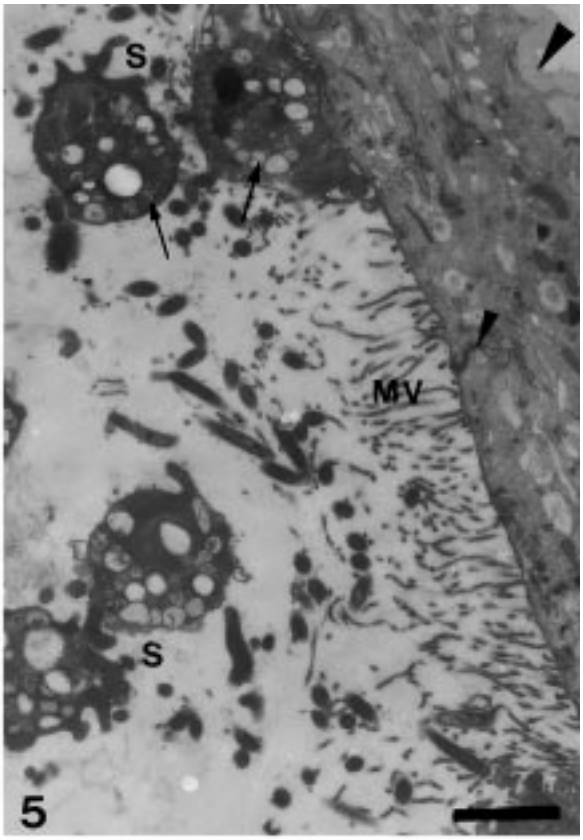

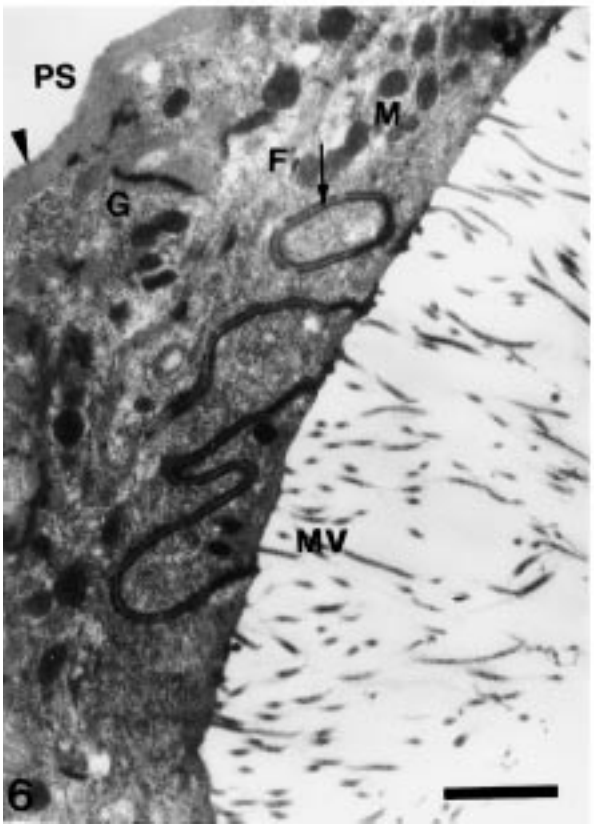

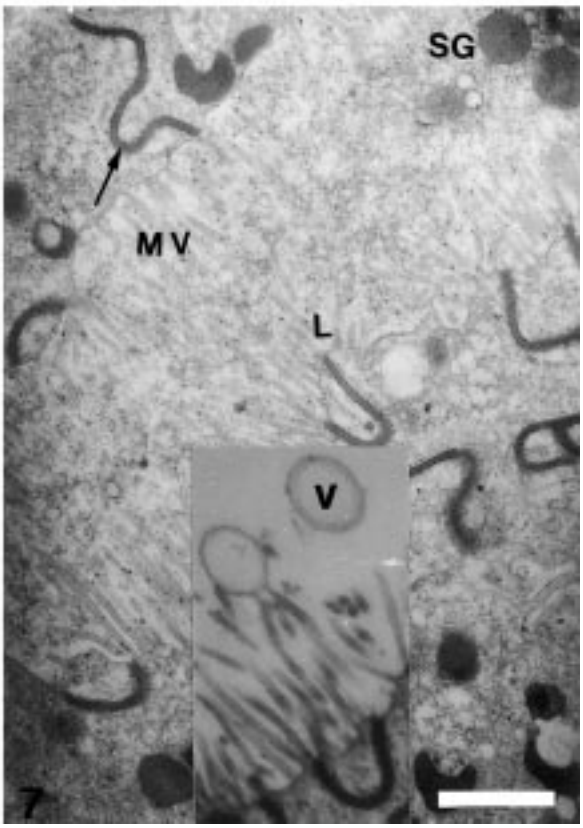

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by a thick membrane and contains various-sized electron-dense granules (300–550 μm i.d.) and membrane-bound vesicles each with a vacuolated matrix (fig. 2). Nerve fibres are closely associated with the oesophageal ducts. Nerve axons are surrounded by a thick layer of homogenous axolemma containing electron-dense vesicles which might be neurosecretory granules. Axons also contain many microtubules, mitochondria, clusters of fine, dense fibres, and cisternae of endoplasmic reticulum (fig. 2).

Valves

An inconspicuous oesophago-intestinal valve, perhaps one-way, directly connects the oesophagus and intestine. Valve cells are almost completely separated from the oesophagus by a basal lamina (fig. 3B). These cells are continuous with the cells of the intestine, and the oesophageal cuticular lining also lines the oesophago-intestinal valve but ends abruptly at the junction of the valve with the intestine. The cytoplasm of each cell consists of a nucleus with nucleolus and fibre bundles beneath the basal lamina connected to the valve cuticle (fig. 3B,C).

After the thickened cuticle of the post-oesophageal lumen wall, the cuticle folds to form the outlet valves, 'valvulae', with attached, controlling oblique muscles. 'Valvulae' open, passing food materials to the intestine during contraction (fig. 3C), and close during relaxation (fig. 3B). At the posterior end (bulb) of the oesophagus, the intestine surrounds the bulb and forms a 'cardia' (fig. 3C).

Cephalo-oesophageal muscles

Eight cephalo-oesophageal muscles, four anterior and four posterior, inserted into the oesophagus below the nerve ring run anteriorly to insert both into the wall of the buccal capsule and into the cuticle (fig. 1A,B). Each muscle consists of contractile and non-contractile portions. The contractile (myofibrillar) substances (actin and myosin filaments) pass from a 'grooved' form to that of a 'closed cylinder' and, as cylinders of fibrillae filled with sarcoplasm, the muscle then runs freely through the body cavity to the point of insertion into the oesophagus (Bird, 1971) (fig. 3C). Ultrastructurally, each muscle cell is surrounded by a well-defined membrane (sarcolemma). The amyofibrillar portion of the sarcoplasm is very dense, containing many mitochondria, glycogen, abundant membrane-bound vesicles, and a round, centrally placed nucleus. The sarcolemma closely contacts the oesophageal basal lamina. Small muscle cells surround the oesophagus at the level of nerve ring, make an intimate contact with the basal lamina through their myofibrillar portion, and are surrounded by nerve ring axons (fig. 1C).

Reproductive system

This species is dioecious with males smaller than females (10–17 mm long, 0.7–0.95 mm wide; 20–24 mm long and 1.2–1.5 mm wide, respectively).

Female reproductive tract

The reproductive tract almost fills the body and folds around the intestinal tract. It comprises paired ovaries, oviduct and uteri (di-delphic), opening into vagina and vulva. Posteriorly each uterus functions as a seminal receptacle, for the temporary storage of spermatozoa. This region is preceeded by a short oviduct, and a long, coiled ovary. Throughout, a basal lamina surrounding the epithelial cells is thick and irregular (Mobarak & Ryan, 1999). The ovary is a long tube, coiled around the intestinal tract 60–90 μm wide, including a posterior part, representing the germinal zone with central rachis and a growth zone, where oocytes are attached to the branched rachis (fig. 4). Anteriorly, the oocytes lie free in the lumen. The ovary is lined with epithelial cells, 5–6 μm deep, each with an outer and an inner zone. The former rests on the basal lamina, 3–4 μm thick, that is highly convoluted and contains electron-dense material within the folds. The outer plasma membrane is highly folded, forming a basal labyrinth occupying the outer zone: this is conspicuous, running deep in the middle of the epithelial cell cytoplasm. The inner zone rests on a thin, apical plasma membrane, lining the ovary lumen, that folds to form a labyrinth, less conspicuous than the outer one. These labyrinths are apparently connected to each other. The cytoplasm of the epithelial cell is rich in mitochondria, dense granules and membrane-bound vesicles. No cell boundary was detected in the ovarian wall but the large, irregular nucleus lies close to the apical plasma membrane. Although no muscle is associated with the ovarian wall, bundles of myofilaments in the outer zone of the epithelial cell represent the contractile element of the ovary. Oocytes filling the ovarian lumen have a large nucleus, filled with thick, dense chromatin, and an irregular membrane. The cytoplasm is filled with many lipid droplets and small, electron-dense granules (fig. 4). The short oviduct was not distinguished from the ovary.

Posteriorly, each uterus forms a seminal receptacle with walls comprising a single layer of cells, 4.5 μm deep, and

Fig. 4. Transmission electron micrograph (TEM) of a transverse section (TS) through ovary showing: ovary lumen with oocytes attached to the rachis (arrowhead); surrounded by epithelial wall (W); oocyte has large, irregular nucleus (N); dense chromatin bodies (arrowed); extensive lipid droplets (LI); and small, dense granules (G), scale bar = 5.0 μm. Fig. 5. TEM of a TS through seminal receptacle showing: epithelial cell apical membrane with microvilli (MV); terminal bar (small arrowhead); basal lamina (large arrowhead); and spermatozoa (S) attached to microvilli and free in the lumen; note spermatozoon peripheral 'vesicles' (arrows), scale bar = 2.0 μm. Fig. 6. TEM of a TS through uterine epithelial cell showing: basal lamina (arrowhead); apical membrane with microvilli (MV); terminal bar (TB) with translucent space (arrowed); mitochondria (M); glycogen deposits (G); fibres (F); and pseudocoelom (PS), scale bar = 0.1 μm. Fig. 7. TEM of a TS through testis rachis showing: microvilli (MV) projecting into the lumen (L) filled with membrane-bound vesicles (V); thick, dense terminal bar attaching to apical membrane (arrowed); and secretory granules (SG), scale bar = 1.0 μm. Inset showing microvilli balloon-like tips.

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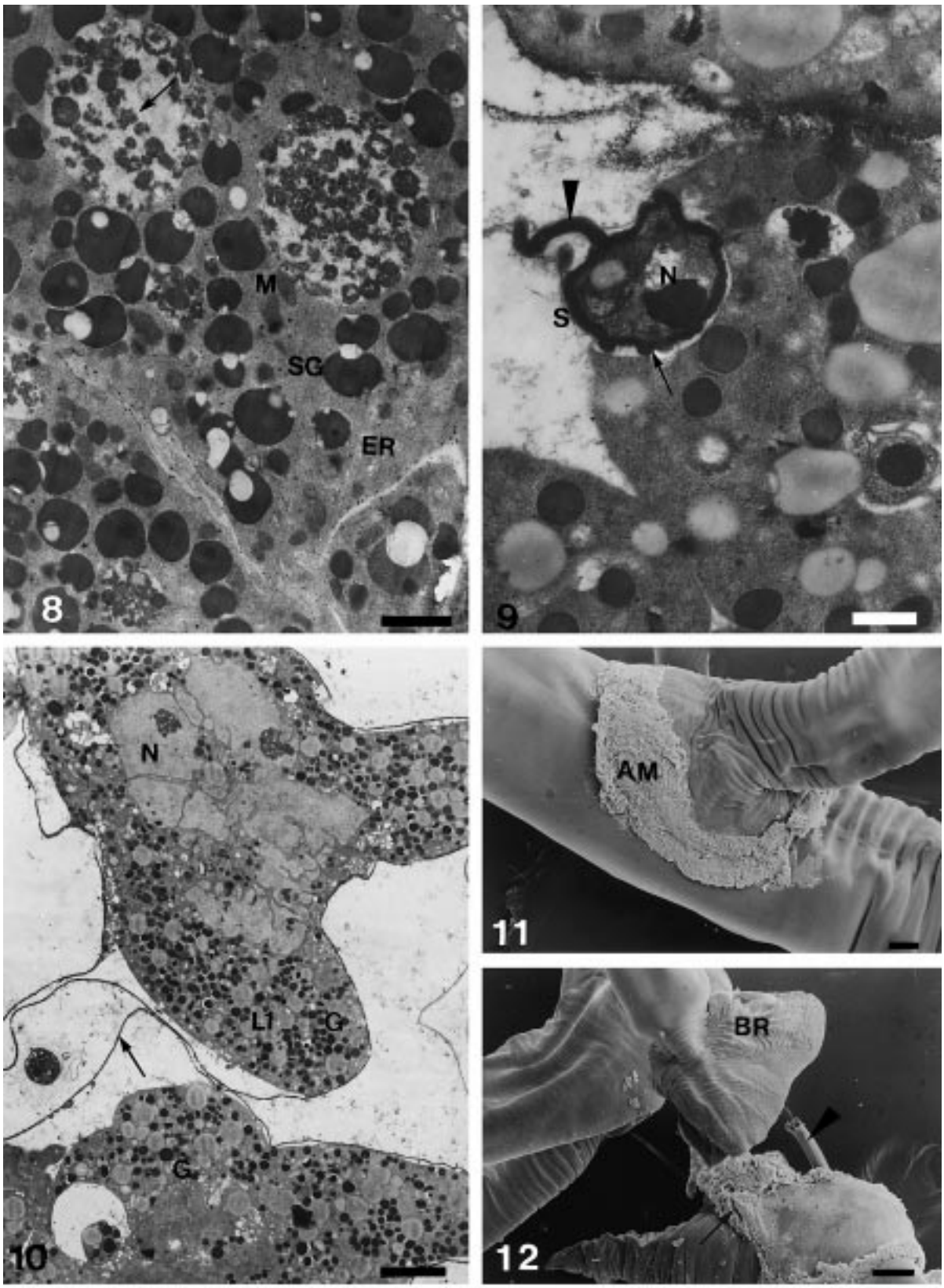

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containing many spermatozoa. The basal lamina of the epithelial cell is thick and convoluted and has no basal labyrinth. The apical plasma membrane extends into the lumen in microvilli-like projections. Spermatozoa usually occur close to this layer. There are no complete cell boundaries between cells, but a terminal bar is attached to the apical plasma membrane. The cell cytoplasm consists of few mitochondria, granules, endoplasmic reticulum, and vesicles. The lumen contains many amoeboid spermatozoa and abundant oocytes. (fig. 5). The few unfertilized oocytes have a thick membrane closely attached to the cell contents with large spaces and darkly-stained lipid droplets. The uterus contains many eggs (up to 45) and is lined by epithelial cells different from those lining the seminal receptacle as follows. Anteriorly the epithelial cells, 3.3 μm deep, lie on a thick, smooth, basal lamina with no labyrinth layer (fig. 6). The uterine apical plasma membrane has microvilli-like projections into the lumen and the terminal bar lies between the apical ends of adjacent cells. Throughout the length of the bar the two plasma membranes are closely apposed, separated by a translucent space. The cytoplasm of the epthelial cells is rich in mitochondria, glycogen deposits, and fibre bundles. The vagina connects the uterus to the cuticle-lined, slit-like vulva.

Male reproductive tract

The male reproductive tract is monorchic, consisting of a single, blind-ending testis, seminal vesicle, vas deferens and ejaculatory duct, opening into the cloaca. The testis is a long tract coiled around the intestine. The adult testis is telogonic or divisible into two regions, a germinal and a growth zone, difficult to distinguish from each other. Anteriorly and in the mid region, testicular cells have two parts (both kidney-shaped) linked by a rachis. The youngest spermatocytes (spermatogonia) appear as elongated, conical cells each with a central large nucleus and a distinct nuclear membrane and nucleolus. Each spermatocyte contains many secretory granules of one apparent type, with abundant endoplasmic reticulum and mitochondria. Each spermatocyte connects with the rachis in a narrow, waist-like constriction (fig. 7).

The rachis cytoplasm contains mitochondria, extensive rough endoplasmic reticulum, and electron-dense granules. The apical plasma membrane of the rachis is elevated to form numerous microvilli-like projections into the lumen. These vary in length and in possessing dilated, balloon-like tips. These round, membrane-bound vesicles are closely attached to the microvilli and are very abundant in the lumen. Thick, dense, fibrous strands attached to the apical plasma membrane of the rachis may represent the terminal bars of rachis cells. Lower down, the spermatocytes surround and are in cytoplasmic continuity with, a cytoplasmic but anucleate rachis (fig. 7, inset). Spermatocytes, at a later stage of development, contain granules that are electron-dense with a pale polar cap, small less-dense granules, and translucent vesicles with small electron-dense material. These vesicles consist of aggregates of small granular material with few mitochondria (fig. 8).

Spermatozoa in the female reproductive tract are, in general, amoeboid. They contain peripheral membrane specialization (vesicles) with microvillar-like projections, numerous translucent vesicles, fibrils, and, non-membrane-bound nucleus. Spermatozoa are intimately enclosed by pseudopodia-like extensions in the lumen of the seminal receptacles (fig. 5).

Oocytes, rich in large lipid droplets and dense granules surrounded by a thick, dense membrane, are penetrated by spermatozoa leading to nuclear division. At fertilization, the spermatozoon membrane becomes dense, thicker with fewer processes, denser pseudopodia, and a more anterior nucleus (fig. 9). Fertilized eggs initiate mitotic division (fig. 10)

Copulation

As in other bursate nematodes, the male *S. vulgaris* holds the female by the copulatory bursa, together forming a Y-shape. Adhesive material, clearly visible, ensures attachment and secures transport of spermatozoa to the vagina. This material left on the vulva may indicate that fertilization has occured (figs 11 and 12). Similar to *Nematospiroides dubius,* the worms lie with their heads in opposite directions as the male grasps the female. A colourless substance is released from the vas deferens and from 'copulation pads' on the vulva, tending to thicken and darken with age (Lee & Atkinson, 1976).

Discussion

Cephalo-oesophageal ligaments

In *S. edentatus*, the mouth, buccal capsule and oesophagus are surrounded at their junction by a tissue with granular plasma and consisting of three rings, each containing nuclei (Immink, 1924). This supports the connection between the capsule and oesophagus and also produces their chitinous parts. The circular band of tissue that usually surrounds the oesophagus at the base of the lip is named the 'arcade' (Immink, 1924; Chitwood & Chitwood, 1974). The present ultrastructural study is consistent with the observations of Immink (1924) as the tissue of the cephalo-oesophageal ligaments shows high metabolic activity, consistent with production of the

Fig. 8. Transmission electron micrograph (TEM) of a transverse section (TS) through testis showing: spermatocyte: translucent vesicles with aggregated material (arrowed); mitochondria (M); extensive endoplasmic reticulum (ER); and polar cap secretory granules (SG), scale bar = 5.0 μm. Fig. 9. TEM of a TS through uterus showing: spermatozoon (S) penetrating oocyte. Note: spermatozoon membrane is dense (arrowed); nucleus (N) without nuclear membrane; and pseudopodium (arrowhead), scale bar = 1.0 μm. Fig. 10. Oocyte nucleus (N) in a state of division; lipid droplets (LI); small, dense granules (G), surrounded by vitelline membrane (arrowed), scale bar = 5.0 μm. Fig. 11. Scanning electron micrograph (SEM) of the male and female during copulation showing the Y-shaped formation; and adhesive material (AM), scale bar = 100 μm. Fig. 12. SEM of provoked separation between male and female showing spicules inserted into the vagina (arrowhead), the adhesive material left around the vulva opening (arrowed) and male bursa (BR), scale bar = 100 μm.

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lining cuticle of the buccal cavity, teeth, dorsal gutter, oesophageal crown and oesophageal lumen. They could also secure and support the connection between the buccal capsule and oesophagus.

Oesophagus

The terminal ducts of oesophageal glands open at various sites in various species of nematodes: in *Nippostrongylus brasiliensis*, the sub-ventral glands open into the oesophageal lumen in its posterior half (Lee, 1968, 1969); in *Necator americanus* they open into the oesophageal lumen posterior to the nerve ring (McLaren, 1974). Food passed into the oesophageal lumen will be immediately subject to further digestion by the enzymes, secreted from the two sub-ventral oesphageal glands (McLaren, 1974). In *S. vulgaris* however, the terminal ducts of the oesophageal sub-ventral gland open into the base of the buccal cavity at the oesophageal crown. These could release a secretion into the buccal cavity to participate in the enzymatic digestion of the mucosal tissue within the buccal cavity. The various opening sites of the terminal ducts may be related to the feeding habitats and the type of food ingested (McLaren, 1974).

In *S. vulgaris* the terminal duct of the DOG runs inside the dorsal gutter and opens to the exterior at the rim 'ring canal' of the buccal capsule, while the secretion of the dorsal gutter canals is released through the dorsal gutter duct which passes through the teeth bases at the base of the buccal capsule (Mobarak & Ryan, 1998b). Similar to *Nippostrongylus brasiliensis*, the DOG dilates into an ampulla which opens through a duct leading to the exterior at the very tip of the mouth (Lee, 1969). In the hookworms *Necator americanus* and *Bunostomum trigonocephalum* the terminal duct of the DOG passes through the dorsal cone and opens directly in the buccal cavity (McLaren, 1974; Wilfred & Lee, 1981). In *Haemonchus contortus*, the DOG duct continues medially through the lancet, opening onto its medio-ventral surface. Thus, the anticoagulant and proteolytic enzymes secreted by the gland will be more efficiently used for extra-corporeal digestion followed by ingestion. Furthermore, the direct injection of these enzymes into the sub-muscularis mucosa could evoke antigen-antibody and cellular responses in heavily infected sheep (Weise, 1977). The buccal capsule of *Pontonema vulgaris* (Enoplida), contains three teeth, through which open the ducts of the dorsal and the two sub-ventral oesophageal glands (Jennings & Colam, 1970).

In general, the position of the external opening of the DOG may have important implications for the role of this gland during feeding and digestion, because it secretes onto the mucosal epithelial tissue. Histolytic enzymes present in the secretion will break down host tissue cells around the mouth. In contrast, if the duct opens into the lumen of the oesophagus, the glandular secretion would be pulled back along the lumen of the oesophagus, by oesophageal pumping action, and would not be effective in extracorporeal digestion (Lee, 1969).

Dorsal oesophageal gland ampulla

The DOG of *S. vulgaris* is well developed and dilates into an ampulla lying beneath the dorsal gutter. The ampulla cytoplasm is an extension of the gland cytoplasm and is rich in cytoplasmic organelles but has few myofilaments. McLaren (1974) reported one supporting cell surrounding the terminal duct in *N. americanus* as containing numerous mitochondria. In *S. vulgaris*, the lumen of the duct in the oesophageal ampulla is convoluted with two large, kidney-shaped, lateral supporting cells, rich in whorl-shaped endoplasmic reticulum and various cytoplasmic oganelles suggesting a high degree of synthetic activity (Mobarak & Ryan, 1998b).

Oesophageal secretion

The present study shows that in *S. vulgaris*, in addition to the action of oesophageal muscular contraction, microtubules within the DOG duct could facilitate the movement of secretory granules along the length of the gland. Also nerve axons closely associated with the duct suggest neural control of the duct secretions. Such axons are also reported from *N. americanus* (McLaren, 1974).

The forward movement of secretory granules is probably facilitated by the array of longitudinally oriented microtubules, common in oesophageal gland extensions of plant parasites e.g. *Meloidogyne incognita* (Endo & Wergin, 1988). The association of secretory granules with microtubules during their transport to the cell surface is common in secretory cells (Burgess & Kelly, 1987). Spherical secretory granules formed in oesophageal glands vary in size and electron density among genera and species of nematodes, and during parasitism (Hussey & Mims, 1990). The abundance of concentric whorls and smaller vesicles of RER, and numerous secretory granules in the cytoplasm of the DOG indicate that it secretes protein (Lee, 1968; Talluri *et al.*, 1986). In the *Toxocara canis* third-stage larva, oesophageal cells are filled with secretory granules that, in addition to a functional feeding mechanism, may secrete histolytic enzymes into the host environment. These would facilitate the penetration of the larva across host tissue (Talluri *et al.*, 1986).

In the present study, granules of various sizes and densities were observed in oesophageal glands, oesophageal ampulla, oesophageal ducts along the length of the oesophagus, and in the terminal duct of the DOG. These granules were not analysed histochemically in this study, but data from other species indicate: the presence of acetylcholinesterase (AChE) and leucine aminopeptidase in *N. americanus* (McLaren, 1974); a non-specific esterase in the region of the oesophagus of the third-stage larve of *Nippostrongylus brasiliensis* where secretory granules are most abundant (Lee, 1969); and non-specific esterase and acid phosphatase activity in the DOG of *B. trigonocephalum* (Wilfred & Lee, 1981). A large molecular weight glycoprotein and acid phosphatase have been identified using monoclonal antibodies from secretory granules in parasitic second-stage juveniles of *M. incognita* (Hussey *et al.*, 1990). The former has been purified, and the latter has been localized in the matrix granules formed in sub-ventral oesophageal glands (Sundermann & Hussey, 1988). There is considerable indirect evidence that these

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enzymes are actively secreted *in vivo* (Ogilvie *et al.*, 1973; McLaren, 1974).

Evidence in this laboratory suggested that the excretory–secretory ESP of *S. vulgaris* is rich in cysteine proteinases (Caffrey & Ryan, 1994). The ultrastructure of the oesophageal secretory granules is consistent with enzyme secretion but such a function requires verification.

Oesophageal lumen

The present study shows the oesophageal lumen is lined with cuticle which differs from the buccal cavity cuticle in that: it dissolves in 1.0 M KOH, is subdivided into four layers, and varies in thickness along its length. Similar to the adult *N. brasiliensis*, the cuticle lines the oesophageal lumen, has an outer membrane, a thin layer of low density, a thicker, dense layer, and varies a little in thickness in various regions (Lee, 1968). It differs from *Pontonema vulgaris*, in which the oesophageal lumen is lined by a smooth cuticle continuous with that lining the buccal capsule (Jennings & Colam, 1970). In *S. vulgaris*, cuticle thickening starts anteriorly just behind the oesophageal funnel and terminates at the posterior region. Transverse ridges or striations in the thickened cuticle may act as flexible or deformable zones, to facilitate bending along its length (Bennet-Clark, 1976). The present study shows the posterior oesophagus contains inlet valves or 'valvulae' or 'cuticular flaps', similar to those of *S. edentatus* and *Ascaris lumbricoides* that project into the lumen. This surface is ridged and provided with oblique muscles to grind food particles and referred to as a 'chewing apparatus' in *S. edentatus* (Immink, 1924). Food particles may be subjected to second stage filtering and concentration as they are passing through the bulb, the ridges on the bulb flaps acting as a strainer in *A. lumbricoides* (Bennet-Clark, 1976).

Oesophageal muscles

The present study shows, in addition to eight cephalo-oesophageal muscles attached to the oesophagus, small coelomyarian oesophageal muscles attached to the outer wall of the basal lamina at the level of the nerve ring. They may represent the attachment for the cephalo-oesophageal muscles that control contractile activity of the oesophagus. Bennet-Clark (1976) reported no evidence of any circumferential fibrous structure at the outer wall of *A. lumbricoides.*

Oesophageal-intestinal valve

The present study has revealed that the oesophageal-intestinal valve (three-lobed) projecting into the intestine of *S. vulgaris* is morphologically similar to those of *S. edentatus* (Immink, 1924), *N. brasiliensis* (Lee, 1968) and *Cyathostoma lari* (Colam, 1971). In *N. brasiliensis*, the cells are almost completely separated from the oesophagus and a basal lamina between the two is joined by a narrow zone continuous with intestinal cells. Although the cuticular lining of the valve also lines the oesophagus, it ceases abruptly at the junction of the intestine with the valve. Each valve cell has a single, large nucleus with nucleolus and radially oriented muscle fibres. The function of this valve is believed to be the prevention of regurgitation from the intestine. Both valves and internal turgor pressure force the oesophageal wall together (Bird & Bird, 1991).

Reproductive tract

As there have been no ultrastructural studies on the male or female reproductive tract of *S. vulgaris*, the present study provides a general description of both systems.

Female reproductive tract

The female reproductive tract in *S. vulgaris* is essentially similar to those of other nematodes studied, e.g. *Ascaris suum, Ancylostoma caninum* (Chitwood & Chitwood, 1974) and *Loa loa* (Weber, 1987). It comprises paired ovaries, oviducts, uteri, a single vagina and ventrally opening vulva. The ovarian epithelial cell in *S. vulgaris* has a large, irregular nucleus, apically located. A thick, convoluted, outer basal lamina gives an irregular appearance to the ovarian tubules. This arrangement in *L. loa* and the extensive infolding of the plasma membrane forming the basal labyrinth, suggest enhanced exchange of nutrients between the ovarian epithelial cells, the intestine (as the reproductive tract coils around the intestine), and pseudocoelom, across the basal lamina (Weber, 1987). No cell boundaries were observed in *S. vulgaris* between epithelial cells, but bundles of myofilaments in the cytoplasm support the ovarian wall. The apical plasma membrane infolds to form a labyrinth continuous with the basal labyrinth, suggesting continuous transport of nutrients across the epithelial cells. Epithelial cell cytoplasm has mitochondria, secretory granules and membrane-bound vesicles, indicating high synthetic activity. Oocytes attached to the rachis have a large nucleus, and a cytoplasm rich in lipid droplets probably acting as a food reserve for further development.

Uterine ultrastructure is different from that of the ovaries. The uterine basal layer is smooth without convolutions, has no labyrinth, and the apical membrane bears microvilli-like projections with which spermatozoa are intimately associated. These might support the spermatozoa during their maturation in the uterus. Similar to *Heterakis gallinarum*, amoeboid spermatozoa are intimately associated with the wall of the seminal receptacle through their pseudopodia-like extensions (Lee, 1971). In contrast to adult *L. loa* (Weber, 1987), microvilli occur in the *S. vulgaris* uterus and not in the oviduct. Uterine epithelial cell cytoplasm comprises mitochondria, glycogen deposits and electron-dense granules, suggesting uterine cells have secretory and storage functions. The uterine lumen is filled with fertilized and non-fertilized eggs: bundles of myofilaments in the wall of the uterine cells with the microvilli may help expel eggs to the exterior, as no muscle layer has been observed surrounding the uterus.

Male reproductive tract

Similar to other nematodes, the male *S. vulgaris* reproductive tract comprises a single testis, seminal

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vesicles, vas deferens and ejaculatory duct, emptying into the cloaca.

Structure and development of spermatozoa

Nematode spermatozoa are classified into four types: ascaroid, strongyloid, dioctophymoid and oxyuroid (Foor, 1970). Little may be gained from the artificial classification of nematode spermatozoa in groups based on their morphology as there is great variability in nematode spermatozoa (Bird & Bird, 1991). In the uterus of *Ascaris lumbricoides* and *Parascaris equorum*, spermatozoa have a clear anterior region which often forms pseudopodia, and a dense posterior region comprising nucleus, refringent body, mitochondria and membranous structures containing microvilli-like processes (Foor, 1970). In spermatozoa of *H. gallinarum*, the membranous strucures are vesicles, containing acid phosphatase, formed by the Golgi complex or endoplasmic reticulum (Lee, 1971). According to Foor (1970) these membranous vesicles in *A. lumbricoides* arise as modification of mitochondria in spermatocytes. In the strongyloid-type and oxyuroid-type of spermatozoa there is a 'head' and 'tail' with the nucleus in the elongate, tail-like, projection. *Ancylostoma caninum* spermatozoa in the seminal vesicle have a tadpole-shape and are extremely polymorphic, or amoeboid after deposition in the female (Foor, 1970).

Strongylus vulgaris spermatocytes are attached to the central rachis, where the apical membrane possesses microvilli. A distended tip of membrane-bound vesicles is in intimate contact with the microvilli, suggesting a transport activity between them and rachis cells. Terminal bars attached to the rachis apical membrane may represent incomplete division between the rachis epithelial cells. Rachis cytoplasm is rich in endoplasmic reticulum and secretory granules suggesting high synthetic activity associated with development and nutrition of spermatozoa. As spermatozoa were studied in the female tract only and not in the seminal vesicles, this precludes a firm categorization of them. Nevertheless, the mature spermatozoa are somewhat similar to those of *A. caninum* and *N. brasiliensis* in the female uterus (Foor, 1970). Spermatozoa of both those species are extremely polymorphic or amoeboid, with a mixing of nuclear and cytoplasmic regions after deposition in the female uterus (Foor, 1970). In *S. vulgaris*, mature spermatozoa in the uterus are amoeboid, contain a nucleus that lacks a nuclear membrane, and are surrounded by membranous vesicles. They have pseudopodia, similar to *Meloidogyne acronea* spermatozoa, which may be required to penetrate the oocytes (Shepherd & Clark, 1983). *Strongylus vulgaris* spermatozoa are different from *Ascaris suum* (ascaroid-type), in lacking a refringent body and in having a clear anterior and posterior region (Bird & Bird, 1991).

Post-insemination changes (activation) in nematode spermatozoa involve an enlargement of the anterior ameboid portion, exocytosis of the membranous vesicles, and development of spermatozoon motility (Bird & Bird, 1991). Spermatozoan activation in *N. brasiliensis* involves the membrane vesicles that line the perimeter of the spermatozoa fusing with the cell membrane and releasing their contents. This may increase the absorptive surface for uptake of uterine fluids, ion exchange, or may facilitate the secretion of substances (Wright & Sommerville, 1984). Activation may occur in *S. vulgaris*, as the spermatozoon membrane becomes thick and dense, the nucleus locates anteriorly, and the membranous vesicles fuse.

Overall, the foregoing results indicate that in terms of oesophageal organization, *S. vulgaris* closely resembles *S. edentatus* and *N. brasiliensis* but with uniqueness represented by the organization of the valves. In terms of the ultrastructure of the reproductive systems, *S. vulgaris* is consistent with that reported from other strongyloid species.

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