Ultrastructural aspects of feeding and secretion–excretion by the equine parasite *Strongylus vulgaris*

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Abstract

Light, scanning, and transmission electron microscopy were employed to provide further data on the putative origins of the immunogenic secretory–excretory product (ESP) of *Strongylus vulgaris* (Looss 1900). The sharply delineated but superficial attachment to the equine caecum by the mouth leaves behind an oval area devoid of epithelial cells. Attachment does not extend deeply enough to reach the muscularis mucosa layer of the equine intestine. The progressive digestion of the ingested plug of tissue (epithelial cells, blood cells and mucous) was visualized. The coelomocytes, floating cells and membranous structures located in the pseudocoelom and intimately associated with the digestive, excretory and reproductive systems, and with the somatic muscles are described. The secretory–excretory system comprises two, ventrally-located, secretory–excretory glands connected to tubular elements. These glands synthesize granules of various sizes and densities that are delineated.

Introduction

The ultrastructure of *Strongylus vulgaris* has been described in detail including that of the buccal capsule with special reference to the dorsal gutter and teeth (Mobarak, 1995; Mobarak & Ryan, 1998b). The buccal capsule is the point of attachment of *S. vulgaris* to the horse caecal epithelial tissue, onto which are poured the immunogenic secretory–excretory products (ESP). The teeth and dorsal gutter are intimately involved in the association between the parasite and host tissue. Through the dorsal gutter, and specifically through the dorsal gutter duct which is connected proximally to the dorsal oesophageal ampulla, pass the dorsal oesophageal gland secretions on to the host tissue (Mobarak & Ryan, 1998b). Also, immunohistochemical studies employed antibodies raised against whole ESP and against combined gelatinolytic subunits of 28–30 KDa. Both antibodies recognized, in addition to buccal capsule sites and amphids, the intestine, excretory gland and ducts, and hypodermis (Mobarak & Ryan, 1998a). There are scant published data derived from electron microscopy of the morphological detail and inter-relationships of these latter structures in *S. vulgaris* (Abdul Rahman & Waddell, 1980).

In nematodes the excretory system, recently redefined as the secretory–excretory or S–E system (Bird & Bird, 1991), shows considerable structural and functional diversity between species (Chitwood & Chitwood, 1974; Wright & Newall, 1976).This system is represented by two basic types, glandular and tubular. The glandular system consists of two S–E glands (renette cells), lying in the body cavity near the base of the oesophagus, and associated with a terminal duct which opens to the exterior through a ventral pore. The tubular system in the Strongyloidea is a H-shaped system with a lateral canal running along the length of the nematode in each lateral cord and connecting ventrally with a small transverse canal. An S–E duct leads from this transverse canal to the S–E pore (Lee, 1965, 1970; Waddell, 1968; Chitwood & Chitwood, 1974).

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https://doi.org/10.1017/S0022149X00700344 Published online by Cambridge University Press

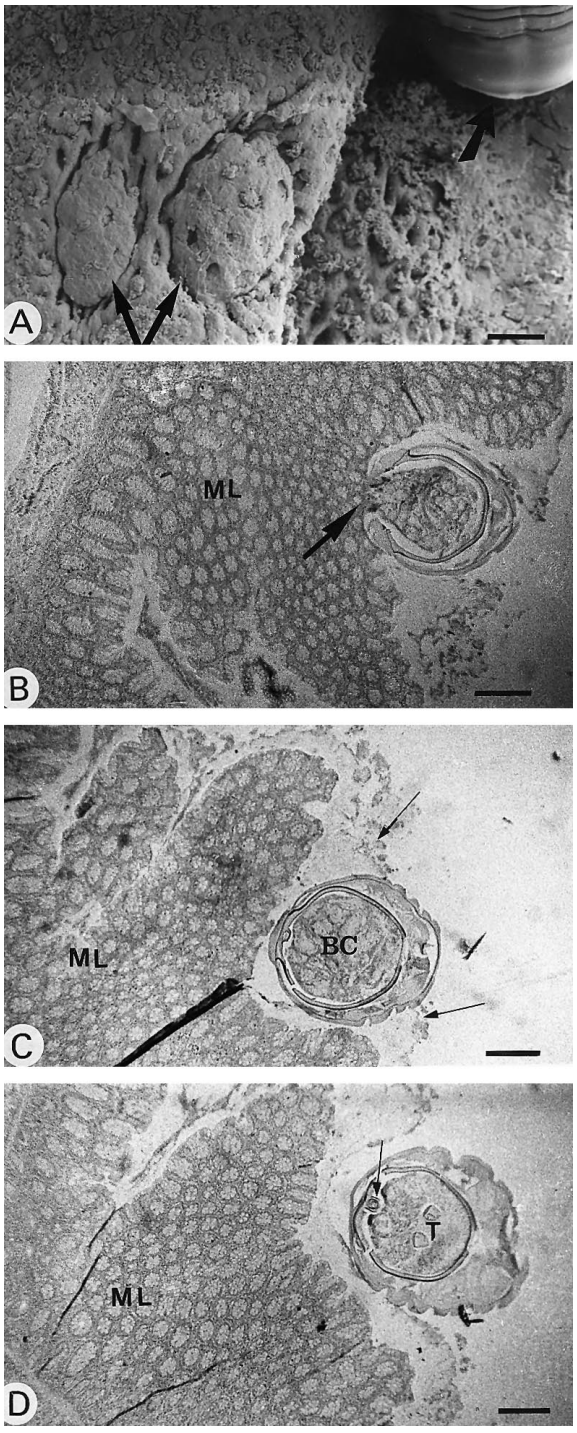

Fig. 1. A, Scanning electron microscopy (SEM) of the worm attached to the horse caecum showing the worm's cephalic end attached (thick arrow) and the naked area on caecal epithelia left after worm detached (arrows). Scale bar = 500 μm. B, C and D, light microscopy (LM) of serial sections of adult worm attached to horse caecum during feeding: B, worm attached superficially to host mucosal layer (ML), and ingested caecal epithelial tissue (arrowed). C, cephalic end surrounded by mucus layer (arrows); buccal cavity (BC) filled with host epithelial tissue. D, buccal cavity with partially homogenized epithelial tissue; teeth (T); dorsal gutter (arrowed). Specimen embedded in paraplast; 5 μm sections stained with hematoxylin/eosin. Scale bar = 500 μm.

Functional diversity is indicated by claims for: an osmoregulatory function (Lee, 1970; Wright & Newall, 1976); an effect on moulting (Rogers & Somerville, 1963); and a secretory rather than an S–E function (Lee, 1970; Romanowski *et al*., 1971; Bird & Bird, 1991). Enzymes and antigenic substances are liberated via the S–E system in many nematodes (McLaren *et al*., 1974; Ogilvie *et al*., 1975; Maizels *et al*., 1983; Maizels & Page, 1990; Bird & Bird, 1991).

Ultrastructural and histochemical studies of the S–E glands in various nematodes have detected similarities including, abundant golgi complexes, rough endoplasmic reticulum, and secretory granules (Wright & Newall, 1976). The S–E gland is active, and a secretory product, e.g. proteases, esterases, 'leucine' aminopeptidases and acetylcholinesterase (AChE) occurs in many nematodes (Lee, 1970; Ogilvie *et al*., 1973; McLaren *et al*., 1974). The transverse canal and S–E sinus have the same ultrastructural morphology as the lateral S–E canal. The nucleus is located in only the sinus. The lumen of the sinus, irregular and lined by a thin layer of cuticle, is connected to the S–E pore by a terminal duct which is lined with a thicker cuticle. Close to the S–E pore the terminal duct is surrounded by nerve axons (Waddell, 1968; McLaren, 1974; Bird & Bird, 1991).

In the S–E system of both the 4th-stage larva and adult *S. vulgaris*, as studied by light microscopy, the tubules and glands differ from those described for other nematodes in that transverse sections reveal a pair of S–E canals in each lateral cord, i.e. a straight canal and a winding one (Enigk & Grittner, 1952). The winding canal starts blindly midway between the nerve ring and the anterior tip of the nematode and winds backward in the lateral cord toward the tail, where it joins the straight canal just before the anal opening. The straight canal begins blindly near the tip of the tail and runs forward to join with the other straight canal, also running

Fig. 2. Transmission electron microscopy (TEM) of a transverse section showing pseudocoelomic cavity: A, between oesophagus (OES) and secretory–excretory (S–E) gland (SEG); cavity fillled with membranous structures (MS); lipid droplet (LI); and small dense granules (G). Scale bar = 2.0 μm. B, TEM of pseudocoelomic cavity between intestine (IN), ovary (OV) and somatic muscle (thick arrow) showing: membranous cells (MC); and fixed coelomocyte attached to intestine (thin arrow). Scale bar = 5.0 μm. C, higher magnification of fixed coelomocyte in the pseudocoelom (PS) in intimate contact with the intestine (IN) showing: coelomocyte plasma membrane (arrowed); mitochondria (M); electron-dense granules (G); fibres (F); lamellar membrane cell (arrow head); endoplasmic reticulum (ER) and intestinal basal lamina (BL). Scale bar = 1.0 μm. D, ovary epithelial wall consists of: irregular outer basal lamina (arrowed); nucleus (N); basal labyrinth (LA); mitochondria (M); fibres (F); membrane-bound vesicles (V); inner plasma membrane (arrowhead) lining the lumen containing oocytes (OC); lipid droplet (LI); and intestine (IN). Scale bar = 2.0 μm.

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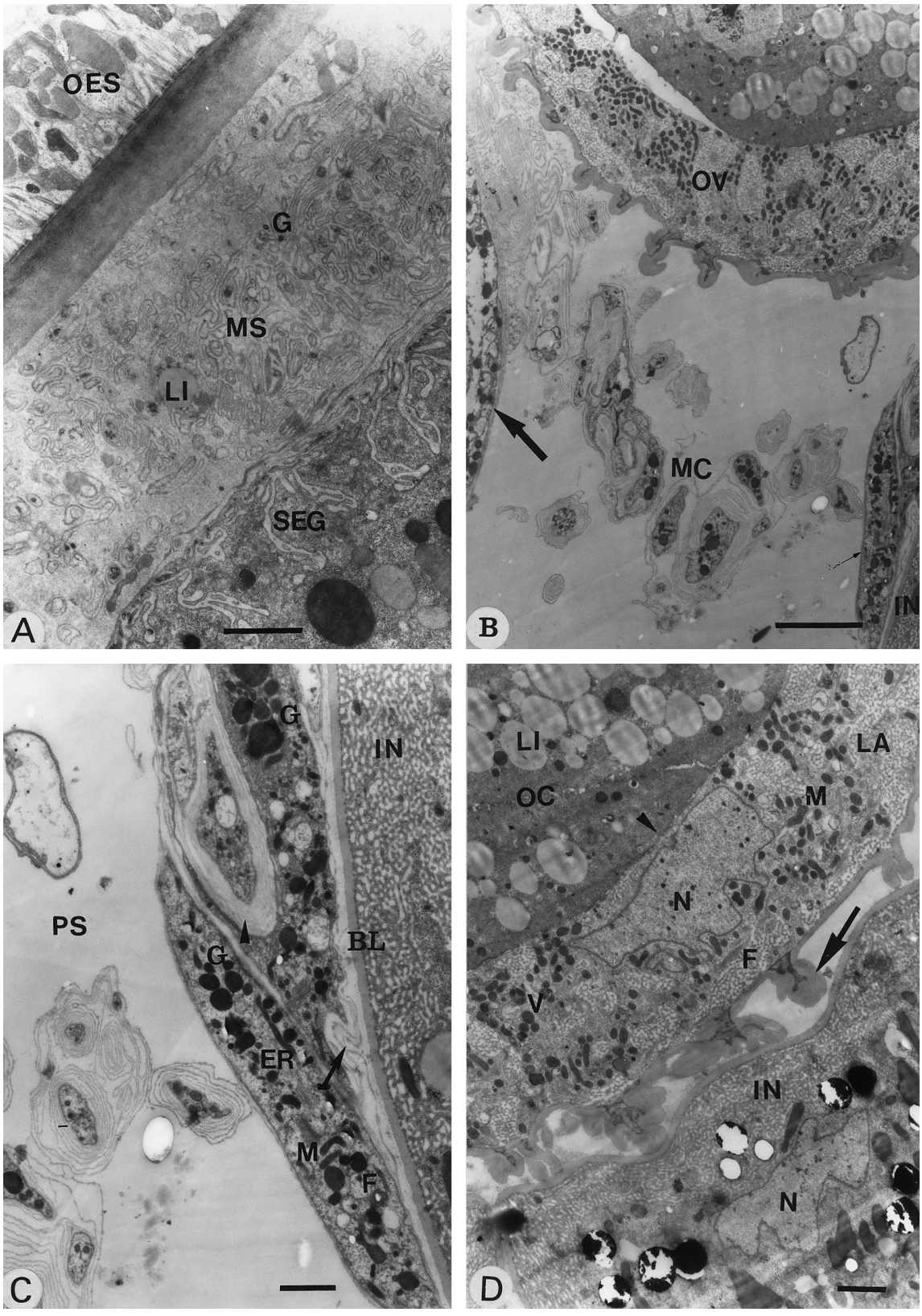

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forward in the opposite lateral hypodermic cord, to form an S–E vesicle just prior to the S–E pore. Substances moving posteriorly in the winding canal and anteriorly in the straight canal could enter these canals through fine cytoplasmic processes, which occur at irregular intervals and connect the canals to hypodermal cells. Secretory–excretory glands vary in size, being largest in the 4th-stage larva during the active migratory phase, and it was suggested that gland granules are end products of metabolism. Proteolytic enzyme activity was not detected in medium containing 200 dissected S–E glands titrated in Tyrod's solution at pH 4.5 and 7.5 (Enigk & Grittner, 1952). The present study employed scanning and transmission electron microscopy to further investigate feeding, and S–E and related structures and processes, with special reference to possible sources of the ESP.

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 1998b).

Results

Attachment and feeding

Both sexes attach firmly to the horse caecum by the mouth which is surrounded by the external leaf crown and four papillae. Apparently the worm does not burrow into the caecum, but attaches via the cephalic end leaving the body free in the host lumen (fig. 1A). This attachment, although pronounced, is shallow, resulting in an oval, naked area devoid of epithelial cells (fig. 1A). Serial histological sections of a paraplast-embedded worm attached to the caecum in fig. 1B, C and D quantify the superficial attachment of the worm's cephalic end to the caecal epithelial layer. It is not deep enough to reach the muscularis mucosa layer of the horse intestine. The worm feeds by drawing a plug of tissue (epithelial cells, blood cells and mucous) into the buccal cavity.

Inside the buccal cavity, the contents could be homogenized mechanically by the action of the teeth, aided by enzymes from the oesophageal glands and a secretion through the dorsal gutter duct. The contents of the buccal cavity comprise a mixture of finely homogenized and non-homogenized particles.

Body cavity (pseudocoelom)

The pseudocoelom has large, fixed cells known as coelomocytes, a network of membranes supporting visceral organs, and free-floating bodies that may be membrane cells. The cavity space, between the end of the oesophagus posterior region and the anterior intestinal cells 'cardia', is filled with an extensive membranous structure lying in intimate contact with the basal lamina of the oesophagus and intestine. Among these membranous structures are few electron-dense granules and lamellar bodies. The space between the oesophagus and the S–E glands is also filled with these membranous structures, and lipid droplets were observed (fig. 2A). Mid-posteriorly, the cavity between the intestine, muscles, and reproductive tract is filled with lamellar membranes and free-floating membrane cells (fig. 2B). The cells are irregularly-shaped, usually longer than wide, and structurally composed of two different parts: the central part with cell organelles, e.g. mitochondria, endoplasmic reticulum, glycogen deposits, and variously sized electron-dense granules surrounded by a plasma membrane; the second part consists of granular lamellar membranes that surround the whole structure (fig. 2B). A large coelomocyte lying in intimate contact with the intestinal basal lamina is elongated with a tri-laminated plasma membrane (fig. 2C). The cytoplasm consists of many electron-dense granules, mitochondria and endoplasmic reticulum. Within the coelomocyte, a small lamellar or membranous cell might be an early stage of these cells before their release to the pseudocoelomic cavity (fig. 2C). The reproductive tract of *S. vulgaris* is coiled arround the intestinal tract. The wall of the ovary (outer plasma membrane) is highly folded forming a basal labyrinth occuping the outer zone of the ovarian epithelial cell wall. These foldings are in intimate contact with the intestinal basal lamina and might be another way of transporting materials (nutrients) from the intestine (fig. 2D).

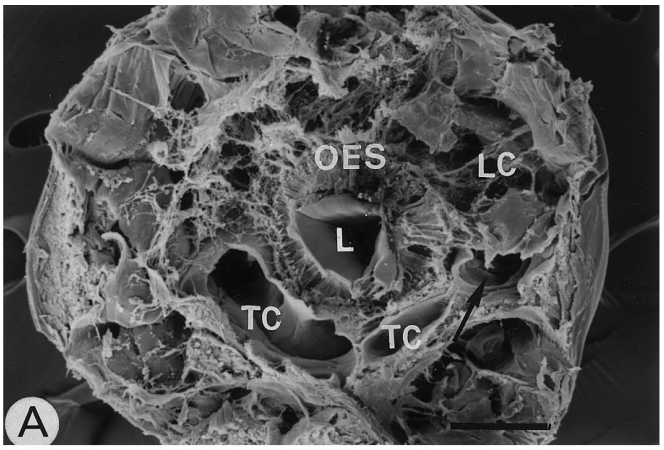
Secretory-excretory system

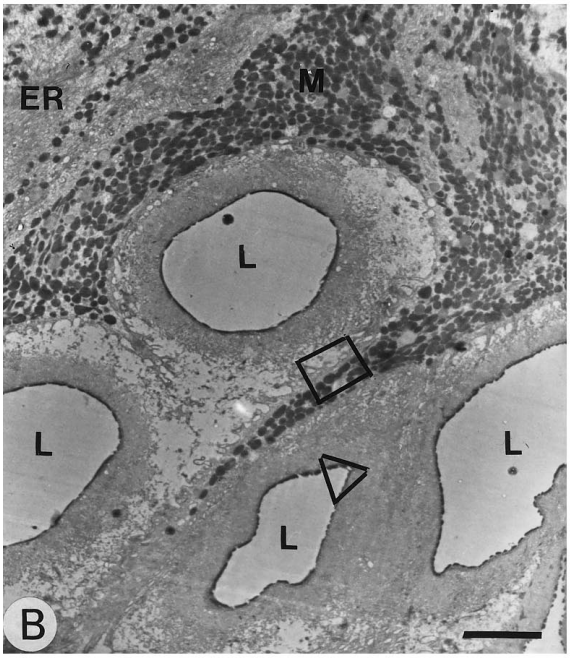
This H-shaped, tubular system consists of canals and two sub-ventral glands leading to a sinus and a terminal pore. This pore, situated ventrally one third of the distance from the anterior end, is round (32.7 μm diameter), cuticularly lined and connected to the sinus via the terminal duct.

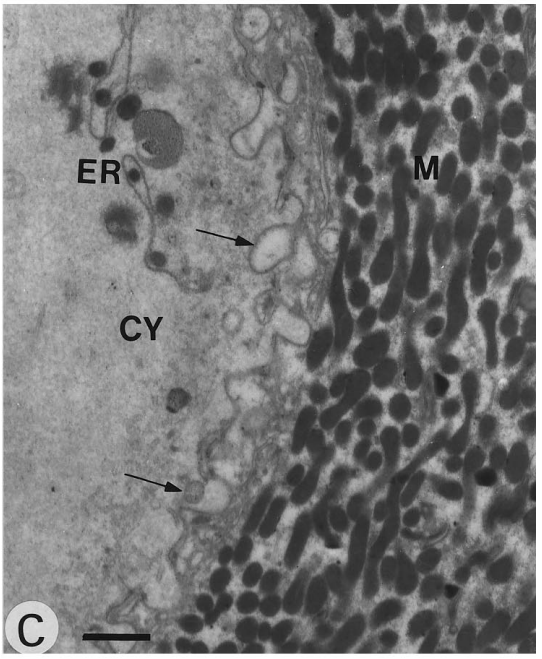
Secretory–excretory canals

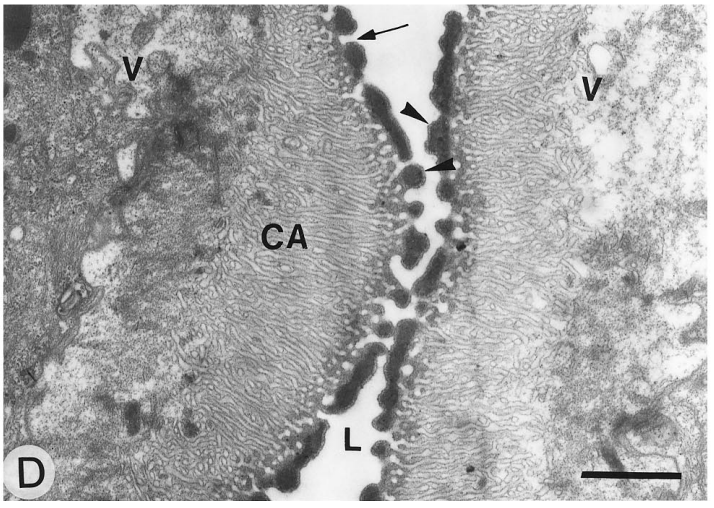
Two pairs of longitudinal canals carried in the lateral hypodermal cords are linked by a transverse canal

Fig. 3. A, SEM of transversely cut surface at the anterior oesophageal region showing: oesophagus (OES) with tri-radiate lumen (L); two ventrally-located transverse S–E canals (TC) connected to the lateral S–E canal (arrowed) in the lateral cord (LC). Scale bar = 100 μm. B, TEM of a transverse section through lateral cord showing: four lumina of lateral S–E canal in the lateral hypodermal cord (L); hypodermal mitochondria (M); and endoplasmic reticulum (ER). Scale bar = 4.0 μm. C, detail of the rectangular area showing: exo/pinocytosis (arrows) through the cell membrane; canal cell cytoplasm (CY); short endoplasmic reticulum (ER); and abundant hypodermal mitochondria (M). Scale bar 1.0 μm. D, detail of the triangular area showing: lateral S–E canal lumen (L) crenellated (square) and crenulated (round) electron-dense membrane (arrowheads); canaliculi (CA) congregated around the lumen; and vesicles opening into the lumen (arrowed); and membrane-bound vesicles in the cytoplasm (V). Scale bar = 1.0 μm.









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(fig. 3A), connected to the S–E sinus. Usually two distinct canals are visible in each lateral cord, a straight and a convoluted one. The convoluted canal extends from the base of the buccal capsule posteriorly for most of the worm's length to join the straight canal at the caudal region, forming one canal. Usually more than three lumina were observed in the transverse sections through the worm mid-body region and only one lumen anteriorly or caudally (fig. 3B).

Each lateral canal consists of an elongated cell embedded in the cytoplasm of the lateral hypodermal cord. The lining of the cell lumen is a crenulated (round) and crenellated (square) electron-dense plasma membrane with a very irregular outline (fig. 3D). Numerous membrane-bound vesicles aggregate around the lumen, apparently to form canaliculi that release their contents across the plasma membrane into the lumen (fig. 3D). The cell contains few organelles, e.g. mitochondria and endoplasmic reticulum in the cytoplasm but these are numerous in the cytoplasm of the hypodermis adjacent to the lateral canal (fig. 3C). The hypodermal cytoplasm and the S–E canals are separated by membranes (fig. 3C). There is active pinocytosis/exocytosis in which the hypodermal mitochondria may be engaged (fig. 3C). A few short strands of rough endoplasmic reticulum occur in the cell cytoplasm. No nuclei were observed in the canal walls.

Secretory–excretory glands

In the pseudocoelom lie two long glands situated ventrally in the posterior region of the oesophagus, extending down to the anterior part of the intestine. Intimately associated with both the transverse canal and the S–E sinus, each gland has a narrow, anterior portion connected to the sinus and a wider posterior portion lying free in the pseudocoelom.

Secretory–excretory glands appear as thin-walled sacs, containing granules distributed along the length of the gland and large posteriorly-located nuclei with prominent nucleoli (fig. 4A) Posteriorly, the granules are within a network of tubular membranes (fig. 4B) indicative of a secretory function, while the anterior part contains many granules of various sizes (about 3, 5, and 15 μm diameter) and electron densities (fig. 4C). These are large, membrane-bound and dense or translucent. Anteriorly, the granules are much more numerous, almost filling the cytoplasm, surrounding small blind-ending canals. The gland contains glycogen deposits, and rough endoplasmic reticulum distended into cisternae with granular material. From the covering membrane irregularly branched trabeculae ramify through these granules (fig. 4D).

The S–E sinus leads to the terminal S–E duct (3.6 μm diameter), lined with thick membrane and surrounded by canaliculi opening to the lumen. The outer plasma membrane is supplied with few nerve axons. Duct cytoplasm has some dense and translucent membrane-bound granules and the surrounding cytoplasm is rich in smooth endoplasmic reticulum (fig. 5A). The terminal duct lumen is 1.8 μm in diameter with a thick cuticular lining, surrounded by canaliculi. Thick, dense fibre bundles occur on the duct periphery attached to the duct lumen membrane (fig. 5B).

Discussion

Body cavity (pseudocoelom)

Although shallow, the penetration by *S. vulgaris* into the caecal wall seems sufficient to introduce ESP at the point of attachment, specially through the naked zones where the mucosal layer has collapsed. This could be relevant for the detection of ESP in equine blood.

In this study, coelomocyte ultrastructure reveals a large cell (giant coelomocyte) with the plasma membrane in intimate association with the intestinal basal lamina, and with floating structures in the pseudocoelom. It contains many electron-dense granules (either secretory or S–E) and lamellar membranes, suggesting high metabolic activity. This cell might contain/produce enzymes, or waste products. Lee (1962) detected esterase activity in the giant coelomocytes of *Ascaris lumbricoides*. In *Trichinella spiralis,* a 37 KDa antigen was detected only in the pseudocoelom (Silberstein & Despommier, 1984). Enzyme and immunohistochemistry studies are required to identify the nature and function of these granules in *S. vulgaris*.

Concentric-whorled membranes, having a granular appearance resembling the ribosomes of rough endoplasmic reticulum, float in the pseudocoelom between somatic muscles, reproductive tract and intestine. They might be produced by the fixed giant coelomocytes as they are in close proximity to their plasma membrane. Alternatively, they might carry secretions absorbed through the intestinal basal lamina for transportation to other organs.

Coelomocytes of all nematodes are considered homologous having similar functions, absorptive or phagocytic (Chitwood & Chitwood, 1974). They are comparable to the fixed histocytes of vertebrates and as the nematode body fluid flows by them, they may purify it. Although their physiological role remains the subject of speculation, some evidence suggests they may have an S–E function (Wright & Newall, 1976; Bird & Bird, 1991). As coelomocytes are active cells these are probably essential to nematodes (Peregrine, 1973). Their various shapes include: branched cells in *Ascaris*; stellate cells with pigmented granules in *Strongylus equinus;* strand-like organs in *Ancylostoma duodenale*, similar to those of

Fig. 4. A, LM of transverse section through: S–E gland (SEG) showing: nucleus (N); pseudocoelom (PS) filled with material (arrowed) between the gland and intestine (IN); and somatic muscle (SM). Scale bar = 10 μm. B, SEM of S–E gland showing: posterior end, granule formation from the tubular membrane (arrowed). Scale bar = 10 μm. C, anterior end, with variously-sized granules (G). Scale bar = 10 μm. D, TEM of a transverse section through anterior S–E gland showing: covering membrane (arrow head); trabeculae (TR) with small membrane-bound granules (arrowed); variously sized and density granules (G); short strands and cisternae of endoplasmic reticulum (ER); glycogen deposits (GL); and pseudocoelom (PS). Scale bar = 1.0 μm.

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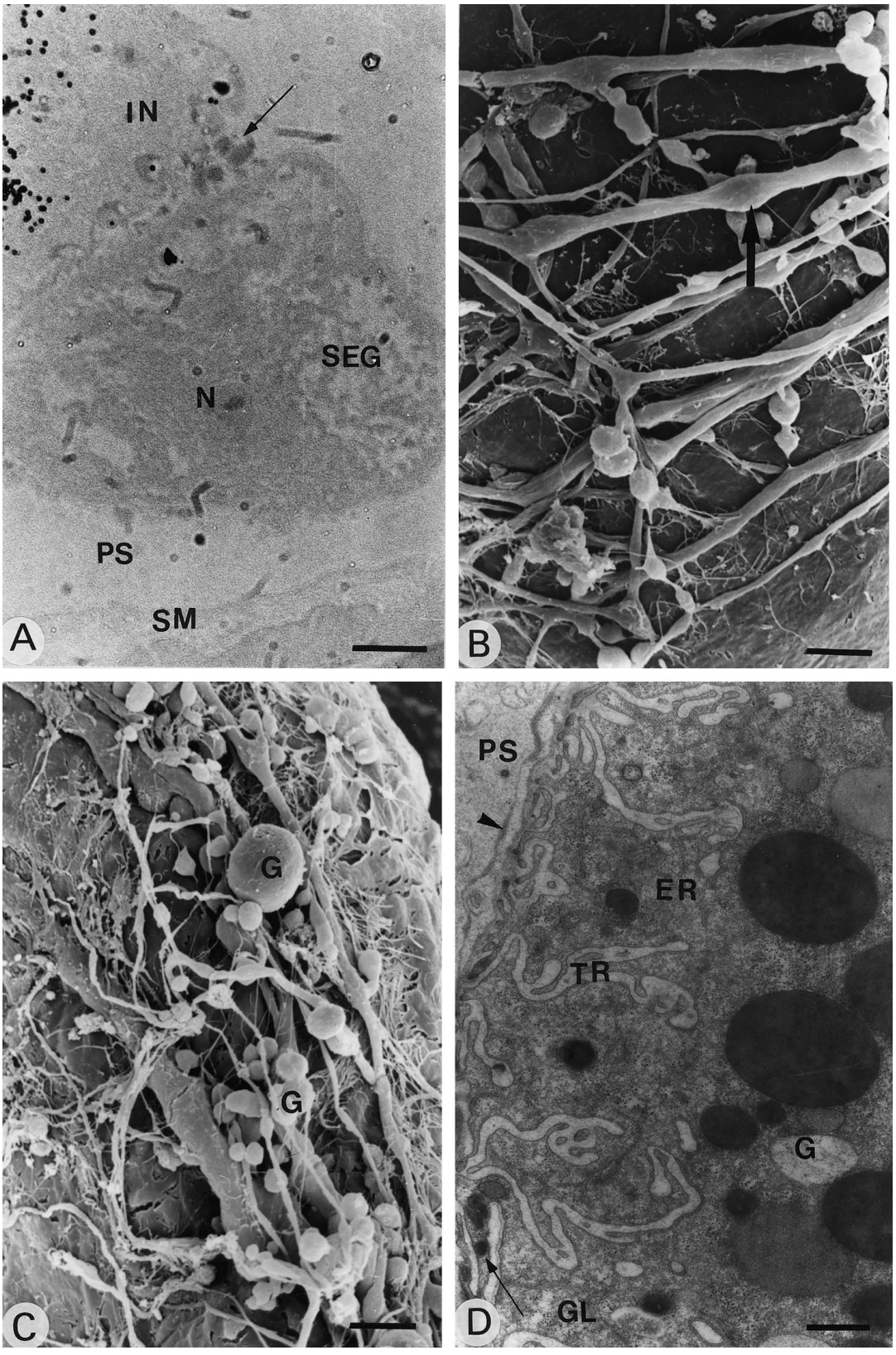

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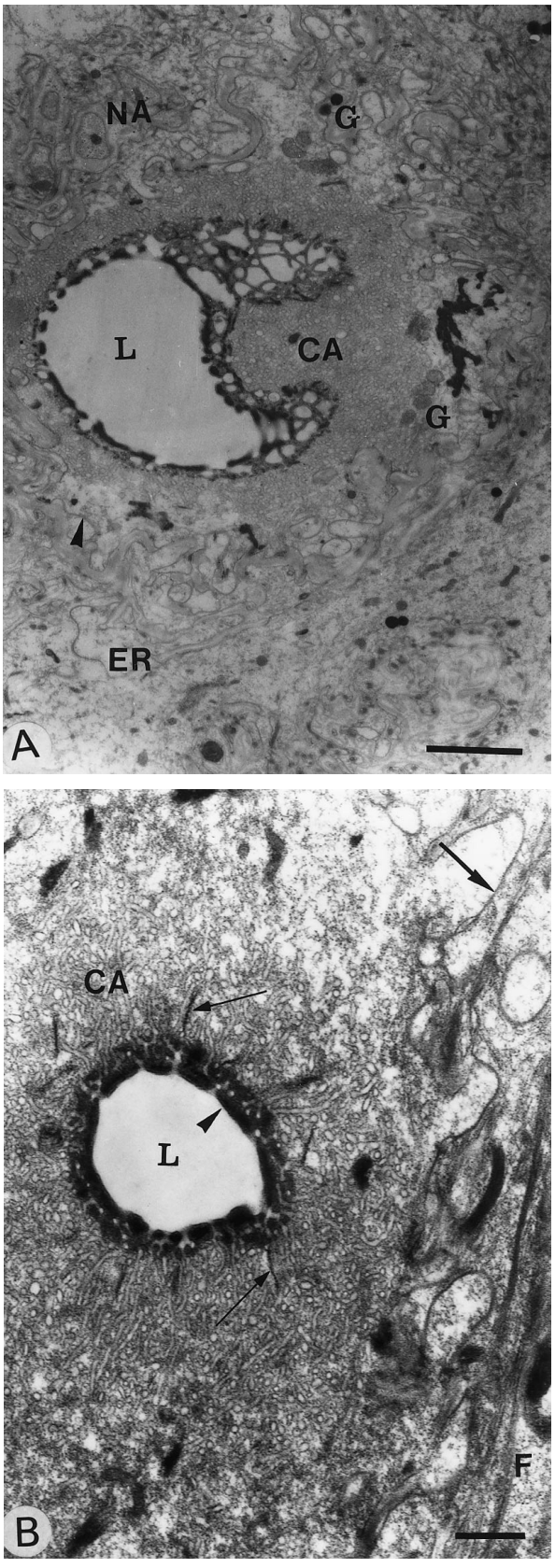

ascarids but containing refractive particles; and others are ovoid or branched (Chitwood & Chitwood, 1974).

In *S. vulgaris*, membranous structures were most abundant between the oesophagus and S–E glands, and between the oesophagus and somatic muscle sarcolemma, and less extensive between the oesophagus and intestinal 'cardia'. Accordingly, these membranes might be a connective tissue providing support and rigidity and may act in transporting materials between the body cavity and these organs. Ultrastructural details of the reproductive tract wall of *S. vulgaris* shows another possible mechanism for transporting materials between the intestine and the reproductive tracts. The folds in the reproductive tract wall increase its surface area and the labyrinth could be channels for transportation of nutrients. In the body cavity of *Trichuris vulpis*, irregularly folded sheets of basal lamina-like material are found between the surface of reproductive and digestive tracts and the body wall. These extracellular coating materials could be considered as connective tissue, having a supportive or suspensory role. Their discontinuous nature does not subdivide the body cavity into compartments and hence would not impede movement of fluid through the body cavity (Wright *et al*., 1972).

Secretory–excretory system

The present data confirm and extend earlier findings on the 4th-stage larva and adult *S. vulgaris* and agree with the description of the S–E system (tubules and glands) (Enigk & Grittner, 1952). The S–E system of *S. vulgaris* is of the rhabditoid type (Chitwood & Chitwood, 1974) and its ultrastructural morphology is very similar to that described for other nematodes especially the hookworm, *Necator americanus* (McLaren, 1974).

In addition, the present ultrastructural study shows the S–E glands of *S. vulgaris* as highly active cells, containing numerous granules of various sizes and densities that may be enzymatic. The gland cell, with its thin, outer membrane and the trabeculae ramifying through the glandular cytoplasm may be involved in absorbing and transporting nutrients from the pseudocoelom to the gland cell used in granule formation. This takes place at the posterior end of the glands and granules pass forward to the anterior site of the gland to be excreted through the S–E pore. Similar to *N. americanus*, the glands are connected to the transverse S–E canals. This indicates the secretions either pass back to the lateral canals or into the sinus to be released by the terminal duct via the S–E pore. The presence of nerve axons and fibrillar bundles around the terminal duct where it

Fig. 5. TEM of a transverse section: A, at the origin of the terminal S–E duct showing: canaliculi (CA) surrounding the lumen (L); cytoplasm of the canal wall containing dense and translucent granules (G); plasma membrane (arrowhead); nerve axons (NA) lying close to the duct, endoplasmic reticulum (ER). Scale bar = 2.0 μm. B, at the end of the terminal S–E duct showing: lumen (L) lined with thick, dense cuticular layer (arrowhead); dense fibre bundles connected to the lumen wall (arrows); S–E duct plasma membrane (thick arrow); fibres (F); and canaliculi (CA). Scale bar = 0.25 μm.

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approaches the pore suggests neuro-mechano co-ordination of release in *S. vulgaris*. A similar finding was reported from *N. americanus* (McLaren, 1974).

In general, the nematode S–E system has many functions: osmoregulation, ion regulation and secretion, in addition to excretion (Mehlhorn, 1988; Bird & Bird, 1991). Enigk & Grittner (1952) suggested that the granules in S–E glands of *S. vulgaris* are an end product of metabolism. In *Nippostrongylus brasiliensis*, histochemical tests have shown the contents of these glands as having non-specific esterase and aminopeptidase activities: it was suggested the enzymes are located in the secretory granules of the gland (Lee, 1970). In *Stephanurus dentatus*, S–E gland cells are composed mainly of protein, few lipids and glycogen (Romanowski *et al*., 1971). Proteases are liberated from the pore of *N. americanus*, and AChE, incubated in medium containing acetylthiocholine iodide, was associated with the S–E glands of *Trichstrongylus colubriformis* (McLaren et al., 1974). In *N. brasiliensis*, AChE was found in only one of the two granule types in the S–E glands (McLaren *et al*., 1974). There is little doubt that these enzymes are immunogenic and probably of great importance for the survival of the parasite (Pritchard, 1986). Immunohistochemical studies using light microscopy have shown that S–E glands of *S. vulgaris* are immunogenic and a strong reaction was observed in the lateral cords, where the S–E canals run (Mobarak & Ryan, 1998a). Further clarification will be enabled by the use of more specific techniques like immunogold staining to identify the constituents of these glands and their canals in *S. vulgaris*.

References

Abdul Rahman, S. & Waddell, A.H. (1980) Comparative study of the ultrastructure of the intestine of species of the genus *Strongylus* and species in subfamily Cyathostominae from the horse. *Mysore Journal of Agricultural Sciences* **14**, 549–556.

Bird, A.F. & Bird, J. (1991) *The structure of nematodes*. 2nd edn. London, Academic Press.

Caffrey, C. & Ryan, M.F. (1994) Characterisation of proteolytic activity of excretory–secretory products from adult *Strongylus vulgaris*. *Veterinary Parasitology* **52**, 285–296.

Chitwood, B.G. & Chitwood, M.B. (Eds) (1974) *An introduction to nematology*. 213 pp. Baltimore, Maryland, Monumental Printing Co.

Enigk, K. & Grittner, I. (1952) Zur Morphologie von *Strongylus vulgaris* (Nematoda). *Zeitschrift für Parasitenkunde* **15**, 267–282.

Lee, D.L. (1962) The distribution of esterase enzymes in *Ascaris lumbricoides*. *Parasitology* **52**, 241–260.

Lee, D.L. (1965) The cuticle of adult *Nippostrongylus brasiliensis*. *Parasitology* **55**, 173–181.

Lee, D.L. (1970) The fine structure of the S–E system in adult *Nippostrongylus brasiliensis* (Nematoda) and a suggested function for the S–E glands. *Tissue and Cell* **2**, 225–231.

Maizels, R.M. & Page, A.P. (1990) Surface associated glycoproteins from *Toxocara canis* larval parasites. *Acta Tropica* **47**, 355–364.

Maizels, R.M., Meghi, M. & Ogilvie, B.M. (1983) Restricted sets of parasite antigens from the surface of different stages and sexes of the nematode *Nippostrongylus brasiliensis*. *Immunology* **48**, 107.

McLaren, D.J. (1974) The anterior glands of adult *Necator americanus*. 1. Ultrastructural studies. *International Journal for Parasitology* **4**, 25–37.

McLaren, D.J., Burt, J. & Ogilvie, B.M. (1974) The anterior glands of adult *Necator americanus* (Nematoda: Strongyloidea). II. Cytochemical and functional studies. *International Journal for Parasitology* **4**, 39–46.

Mehlhorn, H. (1988) *Parasitology in focus. Facts and trends*. Berlin, Springer-Verlag.

Mobarak, M.S. (1995) Ultrastructural and immunological studies of the equine nematode *Strongylus vulgaris* (Looss 1900). PhD thesis, University College Dublin.

Mobarak, M. & Ryan, M.F. (1998a) An immunohistochemical investigation of the adult stage of the equine parasite *Strongylus vulgaris*. *Journal of Helminthology* **72**, 159–166.

Mobarak, M. & Ryan, M.F. (1998b) Ultrastructure of the buccal capsule of the equine nematode *Strongylus vulgaris* with special reference to the dorsal gutter. *Journal of Helminthology* **72**, 167–177.

Oglivie, B.M., McLaren, D.J. & Worms, R.J. (1975) Studies with *Necator americanus* maintained in hamsters. pp. 55–60 in *Nuclear techniques in helminthology research*. International Atomic Energy Agency, Vienna.

Ogilvie, B.M., Rothwell, T.L.W., Bremner, K.C., Schnitzerling, H.J., Nolan, J. & Keith, R.K. (1973) Acetylcholinesterase secretion by parasitic nematodes. I. Evidence for secretion of the enzyme by a number of nematode species. *International Journal for Parasitology* **3**, 589–598.

Peregrine, P. (1973) Observations on arcade cells and pseudocoelomocytes in some oxyuroid nematodes parasitic in cockroaches. *Parasitology* **66**, 165–180.

Pritchard, D.I. (1986) Antigens of gastrointestinal nematodes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 728–734.

Rogers, W.P. & Sommerville, R.I. (1963) *Advances in Parasitology* **1**, 109–177.

Romanowski, R.D., Thompson, D.E. & Madden, P.A. (1971) The secretory nature of the S–E gland cells of *Stephanurus dentatus*. 1. Morphology and histochemistry. *Proceedings of the Helminthological Society of Washington* **38**, 143–149.

Silbertstein, D.S. & Despommier, D.D. (1984) Antigens from *Trichinella spiralis* that induce a protective response in the mouse. *Journal of Immunology* **132**, 898–904.

Waddell, A.H. (1968) The S–E system of the kidney worm *Stephanurus dentatus* (Nematoda). *Parasitology* **58**, 907–919.

Wright, D.J. & Newall, D.R. (1976) Nitrogen excretion, osmotic and ionic regulation in nematodes. pp. 163–201 *in* Croll, N.A. (*Ed*.) *The organisation of nematodes*. London, Academic Press.

Wright, K.A., Dick, T.A. & Hamada, G.S. (1972) The identity of pseudocoelomic membranes and connective tissue in some nematodes. *Zeitschrift für Parasitenkunde* **39**, 1–16.

(Accepted 12 November 1998)