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Fine structure of egg-forming complex ducts, eggshell formation and supporting neuronal plexus in progenetic *Diplocotyle olrikii* (Cestoda, Spathebothriidea)

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Abstract

Ultrastructural descriptions of the oviduct, fertilization canal, seminal receptacle, ovovitelline duct, vitelline reservoir, ootype, Mehlis' gland, proximal uterus, and neurosecretory elements associated with egg-forming ducts are given for the progenetic spathebothriidean tapeworm, *Diplocotyle olrikii* from the body cavity of *Gammarus oceanicus*. The functional significance of cortical granules of the oocyte, as necessary elements for joining vitelline material to an oocyte in the ovovitelline duct, is established. The proximal ootype has a vesicular epithelium and is the site of initial, nascent eggshell formation. Precursors of nascent eggshell are vesicles, synthesized in both the proximal ootype wall and vitelline cytoplasm that become associated with the newly formed shell. Major shell structure comes from subsequent deposition of shell globules from a disintegration of vitelline clusters. Mehlis' gland has a single secretory cell type. Secretory granules from Mehlis' gland become associated with the developing egg that passes through to the distal ootype and proximal uterus where egg-formation is completed. It is not known, however, whether Mehlis' gland secretion promotes breakdown of free vitelline cells, liberation of shell globules, confluence of shell globules on the developing eggshell or provides further structural components for the shell. Despite some differences in ootype morphology, the basic process of eggshell formation in *D. olrikii* may share much in common with the Pseudophyllidea and Caryophyllidea. Small vesicles and dense-core vesicles are in nerve terminals near duct musculature. Nerve terminals with large dense vesicles are described near, in, and within the seminal receptacle, fertilization canal and distal ootype. The possible physiological effects of exocrine neurosecretions are discussed.

Key words

Cestoda, *Diplocotyle olrikii*, egg-forming complex ducts, eggshell formation, neuronal plexus, cortical granules, Mehlis' gland

Introduction

The order Spathebothriidea represents a basal group of tapeworms closely related to both the monozoic Caryophyllidea and polyzoic Pseudophyllidea. Unlike other polyzoic eucestodes that have a strobila body type with proglottisation and distinct segmentation, spathebothridians have a polypleuroid body, i.e. with proglottisation but no external segmentation. At the life cycle level, progenesis, rare among other polyzoic cestodes, is common in two genera, *Cyathocephalus* Kessler, 1868 and *Diplocotyle* Krabbe, 1874, with species often producing eggs within the body cavity of their amphipod intermediate host (Sandeman and Burt 1972, Leontovich and Valovaya 1989, Protasova and Roytman 1995, Okaka 2000). It is not known, however, if other conspicuous differences, as evident above in the gross morphology and life cycles, are also reflected at the ultrastructural level of the reproductive systems of spathebothriids. Such new information may provide additional characters at the ultrastructural level for better assessing the phyletic relationships of the Spathebothriidea within the Eucestoda. Our previous ultrastructural studies have been on the male and female reproductive organs and

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ducts of dixenous *Cyathocephalus truncatus* (Poddubnaya *et al*. 2005a, b); ovarian tissue of monoxenous (progenetic) *Diplocotyle olrikii*, (Poddubnaya *et al*. 2005c) and vitellogenesis in both species (Bruňanská *et al*. 2005). The present ultrastructural study on progenetic *D. olrikii* also focuses on the female system and includes the complex of the female gonoducts with Mehlis' gland, the assembly and production of eggs, and the possible regulation of egg formation by neurosecretory elements.

Materials and methods

Large progenetic procercoids of *Diplocotyle olrikii* (Krabbe, 1874) were obtained from the body cavity of amphipods, *Gammarus oceanicus* from the White Sea. The worms were cut into small pieces, fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 6 h at 4°C, and postfixed in 1% OsO_4 in 0.1 M cacodylate buffer for 1 h at 4°C. The material was dehydrated in a graded series of acetone, and embedded in Araldite and Epon. Semithin sections were cut on a Reichert ultramicrotome, stained with methylene blue and examined by light microscopy for identification of reproductive organs. Ultrathin sections were stained with uranyl acetate and lead citrate. They were examined in a JEOL-100 C transmission electron microscope (TEM) operated at accelerating voltage of 80 kV.

Results

After mature oocytes leave the ovary, structures involved in egg formation in *Diplocotyle olrikii* are ducts that include: oviduct, fertilization canal, seminal receptacle, ovovitelline duct with vitelline reservoir, ootype with Mehlis' gland, and proximal uterus. Common to all of these ducts is a much invaginated, nucleated syncytial epithelium that rests on a thin layer of fibrous basement material on a basal plasma membrane (see Conn 1993a for review of basal matrices). Circular and longitudinal muscles attach to the fibrous layer though their number may vary among the ducts. Nevertheless, in spite of some basic similarities, each duct has its own distinctive architecture.

Oviduct

The oviduct varies gradually along its length. A short distance from the ovary, the epithelial lining is thin and has short, blunt apical folds and is enclosed by a single layer of muscles (Fig. 1A). Further on, the epithelium becomes thicker and the surface has prominent lamellae (Fig. 1B). The epithelial cytoplasm has numerous mitochondria, free ribosomes, sparse rough endoplasmic reticulum, and Golgi elements (Fig. 1B, C). Numerous vesicles in close association with Golgi stacks are throughout the epithelium (Fig. 1B, C). Nuclei are evident (Fig. 1B, D) and the duct lumen is filled with vesicles (Fig. 1B). Up to five layers of muscles are present outside of the basal matrix (Fig. 1D). At the distal end of the oviduct, the lamellae are long, branched and form a dense, sieve-like mesh (Fig. 1E) that frequently contains spermatozoa. There are one or two layers of muscles. Nerve cells are most evident in the distal and adjacent proximal section of the oviduct (Fig. 1F). The neuronal cell bodies have large euchromatic nuclei; their lucent cytoplasm contains mitochondria, filamentous structures, neurotubules, clusters of ribosomes, and an occasional profile of endoplasmic reticulum (ER) (Fig. 1F). Neuropile with nerve terminals filled with small clear vesicles or a few neurotubules and filamentous structures, are in close anatomical relationship to the muscles (Fig. 1F). At the end of this section of the oviduct is the opening from the seminal receptacle.

Seminal receptacle

Short apical lamellae and large basal nuclei are characteristic features of the epithelial cytoplasm of the enlarged terminal part of the vagina tube (Fig. 2A). A single layer of muscles is arranged beneath the epithelium. Nerve terminals, with large, dense vesicles (105–310 nm in diameter) are evident between spermatozoa in the duct lumen (Fig. 2B). The seminal receptacle joins the oviduct that now becomes the fertilization canal.

Fertilization canal

The epithelial syncytial cytoplasm of the fertilization canal bears numerous lamellae, most of which bend to its luminal surface (Fig. 2E-G). The cytoplasm contains rare large nuclei, numerous mitochondria, and free ribosomes (Fig. 2C). Sparse fibres of circular muscles are associated with the canal (Fig. 2C). Proximal to the epithelium are found nerve cell processes filled with large dense vesicles, well developed granular endoplasmic reticulum, mitochondria, neurotubules, filamentous structures, and free ribosomes (Fig. 2C). The terminal parts of nerve processes, containing large dense vesicles, are found in the canal epithelium where they taper to form duct-like structures with longitudinally orientated microtubules (Fig. 2D, F). Within the canal lumen may be seen a membrane-bound accumulation of these large dense vesicles (Fig. 2F, G), nerve processes with large lucent vesicles, measuring approximately 75 nm (Fig. 2E), and with small vesicles (Fig. 2G) as well as the mature oocyte and spermatozoa (Fig. 2C, G).

Vitelline ducts and vitelline reservoir

The vitelloduct epithelium has lamellae and cilia (Fig. 3B). Nuclei are within the epithelium. The cytoplasm contains free ribosomes, mitochondria, sparse ER cisternae, Golgi dictyosomes and vesicles (Fig. 3B, C). The outer plasma membrane of the vitelline reservoir also bears lamellae; however, no cilia were observed. A dense network of nerve cell processes innervates the surrounding layer of circular muscles of the vitelline ducts and vitelline reservoir (Fig. 3A, C). Nerve terminals, composed of a neuropile, are in close anatomical relationship between nerve and muscle (Fig. 3C). Small vesicles

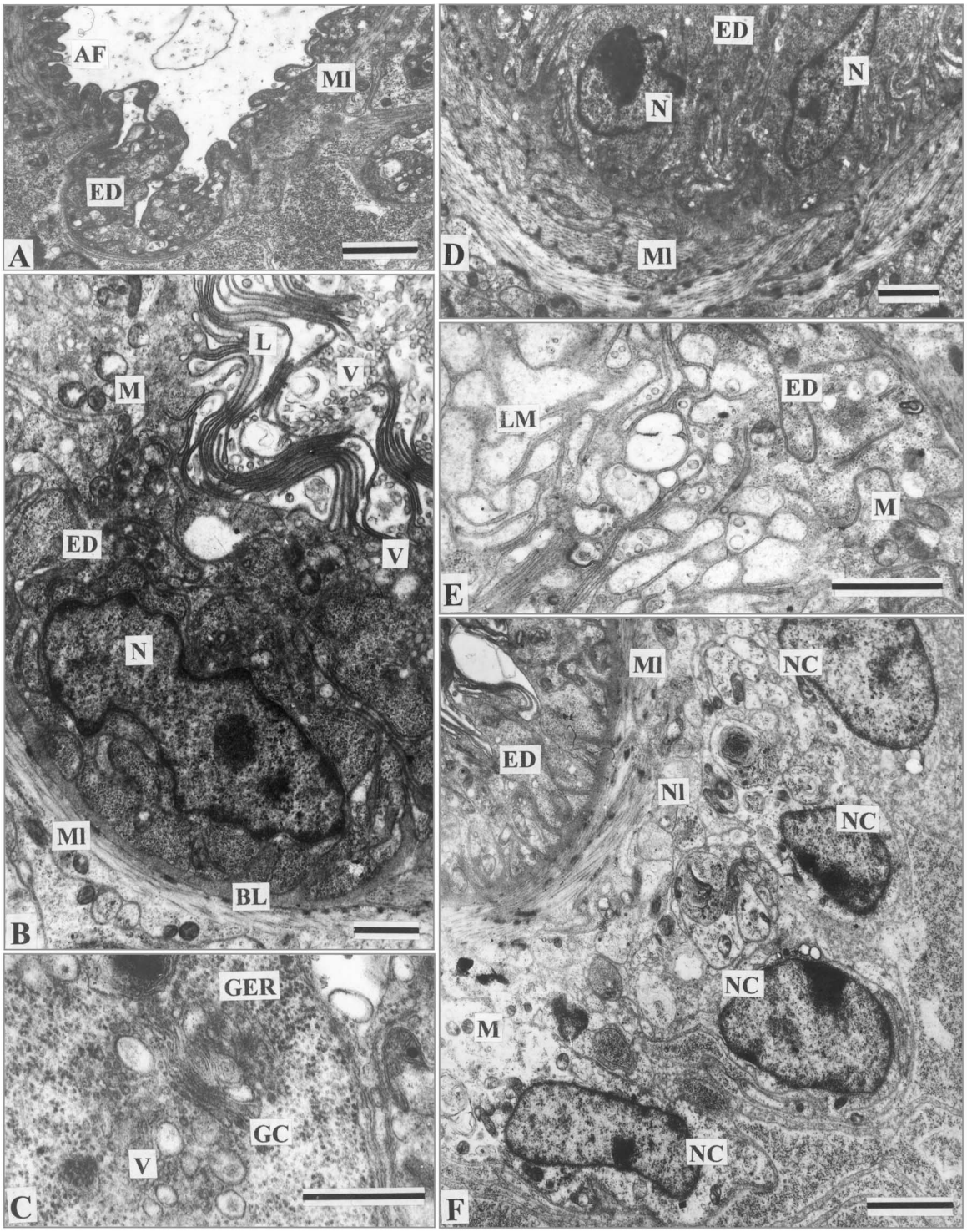

Fig. 1A-F. The morphology of the oviduct epithelial lining in *Diplocotyle olrikii*: **A.** Oviduct wall near ovary. **B.** Middle portion of the oviduct epithelium. **C.** Vesicular material from Golgi complex within epithelial wall. **D.** Oviduct wall, surrounding by five layers of muscles. **E.** Lamellar mesh within distal luminal portion. **F.** Nerve cells around oviduct wall. Scale bars = 2 μm (A, D, F), 1 μm (B, E), 0.5 μm (C). **Abbreviations to all figures:** AF – apical fold, BL – basal matrix, C – cilia, CD – desmosome, CG – cortical granules, Cl – cluster of vitelline globules, D – duct of Mehlis' gland cells, DC – disintegrated vitelline globules, DO – distal ootype, ED – syncytial epithelium, ES – eggshell, GC – Golgi complex, GER – granular endoplasmic reticulum, HS – homogeneous fibrous substance, L – lamellae, Lc – lacuna, LDV – large dense vesicles, LLV – large lucent vesicles, LM – lamellar mesh, M – mitochondria, Mc – microtubules, ME – Mehlis' gland extension, MG – Mehlis' gland cell, Ml – muscles, MO – mature oocyte, N – nucleus, NC – nerve cell, NE – nascent eggshell, Nl – neuropile, NP – nerve process, NT – nerve terminal, PO – proximal ootype, PS – plate-like structures, PU – proximal uterus, SG – secretory granules, Sp – spermatozoa, V – vesicles, Vc – vacuole, VD – vitelline duct, VG – vitelline globule, VM – vitelline material

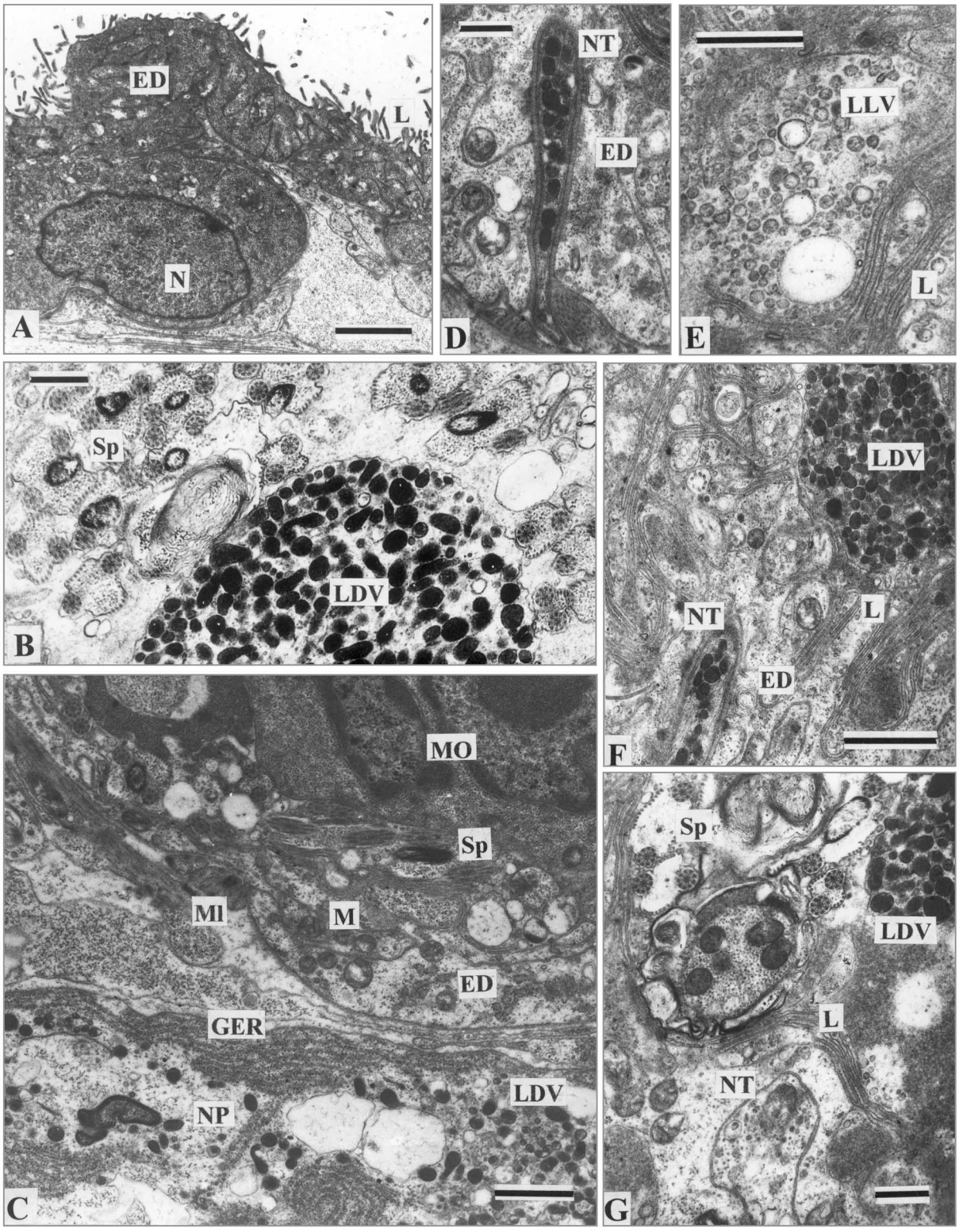

Fig. 2A-G. Fine structure of seminal receptacle and fertilization canal of *Diplocotyle olrikii*: **A.** Seminal receptacle epithelium with large basal nuclei. **B.** Seminal receptacle lumen with spermatozoa and nerve terminal filled with large dense vesicles. **C.** Fertilization canal with mature oocyte, spermatozoa within lumen and nerve process with large dense vesicles exterior to the epithelium. **D.** Fertilization canal wall with terminal part of nerve process filled with dense large vesicles. **E.** Fertilization canal lumen and nerve terminal filled with large lucent vesicles. **F.** Nerve terminals in the epithelial canal cytoplasm and within duct lumen. **G.** Fertilization canal lumen, containing nerve terminals filled with small vesicles, dense large vesicles and spermatozoa. Scale bars = 2 μm (A), 0.5 μm (B, E, G), 1 μm (C, D, F)

can be recognized in these nerve terminals. The vitelline reservoir connects to the distal end of the fertilization canal, forming the short, straight ovovitelline duct.

Ovovitelline duct

The nucleated syncytial epithelium of the ovovitelline duct has prominent lamellae and numerous vesicles that empty into

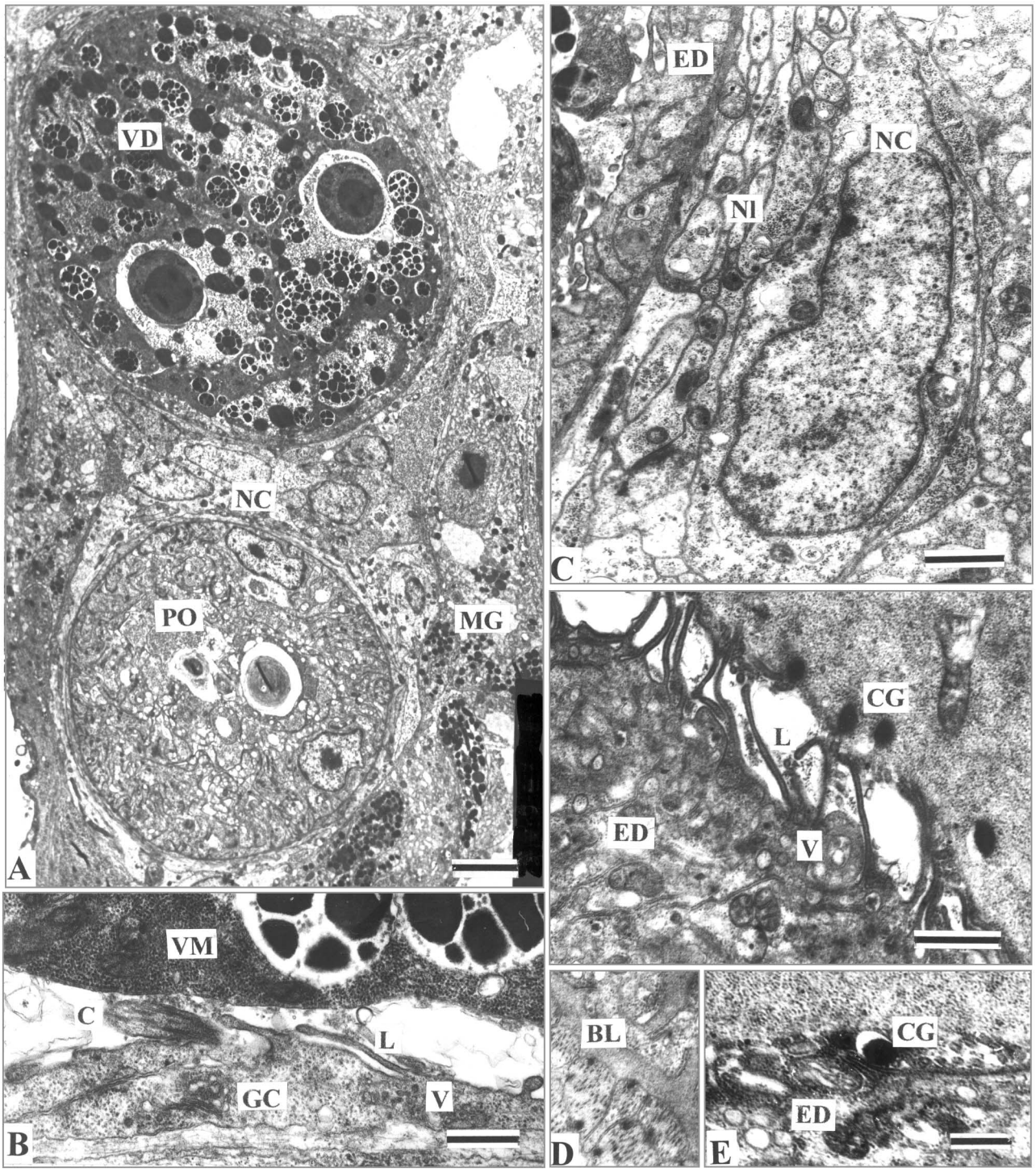

Fig. 3A-E. Ultrastructure of the vitelline ducts and ovovitelline duct of *Diplocotyle olrikii*: **A.** Overview of part of the egg-forming apparatus, showing portions of vitelline duct and proximal ootype. **B.** Epithelium of the vitelline duct bearing lamellae and cilia. **C.** Nerve cell and neuropile around epithelial wall of the vitelline duct. **D.** Ovovitelline duct with fertilized oocyte containing cortical granules. **E.** Fragment of ovovitelline duct lumen with free cortical granule outside of fertilized oocyte. Scale bars = 0.5 μm (A, B, D, E), 1 μm (C)

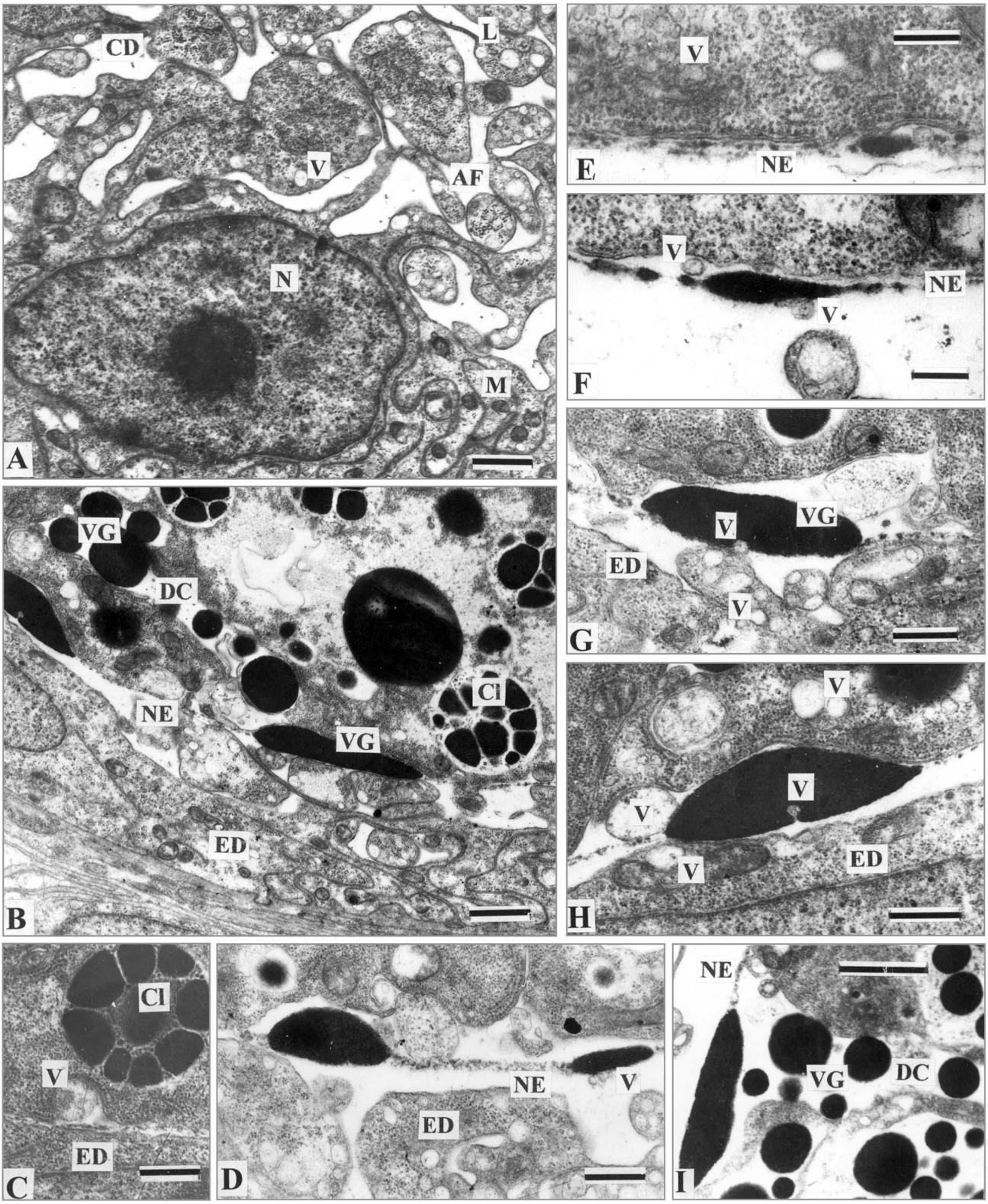

Fig. 4A-I. Proximal ootype wall and newly formed eggshell in *Diplocotyle olrikii*: **A.** Proximal ootype wall with nucleus and long apical folds. **B.** Portion of proximal ootype with transparent membrane around oocyte-vitelline cells complex in lumen. **C.** Vesicle inside of egg near newly formed shell. **D.** Rough egg contour with separate deposition of shell globules. **E.** The nascent eggshell and the presence of vesicles in vitelline cytoplasm. **F.** Liberated vesicles outside and inside of the nascent eggshell with the first deposition of vitelline globules. **G.** Vesicles in proximal ootype epithelial fold, and separate vesicle within formed eggshell. **H.** Vesicles inside egg and in shell globule material. **I.** The release of the vitelline globules by disintegration of the cluster. Scale bars = 1 μm (A-C, I), 0.5 μm (D, G, H), 0.2 μm (E, F)

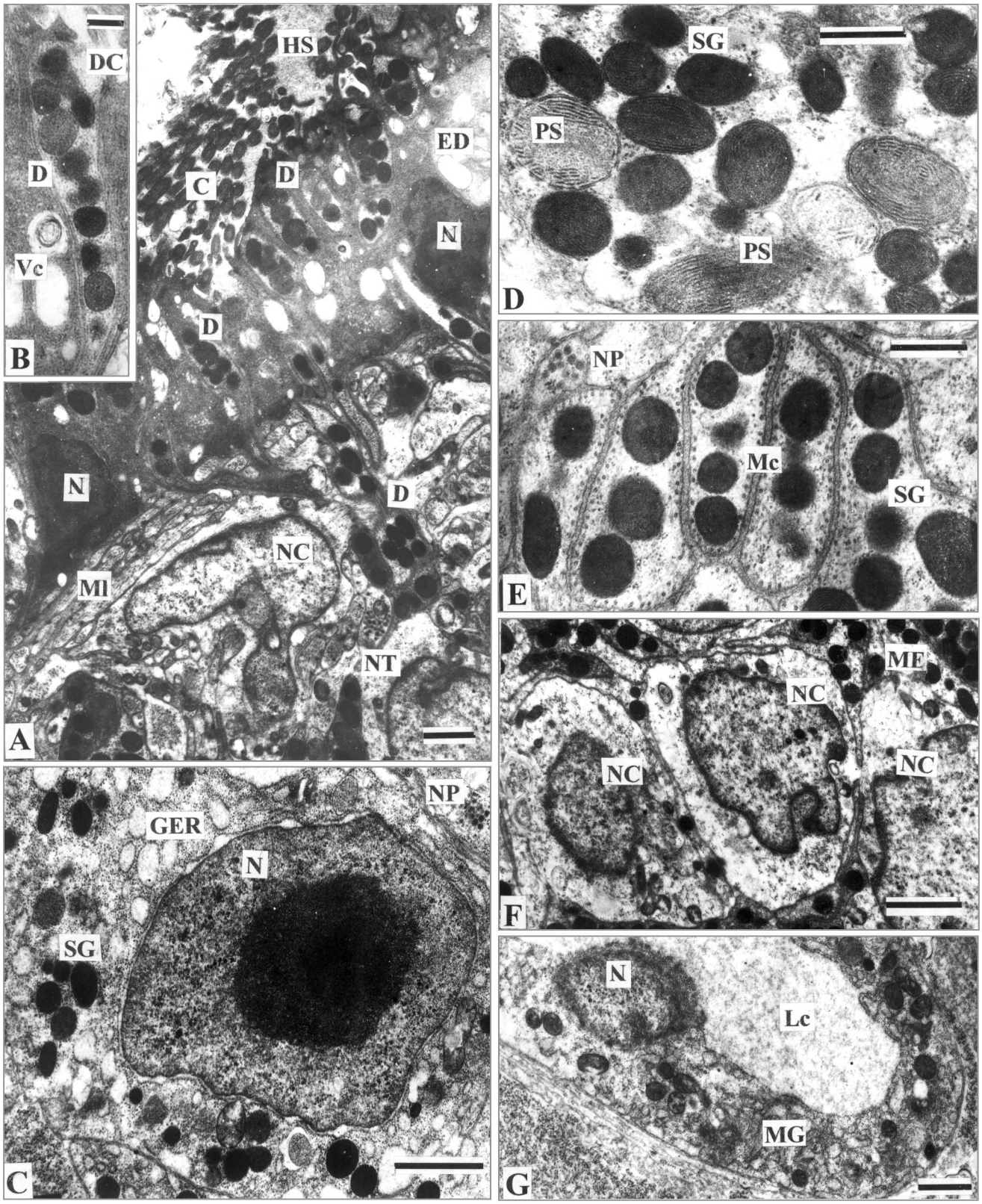

Fig. 5A-G. Middle ootype portion and Mehlis' gland cells in *Diplocotyle olrikii*: **A.** Penetration of epithelial lining of the middle ootype by Mehlis' gland ducts. **B.** Mehlis' gland duct filling with secretory granules. **C.** Perinuclear cytoplasm of the Mehlis' gland cell. **D.** Various states of condensation of plate-like structures into Mehlis' gland granules. **E.** Terminal parts of Mehlis' gland extensions with peripheral ring of microtubules. **F.** Nerve cell bodies between Mehlis' gland extensions. **G.** Late stage of Mehlis' gland secretory activity with expanding lacunary space. Scale bars = 1 μm (A-C, F, G), 0.5 μm (D, E)

the duct lumen (Fig. 3D) that contains vitelline cells and fertilized oocytes. Cortical granules are visible in the lumen and within an oocyte (Fig. 3D, E). Two layers of muscle fibres are present beneath the basal matrix. There is a close relationship

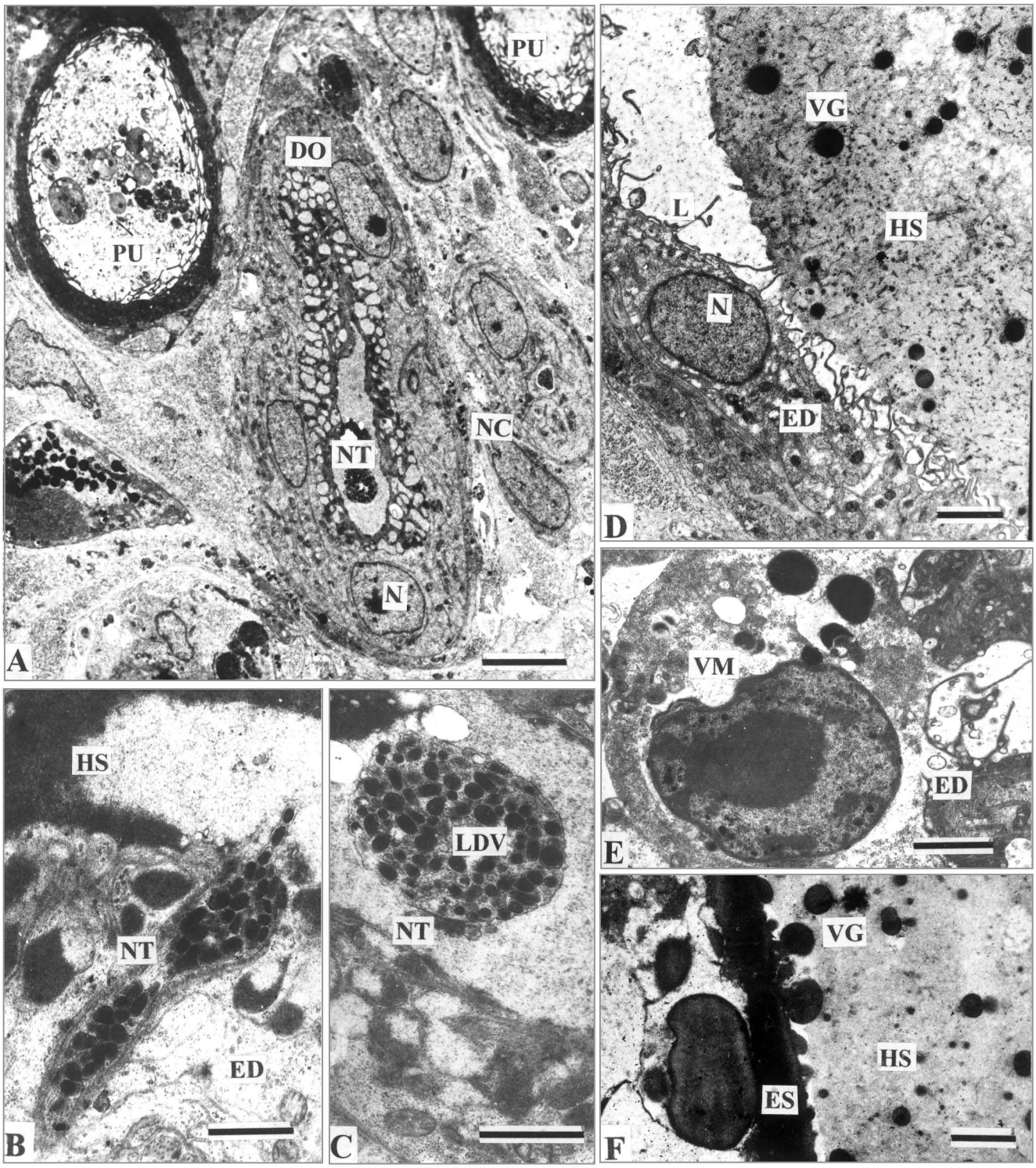

Fig. 6A-F. Fine structure of the distal ootype portion and proximal uterus of *Diplocotyle olrikii*: **A.** Overview of the distal ootype and portion of the proximal uterus. **B.** Distal ootype epithelium and nerve terminal with large dense vesicles and lumen with different consistencies of homogeneous material. **C.** Distal ootype lumen with nerve terminal filled with large dense vesicles. **D.** Lumen of proximal uterus with free shell globules imbedded in homogeneous fibrous matrix. **E.** Fragment of the vitelline cytoplasm in proximal uterine lumen. **F.** Deposition of vitelline globules inside and outside of the eggshell within lumen of proximal uterus. Scale bars = 5 μm (A), 1 μm (B, C, F), 2 μm (D, E)

between neuropile and the musculature of the ovovitelline duct wall. The distal end of the ovovitelline duct becomes modified to form the ootype.

Ootype: *Proximal part*

The luminal plasma membrane of the anterior part of the ootype is elevated to form a network of long apical folds having few lamellae (Fig. 4A). Where two folds come into contact with each other, the adjacent plasma membranes are conjoined by a desmosome (Fig. 4A). Large nuclei are often seen in the ootype epithelium (Fig. 4A). The cytoplasm contains numerous electron-lucent vesicles that are in close association with the luminal plasma membrane (Fig. 4A, G, H). The ootype epithelium contains mitochondria, free ribosomes, granular endoplasmic reticulum and Golgi complexes that are involved in secretion. In the lumen of the proximal part of the ootype there are free vesicles, as well as vesicles within small fragments of constricted cytoplasm (Fig. 4A, B, D, F, G). Associated with the epithelium is a layer of muscles and a net of terminal processes of nerve cells.

Newly formed eggshell

Further along the proximal ootype portion, a transparent membrane, the nascent eggshell, first becomes apparent around the oocyte – vitelline cell complex (Fig. 4B). A variably thin layer begins to form the contour of an egg (Fig. 4D, I). Outside of the nascent eggshell are vesicles liberated from the ootype wall (Fig. 4B, D, G); vesicular material is also on the inside of the newly forming shell (Fig. 4C, F, H). Inside the egg, the membrane of vitelline clusters disintegrates, releasing the vitelline globules (Fig. 4B, I) that subsequently migrate and deposit on the developing eggshell (Fig. 4B, D-I).

Ootype: *Middle part and Mehlis' gland*

The presence of numerous cilia and Mehlis' gland are distinctive features of this portion of the ootype (Fig. 5A, B). The syncytial cytoplasm has numerous nuclei, free ribosomes, and vacuoles filled with membranous structures (Fig. 5A, B). Beneath the basal matrix are two layers of muscle fibers, nerve cells and a supporting nerve plexus (Fig. 5A).

Mehlis' gland in *D. olrikii* has only one type of secretory cells and it lies at different distances and depth from the ootype wall. Its cytoplasm contains abundant granular endoplasmic reticulum with moderately dilated cisternae, numerous mitochondria, Golgi bodies, and a large number of free ribosomes (Fig. 5C). Extensive, isolated lacunae may occur between cisternae of endoplasmic reticulum. Within a cell, a large lacuna may crowd a degenerate nucleus and cytoplasmic organelles to the cell periphery (Fig. 5G). Ovoid or spherical secretory granules, $0.46 \pm 0.03 \times 0.4 \pm 0.02$ μm in diameter are distributed throughout the perinuclear cytoplasm and cytoplasmic extensions (Fig. 5A-E). Some of these granules contain plate-like structures embedded in an amorphous matrix, whereas others have dense, tightly packed, plate-like material (Fig. 5D). Such variation may reflect various stages of their development. These granules of different condensation are conveyed to the middle ootype lumen through long ducts or cytoplasmic extensions (Fig. 5A) whose plasma membrane is lined with prominent microtubules (Fig. 5E) in the terminal part that penetrates the ootype epithelium. Septate junctions connect the ducts to the adjacent ootype epithelium near the point of discharge into the ootype lumen (Fig. 5B). Before emptying into the lumen, the granules lose their integrity and become dissociated to a finely homogeneous fibrous substance (Fig. 5A). Numerous nerve cell bodies (Fig. 5F) and their cell processes are distributed throughout the Mehlis' gland elements. Nerve terminals, containing dense-core vesicles, are found among the extensions of secretory cells and throughout the musculature of the ootype wall (Fig. A, C, E).

Ootype: *Distal part*

The distal part of the ootype is characterized by thin lamellae on the apical surface (Fig. 6B, C), a homogeneous fibrous material of differing consistencies filling the lumen (Fig. 6A-C), sparse fibres of circular muscles, and nerve processes filled with large dense vesicles under the epithelium. Nerve terminal processes with neurosecretions penetrate the ootype epithelium (Fig. 6B). Within the ootype lumen may be seen a fragment of neuron cytoplasm containing neurosecretions (Fig. 6C).

Proximal uterus

The thin uterine epithelium contains few nuclei, has a lamellar apical surface and is surrounded by a circular layer of muscles (Fig. 6D). In the lumen there is a high concentration of free shell globules embedded in a homogeneous, fibrous matrix (Fig. 6D), fragments of vitelline cytoplasm with nuclei, free ribosomes, endoplasmic reticulum, and shell globules (Fig. 6E). Deposition of material from vitelline globules continues here as shown by globules being added to both the inside and outside of the shell (Fig. 6F).

Discussion

The process of producing a complete egg in *D. olrikii* involves adaptations and modifications of the oviduct to proximal uterine wall with regulation by a complex of nerve cells and neurosecretions that bring together the egg components. Our study helps to better understand how that process is accomplished.

Movement of reproductive material and duct neuromusculature

Movement of mature oocytes from the *D. olrikii* ovary is effected by contraction of the oviduct musculature that is well supplied by muscle fibres and nerve terminals. Our research shows that the oviduct is surrounded by numerous nerve cells,

forming an extensive neuronal plexus of cell bodies along the duct wall. Undoubtedly, this rich oviduct innervation in *D. olrikii* has an important role in regulating contraction of muscles active in the handling of mature oocytes to the fertilization canal. According to Halton *et al*. (1998), egg formation in monogeneans involves a highly ordered series of rhythmic contractions of the muscles in the duct walls. Nerve terminals in the neuropile release sites at the muscles of the oviduct, vitelline ducts, vitelline reservoir, ovovitelline duct, proximal and middle portions of the ootype, and the proximal uterus in *D. olrikii*, contain neurosecretory small vesicles and dense-core vesicles as dominating types. Close association of nerve and muscle cells and the presence of similar types of vesicles in the nerve terminals along the egg-formation tube are found in parasitic flatworms (Halton *et al*. 1991, 1997, 1998; Gustafsson 1992; Specian *et al*. 1979; Reuter and Halton 2001; Gustafsson *et al*. 2002; Halton 2004). According to Halton *et al*. (1997), for trematodes, there are both aminergic (dense-core vesicles) and peptidergic (large dense vesicles) immunoreactivities in the nerve plexuses that innervate the various ducts, including oviduct, vitelline duct, ootype and uterus of the fluke's female system; there are relatively few accounts of cholinergic nerves associated with the reproductive system of trematodes.

Fertilization and neural regulation

Fertilization in *D. olrikii* also occurs in the fertilization canal, as recently described for *Cyathocephalus truncatus* by Poddubnaya *et al*. (2005b). It cannot occur sooner because oviductal lamellae serve as a barrier to spermatozoa moving up into the oviduct towards the ovary. The presence of a dense, sieve-like mesh of branching lamellae in the oviduct nearest the seminal receptacle opening would tend to support this conclusion. The presence of mature oocytes and spermatozoa in the fertilization canal in *D. olrikii*, as well as finding sperm axonemes within an oocyte in *C. truncatus* (Poddubnaya *et al*. 2005b), indicate that fertilization in the Spathebothriidea occurs in the fertilization canal.

Neurosecretions along the epithelium of the ducts with interrupted muscle layers may have major physiological effects different from modulating muscle activity. Some of these effects may occur during the fertilization process in *D. olrikii*. Our observations indicate that the nerve terminals on the epithelium of the fertilization canal have three types of vesicles: large dense, large lucent and small clear vesicles. Nerve terminals filled with large dense vesicles release their exocrine neurosecretions into the seminal receptacle and fertilization canal where they may serve as neuroregulators, possibly to control the amount of spermatozoa released into the fertilization canal. Halton *et al*. (1997) have identified peptidergic neurons with large dense vesicles in the nerve plexuses that innervate gonoducts in trematodes. To what extent the other large lucent and small clear neurosecretory vesicles of *D. olrikii* may be involved in the fertilization process is unknown. It would appear, however, that control of fertilization in *D. olrikii* may be a complex process.

Combination of fertilized ovum with vitellocytes, functional significance of cortical granules

Fertilized ova pass on to the ovovitelline duct. Vitellocytes from the vitelline reservoir enter the ovovitelline duct, regulated by the contraction of neuromusculature similar to that of the oviduct wall. The combination of a fertilized ovum with numerous vitelline cells takes place in the ovovitelline duct, with vitelline material from the vitelline reservoir. In the ovovitelline duct vitellocytes start to attach to a fertilized ovum by thin filiform extensions and prominent elongate lipid droplets of the external boundary of vitelline material, as has been demonstrated for *C. truncatus* (Poddubnaya *et al*. 2005b). An earlier study on *D. olrikii* (Poddubnaya *et al*. 2005c) postulated that this joining of vitelline material to the ovum was dependent on the discharge of cortical granules from the ovum. The discovery of free cortical granules within the ovovitelline duct lumen in the present study would tend to support this postulated role for cortical granules in *D. olrikii*. Whether it is the only role remains to be seen.

Shell formation and Mehlis' gland

The initiation of shell formation around the fertilized ovum and vitellocytes first becomes apparent in the proximal part of the ootype, before contact with Mehlis' gland secretions. More recent investigations on the mechanism of new eggshell formation in the trematodes, *Schistosoma mansoni* (Wells and Cordingley 1991) and *Fasciola hepatica* (Threadgold 1982, Colhoun *et al*. 1998) and the cestode *Hymenolepis microstoma* (Schmidt 1996) have suggested that the precursor of the first thin eggshell is a complex of enzyme phenol oxidase with protein. The egg capsule of trematodes and at least some cestode orders consists of sclerotin, the synthetic pathway of which includes proteins and phenolic compounds (Smyth and Clegg 1959, Burton 1963). Eggshell precursor enzymes, synthesized by the vitelline cells and stored in the vesicles as well as in the membrane-bound shell globule clusters (Wells and Cordingley 1991), are also present in the space around newly formed shell (Schmidt 1996). According to Wells and Cordingley (1991) the vesicles also contain a proteolytic enzyme that activates the prophenol oxidase that triggers the tanning process. Our study suggests that possibly similar precursors or enzymes, active in the beginning of eggshell formation, may be synthesized within the ootype epithelium and stored in the vesicles of the proximal ootype. In this way, these ootype vesicles are active at the initial stages of eggshell formation by providing the enzymes required for deposition and subsequent coalescence of the shell globules. Indeed, our data for *D. olrikii* indicate that vesicles of the proximal ootype epithelium and vitelline cell cytoplasm are both associated with the newly formed, nascent shell. It has also been recently established by histochemical investigations of the Mehlis' gland secretion in three schistosome species that a main component of secretion is membrane-bound enzymes, glycosyl transferases, which are initiated within Golgi cisternae of glandular cytoplasm and are observed within vesicles of the distal ootype wall (Moczoń *et al*. 1992; Moczoń and Świderski 2000, 2002).

The reason that eggshell formation starts in the proximal ootype, with its secretory epithelium, is related to the structure of Mehlis' gland. A secretory function for the ootype epithelium in trematodes and monogeneans has often been suggested where there is only one type of secretory cells in Mehlis' gland (Gönnert 1955, Ebrahimzadeh 1966, Rohde and Ebrahimzadeh 1969, Erasmus 1973, Awad and Probert 1990, Moczoń and Świderski 2002). One can hypothesize that the ootype epithelium takes over the function of the absent second cell type of Mehlis' gland. As the present study has revealed, there is only one type of secretory cells in the Mehlis' gland of *D. olrikii*, a condition shared by the closely related, pseudophyllidean species, *Triaenophorus nodulosus* (Korneva 2001, 2002), *Diphyllobothrium latum* (Poddubnaya 2002), and *Eubothrium rugosum* (Poddubnaya 2003a). Of these species, specific data on the morphology of the ootype tube are only available for *D. latum*, which, like *D. olrikii*, has a vesicular epithelium in the proximal ootype (Poddubnaya 2002). With regard to the closely related Caryophyllidea, two types of Mehlis' gland have been reported for the monoxenous *Archigetes sieboldi* by Poddubnaya (2003b). In a number of other Digenea and Monogenea that have this more common, two-secretory cell Mehlis' gland, one type of cell produces membrane-bound vesicles and the other a secretion such as mucus (Burton 1967, Bogitsh 1970, Threadgold and Irwin 1970, Stranock and Halton 1975, Holy and Wittrock 1986, El-Naggar *et al*. 1990). Early research on the caryophyllideans *Caryophyllaeus laticeps* and *Caryophyllaeides fennica*, utilizing histochemical tests for proteins, phenols and polyphenol oxidase, has shown that eggshell formation is similar to that of *D. latum*, *Ligula* and *Schistocephalus* (Mackiewicz 1968). It would thus appear that despite some differences in ootype morphology, the basic process of egg formation in *D. olrikii* may share much in common with the Pseudophyllidea and the Caryophyllidea.

Our data do not support the hypothesis that Mehlis' gland is responsible for secreting a substance that forms a basic shell membrane in *D. olrikii*, since eggshell formation is in progress before reaching Mehlis' gland. Nor does it clearly indicate what that function is in this spathebothriidean species. It is apparent that Mehlis' gland secretions, in the form of granules, appear to dissociate in the middle ootype lumen and pass through to the distal ootype that is filled with fibrous material of different electron density. This material, along with free shell globules, fragments of vitelline cytoplasm with nuclei, free ribosomes, endoplasmic reticulum, and shell globules embedded in a fibrous substance, is also in the proximal uterus. Whether the Mehlis' gland secretion promotes breakdown of free vitelline cells, the liberation of shell globules, confluence of the shell globules on the developing eggshell or provides further structural components for the eggshell is not known. It would appear, nevertheless, that in *D. olrikii*, the eggshell completes its formation in the proximal uterus. The uterine epithelium has been shown to contribute to the final eggshell in several cyclophyllidean species (Conn 1985a, b, 1987, 1993b; Conn and Forman 1993).

Regarding the roles of Mehlis' secretion in reproductive processes, it has been assumed that the secretion: provides a primary membrane which becomes reinforced on the inside by the coalescence of vitelline cell globules; is responsible for the release of the globules from the vitelline cells; causes liberation of the globules; serves to lubricate the capsule-filled uterus, and; hardens the shell as it passes through the uterus (Burton 1963, 1967; Irwin and Threadgold 1972; Eklu-Natey *et al*. 1982a, b; Swiderski *et al*. 1982, 2004; Smyth and Halton 1983). As we have seen, however, the role of Mehlis' gland may vary in different species, depending on whether or not one or two types of secretory cells are present. Furthermore, differences may be evident in the secretion itself, even when closely related spathebothriidean genera share the same type of Mehlis' gland. For example, at the TEM level, the secretory granules of *Cyathocephalus* are electron-dense and round (Poddubnaya *et al*. 2005b), whereas in *Diplocotyle* they are plate-like structures, similar to those of a monogenean, *Diplozoon paradoxum* (Stranock and Halton 1975).

As egg production progresses, there are changes in the structure of Mehlis' gland. One of these in *D. olrikii* is the gradual appearance of conspicuous lacunae, the result of the autolytic process characteristic of holocrine secretion. The holocrine nature of Mehlis' gland cells of *S. mansoni* has been acknowledged by Erasmus (1973). As proposed by Thorsell and Björkman (1965) for *F. hepatica*, the length of the secretory process and life of Mehlis' gland cells is limited and accompanied by autolysis of cell cytoplasm that eventually leads to an accumulation of secretion.

The presence of numerous somata and nerve terminals around the Mehlis' gland cells and near the secretory ducts suggests that Mehlis' gland secretion is highly nerve regulated in *D. olrikii*. In the monogenean, *Diclidophora merlangi* and the digenean *Fasciola hepatica*, pancreatic polypeptide, polypeptide YY, and FMREamide have been reported to be present along ootype/Mehlis' gland and uterus (Magee *et al*. 1989, Maule *et al*. 1990).

The nerve processes of the distal ootype wall are similar to those of the seminal receptacle and fertilization canal. Their exocrine secretion is characterized by large dense vesicles (105–310 nm in diameter) visible in the duct lumen. These neurosecretions may have major regulatory effects, not modulating muscle activity. The secretion of the large dense vesicles may serve as a neuroregulator to control the passage of eggs from the distal ootype into the proximal uterus where the eggshell is completed.

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