DISTRIBUTION AND ULTRASTRUCTURE OF TWO TYPES OF SCOLEX GLAND CELLS IN ADULT *PROTEOCEPHALUS MACROCEPHALUS* (CESTODA, PROTEOCEPHALIDEA)

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Summary :

In the scolex-neck region of the adult *Proteocephalus macrocephalus* two types of eccrine gland cells are present. The first type of gland cells, localized in the frontal part of the scolex only, contains large, more or less round electron lucid granules. The second type of unicellular glands produces large electron dense granules. These electron dense granular gland cells are localized primarily in the neck region, only few are present in the scolex apex. The secretion of both types of gland cells is concentrated in the ducts opening to the exterior. The ducts are fixed to the plasmalemma of the tegument by septate junctions. The function of both types of gland cells is discussed.

KEY WORDS : *Proteocephalus macrocephalus*, scolex glands, ultrastructure.

Résumé : DISTRIBUTION ET ULTRASTRUCTURE DES DEUX TYPES DES CELLULES GLANDULAIRES DU SCOLEX CHEZ L'ADULTE DE *PROTEOCEPHALUS MACROCEPHALUS* (CESTODA, PROTEOCEPHALIDEA)

Dans la région du cou et du scolex de l'adulte de *Proteocephalus macrocephalus*, deux types de cellules glandulaires sont présentes. Le premier type de cellules glandulaires, localisées uniquement dans la partie frontale du scolex, contient de volumineux granules arrondis plus ou moins transparents aux électrons. Le deuxième type de glandes unicellulaires produit de volumineux granules denses aux électrons. Ces cellules glandulaires granuleuses denses aux électrons sont principalement localisées dans la région du cou, peu sont présentes dans l'apex du scolex. La sécrétion des deux types de cellules glandulaires est concentrée dans les canaux s'ouvrant sur l'extérieur. Les canaux sont raccordés au plasmalemma du tégument par des embranchements cloisonnés. La fonction des deux types de cellules glandulaires est discutée.

MOTS CLÉS : *Proteocephalus macrocephalus*, glandes du scolex, ultrastructure.

INTRODUCTION

The importance of the study of gland cells of proteocephalidean cestodes is done for the reason that the localization, quantity, and form of these unicellular glands is useful in their systematics. This paper is a continuation of the ultrastructural studies of the scolex glands of cestodes from eels in our country (Žd'árská & Nebesářová, 1995, 1997). The scolex glands of *P. macrocephalus* have not yet been studied in detail using transmission electron microscopy. Earlier have been detected only the intrategumental parts of two types of gland ducts by Žd'árská & Nebesářová (1995) in *P. macrocephalus* and by Stoitsova *et al.* (1995) in *P. osculatus*.

The proteocephalidean species under investigation possesses an apical sucker in addition to what two types of gland cells are present. These gland cells are not localized inside the apical sucker, but only surround it. In our opinion, when some glands are

concentrated around the apical sucker, they cannot be included in our understanding of the apical organ. In the systematics of Proteocephalidea it would be useful to distinguish the apical sucker, which is a muscular formation, from the apical organ, which is a glandular or glandulo-muscular formation, located in the centre of the scolex apex. Our TEM studies of *P. macrocephalus* have shown that the structure of the apical sucker is identical with the structure of the lateral suckers (details will be published in a separate paper). Four mechanisms of secretory material release have been described in tapeworms - eccrine, apocrine, microapocrine and holocrine. The secretory granules of both types of scolex gland cells of *P. macrocephalus* are released by an eccrine-like mechanism, as only the secretory granules pass through the ducts opening at the surface of the tegument distal cytoplasm. This mechanism of releasing secretory material has also been suggested for the scolex glands of adult *Khawia sinensis* by Kuperman & Davydov (1982), *Caryophyllaeus laticeps* by Richards & Arme (1981) and Kuperman & Davydov (1982), the genus *Diphyllobothrium* by Öhman-James (1973), Andersen (1975), Gustafsson & Vaihela (1981) and Kuperman & Davydov (1982), *Eubothrium crassum* by Arme &

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Threadgold (1976), *Bothriocephalus claviceps* by Žd'árská & Nebesářová (1997), *Proteocephalus percae* by Andersen (1979) and Kuperman & Davydov (1982), *P. torulosus* and *P. exiguus* by Kuperman & Davydov (1982), *P. osculatus* by Stoitsova *et al.* (1995), *Grillotia erinaceus* by Davydov & Biserova (1985), *Tentacularia coryphaenae* by Farooqi (1986) and *Trilocularia acanthiaevulgaris* by McCullough & Fairweather (1989). An apocrine process has been suggested for the genera *Eubothrium* and *Triaenophorus* by Kuperman & Davydov (1982), for the genus *Bothriocephalus* by Kuperman & Davydov (1982) and Žd'árská & Nebesářová (1997), and for *Grillotia erinaceus* by Davydov & Biserova (1985). A microapocrine mechanism has been described for *Huntarella nondulosa* by Hayunga (1979), *Caryophyllaeus laticeps* and *Khawia sinensis* by Kuperman & Davydov (1982), *K. sinensis* and *K. armeniaca* by Davydov & Poddubnaya (1988) and for *Bothriocephalus claviceps* by Žd'árská & Nebesářová (1997). A holocrine mechanism has been envisaged for secretions from the rostellar glands of *Echinococcus granulosus* by Thompson *et al.* (1979). In our opinion the taxonomic work on proteocephalidean tapeworms should include a precise description of glands of the scolex apex, and especially the glands in the apical organ, if this organ is present.

The object of our study was to determine the types and distribution of gland cells on the scolex of *P. macrocephalus* at the electron-microscopical level. From this point of view our paper may provide some insights to taxonomic relationships and specific functions of the various regions of the scolex.

MATERIAL AND METHODS

Adult specimens of *Proteocephalus macrocephalus* (Creplin, 1815) isolated from the intestine of *Anguilla anguilla* (Linnaeus, 1758), collected from the river Lužnice, (Tábor, Czech Republic) were washed in saline, fixed in 3 % glutaraldehyde in 0,1 M cacodylate buffer (pH 7,2) for 2 h at 4 °C, post-fixed for 2 h at 4 °C in 1 % OsO₄, dehydrated through a graded alcohol series and embedded in Durcupan via acetone. Series of ultrathin sections were cut with a Reichert-Jung Ultracut E ultramicrotome, post-stained with uranyl acetate and lead citrate, and examined in a Philips 420 electron microscope at 80 kV. Semi-thin sections were stained in toluidin blue.

RESULTS

In the scolex-neck region of *P. macrocephalus* two types of gland cells were detected.

ELECTRON LUCID GRANULAR GLAND CELLS

These unicellular glands (Figs. 1, 2, 3, 5, 6) are localized on the frontal part of the scolex around the apical sucker. A large nucleus with a nucleolus occupies the central or basal region of the cell. The perikarya contain an extensive granular endoplasmic reticulum (Figs. 3, 5), several Golgi complexes and few mitochondria. The cisternae of the granular endoplasmic reticulum at the periphery of the perikarya are generally parallel to the cell surface (Figs. 3, 5). The secretory granules are electron lucid with fine textured filaments or flocculent material. They are round-oval (1,200-1,500 nm), and usually somewhat irregularly compressed due to tight packing (Figs. 2, 3, 5, 6, 7). The electron lucid secretion granules are concentrated mainly in the ducts. The granules merge near the duct opening (Figs. 2, 6). The ducts are enforced by microtubules (Fig. 4). The ducts, opening to the exterior, are connected with the plasmalemma of the tegument by a ring-like septate junction opposite to the dense collar. The perikarya are surrounded by some thin muscle fibers (Figs. 3, 5).

ELECTRON DENSE GRANULAR GLAND CELLS

These glands (Figs. 1, 5, 8, 9) are primarily localized in the region of the scolex behind the suckers, and in

Fig.1. – Distribution of two types of unicellular glands in the scolex and neck region of *P. macrocephalus*. AP: apical sucker; S: lateral sucker; ■: electron lucid granular gland cells; ■: electron dense granular gland cells.

Figs. 2-5. – Scolex gland cells of adult *P. macrocephalus*. Fig. 2. Distribution of the electron lucid granular gland cell ducts opening on the frontal scolex part. T: tegument with filiform microtriches. $\times 4,300$. Fig. 3. Perikaryon of an electron lucid granular gland cell. N: nucleus, ER: granular endoplasmic reticulum, MG: electron lucid secretory granules, MF: thin muscle fibers. $\times 1,370$. Fig. 4. Cross section through the duct of an electron lucid granular gland with peripherally located microtubules (MT). $\times 39,000$. Fig. 5. Section through the perikarya of both types of gland cells in the region behind the apical sucker. A: gland with electron dense granules (DG), B: gland with electron lucid granules (MG). ER: granular endoplasmic reticulum, G: Golgi complex, MF: single muscle fibers. $\times 10,700$.

Figs. 6-9. – Scolex and neck gland cells of adult *P. macrocephalus*. Fig. 6. Isolated duct of an electron dense granular gland cell (A) among numerous electron lucid granular gland cell ducts (B) penetrating the tegument (T) of the frontal scolex part. E: excretory duct. $\times 5,000$. Fig. 7. Serial section to Fig. 6. Detail of a duct of the gland with electron dense granules (A) in the frontal scolex part. The duct penetrates the muscular layer (M), basal lamina (BL) and tegument (T). The duct is enforced by microtubules (MT). B: electron lucid granular gland cell duct. $\times 17,600$. Fig. 8. In the neck region are glands with electron dense granules only (A, B, C). Their ducts (D) open on the tegument surface among blade-like microtriches (M). $\times 6,400$. Fig. 9. Perikaryon of the electron dense granular gland cell (DG) from the neck region. Labels include N: nucleus, MF: thin single muscle fibers, ER: granular endoplasmic reticulum. $\times 10,700$.

the neck-region. Very seldom occur these glands among the electron lucid granular gland cells in the frontal scolex part (Figs. 6, 7). The basal parts of the perikarya form some lobes (Fig. 9). Among these lobes thin muscle fibers (Figs. 5, 9) are distributed. The perikarya of these glands contain a large nucleus with a nucleolus, granular endoplasmic reticulum, Golgi complexes and few mitochondria (Figs. 5, 9, 12, 13). The cisternae of the granular endoplasmic reticulum are at the periphery generally parallel to the surface (Fig. 12) or form concentric systems (Fig. 13). Mature secretory granules are large (400–500 nm), round, but usually irregularly compressed, mainly in the ducts (Figs. 6, 7, 8, 10, 11). The granules are concentrated in the upper part of the cells and in the ducts. In the duct wall there are many microtubules (Fig. 10). The plasmalemma of the duct, provided at the opening with a dense collar, is connected with the plasmalemma of the tegument by a ring-like septate junction (Fig. 11). The ducts open to the exterior between the microtriches at the surface of the tegument (Figs. 7, 8, 11).

DISCUSSION

Earlier some authors have not paid attention to the difference between the apical sucker and apical organ. Scholz (1989) described in *Proteocephalus* species the apical sucker as – terminal “sucker” (apical organ) –, however, in a recent paper Scholz *et al.* (1998) distinguish strictly the apical sucker and gland cell distribution around it or in other parts of the scolex. De Chambrier *et al.* (1996) have used in *P. regoi* the term “sucker-like apical organ” and have detected around it gland cells opening to the surface of the scolex apex by narrow ducts.

In total preparation stained with carmine, used in systematic determinations, no problems appear when the apical sucker is well developed. There will be problems with the species where the apical sucker is atrophied or absent, and the glands appear as a glandular formation in the centre of the scolex apex. Such formation present in *P. torulosus* has been described by Scholz (1989) as “glandular apical organ” however, in a recent paper (Scholz *et al.*, 1998), where the presence and distribution of scolex gland cells in the *Proteocephalus* tapeworm have been evaluated, only cumulation of gland cells in the apex centre is mentioned.

In the present study the whole cytons of electron lucid granular glands are described for the first time in the scolex of an adult proteocephalidean tapeworm with the aid of transmission electron microscopy. In *Proteocephalus macrocephalus* the electron lucid granular gland cells, localized on the frontal part of the scolex around the apical sucker, contain in the perikarya and

ducts electron lucid granules. The ducts are enforced by microtubules and fixed at the opening to the plasmalemma of the tegument by a septate junction. The structure of these granules in *P. macrocephalus* corresponds with the electron lucid granules of the goblet cells from the digestive and respiratory system of vertebrates (Fawcett, 1966; Ross & Romrell, 1989). However, in *P. macrocephalus* the mode of releasing the secretion from these unicellular glands is not apocrine, but eccrine. In apocrine elimination of the secretion the apical parts of the cells with their accumulated secretory materials are broken off to form a part of the secretion. In eccrine elimination the secretion is a product of the cell but no part of the cell itself is extruded with the secretion. In the tapeworm species under investigation the electron lucid granules only are discharged through the pore to the surface of the tegument. It seems that the secretion globules on the scolex surface of *P. macrocephalus*, illustrated by Andersen (1979) in scanning micrographs (Figs. 15 and 16), were expelled from electron lucid granular gland cells.

There are no earlier descriptions of the whole cytons morphologically resembling the electron lucid granular glands of *P. macrocephalus*. Only two papers describing the ducts or sacs with electron lucid granules in the scolex tegument are available. The ultrastructural results of gland ducts of Stoitsova *et al.* (1995) in *P. osculatus* and gland sacs of McKinnon & Burt (1985) in *Haplobothrium globuliforme* differ from the *Proteocephalus* species under investigation in that the electron lucid granules are smaller, the ducts are not enforced by microtubules and at the duct openings no septate junctions are present.

Unlike the electron lucid granular scolex glands the ultrastructure of the electron dense granular scolex glands is well known in adults of other *Proteocephalus* species. Andersen (1979) observed electron dense granular gland cells in *P. gobiorum*; Coggins (1980) in *P. ambloplitis*; Andersen (1979), Kuperman & Davydov (1982) in *P. percae*; Kuperman & Davydov (1982) and Kuperman (1988) in *P. exiguus* and *P. torulosus*. This type of eccrine scolex gland cells has been described on the ultrastructural level not only in adults of the order Proteocephalidea but in four other orders, in Caryophyllidea by Richards & Arme (1981), Kuperman & Davydov (1982) and Davydov & Poddubnaya (1988); in Trypanorhyncha by Davydov & Biserova (1985) and Farooqi (1986); in Tetracyphallidea by McCullough & Fairweather (1989), and in Pseudophyllidea by Öhman-James (1973), Andersen (1975), Arme & Threadgold (1976), Kuperman & Davydov (1982), Kuperman (1988) and Žďárská & Nebesářová (1997).

Distribution of the thin single muscle fibers around the perikarya of both types of unicellular glands in *P. macrocephalus* has not been mentioned in any ces-

Figs. 10-13. – Neck gland cells of adult *P. macrocephalus*. Fig. 10. Cross section through the duct of an electron dense granular gland (DG) with clearly visible microtubules (MT) enforcing the periphery of the duct. $\times 29,900$. Fig. 11. Longitudinal section through the duct of the gland with electron dense granules (DG). Note the septate junction (SJ) and dense collar (C) opposite the junction. T: tegument, P: pore, M: blade-like microtriches. $\times 46,800$. Fig. 12. Detail of the perikaryon of an electron dense granular gland (DG) with cisternae of granular endoplasmic reticulum (ER) arranged parallel to the cell surface. G: Golgi complex, MI: mitochondria. $\times 29,900$. Fig. 13. Detail of a concentric system of granular endoplasmic reticulum (ER) in the perikaryon of the gland with electron dense granules. N: nucleus. $\times 24,000$.

tode. In our opinion these muscle fibers probably help to express the secretory granules from the perikaryon to the duct.

The structure and functions of the scolex glands in adult cestodes have been reviewed by McCullough & Fairweather (1989). The explanation of the functions of both types of glands in *Proteocephalus macrocephalus* studied by us remains only at the theoretical level. At this point we can only speculate as to the functions of the two gland cell types. On the basis of the fact that the electron lucid granular gland cells are restricted to the anterior region of the scolex, we can assume that these gland cells have a specific role, perhaps their secretion facilitates the movement and attachment of the scolex between the villi of the eel's intestine, or isolates the parasite from the intestinal tissue to decrease the immune response.

Based on their position, primarily in the neck region of the scolex, the electron dense granular gland cells may produce secretion that aid in attachment as it has been proposed in other cestodes by several workers (Befus & Freeman, 1973, Andersen, 1975, Farooqi, 1958, 1986, Gustafsson & Vaihela, 1981, Hayunga, 1979, Kuperman & Davydov, 1982, Öhman-James, 1973, Thompson *et al.*, 1979, McCullough & Fairweather, 1989, Bockerhoff & Jones, 1995, de Chambrier *et al.*, 1996). It is possible that these glands also have proteolytic and protective functions (Thompson *et al.*, 1979, McCullough & Fairweather, 1989, Bockerhoff & Jones, 1995). In some studies it has been proposed that the secretion may protect the worm against the host's digestive enzymes or its immune response (Thompson *et al.*, 1979, Kuperman & Davydov, 1982, Rickard 1983, Farooqi, 1986, McCullough & Fairweather, 1989).

It would be of great value to know the difference between the pathological changes in the intestine around the scolex of this species, and the proteocephalid species which lack the electron lucid granular scolex glands. This problem needs further investigation.

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