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HISTOFLUORESCENT AND ULTRASTRUCTURAL DEMONSTRATION OF BIOGENIC AMINES IN DAUGHTER SPOROCYST OF *DIPLOSTOMUM PSEUDOSPATHACEUM* NIEWIADOMSKA, 1984 (DIGENEA)

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SUMMARY

The aminergic neurons in *D. pseudospathaceum* daughter sporocysts were studied by fluorescence histochemistry and electron microscopy. Green fluorescence showing the presence of catecholamines was present in the central and peripheral nervous systems. Ultrastructurally different types of vesicles were identified on the basis of their size and morphology. Their possible function was discussed.

RÉSUMÉ : **Mise en évidence ultrastructurale et par histofluorescence des amines biogènes dans le sporocyste-fils de *Diplostomum pseudopathaceum* Niewiadomska, 1984 (Digenea).**

Les neurones aminergiques du sporocyste-fils de *Diplostomum pseudospathaceum* ont été étudiés par des techniques d'histochimie fluorescente et de microscopie électronique. Une fluorescence verte observée dans le système nerveux central et périphérique indique la présence de catécholamines. Trois types de vésicules diffèrent par leur morphologie et par leur taille. Leur fonction est discutée.

INTRODUCTION

The neuronal mediators (acetylcholine, biogenic amines and neuropeptides) have been detected in the nervous systems of many digenean species. The distribution of AChE activity was used in the investigation of the morphology of the nervous system, first of all, in adults, but only rarely in cercariae and metacercariae. Some papers deal only with the nervous system morphology of the sporocyst generation (DiConza and Basch, 1975; Théron and Fournier, 1982; Matthews, 1980 ; Choubisa, 1989 ; and Niewiadomska and Moczoń, 1990). Numerous studies demonstrate the occurrence and distribution of biogenic amines mainly in the hermaphrodite generations, especially of *Schistosoma mansoni* and *S. japonicum* (Chou *et al.*, 1972; Machado *et al.*, 1972) and *Fasciola hepatica* (Bennet and Gianutsos, 1977; Shishov *et al.*, 1987; Fairweather *et al.*, 1987). There are also some studies on the cercariae of different species (e. g. Nezlin and Rybakov, 1988) and the rediae of Echinostomidae and *F. hepatica* (Shishov and Kanev, 1986; Shishov *et al.*, 1987) but no observations were made on the daughter sporocysts. The aim of this study is to demonstrate the biogenic amines in the nervous system of the daughter sporocyst of *Diplostomum pseudospathaceum* Niewiadomska, 1984 to complete the investigations of Niewiadomska and Moczoń (1990) on AChE activity in this generation.

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MATERIALS AND METHODS

Daughter sporocysts of *D. pseudopathaceum* were removed from naturally infected *Lymnaea stagnalis* L.

I. HISTOFLUORESCENCE

For histofluorescence of biogenic amines a modified glyoxalic acid technique (SPG method after De la Torre and Surgeon, 1976) was used. About 50 living fragments (anterior, middle ad posterior) of long filiform sporocysts were dipped three time for 1 sec in glyoxylic acid solution (20-22° C), dried in cool air on microscopic slides for 5-20 min, reacted in an oven at 80° C for 5 min, mounted in paraffin oil or Entallan, coverslipped and placed at a hot plate (80° C) for 90 sec. The fluorescence specificity was tested by pretreatment with reserpine (10^{-4} %/24 h) to deplete monoamines from neurons (Fuxe, 1965). A histochemical test with 0.01 sodium borohydride ($NaBH_4$ in 95 % isopropanol for the reduction of fluorephores (Corrodi *et al.*, 1964) was also used as well as distilled water causing disappearance of specific fluorescence of catecholamines and 5-HT dihydroderivatives. Specimens were studied with an incident fluorescence microscope Jena-Med (Carl Zeiss) equipped with light pressure mercury lamps (Osram

HBO 200 W). The filters used were: exciting filter (max. ex. 400-410 nm) and yellow barrier filter (with high absorbtion below 480 nm). Photo micrographs were taken with ORWO Mikroaufnahmefilm MA-8.

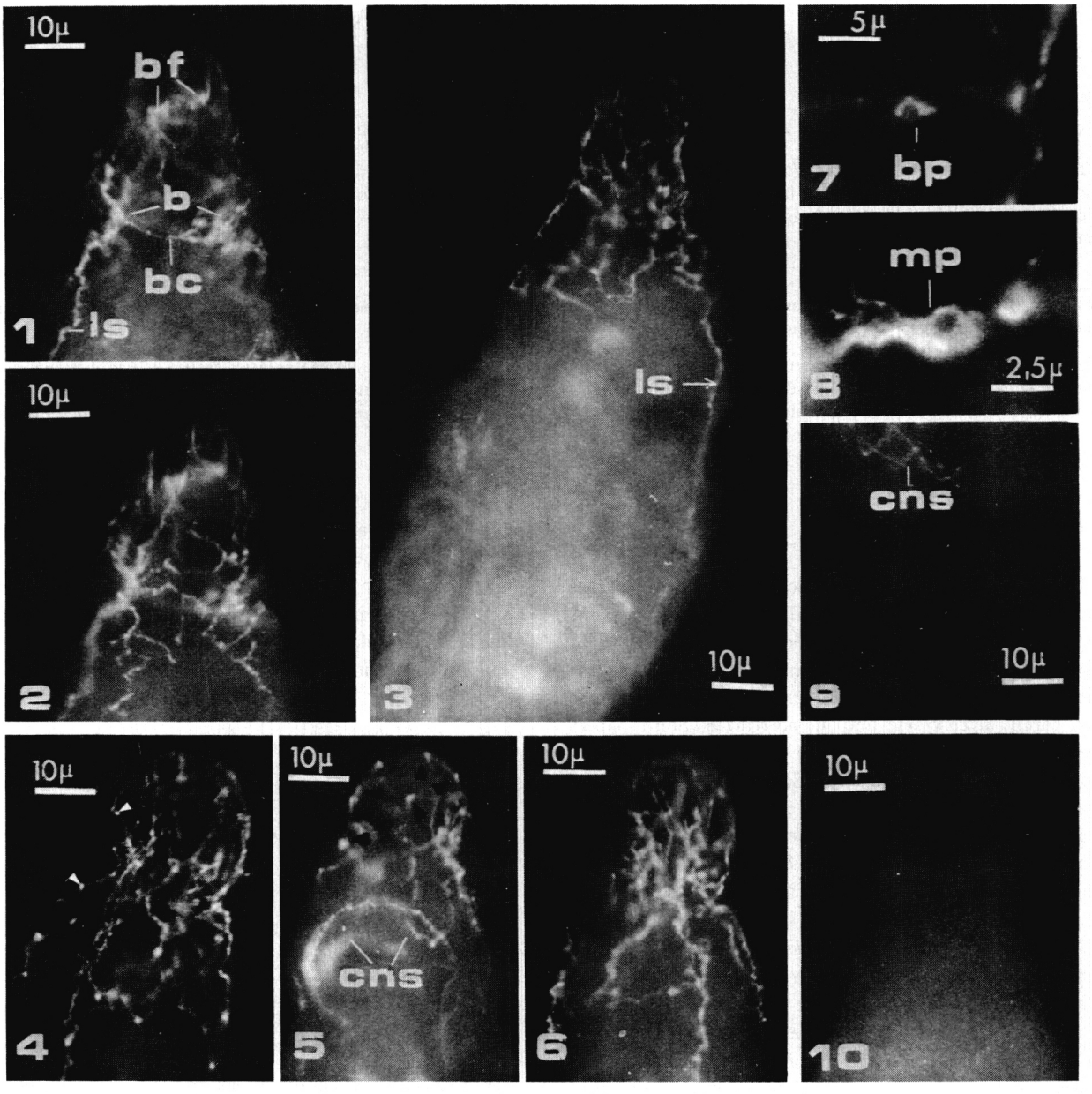
II. Ultrastructure

Sporocysts were treated routinely for electron microscope investigations (see Czubaj and Niewiadomska, 1988). Thin sections were cut on a LKB Ultratome III and viewed with a Jeol 100 B electron microscope.

RESULTS

I. Histofluorescence

The SPG method causing green fluorescence of the nervous system of *D. pseudospathaceum* daughter sporocysts



Figs. 1-10. — Monoamine fluorescence in daughter sporocyst of *Diplostomum pseudospathaceum*. *Fig. 1.* Anterior tip showing central nervous system. *Fig. 2.* The brain of the same specimen of different focus. The peripheral net is also visible. *Fig. 3.* Other specimen showing the irregular nerve net on the anterior body tip. Note the lateral strand (arrow) extending further than the net. *Figs. 4-6.* Different patterns of the nervous net in various specimens. Note the fluorescent endings below the body surface (arrowheads). *Fig. 7.* Bipolar nerve cell (enlarged section of *fig. 6*). *Fig. 8.* Multipolar nerve cell in peripheral nervous net. *Fig. 9.* Posterior end of the sporocyst body showing no fluorescence. *Fig. 10.* Anterior end of the sporocyst body after treatment with NaB4 (control). Fluorescence is not visible. b, brain; bc, brain commissure; bf, places of bright fluorescence; bp, bipolar nerve cells; cns, cercarial nervous system; ls, lateral strand; mp, multipolar nerve cell.

visualised its general topography. In the anterior extremity two concentrations of fluorescent nerve fibres connected by a thin commissure are present *(fig. 1)*. Each concentration (corresponding to a brain ganglion) give rise to nerve fibres running to the anterior end. They are connected by numerous small commissures forming an irregular net *(figs. 3, 4, 6)*. Some fibres of the net with strongly fluorescent endings terminate just below the body surface *(figs. 4, 5)*. In some specimens, at the anterior body tip, a bright fluorescence symetrically distributed was seen *(fig. 1)*. Posteriorly, from neural masses on the lateral side run two distinct strands and on the dorsal and ventral sides the strands are unclear being included in the loop of nerve fibres *(figs. 1, 3, 6)*. At some distance from the anterior end the net disappears. Sometimes the lateral strands extend a little farther. No fluorescence was observed along the body and posterior end of the sporocyst *(fig. 9)*. In some specimens a distinct fluorescence of the perikarya was visualized. Bipolar nerve cells were most frequently seen but multipolar also were observed *(figs. 7, 8)*. On many whole mounts the cercariae inside the sporocyst body showed fluorescence of the nervous system *(figs. 5, 9)*.

II. Ultrastructure

According to our unpublished data the brain of the daughter sporocyst of *D. pseudosathaceum* is composed of paired ganglia connected by commissure containing numerous nerve fibres. Most of the cell bodies of the cerebral ganglia are scattered around the periphery of the ganglion but they are also found in the neuropile area among the nerve fibres. The brain nerve cell bodies have a large centrally placed nucleus and a small amount of cytoplasm. The perikaryon is filled with mitochondria, Golgi complexes, weak developed rough endoplasmic reticulum, ribosomes, microtubules and a variety of vesicles *(figs. 11-13)*. Three morphologically distinct types of vesicles ar found in the cytoplasm of the perikaryon and the neuropile processes *(figs. 12-14)*: (1) small electron-lucent vesicles (axis between 30 and 50 nm); (2) dense-core vesicles (axis between 50 and 110 nm) which have a clear rim separating the electron dense content from the vesicle membrane; and (3) large electron-lucent vesicles (axis between 60 and 100 nm).

The peripheral nerve systems is not rich in body cells (the same picture showed histofluorescence) and consist mainly of nerve processes lying under the muscle layer or among the contractile parts of muscle cells individually or in small concentrations *(figs. 15, 16)*. The cytoplasm of these processes contain two types of vesicles: small electron-lucent, and vesicles with moderate and dense-core *(fig. 16)*. The sensory ending showed in the dendrite two type of vesicles: large oval and sphaeroid electron-lucent vesicles, and dense-core vesicles *(figs. 17-20)*.

DISCUSSION

The histofluorescence SPG method visualised a general topography of the aminergic system of the *D. pseudospathaceum* daughter sporocyst. The comparison with cholinergic ones described by Niewiadomska and Moczoń (1990) pointed out a similar picture of localization. The green fluorescence which was observed in *D. pseudospathaceum* sporocysts indicate the presence of catecholamines which were demonstrated in hermaphroditic and parthenogenetic generations of some Digenea species (see discussion Shishov *et al.*, 1987). The lack of yellow fluorescence may be interpreted as indicating a very low amount of indolamines (mainly serotonine) as was shown by Reuter *et al.* (1980) for the free living turbellarian, *Microstomum lineare*.

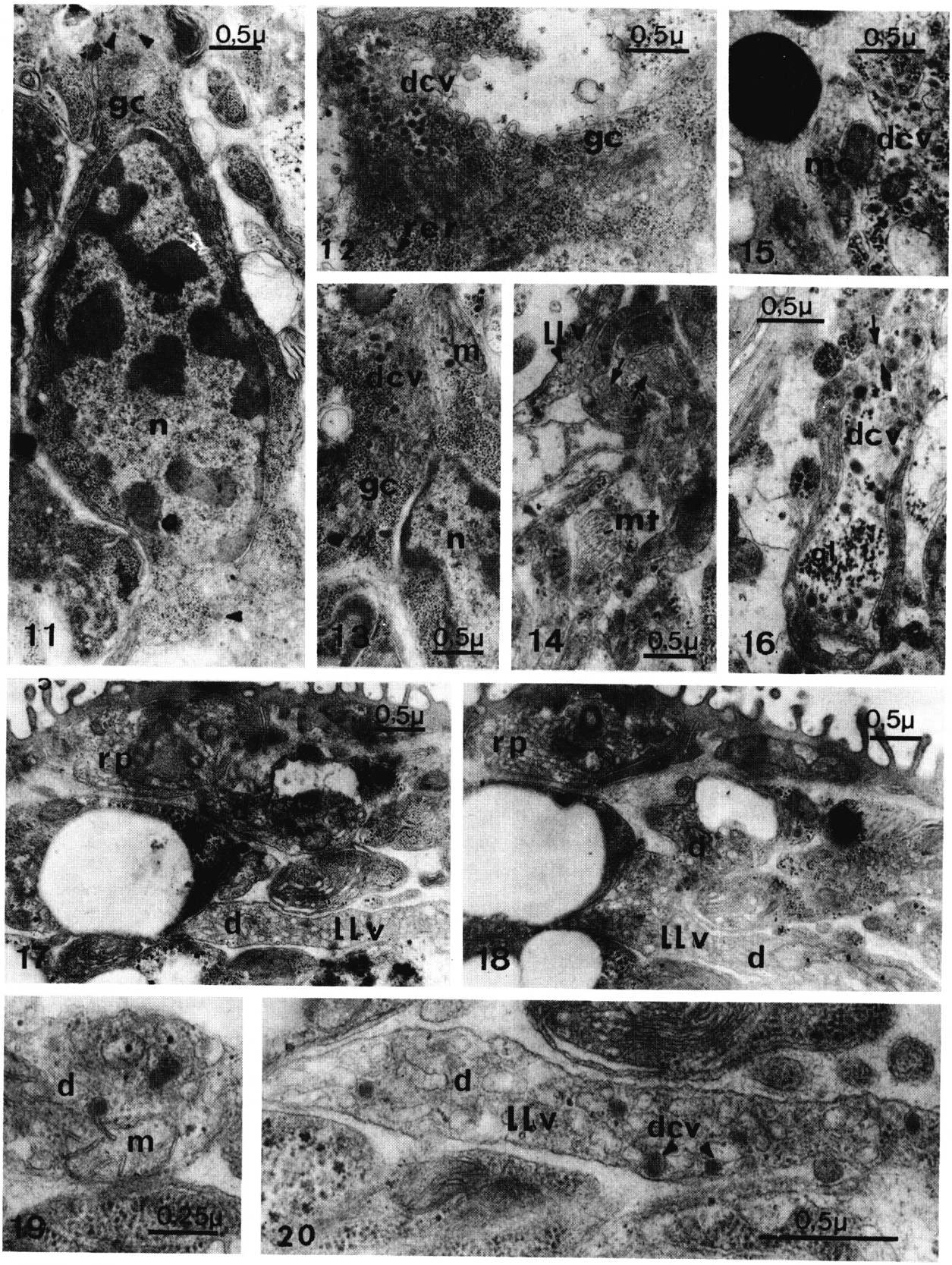
The ultrastructural picture of the nervous system showed vesicles of three different types. Two of them, namely small electron-lucent and dense-core can be morphologically determined as cholinergic and catecholaminergic, respectively. In the daughter spoocyst of *D. pseudospathaceum* the nervous system stains positively for cholinesterase (Niewiadomska and Moczoń, 1990) and the first type of vesicles resembles the vesicles from acetylcholinesterase containing planarian nerves (Czubaj, 1979) or monogenean *Gastrocotyle trachuri* (Shaw, 1982). Thus these vesicles in *D. pseudospathaceum* sporocyst may contain acetylcholine although similar vesicles are known to contain other transmitters in other invertebrate (*e. g.* Botham *et al.*, 1979).

The dense-core vesicles of platyhelminths are regarded as a storage sites of biogenic amines (Reuter *et al.*, 1980; Dei-Cas *et al.*, 1980; Trawicki *et al.*, 1988). In the *D. pseudospathaceum* sporocyst the distribution of fluorescing cells and fibres correspond with the ultrastructural findings of neurons containing dense-core vesicles.

The third type of vesicles—large electron-lucent—are connected with sensory endings. They were visible in the receptive part of the dendrite, and externally between the cilium an collar of examined sporocyst species (unpublished data) and in *Notocotylus attenuatus* rediae (Czubaj and Niewiadomska, 1988). The presence of large electron-lucent and dense-core vesicles in the dendrite of sensory cell may be interpreted either as vesicles of different chemical nature, or one population of vesicles at different stages of development. It is possible that the contents of these vesicles take part in a chemoreceptive function of sensory cells.

Both histofluorescence and ultrastructural methods confirm the presence of an aminergic nervous system in the daughter sporocyst of *D. pseudospathaceum* besides the cholinergic one described by Niewiadomska and Moczoń (1990). Neurotransmitters of both the systems play the basic role in neurophysiology of trematodes. Catecholamines and serotonine has been suggested as a possible excitatory, and acetylcholine as inhibitory transmitters (Sukhdeo and Mettrick, 1987).

The aminergic nervous system, the same as cholinergic,



FIGS. 11-20. — Ultrastructure of the nervous system of the daughter sporocyst of *Diplostomum pseudospathaceum*. *Fig. 11.* Perikaryon of the brain nerve cell with vesicles (arrowheads). *Figs. 12-13.* Parts of the brain nerve cell. *Fig. 14.* Brain neuropile. *Figs. 15-16.* Parts of nervous cell in peripheral nervous system (15) and nerve process (16) lying below the muscle layer. *Figs. 17-20.* Successive profiles of the sensory cell. *Figs. 19 and 20* show enlarged details of sensory cell dendrite. d, dendrite; dcv, dense-core vesicles; gc, Golgi complexes; gl, glycogen; llv, large lucent vesicles; m, mitochondrion; mc, muscle; mt, microtubules; n, nucleus; r, ribosomes; rer, roughendoplasmic reticulum; rp, receptive part of sensory cell; arrows, small lucent vesicles.

does not reflect the typical orthogon of hermaphroditic generation (see Niewiadomska and Moczoń, 1982, 1984, 1987). The latter consists of the paired cephalic ganglia connected by the commissure and three pairs of the nerve trunks (of which ventral one are most developed) connected by numerous commissures. Perhaps one could look for some similarity with the nervous system of some « Turbellaria » as such features as net-like structure, the site under the tegument (between the cytons and muscle cells), and the limitation to the anterior sporocyst tip, would be interpreted as primitive. It corresponds also with our observations showing the presence of both cholinergic and aminergic vesicles in one nerve cell—the feature characteristic for Catenulida (Moraczewski *et al.,* 1977) the free-living group having a primitive phylogenetic status (Ehlers, 1988).

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