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Electron microscope observations of neurosecretion in Fascicola hepatica

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Zoologia. — *Electron microscope observations of neurosecretion in* Fascicola hepatica(*). Nota di MARIO GRASSO e ANTONIO QUAGLIA, presentata(**) dal Socio P. PASQUINI.

RIASSUNTO. — Precedenti indagini eseguite al microscopio ottico hanno messo in evidenza la presenza di cellule nervose infarcite di granuli Gabe–positivi sia nel plesso cerebrale che nei cordoni nervosi ventrali posteriori di *Fasciola hepatica*. Nel presente lavoro questi risultati sono stati controllati con osservazioni al microscopio elettronico. Infatti, si è potuto dimostrare che le fibre nervose di tali organi sono provviste di materiale neurosecretorio sotto forma di granuli ovoidali circondati da una membrana di circa 1000 Å di diametro. Inoltre si fa presente che tali granuli si originano nel *perycarion* delle cellule nervose da dove poi migrerebbero lungo gli assoni.

INTRODUCTION

It has been known for several years that neurosecretion processes occur in the nervous system of many Platyhelminthes; they begin at the cerebral ganglia level and extend in the cephalo-caudal direction along the posterior ventral nerve cords. In Trematoda, early observations were made on *Dicrocoelium lanceatum* by Ude [15] who was the first to succeed in bringing out positive-Gomori granules in the nerve cells both in the cephalic pharyngeal ganglia and in the posterior ventral nerve cords. In 1970 Matskási [10] described neurosecretive cells active according to a daily rhythm in the ganglia, in the commissures, in the nerve cords, in the sucker and in the pharynx of the Trematode *Opisthodiscus diplodiscoides*. Gresson and Threadgolds [8], just in *Fasciola hepatica*, studied the structure of some cells, called by Authors " large neurones ", both by the light microscope and the electron microscope. These elements were often observed not in connection with the central nerve system but in the parenchyma of the anterior region of the body, in the pharynx, in the oral and ventral sucker; they have been classified as α and β type cells according to their morphology and interpreted as neurosecretory neurons. Finally, and still using electron microscopy, neurosecretory cells have been studied by Dixon and Mercer [1] in *cercaria* of *Fasciola hepatica* and by Reissing [13] in the adult of *Schistosoma mansoni*.

Previous investigations had been performed by one of us [3, 4] in the adult of *Fasciola hepatica* using the light microscope and employing common staining techniques for neurosecretion (Gomori-Bergman chrome-haematoxylin-phloxine, Alcian blue method by Adams-Sloper later modified by Herlant, and most of all Gabe paraldehyde fuchsin). These methods, although

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very indicative, are not provided with an absolute specificity; they brought out the presence of nerve cells filled with Gabe-positive granules, situated both in the pharyngeal cephalic ganglia and along the posterior ventral nerve cords.

In the present research such observations are checked by a more specific method, i.e. electron microscopy, by which in nerve cells of other Platyhelminthes such as Planarians (Turbellaria-Triclads) in previous researches we succeeded [5, 6, 7] in observing electron-opaque neurosecretory granules surrounded by a membrane, comparable in structure and dimensions (500–2000 Å) to those described by Morita and Best [12] in the same material.

Material and methods

The living material used for this research was taken directly from infested and freshly butchered cow liver; it was fixed for two hours at 0° C in a 2.5% glutaraldehyde solution in 0.09 M sodium phosphate buffer at pH 7.4. After a two hours washing in the same buffered solution, they were post-fixed for an hour at 0° C in 1% osmium tetroxide solution in sodium phosphate buffer at pH 7.4 [11], then dehydrated in aceton and embedded in Araldite. By means of an OM U 2 Reichert ultramicrotome, cross sections were obtained; once stained with lead citrate [14] they were examined by an HS 8 F Hitachi electron microscope at various enlargements.

Results

As is known, the cerebral plexus in *Fasciola hepatica* is localized anteriorly immediately after the oral sucker and is composed of a single nerve mass from which the very long ventral nerve cords extend laterally. These run along the two sides of the entire body of the animal and are linked together by commissures (fig. 1). Observation of these parts of the nervous system in cross section under the electron microscope show the presence of nerve fibers fairly uniform in caliber (0.2–1 μ) which have only a plasmatic membrane (thickness 80 Å) and are separated one from another by very slight irregular spaces (approximately 300 Å) of low density fundamental substance. Their neuroplasma has neurotubules with an approximately 150 Å diameter, several irregular vesicles, glycogen granules and roundish or elongated mitochondria with sparse crests (Plate I, figs. 1 and 2). A certain number of these nerve fibers contains strongly opaque granules, roundish or ovoidal with dimensions varying from 800 to 1000 Å, typically surrounded by a membrane and which can, therefore, be interpreted as neurosecretory granules (Plate I, fig. 3).

Examination of the neurosecretory cells shows that they have conspicuous dimensions, with a shape tending toward ovoidal; their large thick nucleus contains a nucleolus. In their *pericarya* are present a well developed granular

endoplasmic reticulum, several ribosomes either free or in rosette arrangements, glycogen particles and a rather extensive Golgi apparatus. Near the latter numerous vesicles can be seen and above all many granules surrounded

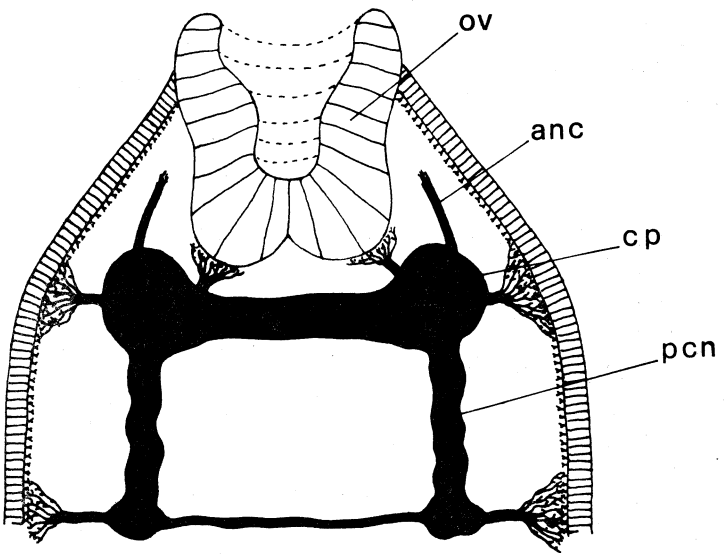

Fig. 1. – Scheme of *Fasciola hepatica* nervous system in frontal view. In black are represented the cerebral plexus (cp), the posterior ventral cords (pcn) united by median commissures and the anterior ventral cords (anc). In white is represented, in the anterior part, the oral sicker (ov), directly innerved by the cerebral plexus.

by a membrane, very much like those seen along the nerve fibers (Plate I, fig. 4). This verifies the well-known fact that neurosecretory substances are synthesized at the Golgi apparatus level and transferred along the axons of the nerve cells.

DISCUSSION

Concluding this brief report on our first results which regard for the present above all the cerebral plexus, it can be said that in the nervous system of *Fasciola hepatica* electron microscope study has demonstrated the presence of uniform caliber nerve fibers endowed with osmium sensitive granulations, often surrounded a membrane, which can be interpreted as neurosecretory material.

Regarding the origin of such granules, it is most probable that they are synthesized in the *pericarya* of the nerve cells and migrate from there along the axons; the observation that granules with the identical characteristics as the granules found in the nerve fibers may be found in the *pericarya* near the Golgi apparatus, and that an electron-opaque material is often found in the Golgi apparatus cisterns, leads particularly to believe that this apparatus is related to the neurosecretory process.

Work is in progress in order to demonstrate the biological role of neurosecretion in *Fasciola hepatica*. In this respect it is noteworthy that other experimental studies have brought out the importance of the nervous system and

of neurosecretion for the sex-maturation of the hermaphroditic genital apparatus in other Platyhelminthes, i.e. Turbellaria–Triclads [2].

Research in this direction has already been started by Harris and Cheng [9] on Trematode *Leucochloridiomorpha constantiae*; for this species the Authors suggest the hypothesis of a possible correlation between neurosecretory activity and gonad maturation, having verified a considerable variation in the quantity of Gabe-positive cells between the *metacercaria* larval phase and the adult animal.

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EXPLANATIONS OF PLATES I–II

Plate I

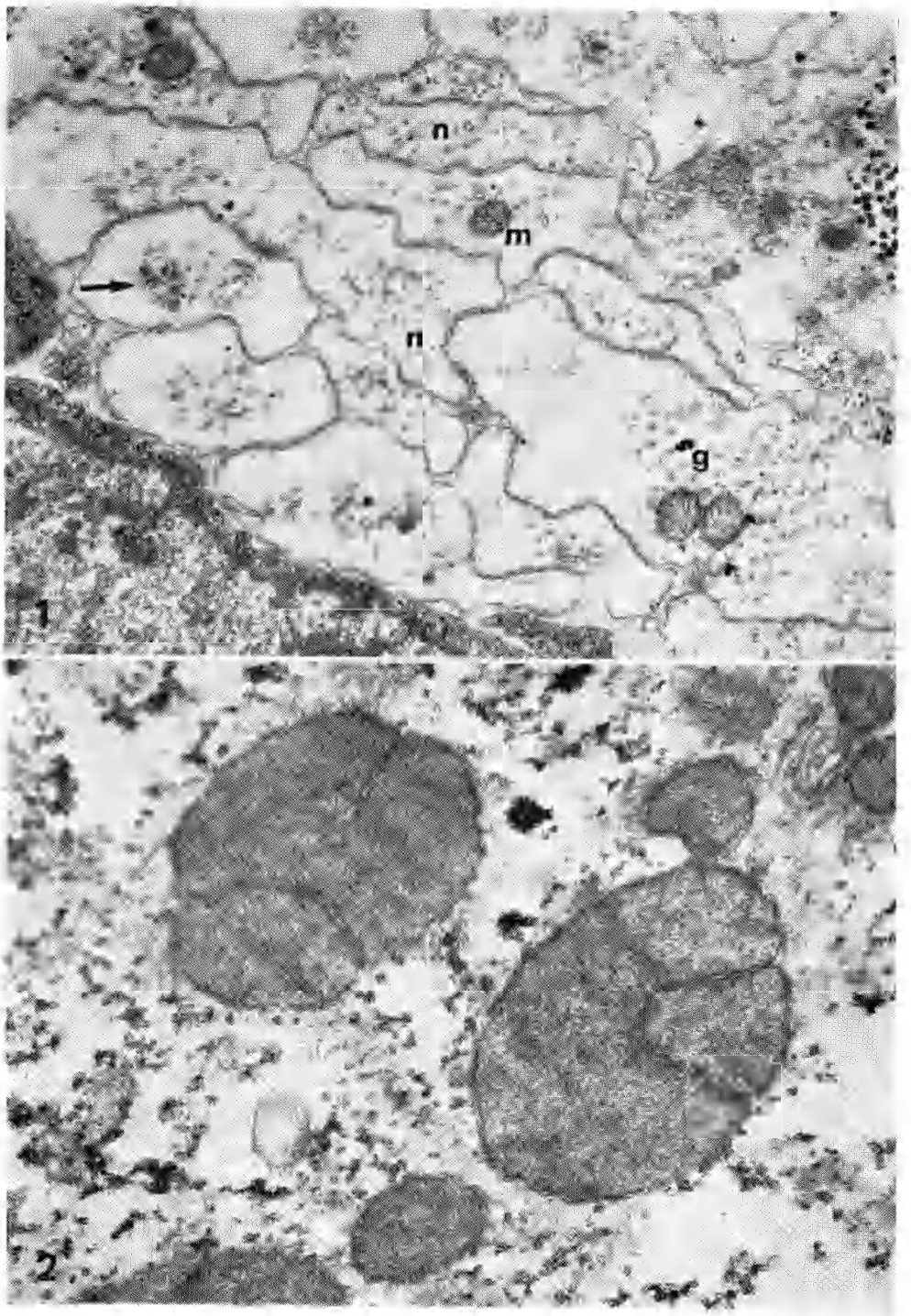
Fig. 1. – Nerve fibres in cross section. Note neurotubules (*n*), mitochondria (*m*), glycogen particles (*g*), and neurosecretory granules (arrow). ×20000.

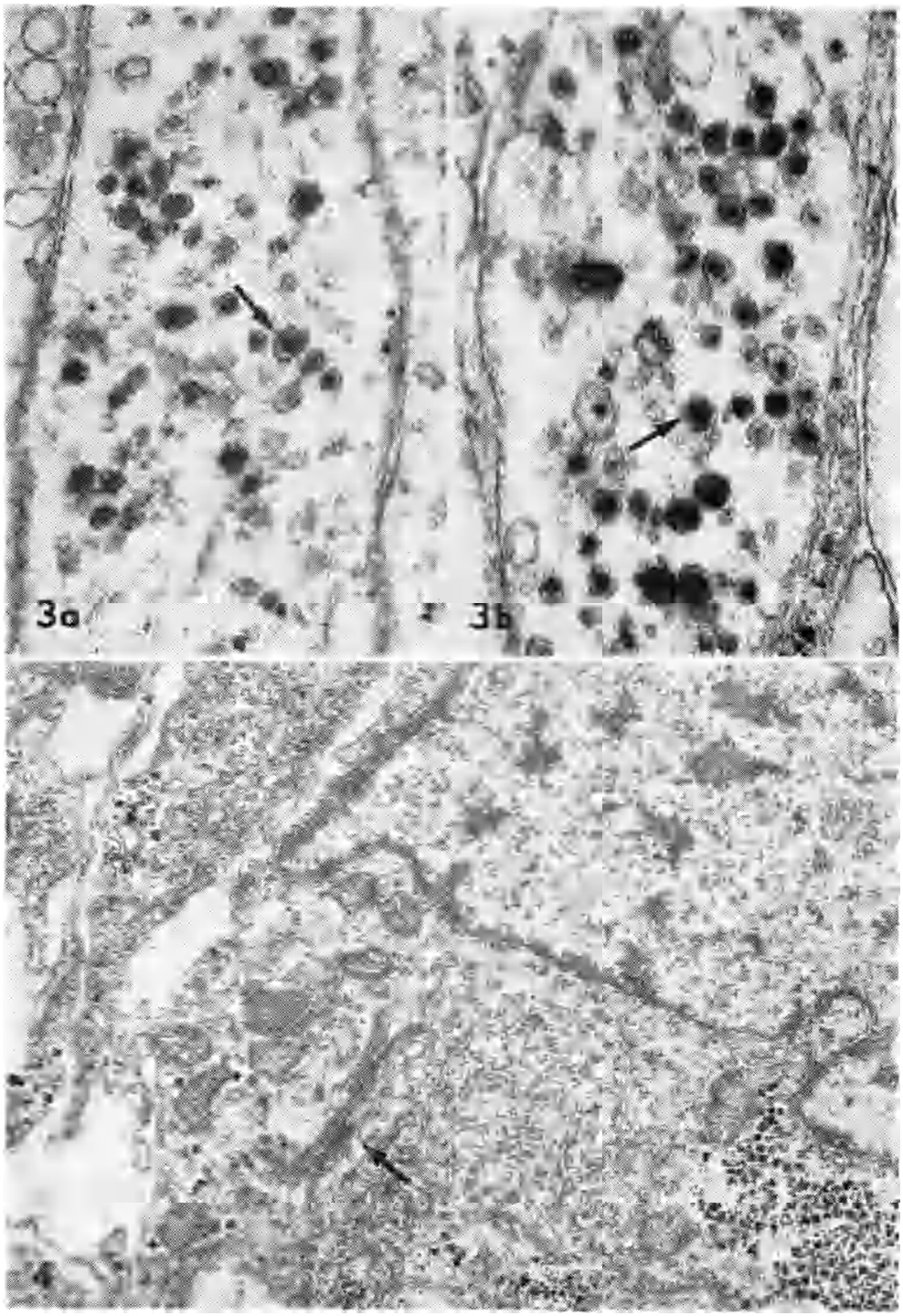
Fig. 2. – High enlargement mitochondria. Note as the number of the crests is very scarce. ×80000.

Plate II

Fig. 3 *a–b*. – Nerve fibres in cross section. Note neurosecretory granules typically surrounded by a membrane (arrows). × 40000.

Fig. 4. – Nerve cell in cross section. Note sparse granules which can be seen around the Golgi apparatus, in the cisterns of which dense material is visible (arrow). ×20000.


n
m
n
g
1
2


3a
3b