

## ULTRASTRUCTURAL STUDIES ON THE GASTROINTESTINAL NERVOUS SYSTEM OF *HELIX POMATIA*

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The gastrointestinal nervous system is made up of neurons, nerve fibres, glia cells and glia fibres. The neurons are large and sharply defined towards the surroundings. They contain many endoplasmic reticules having wide lumen, as well as free ribosomes and ribosomes arranged in rows. Mitochondria and lysosomes are few in number. Neurosecretory granules are characteristic, among which dense-core forms and larger homogeneous oval granules can also be detected. The chromatin is found close to the nuclear membrane in the form of indented lobules. One part of the nerve fibres contains large amounts of neurosecretory granules the other part is prevalent in the dense-core forms. There are added to the nerve fibres of granule content, the agranular fibres containing no granules, as well as glia cells and glia fibres. Many glycogen granules can be found in the latter two. Their characteristic components are the gliogranules.

Key words: gastrointestinal nervous system, *Helix pomatia*.

### Introduction

The intestinal canal of *Helix pomatia* begins at the orifice which is on the ventral side of the head between the two labium tentacles, and leads into the wide pharynx. The short oesophagus opens from here and continues in to the brownish stomach. On this lies the two flattish salivary glands, which cover a large area length-wise and width-wise adhering to the dorsal side on a short section. Both go through a ribbon-like, twisted canal, which protracts on both sides of the oesophagus and leads into the pharynx (ÁBRAHÁM, 1939, 1966, 1967). The stomach is followed by the small intestine which after a short spiral course, enters into the bend of the visceral bursa. Here it turns to the other side and in the form of a dilation resembling the rectum it opens to the outside on the inner side of the lung cavity (ÁBRAHÁM, 1969; SCHMALZ, 1914; THOMAS, 1951).

### Materials and methods

Small pieces were cut from the oesophagus, stomach and intestine of the animals narcotized with chloroform and material were fixed with 0.5% osmium acid following prefixation with glutaraldehyde and embedded in araldite after the usual dehydration. Ultrathin sections were prepared by means of an LKB ultramicrotome and examined under TESLA D 242 and JEOL B 100 electronmicroscopes. The studies were carried out in the Biological Research Institute of the Hungarian Academy of Sciences (Tihany), the Central Research Institute for Medicine of the Hungarian Academy of Sciences (Budapest), the 1st. Institute of Anatomy of the Semmelweis Medical University (Budapest), and the Electronmicroscope Laboratory of the Central Research Institute for Biology of the Hungarian Academy of Sciences (Szeged). During the course of our studies we received help from DR. I. BENEDECZKY, DR. D. SZABÓ, DR. J. HÁMORI, DR. F. JOÓ, and DR. IDA TÓTH, to whom we should also like to express our sincere thanks here.

## Results

The structure of the neurons showed a strange and irregular appearance. Many neurons here been observed by electronmicroscopy, but the author has not seen any similar to these in his own pictures, nor in the literature (SCHARRER and BROWN, 1961; SCHARRER, 1963; KUHLMANN, 1963; SANCHES and BORD, 1958.) The peculiarity, however concerned the cytoplasm; the cell nucleus showed the general and usual neuron nucleus form.

The large amount of endoplasmic cisternae was striking in the cytoplasm (SCHLOTE and HANNEFORTH, 1963). The ribosomes arranged in rows were observable on the boundary of these. Nevertheless, it should also be mentioned that the whole cytoplasm was almost overrun by free ribosomes. The mitochondria belonged to the crystae type their number was relatively low. Many neurosecretory granules of various situations and forms were found in the cytoplasm. Most of them were roundish and smooth, which is the usual form for neurosecretory granules. Nevertheless, dense core forms were also detectable, although extremely rarely. As in the brain of the water beetle (ÁBRAHÁM, 1966, 1967, 1969), (*Dytiscus marginalis*) there were many double and triple groups among the smooth granules. The single groups were circumscribed by capsules. Multivesicular bodies laden with small roundish vesicles were not infrequent in the cytoplasm. The greater part of the nucleus was empty. The chromatin adhered to the nuclear membrane in the form of dense nodules and roughly indented laminae. The two nuclear membranes were found to be uniform laminae the nuclear membrane pores were not observable (Fig. 1).

Among the forms of nerve fibres those found in greatest number contained granules. The latter usually filled the axoplasm completely. Among these there were some which were roundish and elyptic of which some were smaller others larger. They were situated very close to the axolemma and were smooth and homogeneous. Clear vesicles were detectable in the axoplasm mainly near the axolemma, but also in other places, although in smaller quantities. The various kinds of granules were all smooth and their number was particularly high in the area of the stomach and intestine (Fig. 2).

Besides the nerve fibres completely filled with granules, especially in the wall of the intestine, some fibre forms were not infrequent where only a few granules were observable in a rather large area. Sometimes, however, oblong dilatations were detected in the course of the nerve fibres, which occurred again along the fibres. These were filled with roundish granules while no granules at all were seen in the section of fibre linking the two neighbouring dilatations. Since the fibres completely filled with granules were found in the highest quantity the phenomenon could be regarded as a functional state (JUNGSTAND, 1962; KRAUSE, 1960).

Although in smaller numbers such nerve fibres were also found in the nerve fibre plexuses in which, besides mitochondria, dense core vesicles, (or only the latter) could be detected. Their amount was usually low. The fibres containing dense core vesicles seemed to be almost empty, though mitochondria appeared in higher quantities than in the nerve fibres of the plexuses containing smooth neurosecretory granules (Fig. 3).

In the nerve fibre plexuses apart from the nerve fibres comprising neurosecretory granules and dense core vesicles, nerve fibres also occurred which did not contain any kind of granules or vesicles. These fibres are called agranular nerve fibres. On our pictures these are clear formations differing entirely from the other form of fibre. The axolemma was well defined, neurotubules appeared, in large quantities and with



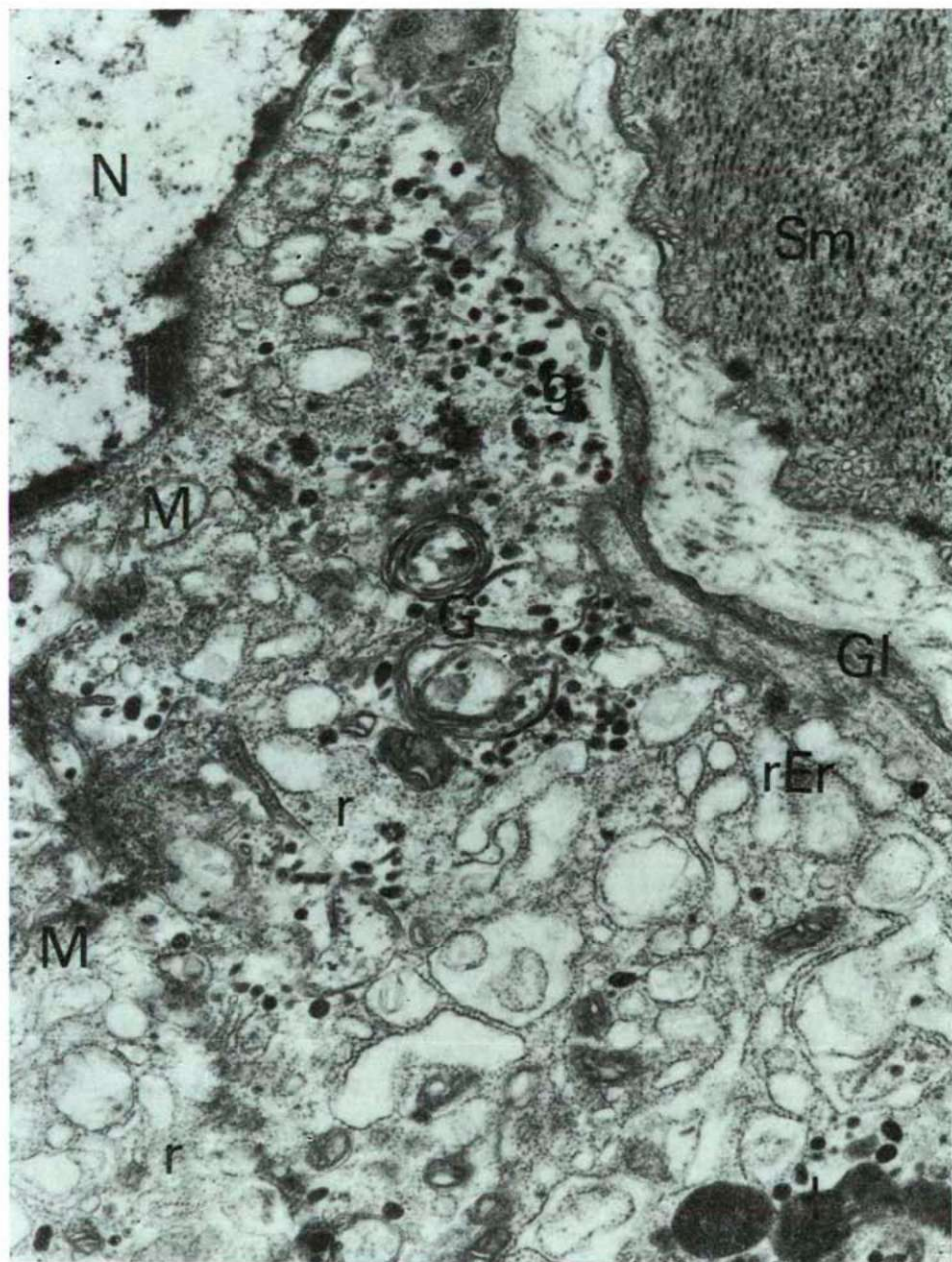


Fig. 1. Detail of neuron from the intestinal wall of snail. The karyoplasm of the nucleus (N) is light, few electron dense heterochromatin are situated along the cell membrane. Neurosecretory granular vesicles (g), wide, rough surfaced endoplasmic reticulum cisternae (rEr) and many ribosomes (r) can be seen in the cytoplasm. The Golgi apparatus (G) is present in the form of concentric sacs. M = mitochondrium, l = lysosome, Sm = smooth muscle cell, Gl = glia process X 20 000.

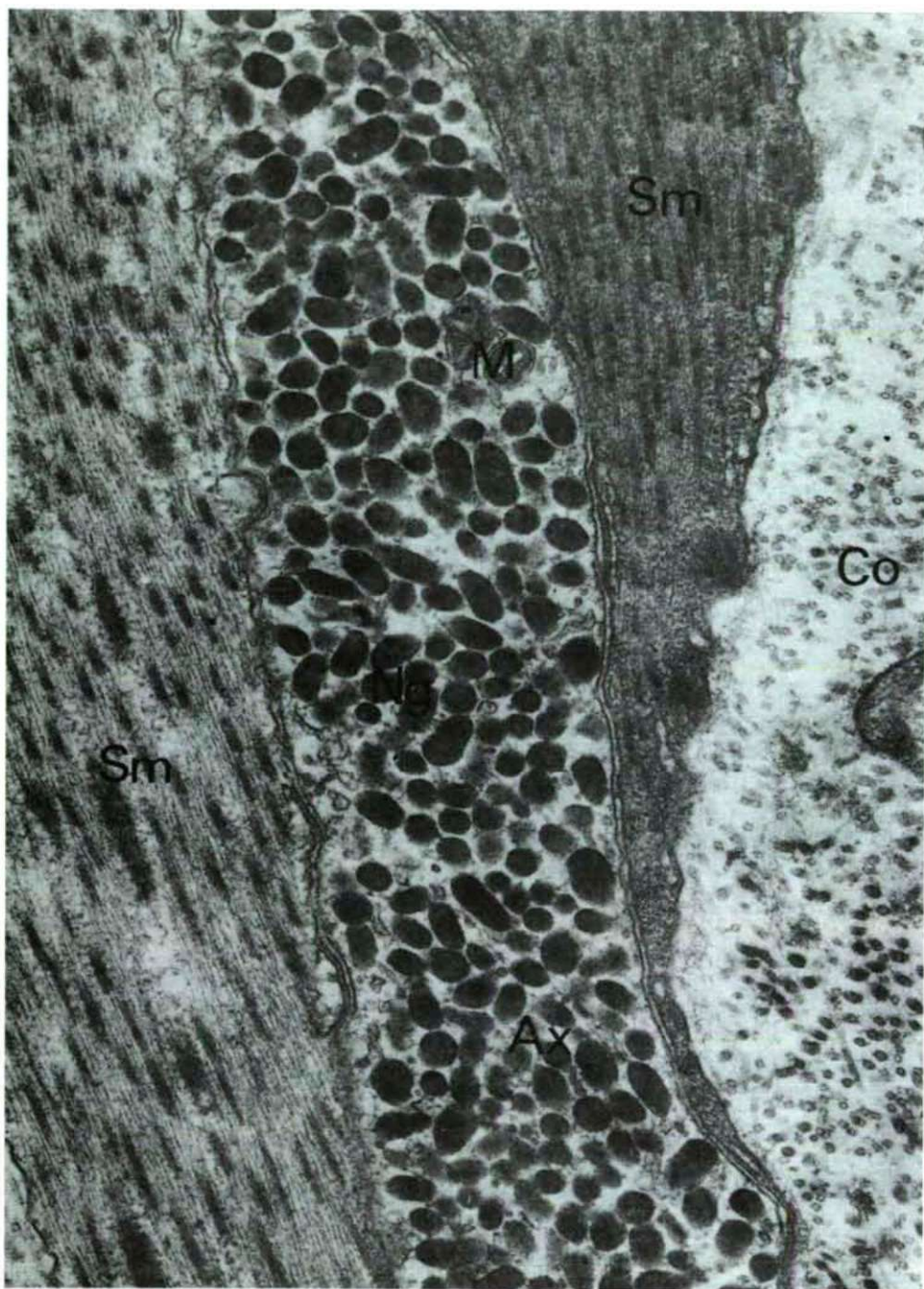


Fig. 2. *Helix pomatia*: nerve fibre from the intestinal wall. Ng = neurosecretory granule, Ax = axon, M = mitochondrion, Sm = smooth muscle, Co = collagen fibres X 20 100.



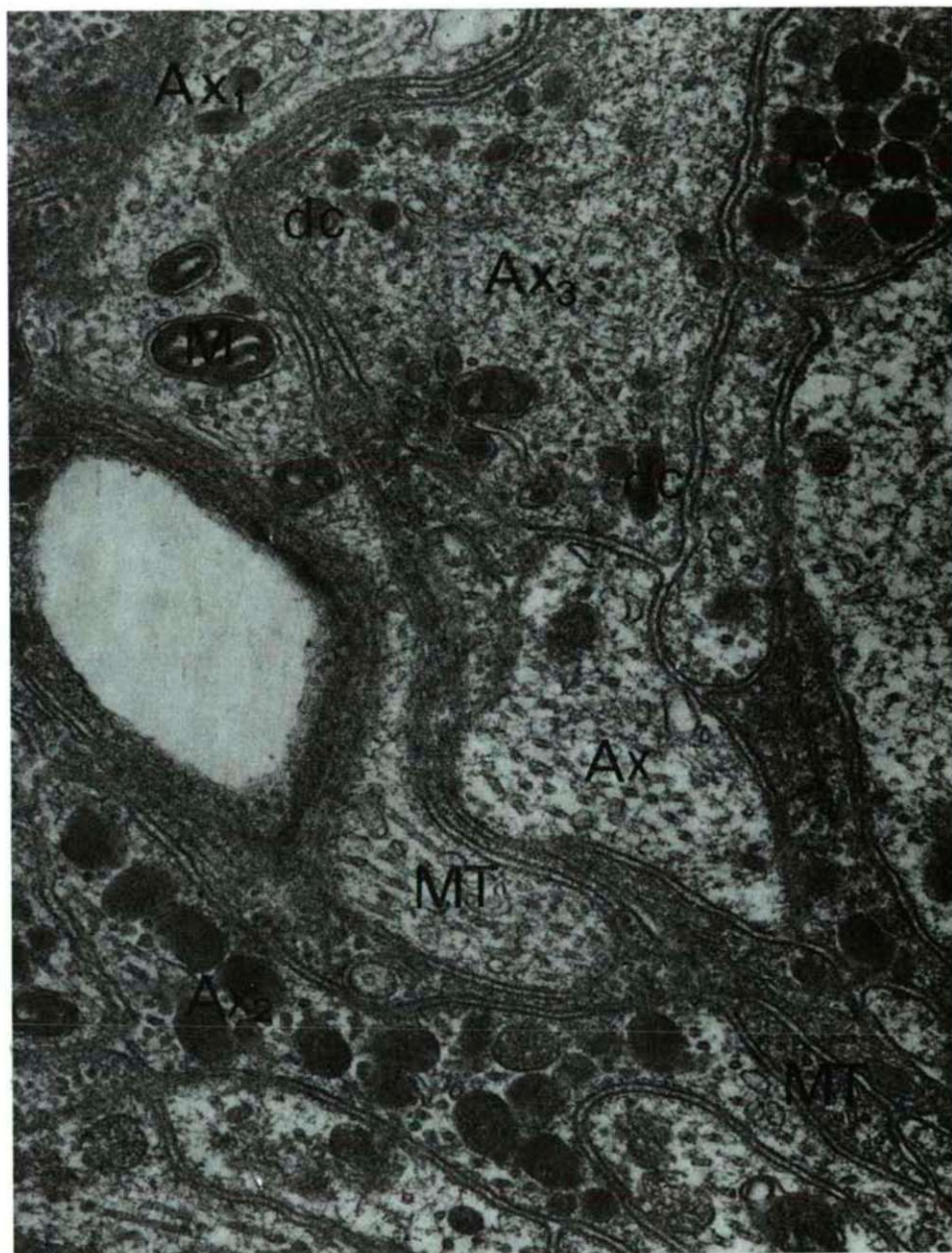


Fig. 3. Cross section of nerve fibres (Ax) in the nerve plexus of snail intestinal wall. In some axons (Ax<sub>1</sub>) one or two mitochondria can be seen apart from the microtubules (MT). In other axons (Ax<sub>2</sub>) electron dense neurosecretory granules and (Ax<sub>3</sub>) dense core vesicles (dc), are observable. (X 48 000).



Fig. 4. Agranular nerve fibres from the wall of the intestine. Ax<sub>1</sub> and Ax<sub>2</sub> = axon cross sections, Mt = microtubules, Co = collagen fibres, Sm = smooth muscle, Gg = gliogranules, g = glycogen granules. X 21 100.





Fig. 5. Glia cell from the intestinal wall of snail. Many heterochromatin can be found in the processing cell nucleus (N). Lipid droplets (L), gliogranules (Gg), glycogen granules (g) and collagen fibres (Co) X 25 000.

unusual sharpness in the axoplasm, both in longitudinal and cross sections. Just the same as the fibres themselves the neurotubules were also very clear. In low number and small areas irregular cavities were observable in the axoplasm. These are held to be the cisternae of the endoplasmic reticulum (Fig. 4).

The neuron and nerve fibres were surrounded by processing glia cells (SCHLOTE and HANNEFORTH, 1963). The cytoplasm was found to be filled with glycogen granules. The thick processes were seen to depart from cells with wide bases, and in the majority of cases they accompanied the nerve fibres in the form of fibres. The cell nucleus was strongly elongated, the chromatin formed large nodules centrally. The glycogen granules and gliogranules were the components of the glia cells and glia fibres. The glycogen granules were polyedric and completely filled the cytoplasm. The gliogranules were homogeneous roundish or ellipsoid small inclusions and indentures were observed on some of them. At times they were seen interlaced with each other (Fig. 5).

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