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ELECTRON MICROSCOPIC STUDY ON THE INNERVATION OF THE GUT-MUSCULATURE IN THE CARP *(CYPRINUS CARPIO)*

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Abstract

Authors studied the innervation of the muscles of the small intestine in lacustrian carp *(Cyprinus carpio)* with the help of electron microscopic method. Studies determined a complex myenteric plexus rich in nerve elements, found between the circular and longitudinal muscle layers. The myenteric plexus was made up of large, continuous neuropil areas, smaller fibre bundles penetrating the muscle layer and single axons. The occurrence of neurons hasn't been detected until now. Axon terminals were frequently found tightly close to the sarcolemma, however, the morphological signs of synaptic specialization were only occasionally observed. Dense-core vesicles with diameters of 60—100 nm were observed most frequently in the axon profiles. In their neighbourhood low amount of pleomorph agranular vesicles also occurred. Neurosecretory granules of varying electron density, with diameters of 200 nm were found in certain axon profiles. On the basis of their electronmicroscopic observations, authors assume that the simultaneous occurrence of aminergic — by the presence of the dense-core vesicles —, as well as peptidergic innervation — by the axon profiles containing neurosecretory granules — is very likely in the enteric nervous system of carp.

Key words: carp, gut musculature, innervation, electron microscopy.

Introduction

Recently interest has turned again towards the triple division (classification) of the autonomic nervous system proposed by LANGLEY in 1921, and within this the concept of the enteric nervous system (ENS).

Several authors (GERSHON et al., 1983; FURNESS et al., 1980) enumerate convincing evidences concerning the separation of the ENS. Each author emphasizes the diversity of the nerves of the ENS from morphological, chemical point of view, more exactly, in respect to their transmitter content. The descriptive studies on the fine structure of the ENS are mostly related to mammalian species (BAUMGARTEN et al., 1970; GUNN, 1968; KOMURO et al., 1982; WILSON et al., 1981), but recently similar studies have been performed in vertebrates of lower order, among them in a few fish species as well. BAUMGARTEN et al. (1973) demonstrated different monoamine-like transmitters in the gut of *Lampetra fluviatilis* with formaldehyde-induced fluorescence, and apart from this, they also gave fine structural description of the intestinal plexuses. Studying the nerve elements of the gut muscle in chondrostean fish, SALIMOVA and FEHÉR (1982) observed the presence of serotonin, dopamine and noradrenaline by histochemical and microspectrophotometric method and they also found nerve fibres of various type and different vesicle content by electron microscope. Histochemical data on teleosts are known on the basis of studies by BAUMGARTEN

(1967) on tench, and by WATSON (1979) on short-spined cottus, plaice and herring. The present paper reports on the results of ultrastructural studies on the enteric nervous system of the lacustrian carp, a teleost species which is general and has great economic significance in Hungary.

Materials and Methods

Studies were performed on mature male and female individuals of lacustrian carp *(Cyprinus carpio* L.*)*, obtained from the Fish-breeding Research Institute in Szarvas. The fish were stunned by blows, the abdominal cavity opened and 1 mm^3 pieces cut with razor blade from the midgut. The tissue samples were prefixed in cold state in 3% glutaraldehyde (pH 7.3) buffered with cacodylate for 4 hours. This was followed by 5 min. washing in cacodylate buffer containing 7.5% saccharose, and then the specimens were postfixed in the mixture of 4% osmium tetroxide: cacodylate buffer in the ratio 1:1 (pH 7.5) for 2 hours. In the course of dehydration in ascending alcohol series the material was contrasted for 30 min. in dark with saturated uranyl acetate dissolved in 75% alcohol. The samples were embedded in Durcupan ACM, and the prepared sections were postcontrasted with lead citrate. The sections were studied and photos were made on JEOL 100 B and TESLA BS 500 electronmicroscope.

Results

The rich plexuses of the nerve fibres of the small intestine in lacustrian carp were found in the connective tissue interstitium between the circular and longitudinal smooth muscle layers. The smooth muscle cells were rather irregular and had various shape. The cell nucleus relatively rarely fell to the plane of the section (Figs. 2 and 5). Both surface of the muscle cells and the cell nucleus were strongly indented. Variously wide, mostly indented intercellular spaces developed among the muscle cells (Figs. 1 and 2). Generally abundant collagen fibre substance could be observed in the intercellular spaces (Figs. 1 and 9), nevertheless, areas without fibres also occurred at places. At certain points the processes of the smooth muscles were found in close contact with each other (Figs. 3 and 6). The sarcoplasma was rich in myofilaments, but poor in mitochondria and other cell organelles. Depending on the plane of the section various amounts of so-called dense bodies (Z fragments) were striking due to their high electron density (Fig. 4). The marginal, myofilament-free parts of the sarcoplasma contained large amount of endocytotic vesicles (Figs. 2 and 4). Various amount of nerve fibre-cross sections were observable in the myenteric plexus among the smooth muscles (Figs. 1, 4). The nerve fibres were frequently covered by glial cell processes (Fig. 1), however, high amount of nerve fibres were also found without glial processes and the axolemmas were attached directly to each other (Figs. 1, 2 and 4). Large amount of parallelly-organized filament bundles occurred in the cytoplasm of some glial cell processes (Fig. 4). No transmitter-storing vesicles were found in the majority of the nerve fibres arranged in large bundles, however, varying amounts of agranular and granular vesicles were observable in the preterminal axons (Figs. 1, 2 and 4). Agranular vesicles were relatively few in the axon profiles, they occurred sporadically as independent (individual) axonprofiles, and were generally located in the neighbourhood of small and large dense-core vesicles. Their cross section was mostly pleomorph, their average diameter was 40 nm. Small and large dense-core vesicles were observed most frequently in the free axon terminals. The average diameter of these were 60 and 100 nm. The nerve fibres contained large

Fig. 1. Detail of myenteric plexus in the smooth muscle of carp gut. Mf=smooth muscle, N=axon profiles in the plexus, T=terminals, Co=collagen fibres, G=glial cell process, M=mitochondrium. ×15 000

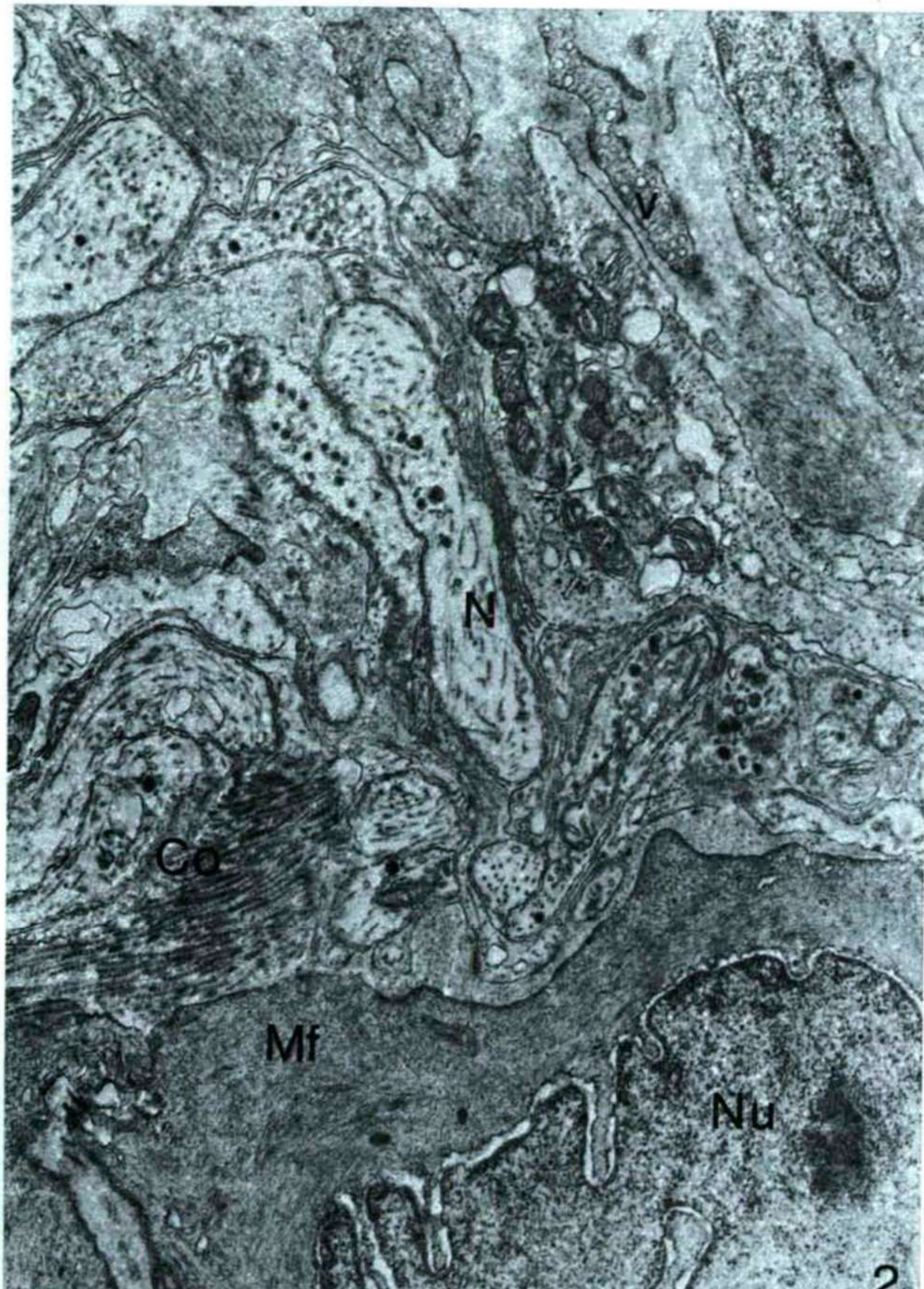

Fig. 2. Great amount of nerve elements (N) can be seen in the intercellular space rich in collagen (Co) between the smooth muscle cells (Mf) of carp gut. The nucleus (Nu) of the smooth muscle cells is of varying shape. Vesicles (v) referring to intensive endocytotic activity are observable on the sarcolemma. ×18 000

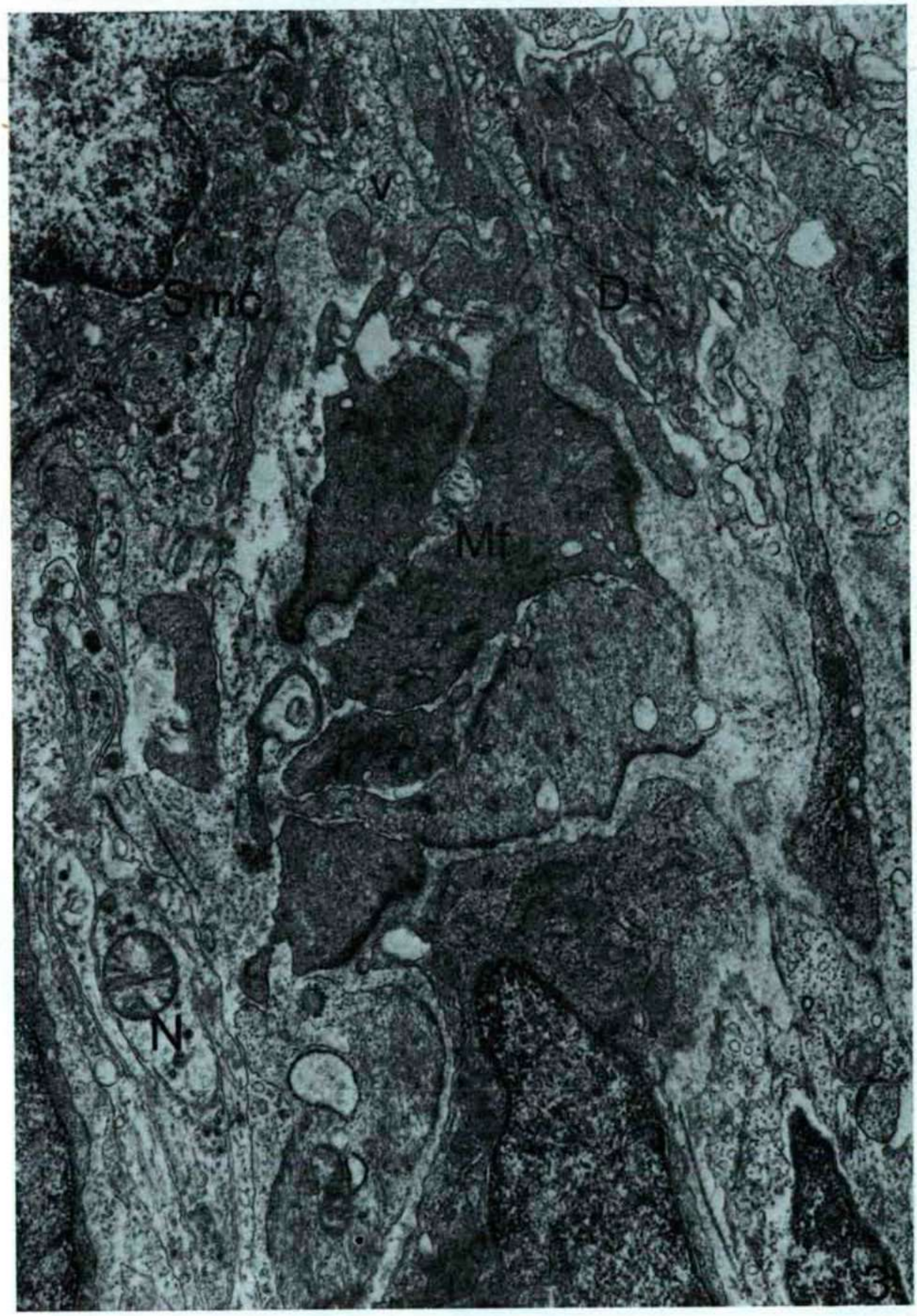

Fig. 3. Longitudinal nerve fibres (N) and cross-section of muscle cells originating from various depth: Mf=apical detail rich in myofilament, Smc=plane of intersection with nucleus rich in cell organelles. Rather large amount of endocytotic vesicles (v) can be seen in certain processes. D=desmosomes. ×18 000

Fig. 4. Nerve bundle (Nb) in carp gut. Varying vesicle populations can be observed in certain axon profiles. The glial cell process (G) contains large number of filaments arranged in bundles. Co = collagen fibres, v = endocytotic vesicles, F = supporting sheath. ×18 000

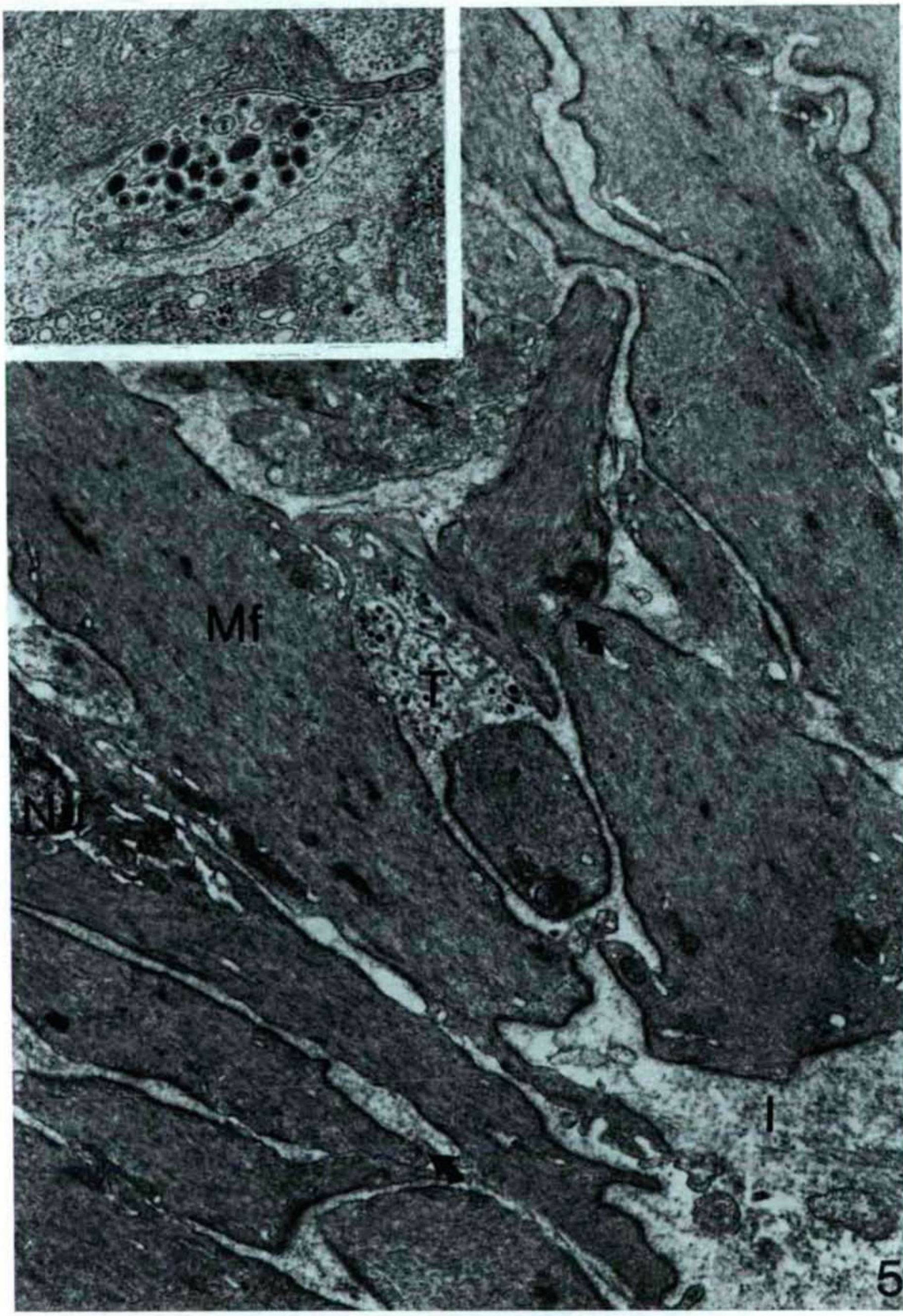

Fig. 5. Cross-section of axon profiles (T) in the direct neighbourhood of the muscle fibres (Mf). The arrow show the desmosome-like junctions of the smooth muscle cells. I=interstitium, Nu=nucleus. ×15 000
Insert: terminal, rich in dense-core vesicles at high magnification. ×40 000

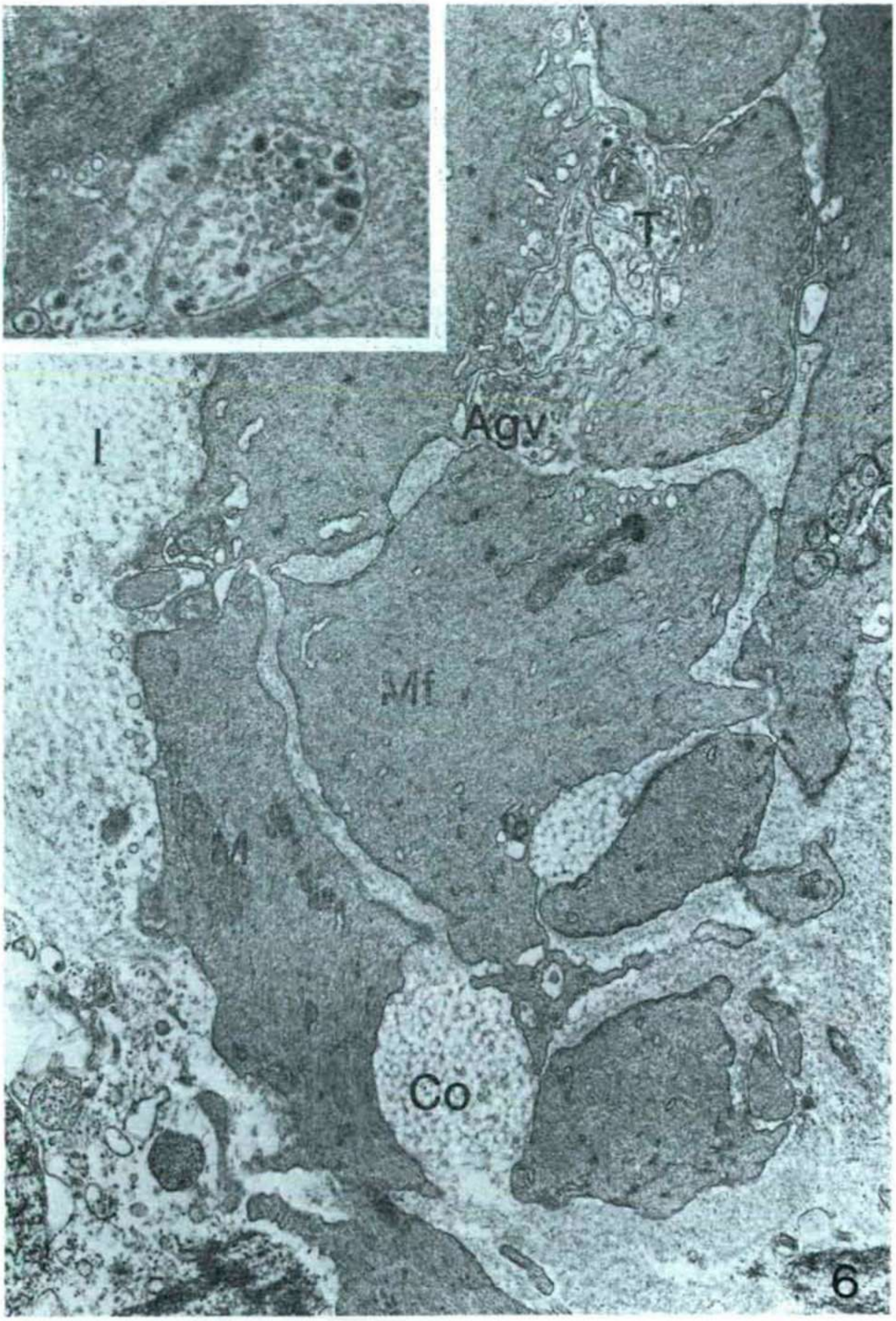

Fig. 6. Nerve fibres (T) in tight contact with the smooth muscle cells of the small intestine in carp. Mainly agranular vesicles (Agv) are situated in certain terminals. Mf=smooth muscle cell, I=interstitium, M=mitochondrium, Co=collagen fields. ×18 000
Insert: the above described terminal type at higher magnification. ×40 000

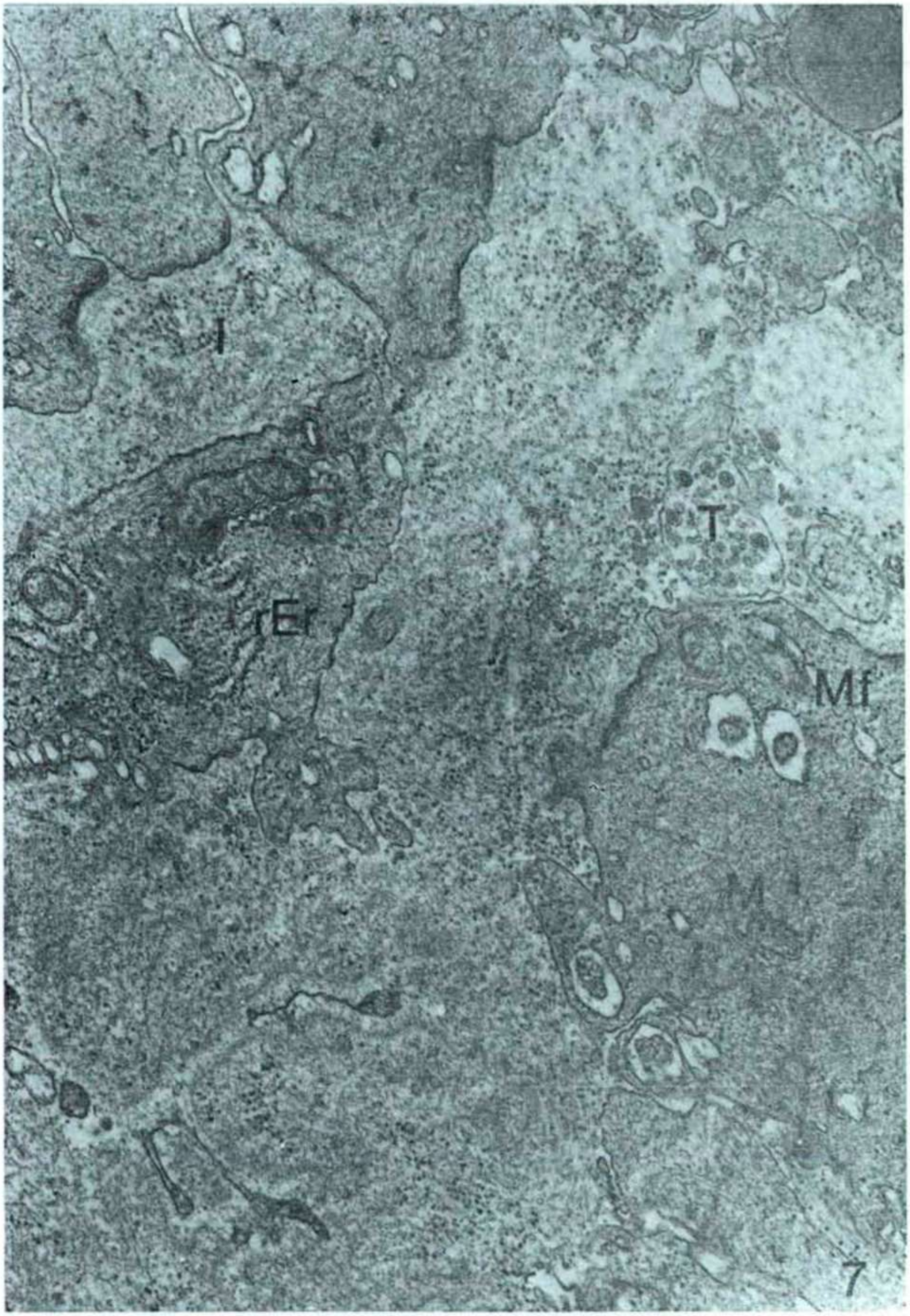

Fig. 7. Axon terminal (T) with dense-core and clear vesicles in the direct neighbourhood of a smooth muscle cell (Mf). The lack of glial covering is obvious on the surface of the axolemma. I=interstitium, rEr=rough surfaced endoplasmic reticulum cisternae, M=mitochondrium. ×25 000

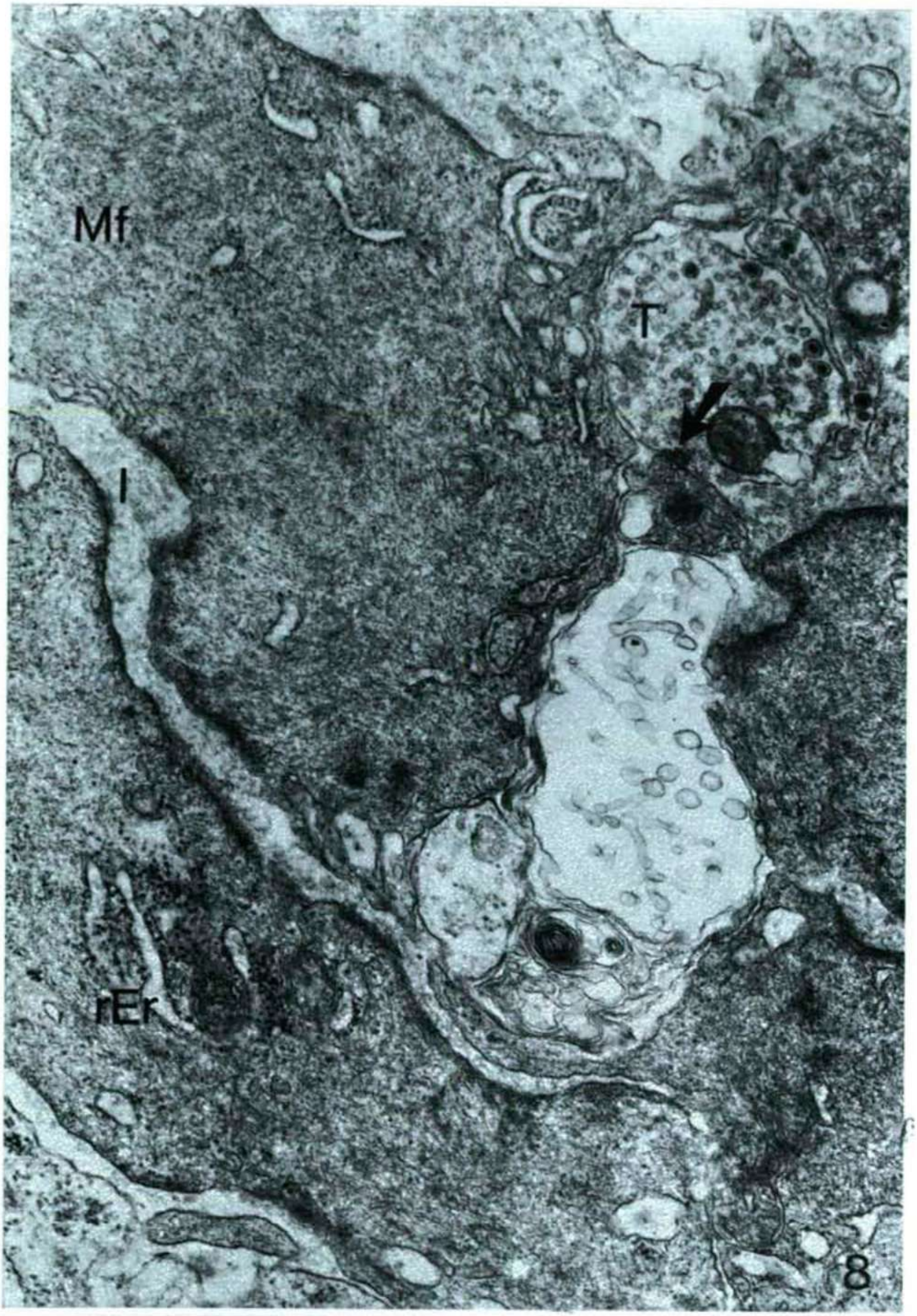

Fig. 8. The axon terminal (T) lying near the cell process of the smooth muscle contains granular and agranular vesicles. The presynaptic thickening refers to the synapse-like contact (arrow). Mf = smooth muscle, I = interstitium, rEr = rough surfaced endoplasmic reticulum. ×28 000

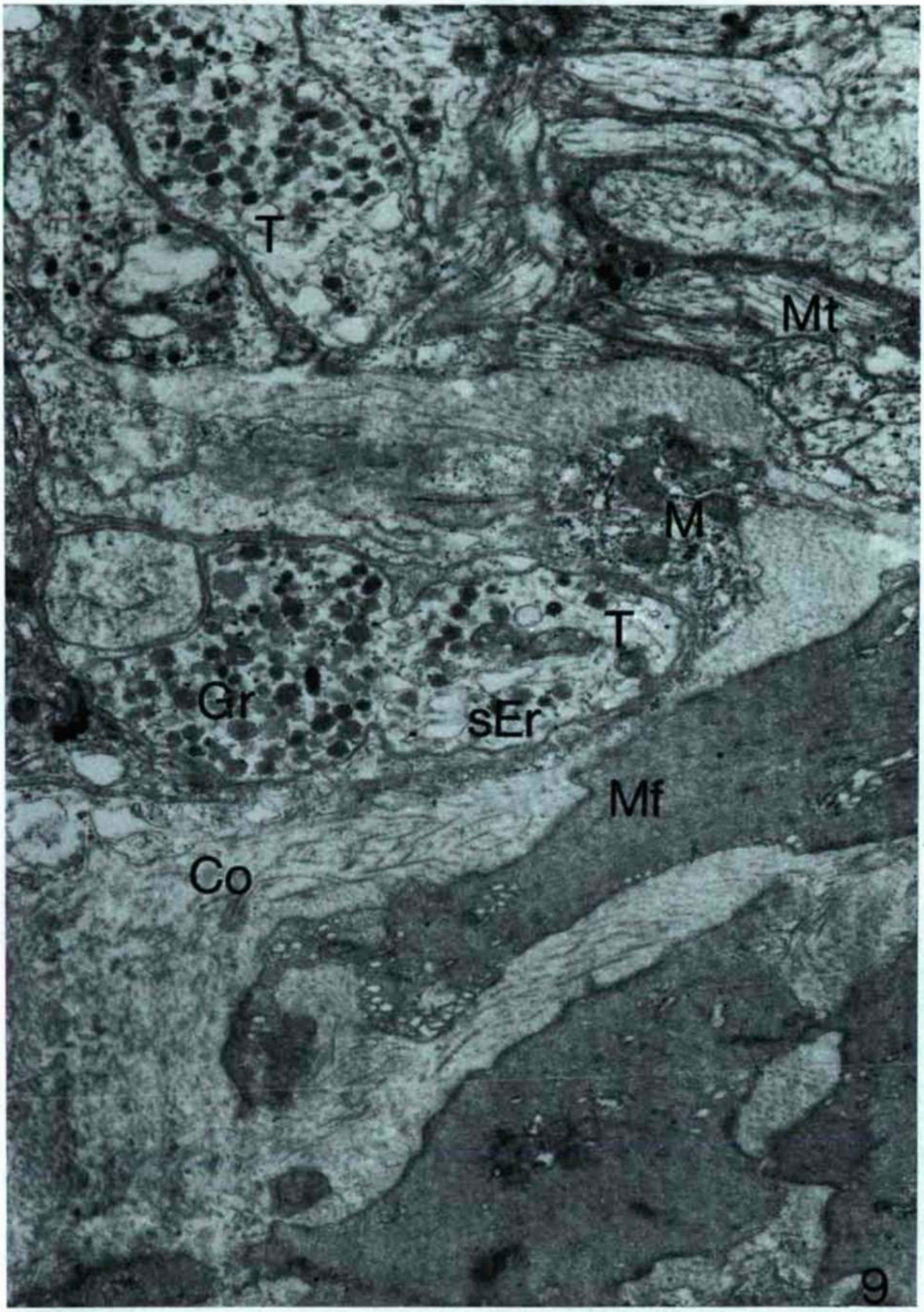

Fig. 9. Nerve fibres (T) filled with granules (Gr) of varying electron density are also found near the smooth muscle cells (Mf). Co=collagen fibres, M=mitochondrium, Mt=microtubuli, sEr=smooth surfaced endoplasmic reticulum. ×16 000

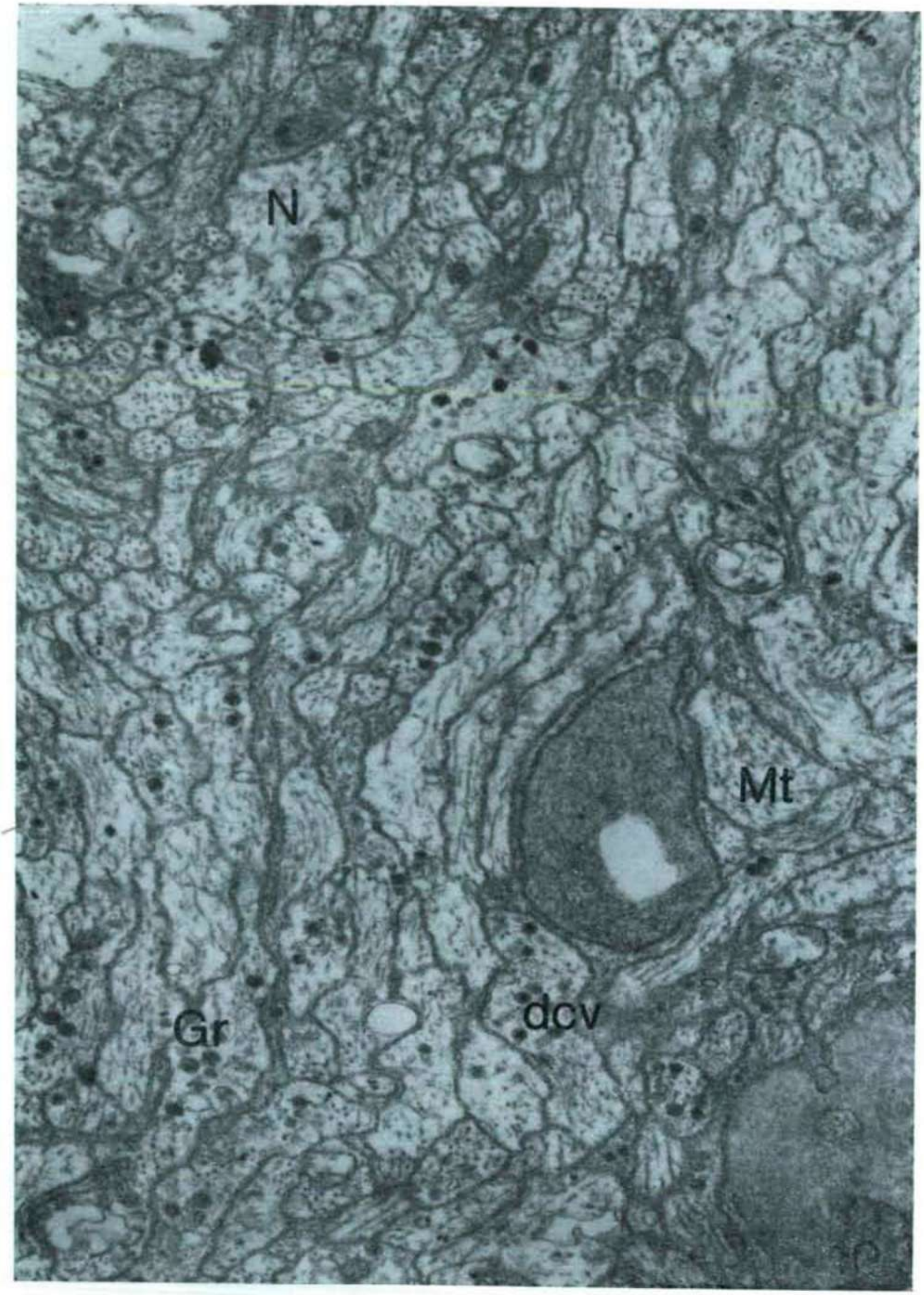

Fig. 10. Compact neuropil-detail from the myenteric plexus of carp. N = nerve fibre, Mt = microtubule, dcv = dense-core vesicles, Gr = neurosecretory granules. ×15 000

amount of microtubuli and neurofilaments. Synaptic junction was not observable despite the fact that the axolemma of certain axons of the nerve fibre bundles were frequently found in tight morphological contact.

In respect to the nerve-muscle relationship, the presence of a few (1—10) axon profiles was also often observed in the wide extracellular space of several muscle cells. Here, the membrane of several axons was found directly besides the sarcolemma of the smooth muscle. (Figs. 5 and 6).

Since in the majority of the axon profiles agranular and granular vesicles were present, it is presumable that the majority of these correspond to axon terminals (Figs. 5 and 7). Axon terminals with presynaptic membrane thickening and vesicle-accumulation referring to neuromuscular junction were very rarely observed (Fig. 8). Here, the postsynaptic membrane thickening of the sarcolemma was only of slight degree.

Apart from the axon types described so far, preterminal axons with granules of neurosecretory type were also seen at certain areas of the myenteric plexus (Fig. 9). The most obvious characteristic of these granules was the varying electron density: completely electron-lucent grains occurred besides rather electron dense ones. The large average diameter (200 nm) of the granules was also striking. The majority of the granules were rounded, some of them were of somewhat irregular shape. The limiting membrane was generally observable as a well distinguishable sharp line, seeming to be slightly indented at places. In this terminal vesicles of agranular type were only found in small number in the neighbourhood of the neurosecretory granules. Microtubule cross sections and a few smooth surfaced endoplasmic reticulum vacuoles were also found in the axon terminals apart from a few mitochondria. On the basis of their fine structural characteristics these granules presumably contain neuropeptides. These axon terminals which could be regarded as peptidergic, are rather close to the sarcolemma of the smooth muscle cells, however, their tight connection haven't been observed yet. Large, compact neuropil areas were frequently found in the myenteric plexus, in which rather various axon profiles were detectable (Fig. 10). The presence of microtubuli and microfilaments was striking in the majority of these profiles, but smaller-larger amounts of granules were also observed in some cases. The majority of these were dense-core vesicles, the smaller part was of neurosecretory type. Intercalary nerve terminals were not found in the extensive neuropil.

Discussion

Reviewing our neurohistological knowledge on the gastrointestinal system it can be determined that the innervation of the mammalian gut muscle is the most clarified (Baumgarten et al., 1970; Burnstock, 1983; Furness et al., 1980; Gabella, 1979; Gordon—Weeks, 1982; Gunn, 1968; Komuro et al., 1982; Wilson et al., 1981). Relatively large amount of data are at disposal in respect to the gastrointestinal tract of insects (Anderson et al., 1977, 1978; Benedeczky et al., 1982) and certain molluscs, too (Fritsch et al., 1977; Halasy et al., 1983; Heyer et al., 1973; Mercer et al., 1981). At the same time, fine structural data are rather rare regarding fish — representing an important developmental level in evolution.

All these justify the importance of studies carried out on fish. It is also surprising that light microscopic studies were mostly performed only from the beginning of the century (BACKMAN, 1917; BOEKE, 1935; CHEVREL, 1893, 1894; LUTZ 1931; PATTERSON et al., 1933; STANNIUS, 1849). The reason for our electronmicroscopic studies was that detailed observations are already at disposal in respect to snail *(Helix pomatia)*, and insects, namely *Periplaneta americana* and *Locusta migratoria* (BENEDECZKY et al., 1982; HALASY et al., 1983). The aim was to clarify the innervation of the gastrointestinal tract of fish, being an important link in evolution.

From the results it seems worthy to emphasize that regarding the anatomical and fine structural features the most important characteristics in teleosts are very similar to those of mammals. It could be determined that the submucous plexus is still relatively undeveloped (WATSON, 1979), while the myenteric plexus is well developed. Both the circular and longitudinal muscle layers are richly innervated, smaller-larger amounts of nerve fibres or nerve bundles occur in almost every plane of section. It is also noteworthy that neurons cannot be found with such frequency as e.g. in the snail gut muscle, where aminergic as well as peptidergic cells can be found in high number (HALASY et al., 1983). This refers to the fact that the local neurons are not so significant as in invertebrates. It is also known that few giant cells of the neurosecretory type have mainly been found only in the hindgut of insect, (REINECKE et al., 1978). On the basis of studies performed on mammals so far (BURNSTOCK, 1983; GABELLA, 1979; KOMURO et al., 1982) it seems that at least 8—10 morphologically various types of nerve terminals can be found in the ENS. Regarding the histtochemical and fine structural studies carried out until now, it cannot be stated thathese 8—10 axon types represent the same number of neurotransmitters. At the same time the chemical, pharmacological and physiological studies (BURNSTOCK, 1983; FURNESS et al., 1980) make the role of at least 16 transmitter-candidates probable in the ENS as well. It must be certain that among the monoamines noradrenaline, dopamine and serotonin play role as mediators (BAUMGARTEN et al., 1970; FURNESS et al., 1980). The physiological role of acetylcholine is also rather unambiguously verified and newer data are also at disposal in respect to the occurrence of purinergic innervation (BURNSTOCK, 1983). A large number of mainly morphological and histochemical data supports the direct role of certain oligopeptides in the regulation of the intestinal activity; so far there are mainly morphological data on the substance-P, VIP, and the possible role of somatostatins and encephalines in mammals (BURNSTOCK, 1983). The estimation of these morphological observations is hindered by the circumstance that the regular physiological and pharmacological study of above peptides has not been performed yet. Accordingly, there is a lack in those most important physiological criteria on the basis of which the peptidergic regulation could be regarded to be proved. Our own studies on fish also seem to verify the considerations presuming multifactoral nerve regulation in mammals. The various types of vesicles were also found by us in the axon terminals of the fish gut muscle (agranular and granular, peptidergic). The role of aminnergic innervation has been supported by many authors, mainly with fluorescence, chromatographic and other studies (BAUMGARTEN, 1967; BAUMGARTEN et al., 1973; SALIMOVA et al., 1982; SANTER, 1977; WATSON, 1979). During the course of our biochemical studies under progress, so far we have been demonstrated the presence of noradrenaline, adrenaline and dopamine in the gut of carp (NEMCSÓK et al., unpublished data). On the basis of these chemical measurements as well as on the above mentioned literary data it can be unambiguously determined that each of the listed biogenic monoamines may play role as neurotransmitter or neurohormone in the gut muscle. It is more difficult to judge the role of the neuropeptides. Even histochemical studies have only occasionally been performed in this field. On the basis of the results obtained on insects (BROWN, 1975; STARRAT et al., 1980) and mammals (BURNSTOCK, 1983; FURNESS et al., 1980) so far, furthermore, concerning the occurrence of peptidergic axon profiles found by us in lacustrian carp, it is likely that the neuropeptides may influence the function of the intestinal muscles in fish as well.

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