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Ultrastructural characteristics of anterior gut innervation of *Gallus gallus*

J. Aisa, P. Parra and M.J. Azanza
Department of Morphology, School of Medicine, University of Zaragoza, Zaragoza,Spain

Summary. The enteric nervous system of the bird's anterior gut is very well developed. Myelin fibres are seen accompanying the nervous trunks up to the mucous layer. Glial cells duplicate the number of neurons in the myenteric plexuses. Their number decreases at the submucous plexuses, but it is always higher than the neurons. Isolated neurons are widely spread in the circular muscle coat accompanying the nervous trunks which can be inter and intrafascicularly located. Direct synaptic contacts with the soma neuronal membranes are very often seen. We have never observed synaptic specializations. The most prominent varicosities either in the peripheric nervous trunk axons or directly laying on the soma membranes are those containing peptidergic or mixed vesicles of cholinergic and peptidergic types. The neurons show big nuclei of different size and shape. Neighbouring smooth muscle cells show abundant caveolae near the nervous elements. Although we have not observed close contacts with glands, thin axon bundles spread near the glandular cells of the mucous layer.

Key words: *Gallus gallus*, Anterior gut, Cholinergic and peptidergic vesicles, Autonomic innervation

Introduction

We have described elsewhere the intrinsic innervation of bird anterior gut: esophagus, crop, proventriculus and gizzard (Aisa, 1986; Aisa et al., 1987a); the postnatal development of proventriculus and gizzard enteric nervous system (Aisa et al., 1987b); and the postnatal development of the esophagus and crop innervation (Aisa et al., 1988). Very few ultrastructural studies have been undertaken to elucidate the fine organization of bird enteric system. The only studies dealing with bird anterior gut ultrastructure are those from Bennet and Cobb (1969) describing the nature of synaptic contacts on cell somas or on their processes in gizzard ganglion cells. Young has described in great detail the catecholaminergic varicosities in the intestine (Young, 1983) and rectum (Young, 1989). The aim of this work was then to extend our research at optic level with the description of the general ultrastructural characteristics of bird anterior gut.

Offprint requests to: Dra. J. Aisa, Cátedra de Biología, Departamento de Ciencias Morfológicas, Facultad de Medicina, C./ Domingo Miral s/n, 50009 Zaragoza, Spain

Materials and methods

Twenty adult chickens were used in this study. The standard method for electron microscopy was applied; fixation in 2.5% glutaraldehyde in Milloning buffer (pH 7.3), post-fixation in 2% OsO_4, stained with 70% uranyl acetate, dehydrated and embedded in Araldyte. The ultrathin sections were contrast stained following the conventional methods.

Results

Neurons and nerves were abundantly distributed through the whole of bird anterior gastrointestinal wall.

The muscularis externa was crossed by thick nervous trunks where preganglionic myelin fibres were mixed with unmyelinated axon fascicules and located at the periphery of the trunks. Myelin fibres were seen reaching the mucosa. Nerve fascicules were accompanied by Schwann cells and surrounded by collagen fibres. Myenteric plexuses were typically located between the two outer muscle coats (Fig. 1). As an exception we might mention the gizzard where the ganglia lay on the surface of the circular muscle coat, immediately below the serosa, since throughout the development the outer longitudinal muscle coat was lost. Glial cells duplicated the number of neurons in the myenteric plexuses. At the level of the submucous plexus the number of glial cells decreased but it was always larger than the neurons.

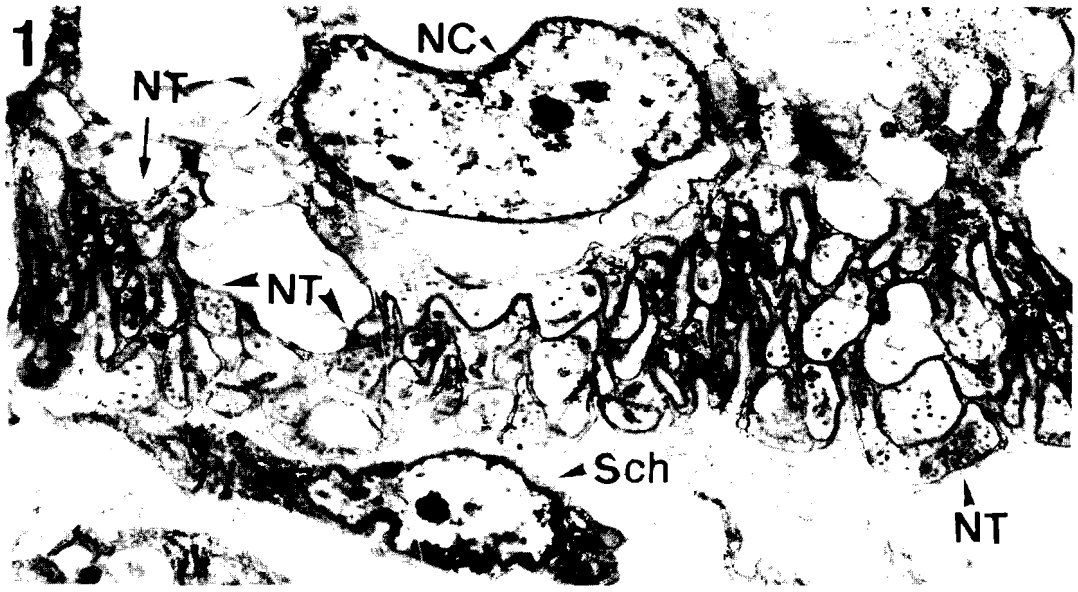

Fig. 1. Crop. Nervous trunk (NT) between the external muscle coats: myenteric plexus. Neuron (NC) and Schwann cell (Sch). × 4,500

Fig. 2. Esophagus. Inter (↟↟) and intrafascicular (↟) nervous trunks (NT) in the circular muscle coat. They are accompanied by nervous cells (NC) and Schwan cells (Sch). Peptidergic varicosities (p) are prevailing. The smooth muscle cells show typical caveolae. × 2,500, × 4,500, × 22,000

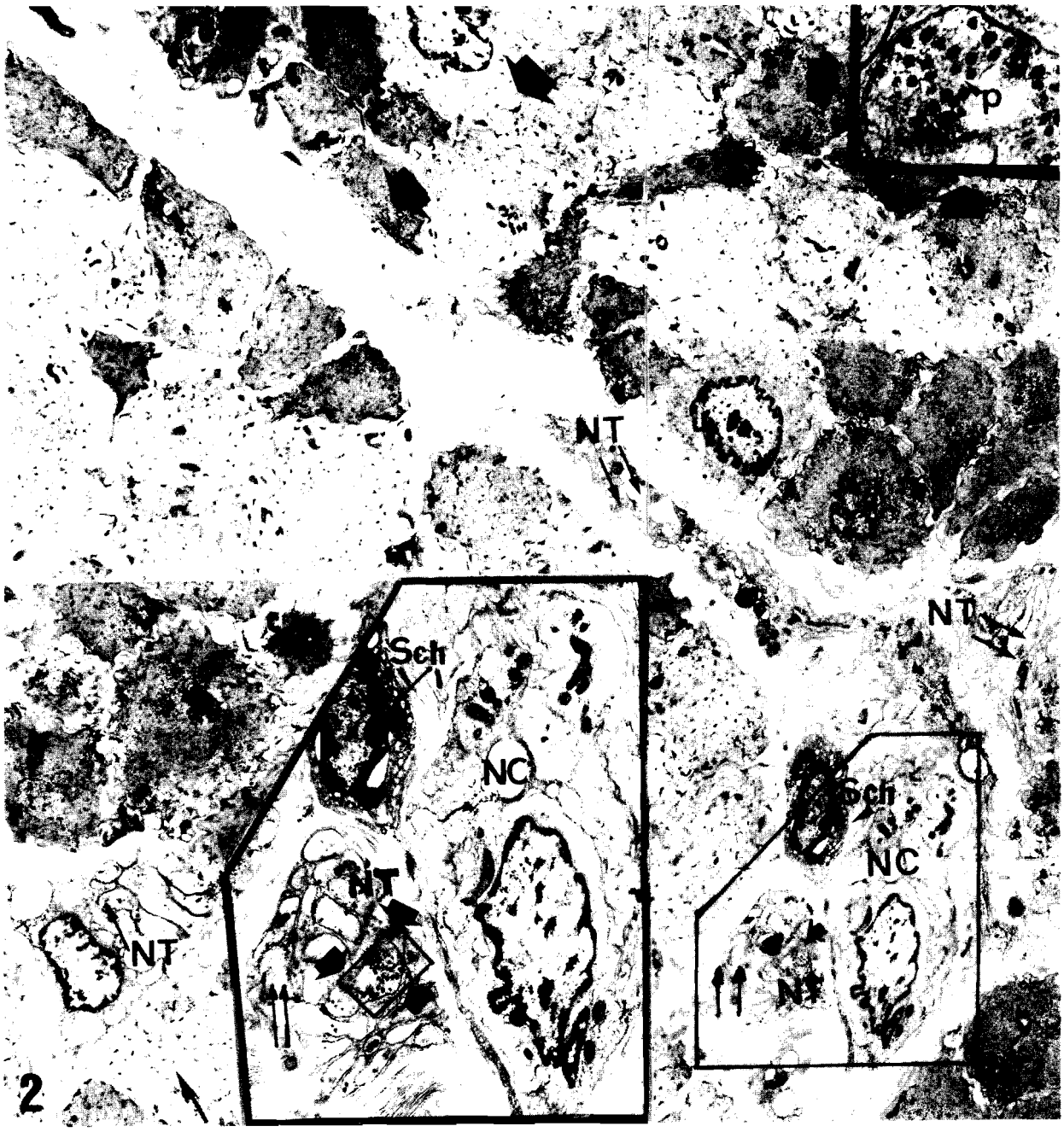

Fig. 3. Esophagus. Nervous trunk (NT) in the submucous layer. Varicosities with peptidergic vesicles (p) are shown. × 22,000

At the gut levels, where the muscle coats are thick enough, nervous trunks accompanied by isolated neurons were located in the connective tissue among the main muscle fascicules (interfascicular trunks) and inside the muscle fascicules (intrafascicular trunks) (Fig. 2).

As the nervous trunks traversed the gut wall they became thinner since they gave branches to the different histological structures they innervate. At the level of the submucous (Fig. 3) and near the muscularis mucosae the nervous bundles were of a medium caliber. Although we did not find close contacts with glandular cells we observed thin axon bundles near the glands (Fig. 4).

Most perypheric axons of the nervous trunks showed varicosities with different types of vesicles: peptidergic (Figs. 2, 3, 5), cholinergic (Fig. 5) and mixed (Figs. 4, 6) being mostly of cholinergic and peptidergic types. Since we did not apply specific ultrastructural methods for catecholamines, we observed very few noradrenergic vesicles.

The neurons showed large nuclei with different shapes: irregular (Figs. 1, 2, 5, 6), almost round (Fig. 7) or oval (Fig. 8) with patent marginal chromatin. They were surrounded by collagen fibres (Fig. 7) or axon varicosities which lay in close contact with the neuronal membranes (Figs. 2, 5, 6, 8). We did not find synaptic membrane specializations either at the level of presynaptic or on postsynaptic membranes.

It is worth noting that neighbouring smooth muscle cells showed abundant typical caveolae that always appeared near the nervous elements constituting a constant characteristic (Fig. 2).

Discussion

The electron microscope images we obtained confirm our results at optic level (Aisa et al., 1987a,b, 1988). It is worth noting the presence of isolated neurons in the circular musculature. These neurons seem to be a prolongation of the myenteric plexus in those portions of the gut where the circular muscle coat is particularly thick, such as the esophagus and gizzard. It has been shown that in human embryonic development some neurons migrate from the myenteric plexus to the submucosa to form the Meissner plexus. In those levels of the gut where the muscle coat is very thick the observed neurons could mediate the bioelectric transmission between Auerbach and Meissner plexuses.

We have observed several types of neurons according to their nuclei shape in the bird gut. The question of neuronal cell types in mammal enteric plexuses is still controversial. Observations on the variability of the neuronal morphology and tentative classifications have been widely described (Gabella, 1971a; Cobb and Burnstock, 1976; Yamamoto, 1977). Because of their position between the muscle layers the ganglion neurons are greatly affected by the mechanical action of the musculature (Gabella, 1982). It has also been suggested that different size and shape could reflect functional differences.

We have observed that the cells located at the submucous plexus are smaller and more heterogeneous in size than those from the myenteric plexus. Similar results have been obtained for mammals (Ohkubo, 1967;

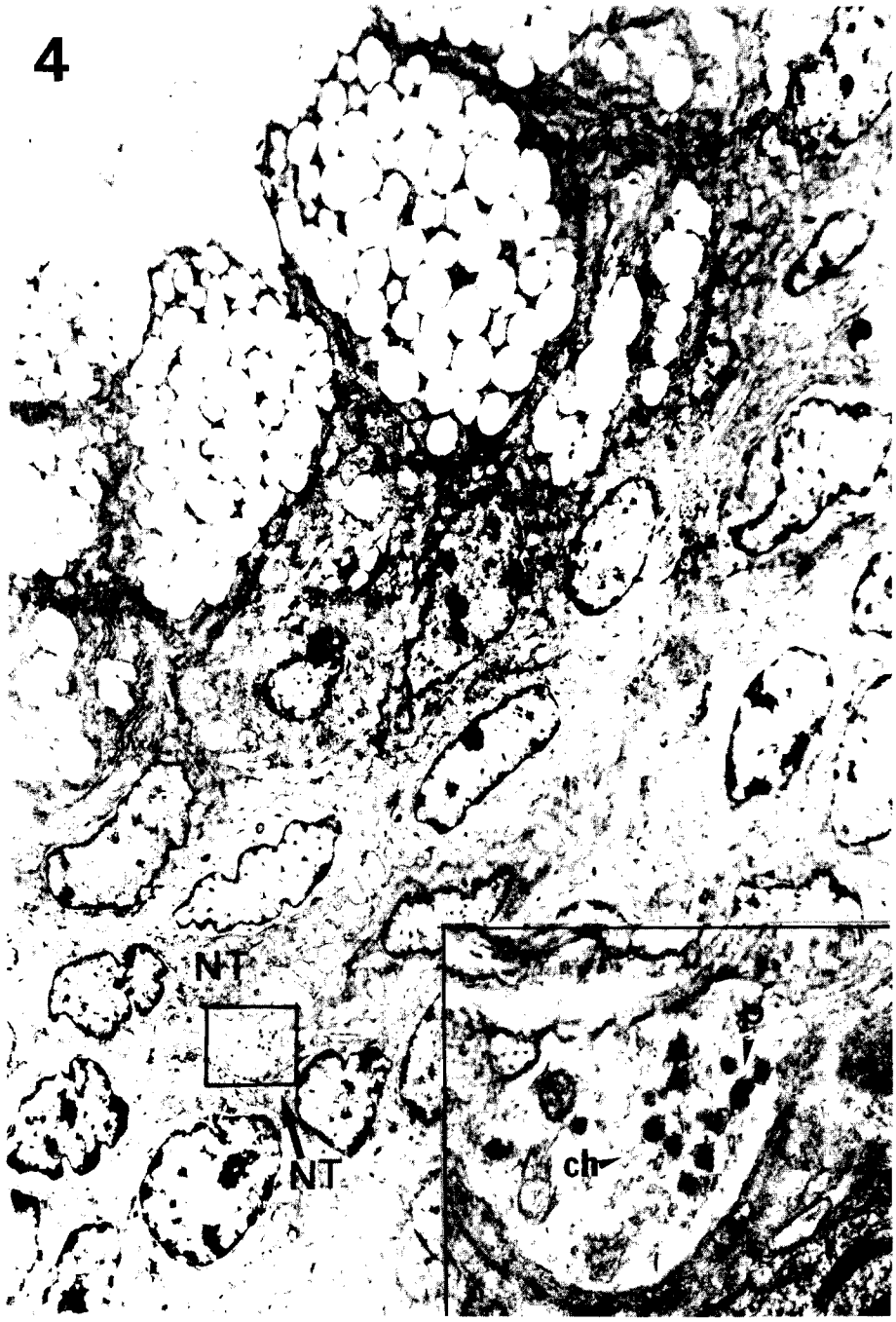

Fig. 4. Esophagus. Nervous trunk (N) in the mucous layer. Mixed varicosities with cholinergic (ch) and peptidergic vesicles (p) are abundant. Thin axon bundles are distributed near the glands. × 3,800, × 19,000

Gunn, 1968). We have not observed neurons in the gizzard submucosa, so this description coincides with that from Bennet (1969).

Glial cells of the intramural ganglia, called «enteric glial cells» by Gabella (1971b, 1981), are readily identified on the basis of their shape, position and ultrastructure. Glial cells are smaller in size and lack the large expansions of cytoplasm which are common in neurons. Some glial cells are spread over the surface of neuronal perikarya but they do not form a complete sheet around them. An important role for glial cells is related to the fact that during mechanical activity the ganglia are exposed to very intense mechanical stress and glial cells are considered to be important in the structural rearrangement of the ganglia (Gabella, 1981).

Blood vessels do not penetrate into the enteric ganglia and it has been reported that there is a blood myenteric plexus barrier similar to the blood brain barrier (Gershon and Bursztajn, 1978). We have not applied specific ultrastructural methods for catecholamines, but at optic level we have shown that the noradrenergic contacts are very rich on blood vessel

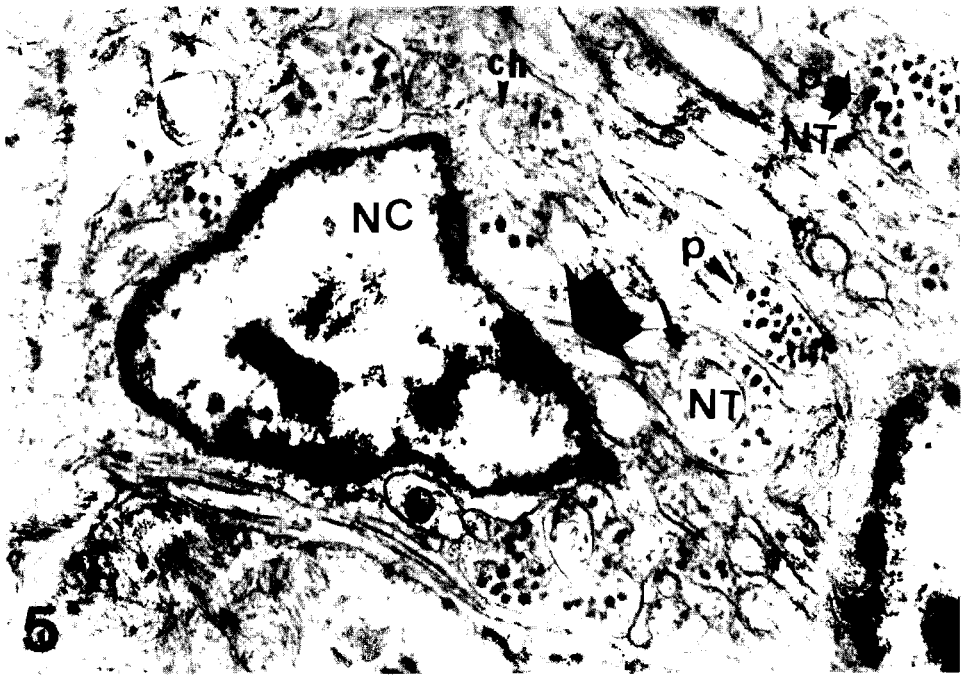

Fig. 5. Proventriculus. Trunk axons (NT) in the circular muscle coat surrounding a neuron (NC) with irregular nucleus, rich in euchromatin. Cholinergic (ch) and peptidergic varicosities (p) are disposed in close contact with the neuronal membrane. × 1,900

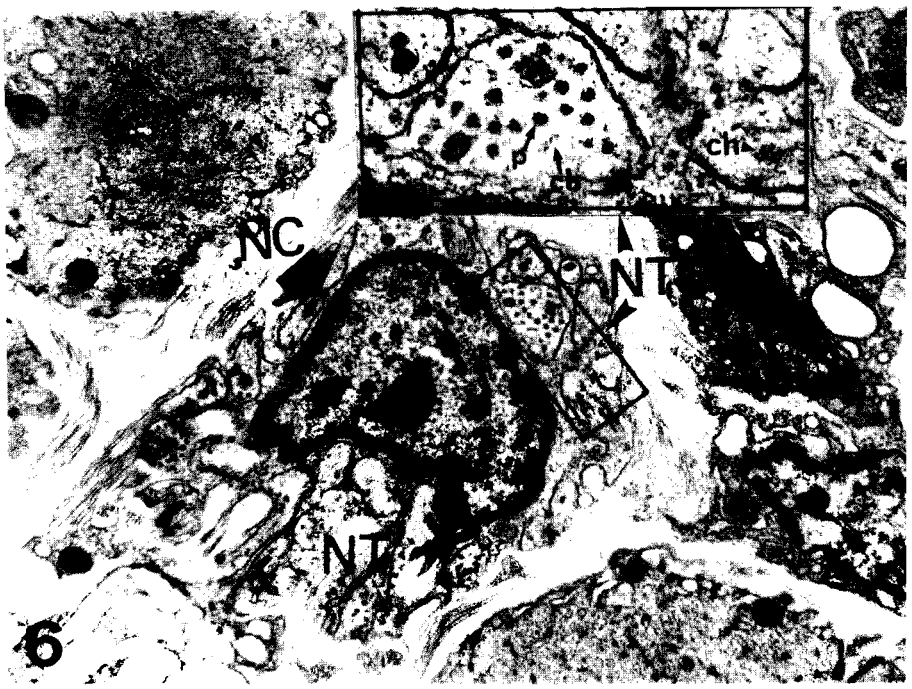

Fig. 6. Esophagus. Neuron (NC) with irregular nucleus in the middle of a nervous trunk (NT) with mixed varicosities: cholinergic (ch) and peptidergic (p) in close contact with the neuronal membrane. × 5,700, × 15,000

walls, are more scarce on myenteric ganglions, and are scarcely spread in the base of the glands. We have furthermore shown at optic level that the noradrenergic plexus distribution pattern is not as continuous as that for cholinergic or even for peptidergic plexuses (Aisa, 1976; Aisa et al., 1988).

The most abundant nerve profiles are those classified as containing small agranular vesicles (AGV) which resemble the structure of established cholinergic nerve terminals. Very common also are those profiles which belong to the large opaque vesicles (LOV), which are considered to contain peptides (Burnstock, 1981; Komura et al., 1982). Our results with optic microscopy show that peptidergic innervation is very rich in bird anterior gut (Aisa et al., 1987a,b, 1988). Very abundant also are the mixed varicosities with vesicles of cholinergic and peptidergic types. The noradrenergic-containing vesicles (SGV type) are relatively scarce in the

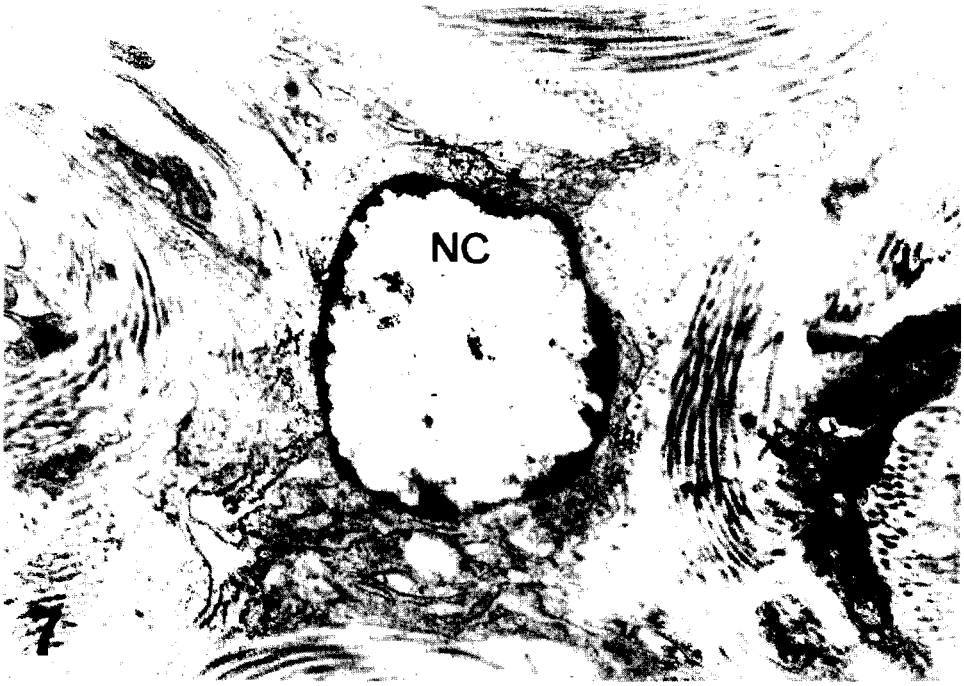

Fig. 7. Esophagus. Neuron (NC) in the submucous layer surrounded by collagen fibres. The nucleus is almost round, rich in euchromatin with patent marginal chromatin. × 5,700

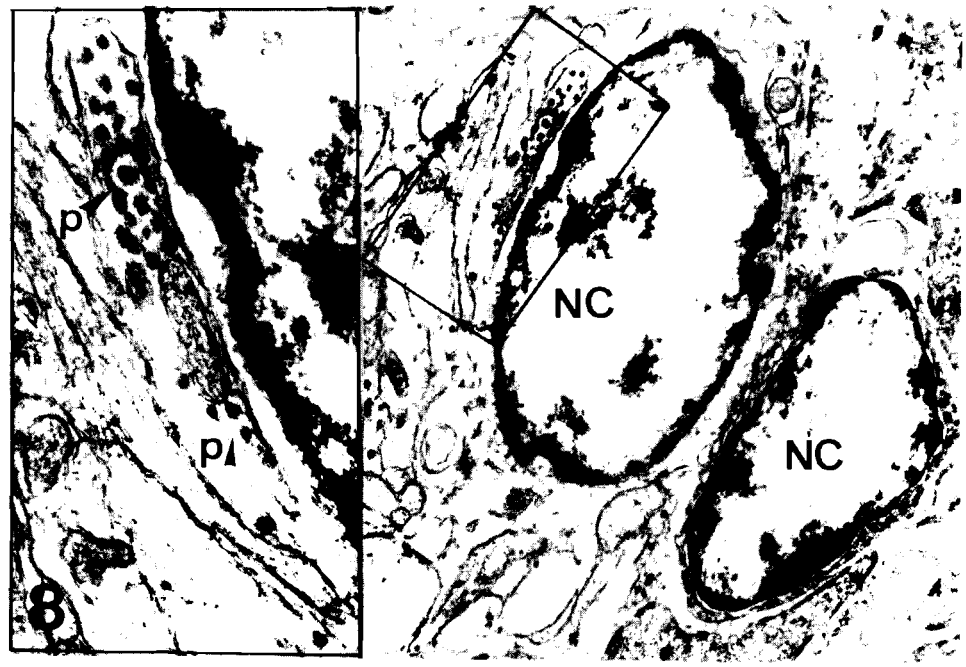

Fig. 8. Crop. Neurons (NC) with oval nuclei, patent marginal chromatin and peptidergic varicosities (p) lying on the neuronal membrane. × 14,200, × 30,000

intestine, according to the Young's descriptions (1983), and also with our results about the noradrenergic innervation distribution at optic level (Aisa et al., 1987a,b, 1988).

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