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CELL TYPES OF THE ENTERIC NERVE PLEXUSES IN THE CHICKEN (*GALLUS DOMESTICUS* L.)

M. Csoknya and I. Benedeczky

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Abstract

The innervation of the esophagus and small intestine was studied with impregnation method. The myenteric plexus located between the concentric and longitudinal smooth muscle layer of the tunica muscularis was found to be well developed and of reticular arrangement in both enteric regions. The Meissner plexus in the tunica submucosa was particularly poor in cells and fibers in the esophagus.

On the basis of affinity to silver salts the neurons of the enteric nerve plexuses could be argentophilic and argentophobic. Both cell types occurred in the small intestinal plexuses, mainly argentophile cells could be found in the esophagus.

On the basis of the cell processes, uni-, bi- and multipolar cells were found in the plexuses. Besides the known Dogiel-types, a new neuron-type was also described among the multipolar neurons.

On the basis of cell-volume values, small and large cells were observable in the small intestinal plexuses, while only small cells were manifested in the esophagus. The volume of the small cells could be ranked till 1600 μm^3, the large cells could be ranked among the domain above 1600 μm^3 volume value.

Key words: chicken, enteric nerve plexus, neuron types, cell volume

Introduction

Numerous data are at disposal regarding the location, structure and junctions of the nerve plexuses found in the intestinal tract of the vertebrates (Christensen et al. 1983; Furness and Costa, 1980; Gunn, 1959; Kolossow et al. 1932.) However, the types of the neurons having role in the nerve plexuses have not been defined unambiguously.

Dogiel (1896; 1899) was the first to classify the cells of these plexuses on the basis of the number and lenght of their processes. Accordingly, Dogiel I., Dogiel II. and Dogiel III. cell types are known. Later on, others also confirmed this type of classification of the intestinal neurons (Hill, 1927; Kolossow et al. 1932; Stach, 1973; 1982a).

Other classifications of the neurons of the enteric nerve plexuses are also known; thus on the basis of affinity to silver salts argentophile and argentophobe neurons can be differentiated. Attempts were made to find correlation between the argentophile and argentophobe cells and the Dogiel cell-types by Honjin et al. (1959) as well as Rintoul (1959) in the case of mammals, and by Michail and Karamandlidis (1967) in the case of birds. The plasma of Dogiel I. type neurons stains lighter than that of the Dogiel II. type ones, but their nuclei stain rather dark.

In the case of chickens, MICHAIL and KARAMANDLIDIS (1967) found the incidence rate of the argentophile and argentophobe cells to be 1:1.

The classification of neurons following silver salts staining is rather difficult since staining is influenced by many factors. Several authors have studied the relationship between the degree of silver affinity and the cholinesterase activity of the neurons (BENNETT, 1969; GUNN, 1968; LEAMING and CAUNA, 1961). On the basis of their studies these authors determined that the argentophile cells show negative, while the argentophobe cells show positive cholinesterase activity. It is known from the work of BENNETT (1969) that the Dogiel I. type cells have strongly positive cholinesterase activity, while the Dogiel II. types show weak cholinesterase activity.

Several authors have classified the neurons of the plexuses on the basis of their size and $AgNO_3$ staining (FEHÉR and VAJDA, 1972; GABELLA and TRIGG, 1984; GUNN, 1959; 1968; HONJIN et al. 1959). These authors have reported on the comparative data regarding the neurons of either the various gut sections of one species or the same gut sections of various species (mostly mammals).

Only few systematic comparative studies by quantitative morphological methods are at our disposal regarding intestinal innervation, and even these mainly pertain to mammals.

The aim of the present study was to classify the neurons of the enteric nerve plexuses found in two sections of the basis of comparative morphological and morphometric data.

Material and method

Studies were performed on the esophageal and small intestinal (with the exception of the duodenum) segments of young, 3–4 weeks old (of 35–40 g weight) roosters. The nerve plexuses of the intestinal tract were examined on sections stained with BIELSCHOWSKY–GROS–CAUNA impregnation of 15–20 μm. (The sections were prepared parallel with the longitudinal axis of the intestinal tract). The diameters of the cells perpendicular to each other were studied by ocular micrometer besides 2000x magnification. Only those cells were measured in which the nuclei were observable. The calculations from the measured data (volume and excentricity values) were done according to PALKOVITS (1962; 1968). 600–600 cells were measured from each plexus.

Results

The myenteric plexus was situated in the connective tissue separating the tunica muscularis layers of the intestinal tract. It was characteristic of its structure that neuron groups were located at the branchings of the large nerve fiber bundles, which could even stand of 10–15 cells in the esophagus, and of 30–35 in the small intestine (Plate I., figs. 1,2,3). The thick bundles became all thinner by means of gradual branchings. Ganglia containing few number of small cells could sometimes be seen along the smaller bundles as well. This latter one was more characteristic to the esophagus.

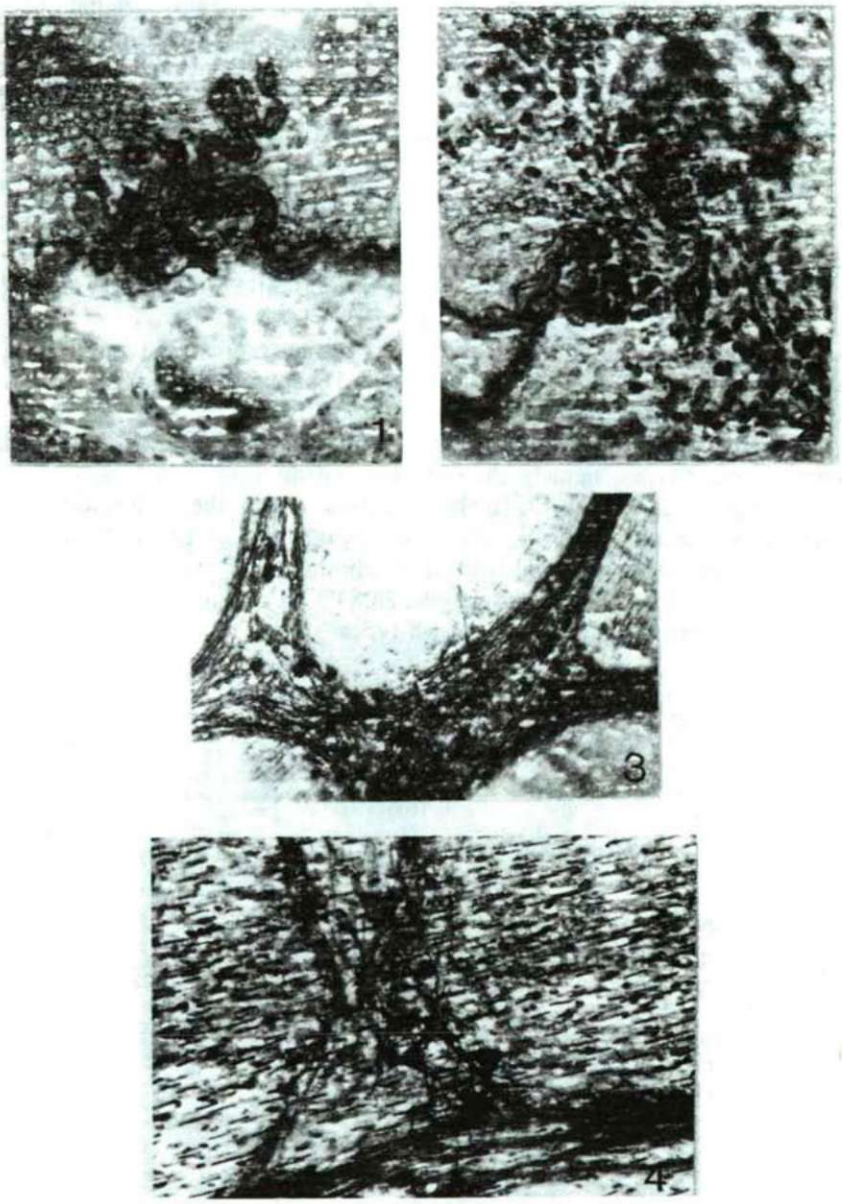

Plate I: figs. 1. and 2.: Detail from the esophageal myenteric plexus. 930x.
figs. 3. and 4.: Ganglia of the small intestinal myenteric plexus. 350x.

Following $AgNO_3$ impregnation the neurons of the plexus were light (argentophobic) or dark (argentophilic). The dark cell type was observable in the esophagus; in the small intestine both types of cells were found in equal number (Plate I., fig. 4.).

The cells could be grouped on the basis of their calculated volume and excentricity values (Plate II). Accordingly, the cells of the small intestinal myenteric plexus could be grouped into two; namely into the small and large cell groups. Small cells were ranked which had volumes till 1600 μm^3; 59, 16% of the measured cells were mostly round, or had strongly elongated elliptic form.

The other group consisted of the large cells amounting to 40.84%, their volume being above 1600 μm^3 (1600–15000 μm^3).

The cells of the myenteric plexus of the esophagus represented a uniform group. These neurons had sizes identical with the first, i.e. small cells of the small intestine on the basis of their size domain.

The number of the neuronal processes also varied. Among the measured cells all morphological types, namely the uni-, bi- and multipolar cells were detectable (Plate III., figs. 1, 2, 3. and 4.). In the myenteric plexus the multipolar cells were the highest in amount, being 88.33% in the esophagus, and 91.82% in the small intestine. One part of these could be ranked among one of the known Dogiel-types, their other part (19.16% in the esophagus, 28.83% in the small intestine), however, could not be compared with either known types.

		Volume		Eccentricity	
		50–1600 u^3 small cells	1600–15000 u^3 large cells	small cells	large cells
small intestine	myenteric plexus	+	+	1:1.0 1:1.5	1:1.0 1:1.2 1:1.4
	submucous plexus	+	+	1:1.0 1:1.2 1:1.3 1:1.5	1:1.0 1:1.2 1:1.3
esophagus	myenteric plexus	+	–	1:1.0 1:1.5	–
	submucous plexus	+	–	1:1.0 1:1.2	–

Plate II: Grouping of the enteric nerve plexus neurons according to volume and excentricity values.

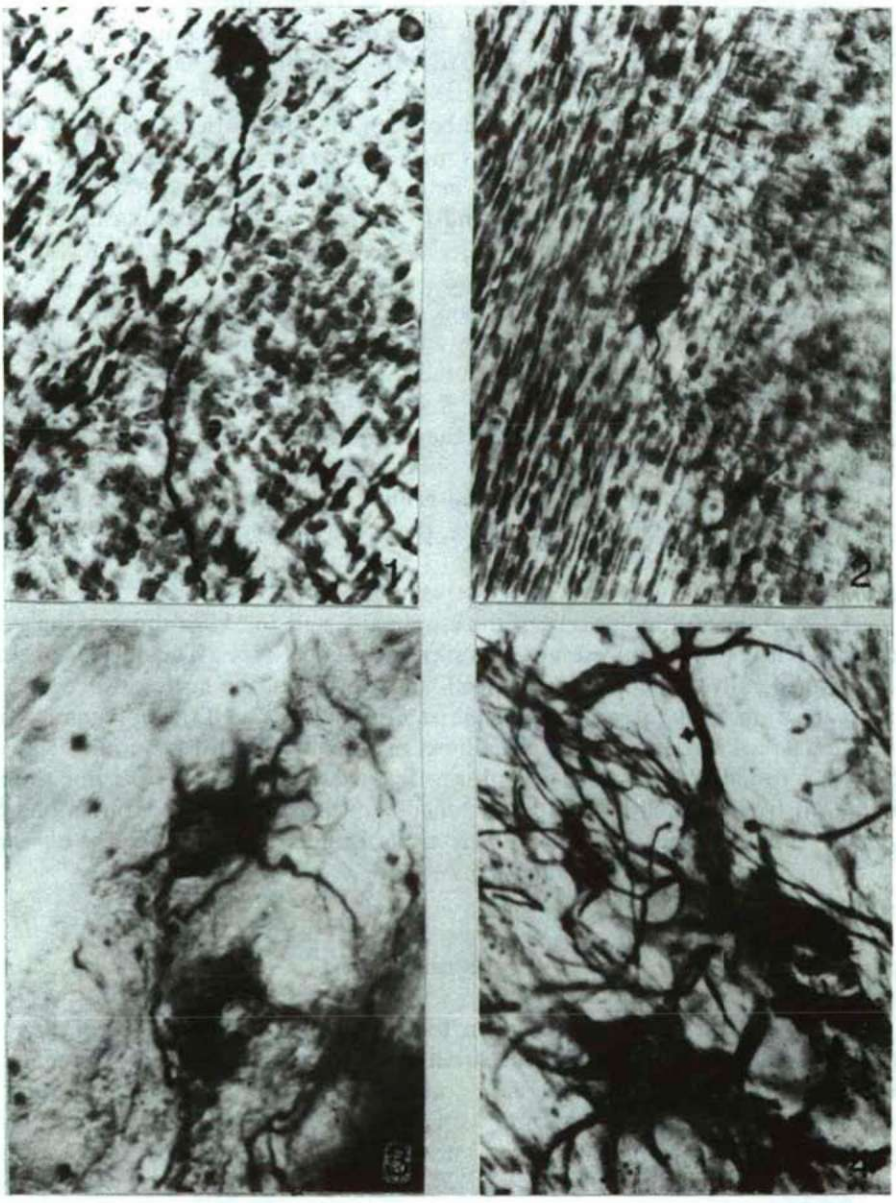

Plate III: figs. 1,2,3. and 4.: Cell types from the small intestinal myenteric plexus. 570x.

From the cell processes only a few could be traced within the ganglia, mainly belonging to the light-staining cells; while those of the darkly staining cells generally left the ganglia, either proceeding towards the neighbouring ganglia, or in most cases towards the inner concentric muscle layer.

The submucous plexus was situated between the concentric muscle layer of the intestinal tract and the tunica muscularis mucosae. Its nerve fiber bundles were thin, containing only a few fibers, and rather small ganglia made up of a few cells. It was characteristic that single cells were found to occur sporadically along the bundles (Plate IV., figs. 1, 2). Single fibers were also detectable besides the small bundles, in general proceeding together with blood vessels.

The Meissner plexus of the esophagus was found to be poor in cells compared to other sections of the intestinal tract, and these few cells were also difficult to stain, thus only 200 cells could be measured from this plexus.

Similarly to the myenteric plexus, the cells of the small intestinal Meissner plexus belonged to two groups; those of the esophagus to one group, all being small cells (Plate II).

The percental distribution of the cell groups in the small intestinal plexus was as follows: the first (small cells) was 37%, the second (large cells) was 63%.

The majority of the cells of the plexus were multipolar here, too (80% in the esophagus, 90.32% in the small intestine) (Plate IV., figs. 3, 4).

25.5% of the multipolar cells of the esophagus, and 38.5% of those of the small intestine could not be grouped into either known Dogiel-types. The nucleus of these cells was generally excentric, thus the cell had pear-form, the nucleus was rarely of central location, and the cell was roundish. Following $AgNO_3$ impregnation the cells stained dark. 4–5, sometimes even more, maximally 10 processes could originate from the cell body. These were of uniform thickness and gradually became thinner moving off from the cell body. It was difficult to differentiate the axon among the processes (Plate IV., figs.3, 4). These were either isolated cells, or could be observed in a rather small ganglia containing few cells.

Discussion

The myenteric plexus is located in the connective tissue layer separating the tunica muscularis layers of the intestinal tract, the inner concentric and outer longitudinal smooth muscles. The developmental stage of the two layers is different in birds (FARNER, 1960; MAGON and MOHAN, 1976). In chickens the inner muscle layer is more developed in the studied intestinal sections.

Reticular arrangement is characteristic to the myenteric plexus, which is very similar to the observations in mammals (CHRISTENSEN et al. 1983; FEHÉR and VAJDA, 1972; HILL, 1972; SCHOFIELD, 1968). The primary thick nerve fiber bundles are the basis of the network-structure, found in the complete lenght of the plexus. These are connected by thinner, secondary bundles. The smaller-larger ganglia are situated at the branchings of these two kinds of bundle systems. Single fibers are rare in the

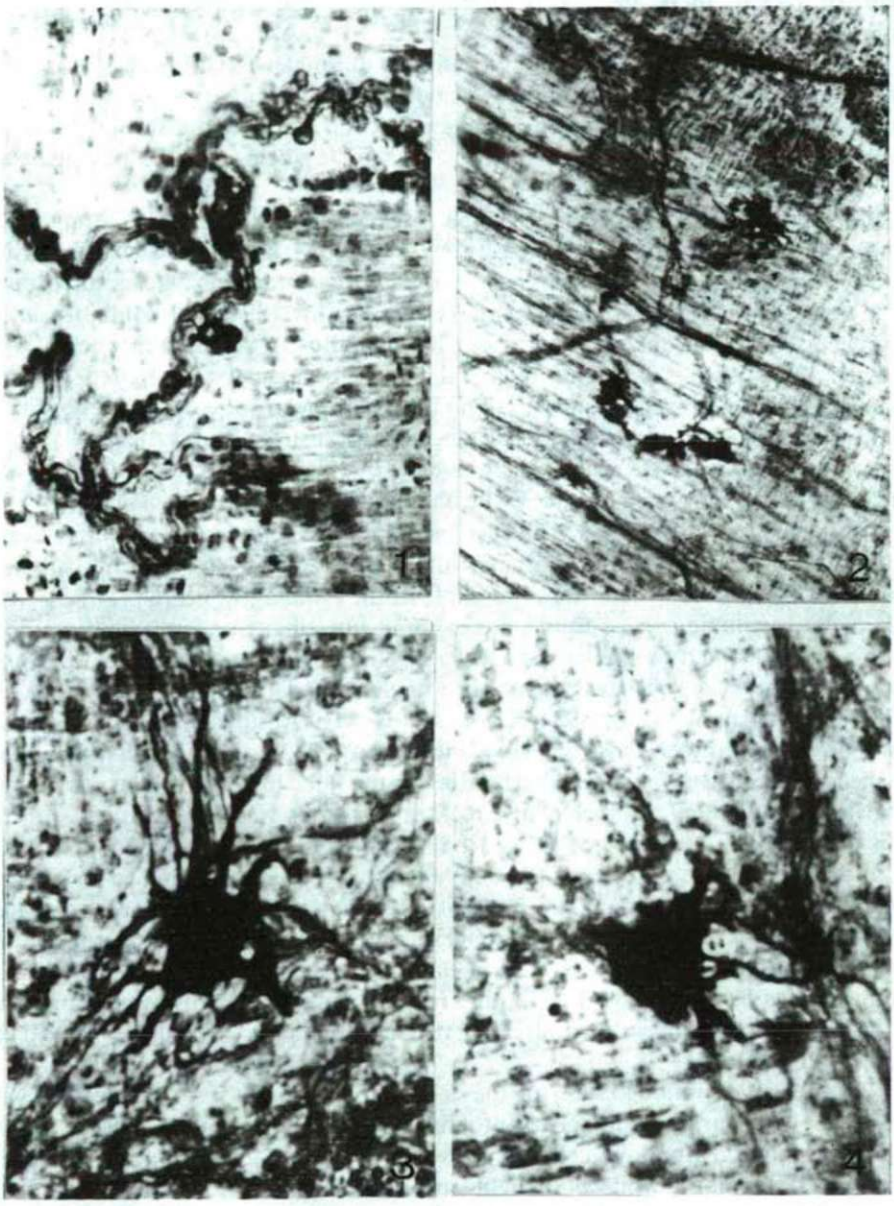

Plate IV: fig. 1.: Detail from the esophageal Meissner plexus.800x.
fig. 2.: Solitary cells of the small intestinal Meissner plexus. 330x.
fig. 3.: and 4.: Characteristic cells of the enteric nerve plexuses. 750x.

myenteric plexus, these are mostly characteristic to the enteric nerve plexuses of mammals.

The submucous plexus is mainly built up of single nerve fibers and bundles containing low number of nerve fibers. Its neurons can be observed singly along the nerve fibers or as few cells (2–3) forming ganglia. The Meissner plexus of the chicken esophagus is particularly poor in neurons and nerve fibers, and the neurons stain weakly (KOLOSSOW et al. 1932).

Comparing the nerve plexuses of the chicken gut with those of the mammals significant deviations can be detected: In mammals the submucous plexus is rich in neurons, these are generally arranged in the form of ganglia. The occurrence of single cells is not characteristic. The nerve bundles are thick and contain a lot of nerve fibers. The neurons and nerve fiber bundles show reticular arrangement, and thus the Meissner plexus of the mammalian intestine can be compared with the arrangement of the myenteric plexus. The Meissner plexus of chicken is morphologically similar to the enteric nerve plexuses of the fishes (BURNSTOCK, 1959) and amphibia (GUNN, 1951), where there are also only few nerve fibers and cells detectable and in the arrangement of which the reticular structure is less recognizable.

In the chicken enteric nerve plexuses small and large cells can be differentiated according to the calculated cell volume values. The regional distribution of these is uneven, since only small cells are observable in the esophageal nerve plexuses, while cells of both volume can be found in the small intestinal plexuses. Similar studies have been performed in the case of species belonging to other vertebrate groups, too, thus in the case of fishes (BURNSTOCK, 1959), and mammals (FEHÉR and VAJDA, 1972; GABELLA and TRIGG, 1984; GUNN, 1968). The results obtained by us can better be compared with those of FEHÉR and VAJDA (1972), on the one hand, because the measurements were performed according to the same method, on the other hand, because the individual number of the measured groups was also identical. On the basis of their calculated volume values, the measured cells were grouped in a similar way however the size domain of the different groups was much smaller in the case of chickens compared to mammals. For chickens the maximal volume of the so-called small cells was 1600 μm^3, this being 15000 μm^3 for cats. From the neurons of the chicken enteric nerve plexus the large neurons can be regarded as the cells having volume values between 1600–15000 μm^3. In the case of mammals even the volume of the medium neurons is much greater, reaching the value of 30000 μm^3 and the large cells 6000 μm^3.

The degree of impregnation of the cells can be brought into connection with their sizes. The darkly staining argentophile cells may be small and large, at the same time the argentophobe cells are only large. In the esophageal plexuses every small cell stains dark, while both light and dark cells can be found in the small intestine. No measurements were performed in respect to the quantitative occurrence of the argentophile and argentophobe cells, nevertheless, relating numerical data are at disposal in the work of MICHAIL and KARAMANDLIDIS (1967), who had found the ratio of the two cell types to be 1:1 in chicken small intestine.

Especially the darkly impregnated cells could be grouped according to the number of their processes. Thus, uni-, bi- and multipolar cells could be differentiated (SCHOFIELD, 1968).The majority of the multipolar cells could be ranked among the known Dogiel cell types. One further type could also be differentiated. These cells are similar to the Stach type IV. cells on the basis of their shape, and to the Dogiel I. type cells regarding the amount of their processes (DOGIEL, 1896; 1899; STACH, 1982b).

Several authors (KÖLLIKER, 1984; KUNTZ, 1922; SCHOFIELD, 1968) do not agree with the known Dogiel kind of classification, according to which the cells type I. are motoric, the type II. are sensory and the type III. are interneuronal cells. These authors dispute the justification of this rigid classification, on the basis of the transitional types. No doubt, the neuron classification entirely on the basis of morphological methods has no longer meets the modern neurobiological requirements, and the characterization of the cells should be supplemented by immuncytochemical and neurophysiological data.

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Addresses of the authors:
M. CSOKNYA
Department of Zoology
Janus Pannonius University
H-7604 Pécs,
P.O. Box 9.
Hungary
I. BENEDECZKY
Department of Zoology
Attila József University
H-6701 Szeged,
P.O. Box 659.
Hungary