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FLUORESCENCE CHARACTERIZATION OF THE NERVE PLEXUSES IN THE SMALL INTESTINE OF THE CHICKEN

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Abstract

The enteric nerve plexuses in the small intestine of the chicken has been investigated in sections and stretch preparations by means of the silver staining and the glyoxylic acid histofluorescence methods. Both techniques revealed the presence of two submucosal plexuses in the small intestine of the chicken. Using the histofluorescence technique some of the nerve cell bodies located in the perivascular plexuses showed monoamine-specific fluorescence. The appearance and distribution of nerve elements in the chicken small intestine has been studied also with electronmicroscope. Profiles containing dense-core vesicles in different sizes have been claimed to contain catecholamines.
Key words: enteric nerve plexuses, catecholamine, chicken, small intestine

Introduction

Most of the structural and functional studies concerning the organization of the enteric nervous system were performed on mammalian intestine (GUNN, 1968; GABELLA, 1979; FURNESS and COSTA, 1980; SCHEUERMAN and STACH, 1984).

Data on the innervation of the alimentary tract of the birds were mostly based on staining techniques as methylene blue and silver methods (ÁBRAHÁM, 1936; CSOKNYA and BENEDECZKY in print; KOLOSSOW et al. 1932; MICHAIL and KARAMANDLIDIS, 1967). Using fluorescence histochemical methods (BENNETT and MALMFORS, 1970) it was shown that the basic pattern of the adrenergic innervation of the alimentary tract in the birds were comparable to that in mammals (COSTA and GABELLA, 1971; STACH, 1984). All these techniques revealed several contrary concerning the organization of the enteric nervous system. Because of the very small number or even the lack of fluorescent nerve cell bodies observed in the alimentary tract of the chicken (READ and BURNSTOCK, 1968; BENNETT and MALMFORS, 1970; ALI and MCLELLAND, 1978) it has been suggested that all the catecholamine (CA) containing axons in the intestine of the domestic fowl arise from extrinsic ganglions. Some authors report on a secondary meshwork of submucosal plexus (ALI and MCLELLAND, 1978). On the bases of ultrastructural studies (BENNETT and COBBS, 1969) the majority of the terminals contained a mixed

population of small and large granular vesicles which suggest that at least some of the nerves handle with catecholamines.

During present work using silver impregnation and glyoxylic acid induced fluorescence methods we tried to clear up some of these contraversaries.

Materials and methods

The small intestine of the 6 days old chicken was investigated. The basic histological structure of the small intestine was studied by hematein-eosin stained paraffin sections. Detection of the enteric nerve plexuses was carried out on sections (30 μm) stained by BIELSCHOWSKY-GROS-CAJAL silver method.

For the histochemical detection of monoamines the sucrose-phosphate-glyoxylic acid (SPG) method (DE LA TORRE and SURGEON, 1976) was applied to wholemount stretch preparates of chicken small intestine. After dissection, the mucosal and muscle layers were separated and incubated in reaction mixture containing 6.8 g sucrose, 3.2 g KH_2PO_4 and 1 g glyoxylic acid (GA) in 100 ml of distilled water at 4°C for 25 min. The layers were then stretched separately on microscope slides, blotted with blotting paper and dried under cool air for about half an hours. Finally the specimens were placed in an oven at 95°C for 4 min, and mounted with liquid paraffin. The preparations were viewed through a Leitz Orthoplan microscope equipped with HBO 50 W super pressure mercury lamp and an E-3 filter block. Tissue blocks for electron microscopy were taken after vascular perfusion with ice-cold KARNOWSKY fixative. Small pieces of the small intestine were kept in the same fixative for 2 hours. After postfixation in 2% osmium teroxid the specimens were dehydrated in ascending ethanol series and embedded in Durcupan. Tissue blocks were contrasted with saturated uranyl acetate in 75% ethanol and sections were recontrasted with lead citrate, then studied under TESLA BS 500 electronmicroscope.

Results and discussion

The basic histological structure of the chicken small intestine is similar to that of mammalian pattern (Table 1.a.). The thick epithelium is characteristic to the absorptive tissues. The muscularis mucosae consist of one longitudinal layer of smooth muscle cells. The submucosa is reduced to a few connective tissue fibres. The muscularis externa has a highly developed inner circular layer and a relatively poorly developed outer longitudinal layer covered by the tunica serosa in the whole length of the small intstine. Both the histochemical and histological methods we

Table 1.

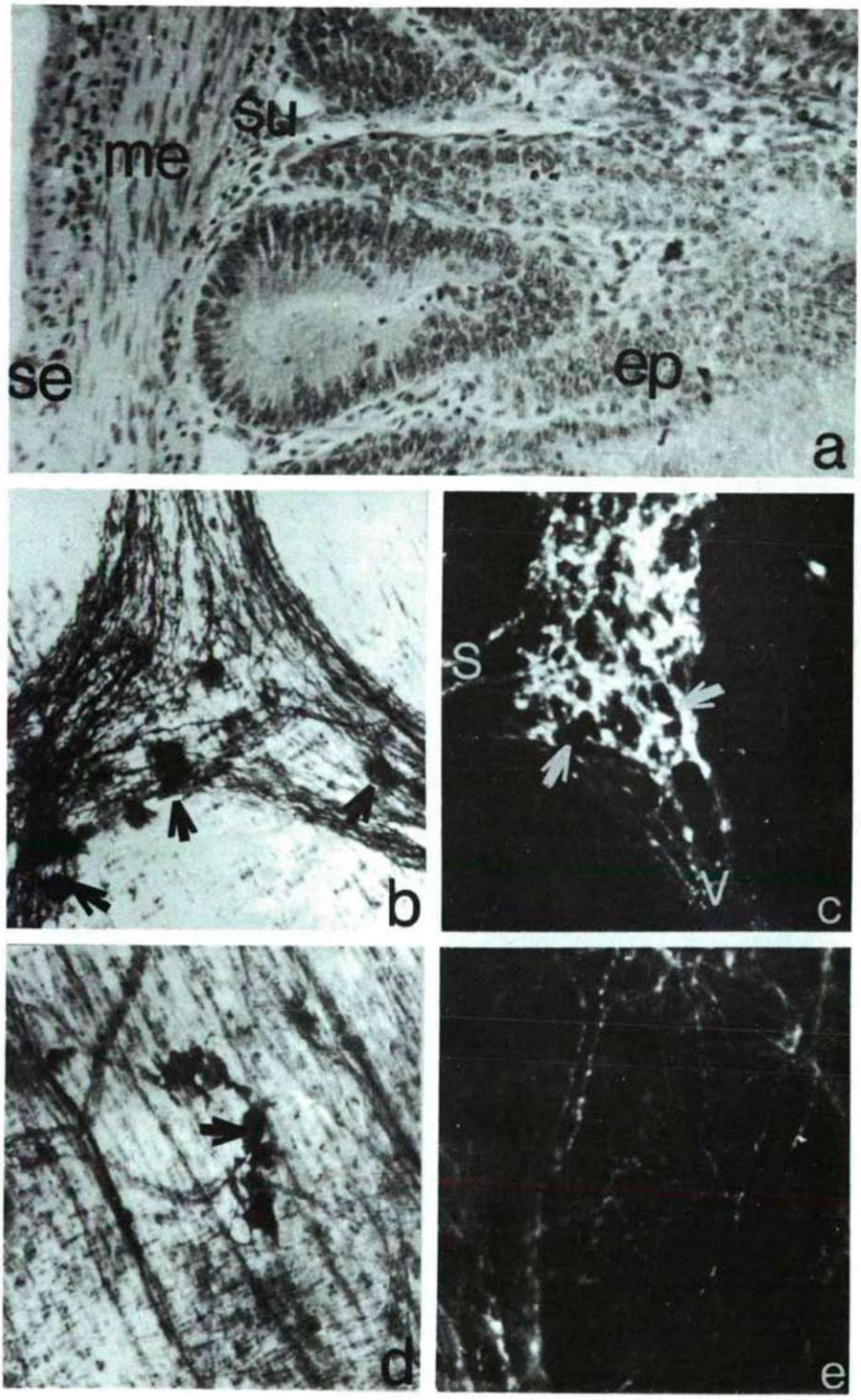
a. Detail of a hematein-eosin stained cross section from the small intestine of the chicken. ep—epithelium, su—submucosa, me—muscularis externa, se—serosa x 450

b. One ganglion in the plexus myentericus after silver impregnation. Arrows indicate the large, mainly multipolar nerve cells. x600

c. Ganglion in the plexus myentericus after GA induced fluorescence. Dense holes (arrows) represent the non-fluorescence cell bodies in the ganglion. s—smooth fibres, V—varicose fibres x600

d. Silver impregnated fibres of the plexus submucosus externus, characterized by ganglions with a few cell bodies (arrows) and a small number of fibres. x 600

e. Fine network of fibers from plexus submucosus externus, with a very intense GA induced fluorescence. x 600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used during the present studies were suitable to visualize the enteric nerve plexuses in the small intestine of the chicken (Table 1.b-e., 2.a-e.). After silver impregnation the nerve fibers of the plexus myentericus appeared as a compact aggregates between the longitudinal and circular muscle layer (Table 1.b.). All the nerve cell bodies were located in ganglions (Table 1.b.c.). Both in the silver impregnated sections and on the GA treated wholemounts two networks of plexus submucosus were identified (Table 1.d.e., 2.a.b.). In the plexus submucosus internus (Table 2.b.) which lies close to the tunica muscularis mucosae the individual nerves could much better be recognized than in plexus myentericus. This plexus forms a regular pattern with fibers running to the mucosa. The plexus submucosus externus can be recognized as a separate entity lying close against the inner side of the circular muscle layer. Most of the nerve cell bodies are also aggregated into ganglions although the sizes of the cells and the ganglions are smaller than in Auerbach's plexus. It was demonstrated with glyoxylic acid that a large part of these nerves were catecholaminergic. The myenteric plexus contains the greatest density of the catecholaminergic fibers (Table 1.c.). At many places in the ganglions large dense holes surrounded by basket-like fluorescent varicose and non-varicose axons have been observed (Table 1.c.). It is highly probable that dense holes related to nonfluorescent enteric nerve cell bodies. The smooth appearance of some nerves and varicosity of others might be the indication of the different role of these nerves. We suppose that the varicose segments represent the active segments in chemical neurotransmission while the smooth have mainly conducting role. The submucosal plexus in stretch preparations were distributed in two discrete plane (Table 2.b.). In plexus submucosus internus almost all the fibers were varicose in appearance (Table 2.b.) The fibers of the plexus submucosus externus have a less intense fluorescence (Table 1.e., 2.b.) which suggests a lower concentration of catecholamine. The fibers of the plexus submucosus internus were always in close connection to the perivascular plexuses (Table 2.d.).

The topographical difference between the two submucosal plexuses might suggest functional difference, too. Our suggestion is that the fibers of the primary or external meshwork may influence mucosal function directly however the fibers of

Table 2.

a. Plexus submucosus internus after silver impregnation. Single nerve cell body is characteristic to this plexus. x 600

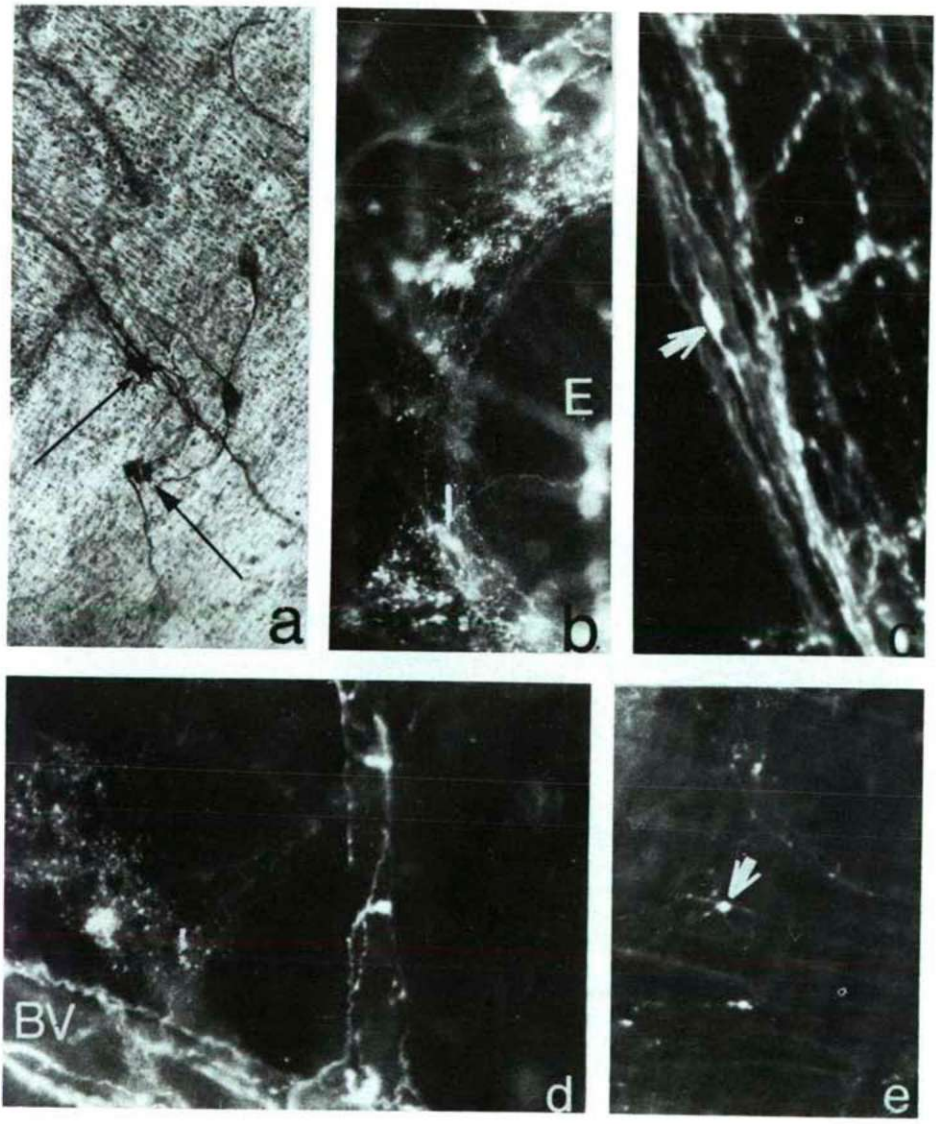
b. GA induced picture of plexus submucosus internus (I) superlayered on the plexus submucosus externus (E). x 600

c. GA induced nerve cell body (arrow) in the perivascular plexus of the chicken small intestine. x 600

d. Close relation of the plexus submucosus internus (I) and blood vessel (BV) in the chicken small intestine. Both the submucosus and the perivascular plexuses show and intensive GA induced fluorescence. x 600

e. Small soliter multipolar cell (arrow) in the intestinal wall, with a well defined GA induced fluorescence. x 800

the inner plexus act by influencing the blood flow. Even though there are a lot of contrary about the ultrastructural appearance of aminergic nerves in the gut, profiles containing dense-core vesicles in different size have been claimed to contain catecholamines (BENNETT and MALMFORS, 1970; GORDON-WEEKS, 1981). Accepting this agreement our present ultrastructural observations indicate the presence of catecholaminergic nerves in both the myenteric and submucosus

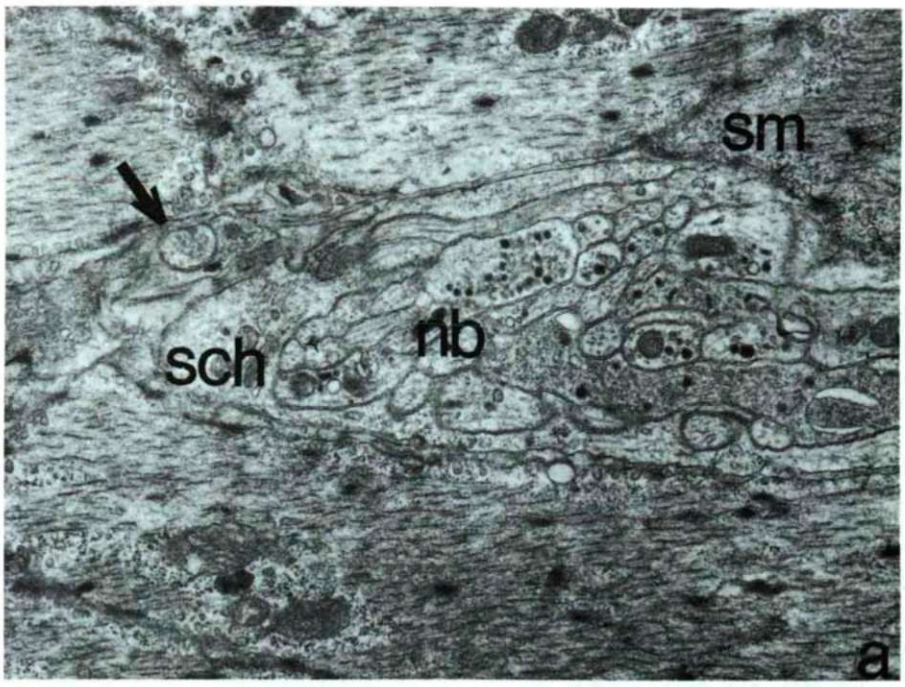


plexuses of the chicken small intestine. In a good agreement with our light microscopic investigations the number of nerve bundles was much higher in myenteric plexus than in submucous plexus. Most of the axons were grouped into bundles (Table 3.a.) lying mainly parallel to the smooth muscle cells. Profiles in nerve bundles were sometimes surrounded by Schwann-cell processes while others were in close association with the muscle cells (Table 3.a.) The axon profiles in both plexuses contained heterogeneous vesicle population even though the dominancy of dense-core vesicles was characteristic. The diameter of these vesicles was around 50—100 Å (Table 3.a.b.). This indicates that most of the nerves in the enteric plexuses handle different kind of catecholamines. Besides transmitter vesicles the axon profiles contain mitochondria and multivesicular bodies. These detailed results allow us to make the conclusion that large part of the nerves in the enteric plexuses in the chicken small intestine are catecholaminergic, and except the perivascular plexuses, where intensively fluorescent cell bodies were noticed all the aminergic nerves are extrinsic in origin.

Table 3.

a. Nerve bundle in the chicken small intestine (nb), surrounded by glial processes (sch). Arrow shows a neuromuscular junction. sm—smooth muscle cell. x 13 000

b. Nerve bundles with different axon profiles in the chicken small intestine. Arrow shows a characteristic profile with heterogeneous vesicle population: dense-core (50—100Å in diameter), small agranular and flattened vesicles can be seen. x 22 000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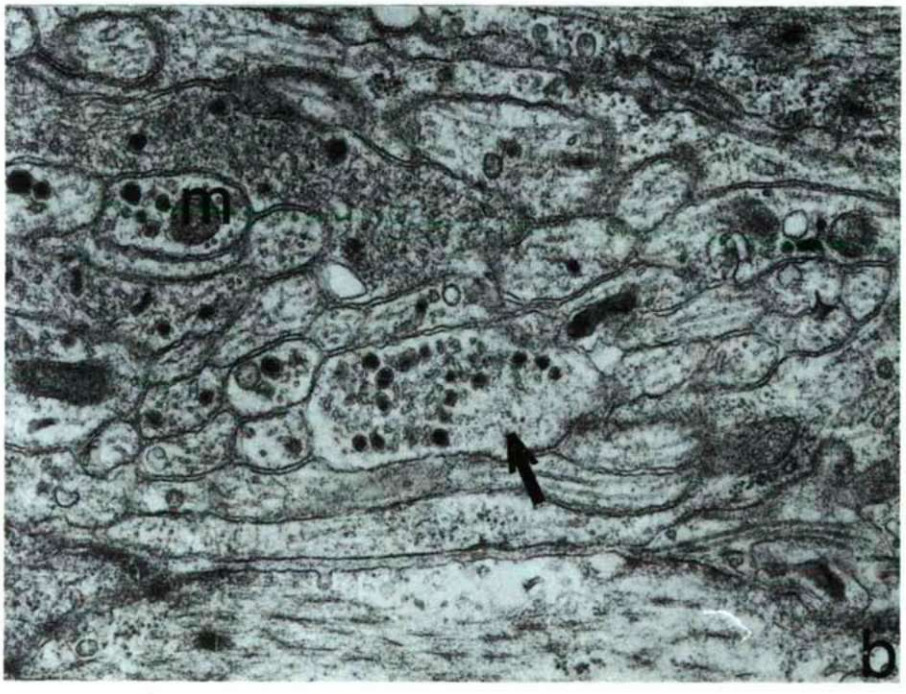
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References

ALI, H.A. and MCLELLAND, J. (1978): Avian enteric nerve plexuses. A histochemical study. — Cell Tissue Res. *189*, 537-548.

ÁBRAHÁM, A. (1936): Beitrage zur Kenntnis der Innervation des Vogeldarmes. — Z. Zellforsch. *23*, 737-745.

BENNETT, T. and COBB, J.L.S. (1969): Studies on the avian gizzard: Auerbach's plexus. — Z. Zellforsch. *99*, 109-120.

BENNETT, T. and MALMFORS, T. (1971): Fluorescence histochemical observation on Auerbach's plexus and the problem on the inhibitory of the gut. — J. Physiol. *218*, 77-78.

COSTA, M. and GABELLA, G. (1971): Adrenergic innervation of the alimentary canal. — Z. Zellforsch. *122*, 357-377.

CSOKNYA, M. and BENEDECZKY, I. (1986): Cell types of the enteric nerve plexuses of the chicken (*Gallus domesticus* L.). — Acta Biol. Szeged, *32*, 93-102.

FURNESS, J.B. and COSTA, M. (1980): Types of nerves in the enteric nervous system. — Neurosci. *5*, 1-20.

GABELLA, G. (1979): Innervation of the gastrointestinal tract. — Int. rev. Cytol. *59*, 129-193.

GORDON-WEEKS, P.R. (1981): Neuroscience properties of nerve endings with small granular vesicles in the distal colon and rectum of the guinea-pig. — Neurosci. *6*, 1-20.

GUNN, M. (1968): Histological and histochemical observations on the myenteric and submucous plexuses of mammals. — J. Anat. (Lond.) *102*, 223-239.

KOLOSSOW, N.G., SABUSSOW, G.H. and IWANOW, J.F. (1932): Zur Innervation des Verdauungskanales der Vögel. — Z. mikr. anat. Forsch. *30*, 257-294.

MICHAIL, S. and KARAMANDLIDIS, A. (1967): Morphologie du plexus myentérique d'Auerbach de l'intestine. — Acta anat. *67*, 424-436.

READ, J.B. and BURNSTOCK, G. (1968): Comparative histochemical studies of adrenergic nerves in the enteric plexuses of vertebrate large intestine. — Comp. Biochem. Physiol. *27*, 505-517.

SCHEUERMANN, D.W. and STACH, W. (1984): Fluorescence microscopic study of the architecture and structure of an adrenergic network in the plexus myentericus (AUERBACH), plexus submucous externus (SCHABADASCH) and plexus submucous internus (MEISSNER) of the porcin small intestine. — Acta anat. *119*, 49-59.

DE LA TORRE, J.C. and SURGEON, J.W. (1976): Histochemical fluorescence of tissue and brain monoamines: results in using the sucrose-phosphate-glyoxylic acid (SPG) method. — Neuroscience *1*, 451-453.