Acta Biologica Szeged 16 (3—4), pp. 139—148 (1970)

HISTOLOGICAL AND HISTOCHEMICAL INVESTIGATIONS OF OESOPHAGUS AND STOMACH OF THE MARSH-FROG (RANA RIDIBUNDA PALL.)

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(Received December lst, 1969)

The attention of anatomists was attracted long ago by the investigation of the gastro-intestinal tract of frogs. In this respect the books of KRAUSE (1923), KÜKENTHAL and MATTHES (1950) are especially worth mentioning. These, apart from treating the anatomical conditions, are summing up also the histological results till then the date of publication. From these, as well as from the other observations examining different species we can point out several unsolved problems in the histological structure especial in the innervation of intestinal tract (GUNN, 1951). A part of them has been elucidated by the help of modern methods, the other part, however, is still awaits solution (FELDBERG, 1952; DONHOFFER, 1959).

We have, therefore, undertaken the tasks to investigate the displacement of plexuses in the intestinal tract of the marsh-frog, the types of the nerve cells of the vegetative plexuses and their synaptic connections, not only by impregnation but also by histochemical method.

Materials and Methods

Our investigations have been carried out on some well developed specimens of marsh-frogs (*Rana ridibunda* PALL.). Their intestinal tract, similarly to that of the other vertebrate groups, divides into oesophagus, stomach, small and large intestines. In this paper we are discussing only the results of our investigations concerning the oesophagus and the stomach.

After section the oesophagus was fixed partly in BOUIN's and CARNOY's fixing mixture and partly in neutral formalin of 10 p. c. The 5—7 μ sections made of a matter embedded in paraffin were stained with HEIDENHAIN's iron-haematoxylin, MALLORY's mixture, and haematein-eosin. A part of the intestinal tracts fixed in formol has been used for the demonstration of cholinesterase, carried out with KOELLE-FRIEDENWALD's procedure (1949), and its form is modified by GEREBTZOFF, COUPLAND and HOLMES (1957), and with a procedure applying thioacetic acid. In both cases DFP and prostigmine were used for separating specific and non-specific esterase. The other part of intestine tracts has been impregnated with the BIELSCHOWSKY—ÁBRAHÁM (1951) and BIELSCHOWSKY—GROS—CAUNA methods after having been fixed in formol for a longer period time.

Discussion of results

Oesophagus

The oesophagus running caudally from the pharynx is comparatively short. On its inner surface longitudinal plicae are found. These are protrusions of the mucous tunic. In the cranial part the epithelium is stratified, in the caudal part it is a simple ciliated cylindrical epithelium. Among the epithelial cells simple salivary glands varying in number can be observed (SALAMON, 1955). The proper tunic is thin, in the vicinity of stomach, however, thicker, containing various quantities of composed tubular glands. Among them a great number of capillaries are found which may run up to the basis of the epithelium. The thickest histological layer of the oesophagus is the muscular tunic. It consists of an inner, thicker, circular and an outer, thinner, longitudinal smooth muscle layer, separated from each other by a connective tissue septum. The longitudinal muscle layer is covered by the external tunic that is rich in elastic fibres, covered from the autside by a simple flatteden epithelium.

At the boundary of the external tunic and the logitudinal muscle layer of the muscular tunic smaller or bigger nerve-trunks are found, with scattered single unipolar nerve cells (Table I, Fig. 1). The trunks have cerebrospinal myelinated fibres and also vegetative ones with SCHWANN's membrane (HARTING, 1934; SOTELO, 1954). The thinner trunks running between the longitudinal and circular smooth muscle layers (Table I. Fig. 2) form the AUERBACH's myenteric plexus (IKEDA, 1954). The nerve cells forming smaller groups in the plexus in spite of the variety of their forms, belong to the DOGIEL's cell types (Table I, Figs. 3, 4). From the two kinds of DOGIEL's cell type the DOGIEL's I occurs in higher numbers. Nerve cells can be observed not only in the area of plexus but also distally from it, at the inner boundary of the circular muscle layer.

One part of the fibres belonging to the myenteric plexus runs through the circular muscle layer into the mucous tunic plexus which contains mainly unipolar nerve cells (SMIRNOW, 1899; Table II, Figs. 1, 2). We consider this plexus to be homologous to MEISSNER's plexus in the submucous tunic of other vertebrates. KRAUSE (1923) found this plexus to be free from nerve cells in the intestinal tract of *Rana esculenta*. As we mentioned before we observed nerve cells in the lamina propria of the oesophagus of the marsh-frog. The myelinated and non-myelinated fibres originating from the plexus, diverge abundantly at the basis of epithelium and among the glandular tubules and end freely (ÁBRAHÁM, 1936; HONJIN, 1951; SOTELT, 1954, Table II, Fig. 3).

Stomach (ventriculus)

The caudal part of the oesophagus continues in the stomach. It is somewhat bent and situated medially. On its inner surface, the plicae of oesophagus are running.

TABLE I

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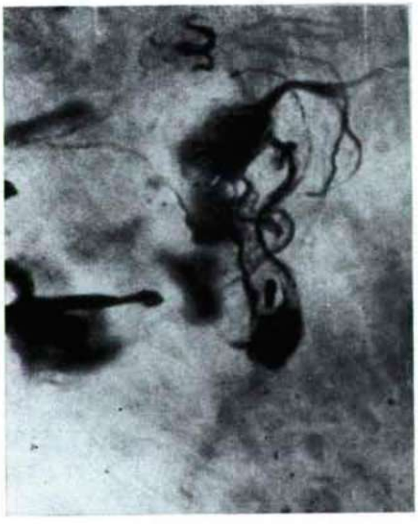

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Fig. 1. *Rana ridibunda:* oesophagus. Mixed nerve-trunk and single unipolar nerve cells in the tunica externa. BIELSCHOWSKY—ÁBRAHÁM's procedure. x 300.

Fig. 2. *Rana ridibunda:* oesophagus. Nerve cells from AUERBACH's plexus. BIELSCHOWSKY—GROS—CAUNA's procedure. x 200.

Fig. 3. *Rana ridibunda:* oesophagus. Cells of types DOGIEL I and II from the plexus myentericus. BIELSCHOWSKY—GROS—CAUNA's procedure. x 400.

Fig. 4. *Rana ridibunda:* oesophagus. Endings on a nerve cell of type DOGIEL I. BIELSCHOWSKY—ÁBRAHÁM's procedure. x 675.

Its histological structure is more complicated than the rest of the oesophagus because the mucous muscular layer appears in the stomach with two layers, as well as the submucous tunic dividing it from the muscular tunic.

In the external histological layer of stomach — the tunica serosa — we find nerve-trunk consisting more or less of fibres that are the thickest at the cardia but, owing to bifurcations, they grow quite thin in the pylorus region. It is very difficult to separate the impregnated myelinated and non-myelinated fibres from each other in the smaller nerve-trunks while the myeline sheath becomes gradually so thin that it could be distinguised from the other vegetative fibres only with a special staining procedure applied with great routine. The smaller bundles bifurcating from the major nerve-trunks run over to the longitudinal muscle layer of muscular tunic, and they are mixed with the fibres of AUERBACH's myenteric plexus which innervate both muscle layers. Another part of fibres end in the mucous tunic and in the muscular mucous tunic.

The plexus myentericus Auerbachii contains nerve cells DOGIEL I and II types but type DOGIEL II appears in greater numbers (Table II, Fig. 4). The nerve cells are set generally one by one, but at the bifurcation, and at the meeting points of the nerve bundles they come in twos (Table III, Fig. 1).

It is not decided, as yet, on the basis of literary date whether the fibres of the myenteric plexus run over to the submucous tunic or not. Our finding reveals that some of the smaller nerve trunks, and single nerve-fibres, too, run over to the submucous tunic (Table III, Figs. 2, 3). This generally takes place beside the smaller vessels and capillaries. We think possible, that these nerve-fibres take part not only in the innervation of blood vessels but also in the glands of the mucosa and submucosa (IWANOW, RADOSTINA, 1937).

In the initial section of the stomach the MEISSNER's plexus contains nerve cells, however, in the fundus and pylorus these are none. We have found a great number of smooth-edged fibres in the nerve trunks and several thicker fibres of meandering course, too, that lost their myeline sheaths. They run, after their dichotomical branching partly to the glandular cells (Table III, Fig. 4), and partly to the simple columnar epithelium. In this way, the end-branches of fibres end on the basal part of the cells (SALAMON, 1955).

Study of the acetylcholinesterase activity

In the plexuses of the oesophagus and stomach the nerve cells and the nerve fibres show a cholinesterase activity of different degrees (DONHOFFER, 1959; Table IV, Fig. 1). In the myenteric plexuses of the fundus and the

Table II

Fig. 1. *Rana ridibunda:* oesophagus. Unipolar nerve cell in the lamina propria. BIELSCHOWSKY—GROS—CAUNA's procedure. x 450.

Fig. 2. *Rana ridibunda:* oesophagus. End-rings on the nerve cells of type DOGIEL I. BIELSCHOWSKY—GROS—CAUNA's procedure. x 400.

Fig. 3. *Rana ridibunda:* oesophagus. Innervation of the glandparenchyma. BIELSCHOWSKY—ÁBRAHÁM's procedure. x 150.

Fig. 4. *Rana ridibunda:* fundus. Nerve cell of type DOGIEL II from the plexus myentericus. BIELSCHOWSKY—ÁBRAHÁM's procedure. x 500.

TABLE II

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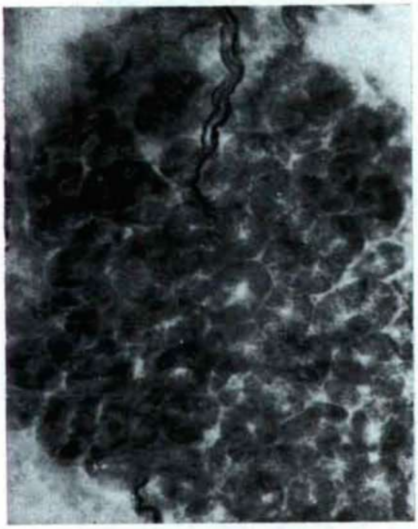

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TABLE III

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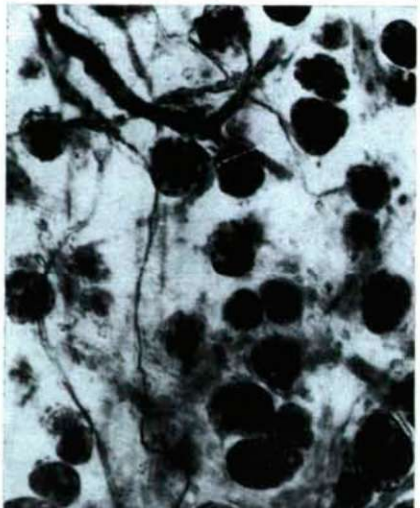


pylorus cell bodies of DOGIEL I and II types showed a weak acetylcholinesterase activity. On the other hand, in the oesophagus and cardia, besides the uni- and bipolar nerve cells, there are also multipolar nerve cells of DOGIEL I types which showed a diffuse positive reaction not only in the cell body but in the cell processes, too. On the basis of this, they may get impulses through the preganglionic fibres of the spinal cord while others from the motor nuclei of the vagus in the medulla oblongata. We try to prove the above mentioned datum by the impregnated preparations made from these intestinal tracts, where we can see numerous end-rings and end-knobs around the cells of DOGIEL I type (Table I, Fig. 4, and Table II, Fig. 2). We consider to be verified the vagal origin of some of these cells if we accept the results of MÜLLER's investigations (cit. ÁBRAHÁM, 1936) concerning the embryonal vagus of vertebrates according to which nerve cells of the central vagal system migrate to the intramular intestinal plexuses. So the acetylcholinesterase appear in some cells in the cranial section of the intestinal tract because they belong to the parasympathetic vagal system and in other cells in consequence of the transfer of stimuli. According to our supposition, the cells of DOGIEL I types may be partly sympathetic, partly parasympathetic motor cells. It was established by OKINAKA et al. (1967), on the basis of demonstrating acetylcholinesterase and monoamino-oxidase in the oesophagus, that in addition to the cholinergic mediation, a strong adrenergic mechanism was effective, as well. The uni- and bipolar nerve cells occuring in these plexuses have sensory function according to our supposition (IWANOWA, 1952).

The nerve-fibres leaving these plexuses show acetylcholinesterase activity in the other histological layers of the oesophagus and stomach, as well. The intensive brown granules among the smooth muscle cells may be the transmitter sites of the neuromuscular stimulation (Table IV, Fig. 2). Dark brown granules appear on the membranes of the glandular and epithelial cells, too (Table IV, Figs. 3, 4). We consider these granules to be the transmitter sites of stimuli of the secretory, and intraepithelial nerve fibres.

We cannot separate histochemically the sensory system of the intestinal tract. Its cause is that the differences between acetylcholinesterase activities of the afferent and efferent cholinergic nerves cannot be demonstrated. A number of investigators have already drawn the attention to that fact. They have established that, e. g., both the sensory nerve fibres of the spinal cord (CSILLIK et al., 1954) and the depressor fibres of vagal origin (ÁBRAHÁM, 1956) similarly show an acetylcholinesterase activity. On this basis we do not exclude the possibility that in the oesophagus and stomach of the marshfrogs sensory fibres of vagal and spinal origin may also occur.

Table III

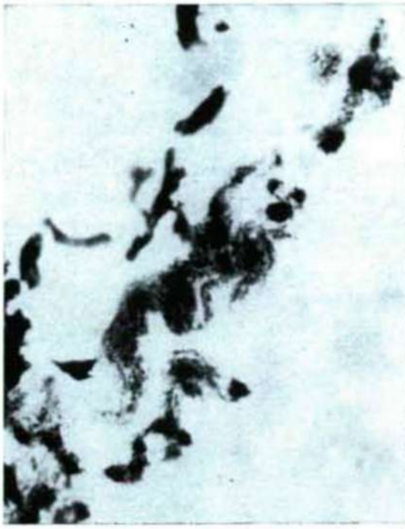
Fig. 1. *Rana ridibunda:* fundus. Nerve cell of type DOGIEL II from AUERBACH's plexus. BIELSCHOWSKY—ÁBRAHÁM's procedure. x 300.

Fig. 2. *Rana ridibunda:* fundus. Innervation of muscle and submucosa. BIELSCHOWSKY—ÁBRAHÁM's procedure. x 200.

Fig. 3. *Rana ridibunda:* pylorus. Innervation of capillaries in the submucosa. BIELSCHOWSKY—ÁBRAHÁM's procedure. x 550.

Fig. 4. *Rana ridibunda:* fundus. Nerve-plexus in the glandular parenchyma. BIELSCHOWSKY—GROS—CAUNA's procedure. x 150.

TABLE IV



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Summary

The results of our examinations carried out on the oesophagus and stomach of the marsh-frog (*Rana ridibunda* PALL.) can be summarized as follows.

1) From the histological layers of the oesophagus, the tunica muscularis mucosae and tunica submucosa are missing. MEISSNER's plexus containing mostly unipolar nerve cells takes place in the lamina propria. In AUERBACH's plexus, besides the unipolar nerve cells, nerve cells of type DOGIEL I occur in high numbers, on which synaptic end-rings and end-knobs can be observed.

2) In the nerve-fibre plexus of the tunica submucosa of stomach we have not found any nerve cells. The nerve cells of the plexus myentericus AUERBACHII are mostly of type DOGIEL II. We have not seen any synaptic endings on these cells.

3) In the innervation of oesphagus and stomach, apart from the vegetative neurons of the intestinal tract, there take part the cerebrospinal nerve-fibres, as well. These occur in the tunica externa in thicker, in the tunica serosa in thinner nerve trunks.

4) The nerve cells and nerve-fibres of the oesophagus and stomach show an acetylcholinesterase activity of changing intensity. The nerve cells of the plexuses, on the basis of the differences in forms and activites, are partly of sympathetic and partly of parasympathetic in origin.

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Table IV

Fig. 1. *Rana ridibunda:* oesophagus. Acetylcholinesterase activity in the plexus myentericus. KOELLE—FRIEDENWALD's procedure. x 150.

Fig. 2. *Rana ridibunda:* cardia. Acetylcholinesterase activity in the tunica muscularis. Thioacetic-acid procedure. x 200.

Fig. 3. *Rana ridibunda:* oesophagus. Acetylcholinesterase activity in the tunica mucosa. Thioacetic-acid procedure. x 200.

Fig. 4. *Rana ridibunda:* cardia. Acetylcholinesterase activity on the basis of epithelial cells. Thioacetic-acid procedure. x 300.

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