The Myenteric Nerve-Plexus in some lower Chordates.

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With 12 Text-figures.

INTRODUCTION.

The structure and mode of function of the autonomic nervous system in the alimentary canal of vertebrates have been extensively investigated during the last fifty years. These studies have been, with only a few exceptions, directed towards the elucidation of the problem in the higher forms, chiefly the mammals. The resulting diversity of opinion in the interpretation of this structure has led to the general realization that a better knowledge of the conditions obtaining in the less highly organized chordates may prove to be of considerable assistance in arriving at a more accurate conclusion.

Part of the present study, dealing with the intestinal nerve-plexus of fish, was started some time ago at the suggestion of Prof. D. M. S. Watson and carried out under his kind supervision at University College, London, and at the Marine Biological Laboratory, Plymouth. Later, this work was continued in the Department of Zoology, University College, Colombo, and extended to include the enteric nervous system of Amphioxus. My grateful thanks are due to Dr. E. J. Allen for the facilities accorded to me at the Plymouth Laboratory.

PREVIOUS WORK.

Monti (1895), using methylene blue, studied the nerve-plexuses in the digestive tube of the tench. He described the general appearance of the plexuses in detail and noted the presence of nerve-cells in them at the nodal points. According to this author these neurons were all of one and the same type,
possessing short, thick, branching dendrites and an axon. The
dendrites appeared to travel towards the mucosa while the
axon joined a fibre-bundle in which it could not be followed to
its termination. In some preparations there were extremely
complicated clumps of very fine nerve-fibrils of a delicately
varicose appearance at the points usually occupied by nerve-
cells. In such cases the nerve-cells themselves had not taken
up the stain. Nerve-endings on the smooth muscle-cells of the
intestinal wall were found which were in every way similar to
the nerve-endings on the smooth muscle-cells of the higher
vertebrates. Sakussef (1897) used methylene blue and a Golgi
method to study the plexus of Auerbach in Petromyzon
and in fish. He showed that in fish the neurons belonged to both
of Dogiel's types I and II [Dogiel (1896)], and described nerve-
endings on the smooth muscle-cells and sensory endings of
nerve-fibrils on the columnar epithelial cells of the intestine.
He found the condition in Petromyzon simpler than in fish.
In the former, the nerve-fibres in the plexus did not run in
bundles but singly, and the neurons were not grouped into
ganglia. Each neuron had three to four long, branched pro-
cesses among which it was difficult to distinguish an axon.
Müller and Liljestrand (1918) in their study of the autonomic
nervous system in Elasmobranchs described the general appear-
able of the myenteric plexus in the different regions of the gut,
and Müller (1920) showed the occurrence of pericellular nerve-
endings on the neurons of his plexus in Squalus acanthias.
Very recently Boeke (1935) demonstrated the presence of an
enteric nervous system in Amphioxus lanceolatus in
which he found plexuses, comparable with the plexuses of
Auerbach and Meissner of the higher vertebrates, containing
numerous stellate ganglion cells in synaptic connexion with the
nerve-fibres in the plexuses and anastomozing with each other
to form a primitive nerve-net.

MATERIAL AND METHODS.

The material used in this investigation included Amphioxus
and the following fish: Scylliorhinus canicula, Motella
sp., Saccobranchus fossilis, and Ophiocephalus
striatus. The methods employed were the silver nitrate method recommended by Hill (1927), the modified gold chloride method of Gairns (1930), and methylene blue. In using the methylene blue stain the procedure adopted was as follows. The fish was decapitated and pithed. The intestine was removed and washed out with filtered sea-water in the case of the marine forms, with 0.75 per cent. salt solution in the fresh-water forms. It was then filled with a very dilute solution of the stain in filtered sea-water or the salt solution as above, and placed in a shallow glass dish containing a similar solution of the stain. From this vessel the intestine was taken out and exposed to the air for short periods at frequent intervals. The stain took effect after one and a half to two hours of this treatment. After that the intestine was cut open longitudinally and its muscular wall separated off from the mucosa by means of fine forceps. The sheet of muscle was cut into small pieces and the stain fixed by putting these pieces for about half an hour into an 8 per cent. solution of ammonium molybdate. They were then washed for half an hour in three or four changes of distilled water, gently pressed between two glass slides to uncurl, dehydrated by grading through the alcohols, cleared in xylol, and mounted in Canada balsam. Such preparations allowed the plexus to be seen through the thin layer of longitudinal muscles. They kept the stain for about six months, so that it was possible to draw and study them conveniently.

The Amphioxus material available was of unknown fixation but had been preserved in dilute formalin. On treatment with the silver nitrate stain followed by toning with a few drops of 1 per cent. gold chloride in 3 per cent. solution of ammonium sulphocyanide, some good preparations were obtained in which, however, the gold chloride stain appeared to predominate.

**General Structure of the Myenteric Plexus.**

The general appearance of the myenteric plexus (plexus of Auerbach) in fish, already described in great detail by Monti, is represented in Text-figs. 1 and 2. It consists of a network of bundles of non-medullated nerve-fibres which cross each other chiasmatically and of nerve-cells. In Scylliorhinus these
nerve-cells are placed at the points of crossing of the fibre-bundles either singly or in small groups of two or three. Sometimes single nerve-cells are found along the fibre tracts. In the Teleosts studied these nerve-cells are usually to be seen singly in the meshes of the plexus, in this respect approaching the condition of the plexus in Amphioxus, figured by Boeke and depicted here in Text-fig. 3, and also that in Petromyzon as described by Sakussef. In Amphioxus the nerve-fibres

Text-fig. 1.
Part of the myenteric plexus from the intestine of Scylliorhinus canicula.
Text-fig. 2.

Part of the myenteric plexus from the intestine of Motella. $N_I$, nerve-cell of type I; $N_{II}$, nerve-cell of type II; $ax.$, axon.

themselves are not gathered together into such well-formed bundles as in fish, but run for the most part singly or in loose tracts of a few fibres each.
NERVE SUPPLY TO THE PLEXUS.

The nerve-fibres of the intestinal plexuses consist of (a) the processes of the nerve-cells contained within them, and (b) the nerve-fibres supplied to the gut from extrinsic sources. The nerve supply to the viscera in Selachians has been studied by Müller and Liljestrand, Müller, and Young (1933). In Scyliorhinus, according to Young, pre-ganglionic fibres are supplied by the vagus to the oesophagus and cardiac arm of the stomach and even to the anterior part of the intestine; both pre-ganglionic and post-ganglionic fibres are supplied by a pair of anterior splanchnic nerves to the oesophagus, stomach, and anterior region of the intestine; post-ganglionic fibres are supplied to the intestine by a pair of median splanchnic nerves and both pre-ganglionic and post-ganglionic fibres are supplied by the posterior splanchnic nerves to the hind end of the intestine and the rectum. In Teleosts, according to the same author [Young (1930)], probably both pre-ganglionic and post-gan-
glionic fibres are supplied to the gut from the visceralis branch of the vagus as well as from the first splanchnic nerve.

It should here be mentioned in passing that the condition of the first splanchnic nerve in *Ophiocephalus striatus* is intermediate between the conditions described by Chevrel (1887) and Young (1931) in *Uranoscopus*. Chevrel found a separate right and a left splanchnic nerve. Young described a large right splanchnic nerve arising from the first two spinal ganglia and a large commissure running across from the left side ventrally to the aorta to join the right first spinal ganglion. In *Ophiocephalus striatus* the right and left splanchnic nerves arise from the first two spinal ganglia of each side, but the left nerve crosses over to join the right nerve some distance away from the origin of the latter from the sympathetic chain.

**Nerve-cells in the Plexus.**

According to Monti’s description the nerve-cells in the plexus of the tench are all of Dogiel’s type I, but Sakussef observed cells belonging to types I and II from the myenteric plexus of the sterlet and the perch. These two types of cells are described in detail by the latter author so that it is not necessary to deal further in the present instance with their structure.

The ganglion cells in the plexus of *Scylliorhinus* are all of type II. They are unipolar, bipolar, or multipolar, the multipolar cells having from three to eight processes. In the myenteric plexus of Teleosts neurons of both types are represented. In this respect the observations here recorded are in agreement with those of Sakussef.

In the enteric plexuses of *Amphioxus* Boeke has conclusively demonstrated the presence of multipolar ganglion cells. He found, as did Sakussef in the enteric neurons of *Petromyzon*, that in cells of this kind a distinction between axon and dendrites was not to be made, the processes being all alike. He also suggested the possibility of the occurrence of bipolar ganglion cells, but was unable to establish this with certainty. In some cases he found on the walls of the liver and of the post-hepatic small intestine bipolar cells with two processes running in opposite directions; but whether they really
represented a different type, comparable with the second type of Dogiel, it was difficult to say, for in the method he adopted it was always possible that some of the longer processes may
have been cut away. This objection—Boeke's own—to the definite acceptance of the presence of these bipolar cells obviously cannot apply to the method employed in the present investigation. Moreover, these bipolar cells are so plentifully present in every part of this plexus (Text-figs. 3, 9, 10, 11, 12) that there cannot now be any doubt as to their occurrence.

In methylene blue preparations of the plexus of fish there is apparent another system of cells—the 'interstitial cells' of Cajal—between the longitudinal and circular muscle-layers. A portion of this system of cells from Ophiocephalus striatus is drawn in Text-fig. 4. In Scylliorhinus too it has

![PERICELLULAR ARBORIZATION OF A VAGAL FIBRE ON A NERVE-CELL FROM THE MYENTERIC PLEXUS OF SCYLLIORHINUS CANICULA. NII, NERVE-CELL OF TYPE II; PA., PERICELLULAR ARBORIZATION; VF., VAGAL FIBRE.](image-url)
an identical structure. These 'interstitial cells' are of variable shape, with three to four processes, and are arranged syncytially. Each cell is in protoplasmic continuity with one or more of the neighbouring cells either by the union of their processes or by the fusion of a process of one cell directly with the body of a neighbouring cell. The cell processes are branched, but the branches of the processes belonging to the same cell are not anastomosed. Sometimes the cell processes are thin and long, sometimes they bear distinct swellings. Those processes, which do not unite with neighbouring cells, penetrate between the muscles but could not be traced to their terminations.

**Synapses within the Plexus.**

Kuntz (1911) and Müller described the termination of pre-ganglionic fibres in the form of pericellular arborizations on the enteric neurons of fish. These endings were also probably observed by Monti, who described clumps of very fine nerve-fibrils of varicose appearance at the points usually occupied by nerve-cells when the nerve-cells themselves, as commonly
happens in methylene blue preparations, had failed to take up the stain.

Pericellular arborizations are to be found on many of the nerve-cells in the plexus of Scylliorhinus (Text-figs. 5 and 6). Such an arborization is formed by a nerve-fibre running close to a nerve-cell giving off a few branches which in their turn divide into fine fibrils surrounding the nerve-cell very closely, these fibrils being especially prominent at the bases of the dendrites and the axon of the nerve-cell. The main nerve-fibre, meanwhile, continues on its course, probably giving off other branches which form arborizations round several more nerve-cells. On other nerve-cells, however, in the plexus of this fish it has been possible to observe what may be regarded as
a different type of synapse. In these cases a distinctly varicose fibre whirls round and round a nerve-cell, at first some distance away from the cell but later approaching nearer and nearer to lie against the cell-body (Text-fig. 7).

In the plexus of Teleosts pericellular arborizations are to be found on nerve-cells of type II. Occasionally, in addition to the pericellular arborization, another kind of nerve-ending can be observed on the same type of nerve-cell where a nerve-fibre of moderate stoutness can be seen to approach a nerve-cell and terminate on its cell-body in one or two small boutons (Text-fig. 8). A nerve-fibre forming this kind of synapse could not be successfully traced back all the way to its origin; but, from its stoutness and by comparison with a similar kind of ending, to be described later, on the bipolar cells of Amphioxus I am inclined to conclude that it represents the termination of the axon of a neuron of type I.

In the enteric plexus of Amphioxus three distinct kinds of
synaptic connexions are to be observed: (1) synapse between a process of a stellate ganglion cell and a nerve-fibre; (2) pericellular arborization of a nerve-fibre on a bipolar nerve-cell; and (3) termination of a process of a stellate ganglion cell on a bipolar cell. The first of these synapses has been amply described and illustrated by Boeke. But about the other two kinds of synapses, involving as they do the bipolar cells, he was naturally unable to say anything as he was not quite certain of the existence of bipolar cells in the plexus. Text-figs. 9 and 10 show the relation which the stellate ganglion cells bear to the rest of the plexus. In each of these figures one process \( p_2 \) of a stellate ganglion cell is in synaptic contact with a nerve-fibre, presumably pre-ganglionic, and another process \( p_1 \) is seen to terminate on the cell-body of a bipolar nerve-cell. Still another process \( p_3 \) forms an ending round the nucleus of an epithelial cell. Pericellular arborizations on bipolar cells are shown in Text-figs. 11 and 12.

**Discussion.**

Of the extrinsic vagal and sympathetic nerve-fibres which enter the wall of the alimentary tract of fish it is generally agreed that the vagal fibres are pre-ganglionic and that they form pericellular arborizations on enteric neurons. Müller as well as Young (1933) found that in Selachians some of the sympathetic fibres are also pre-ganglionic, but Kuntz (1911) denied the presence of any neurons of sympathetic origin in the enteric plexuses of vertebrates including fish. In the light of Young's recent contributions to our knowledge of the autonomic nervous system in fish we have to accept his confirmation of Müller's observation as to the pre-ganglionic nature of some of the sympathetic fibres supplied to the intestine in Selachians. In Teleosts, however, we cannot be so sure that any pre-ganglionic sympathetic fibres enter the gut-wall. Young (1931) says that in Teleosts, too, some of the sympathetic fibres are probably pre-ganglionic. But it may be that in Teleosts the pre-ganglionic sympathetic fibres terminate on the coeliac ganglion cells, as they are said to do in the higher vertebrates. In Selachians, on the other hand, some pre-ganglionic fibres are apparently continued uninterrupted through the axillary body
A stellate ganglion cell and a bipolar nerve-cell from the enteric plexus of Amphioxus showing synapses. b.g.c., bipolar ganglion cell; e.c., epithelial cell; g.c., stellate ganglion cell; n.f., preganglionic nerve-fibre; p. 1–3, processes of stellate ganglion cell; sy., synapse.
According to Müller, in *Squalus acanthias* the preganglionic vagal fibres end as pericellular arborizations on the enteric neurons. It is here attempted to show that in *Scylliorhinus* a second kind of nerve-ending is to be found in the form of varicose nerve-fibres which wind themselves round the cell-body of some of the enteric neurons (Text-fig. 7). This form of nerve-ending strongly resembles the sympathetic innervation (first sympathetic ganglion of the trunk), and also by way of the posterior splanchnic nerves to terminate on neurons in the enteric plexuses.

**Text-fig. 10.**
For legend see opposite.
TEXT-FIGS. 11 AND 12.
Bipolar nerve-cell from the enteric plexus of Amphioxus showing pericellular arborization of a preganglionic fibre. n.f., preganglionic nerve-fibre; pa., pericellular arborization.
of the blood-vessels and may represent the mode of synapse of a pre-ganglionic sympathetic fibre on an enteric neuron as distinct from the pericellular arborization formed by a pre-ganglionic vagal fibre.

It has been mentioned above that while the enteric neurons of Scylliorhinus are all of type II, those of Teleosts belong to both types I and II. In the latter fish the pericellular arborizations of pre-ganglionic vagal fibres are found on cells of type II, while the cells of type I show no endings on them. These neurons of type I have short, thick, branching dendrites. As far as it has been possible to observe, the dendrites of a nerve-cell of this type do not anastomose with one another or with the dendrites of a neighbouring cell. The only form of connexion these have with the rest of the plexus is probably provided by the synapse of their axons with neurons of type II. Thus some of the neurons of type II may have two kinds of endings on them, namely, the pericellular arborization of a vagal fibre and the bouton-like termination of an axon of a neuron of type I (Text-fig. 8).

Whilst describing the pericellular arborizations of the extrinsic fibres on the enteric neurons, Müller believed that in Selachians the enteric neurons themselves were in a syncytial arrangement forming a real nerve-net. If we accept the nervous nature of the ‘interstitial cells’, the only nerve-net in the alimentary canal is provided by these cells. No connexion between these ‘interstitial cells’ and the true neurons is to be observed. The enteric neurons do not fuse to form a syncytium. In Scylliorhinus there does not appear to be any connexion between them, while in Teleosts a connexion between them is formed by the termination of the axons of cells of type I on cells of type II.

In the enteric plexus of Amphioxus Boeke compares the stellate ganglion cells with the cellular elements of the primitive nerve-net. He believes that these stellate ganglion cells anastomose with each other and that the neurofibrillae of these cells are continuous through their processes. I am unable to confirm that these stellate ganglion cells form such a syncytium. It has been shown earlier that in addition to the stellate ganglion cells
bipolar cells are undoubtedly represented in the enteric plexus of *Amphioxus*, and the mode of synapse between these two kinds of nerve-cells has been described. It appears, therefore, that these enteric nerve-cells of *Amphioxus* are of the same nature as the true neurons of the enteric plexuses of fish and the higher vertebrates. Whether there is in *Amphioxus* any syncytial system of cells comparable with the 'interstitial cells' in the alimentary canal of the higher chordates still remains to be demonstrated. There seems to be greater justification for comparing the stellate ganglion cells of *Amphioxus* with neurons of type I in the higher forms. These nerve-cells have in common the possession of much branched dendrites and the same manner of synapse with neurons of type II. Müller and Kuntz found that the neurons of type II on which pericellular arborizations occur arise in development from the vagus ganglia and later migrate into the gut-wall.

**Summary.**

The arrangement of the myenteric nerve-plexus is simpler in fish, much simpler in *Amphioxus*, than in the higher forms. The nerve-cells in it are not grouped into distinct ganglia at the points of crossing of the fibre-bundles. In *Scylliorhinus* only two or three cells are present at such points, while in Teleosts the nerve-cells are situated in the meshes of the plexus, as is also the condition in *Amphioxus*.

In *Scylliorhinus* these nerve-cells are all of Dogiel's type II; in Teleosts they belong to both types I and II. In *Amphioxus* bipolar nerve-cells are definitely present in addition to the stellate ganglion cells described by Boeke.

Synapses between (a) the pre-ganglionic fibres and the enteric neurons, and (b) the enteric neurons themselves are figured and described.

The 'interstitial cells' of Cajal are present in the muscle-wall of the gut of fish. A connexion between this system and the enteric plexuses is not to be observed.

The enteric plexuses of fish and *Amphioxus* are not in the form of a nerve-net. It is not possible to compare the enteric plexus of *Amphioxus* with the system of 'interstitial
cells' of the higher chordates. The stellate ganglion cells of *Amphioxus* are to be compared with the neurons of type I in the higher forms.

**References.**


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