Histology. — *The autonomic (enteric) nervous system of Amphioxus lanceolatus.* By J. BOEKE (Utrecht).

(Communicated at the meeting of December 16, 1933.)

About the sympathetic nervous system of Amphioxus very little is known, common opinion among the scientists being, that amphioxus does not possess any sympathetic nerves at all. The dorsal roots send out rami dorsales and rami ventrales. From these rami ventrales branch off delicate rami viscerales, running between the myotome and the pterygial muscle; they divide into a ramus descendens and a ramus ascendens. According to LEGROS the postbranchial rami ascendentes run inside the transversal septa, pass to the membranes covering the wall of the enteric canal and follow the course of the veins, running along the dorsal wall of the liver. This is accepted by FRANZ (1927), but although FRANZ accepts the existence of rami viscerales even of the postatrioporic nerves, running to the visceral wall, he never could see a nerve fiber on the entodermal visceral wall; after his opinion the ganglioncells described by VAN WIJHE (see below), which were to be seen in a microphotogram sent him by VAN WIJHE, were nothing but motor endings of the common spinal type on the atrial muscle fibers. Neither DOGIEL nor HEYMANS and VAN DER STRICHT could find real ganglioncells in the dorsal roots, or peripheral sympathetic ganglioncells, and because of this FRANZ entirely denies the existence of a real „sympathetic" of the viscera, „paraspinale Ganglien reiner Eingeweidenerven oder aber ein Grenzstrang fehlen dem Akranier, und so sind seine Eingeweidenerven so wenig „sympathisch" im morphologischen Sinne wie die viscerale Verzweigung des Vagus bei einem Kranioten." (FRANZ, 1927, Page 528). According to YOUNG (1933) in Amphioxus all the visceral nerve fibers run to the viscera through dorsal roots (without peripheral ganglion cells) and this was presumably the original condition (l.c. page 617).

Only VAN WIJHE described in a footnote on page 1550 of his paper on the metamorphosis of Amphioxus in these Proceedings of the year 1913, that he found underneath the atrial epithelium covering the liver and the postbranchial prohepatic intestine a great number of beautiful multipolar ganglioncells, which sent their axiscylinder through the dorsal nerve roots to the spinal cord.

VAN WIJHE never published anything more about these ganglioncells, but he kindly sent me a microphotograph of one of his preparations, which is reproduced here in Fig. 1, and in a letter accompanying the photo VAN WIJHE wrote the following statement (16 November 1933): „on the sympathetic in Amphioxus I never published anything, but I

possess preparations, in which neurones are seen under the atrial epithelium covering the oesophagus and the liver. The ganglioncells show two or three short dendrites and a long axiscylinder (neurite) is seen ascending into the dorsal (septal) nerves. The ganglioncells are similar to the sympathetic cells described in Petromyzon, but their position directly underneath the atrial epithelium suggests that they are analogous to the ganglioncells which form the first two septal (dorsal) nerves under the dorsal covering of the rostrum. In no case they can be motoric, because there are no muscle fibers in the neighbourhood."

The cells which are to be seen in the photograph (Fig. 1), described by VAN WIJHE with their long axiscylinders I know from my own Golgi-preparations, in which a few were impregnated, but I must confess that I had not dared to identify them with sympathetic elements. Yet after having studied the photos and the description of his findings I am sure that the cells mentioned by VAN WIJHE are real sympathetic cells, and that FRANZ is wrong when he thinks that the elements shown in the photograph are merely motor endplates on the muscle fibers of the atrial muscle.

For a series of splendidly impregnated Bielschowsky-preparations, longitudinal sections and cross-sections through adult animals, revealed a quite astonishing abundance of stellate multipolar nerve cells on the wall of the liver and of the adjoining parts of the intestine, the „oesophagus" and the „stomach".

The neurofibrillar structure of these cells and of their processes is very clear and distinct, as is shown in the figures 2—5 of this communication. Coarser fibrillae run through the cellbody from one process to another, but inside the cellbody they are everywhere connected by a number of finer fibrillae forming a distinct network. In several places the processes themselves are broader, flattened out, and in these places the very delicate neurofibrillar network is as conspicuous as inside the cellbody. In short, the multipolar cells show exactly the same neurofibrillar structure as the nerve cells of the spinal cord of Amphioxus as it was described amongst others by me in these Proceedings (Proc. of the meeting of April 1902, page 695). A difference between neurites and dendrites was not to be found, the processes of the cells being all of them of the same structure, as is clearly shown in the accompanying figures. In this they resemble closely the sympathetic cells of the higher vertebrates, in which according to STOEHR in most cases all the processes are so entirely of the same pattern, that a distinction between a long axiscylinder, a neurite, and branched dendrites is impossible. In some cases however I found on the wall of the liver and of the small intestine *bipolar cells* with two processes running in opposite direction (nearly in longitudinal directions) along the wall of the liver, which showed exactly the same form as the cells photographed by VAN WIJHE, reproduced in Fig. 1. In reference to the two kinds of sympathetic cells in the plexus of

Auerbach in the higher vertebrates described by DOGIEL, LAWRENTJEW, VAN ESVELD, KOLOSSOW, SOKOLAW and others, which may be connected with the sympathetic and parasympathetic systems, the existence of bipolar and multipolar cells in Amphioxus may be of interest. Here however they are lying in and part of the same plexus without any further distinction.

The ganglioncells described above are very numerous and are distributed very regularly in a thin layer on the wall of the liver, the oesophagus and the stomach or middle intestine, and form a regular plexus, the processes extending in the thin sheath covering the wall in every direction (viz. always in the same horizontal plane), and intertwining to form plexiform bundles, as is shown in the figures. As far as I could gather from the study of the sections, the processes of the multipolar cells anastomose freely with eachother, but as I could study only thin sections, from 10 to 6 μ, it was impossible to settle this point definitely and without doubt. At all events, as is shown in the figures, the cell-bodies often are lying in the knot-points of the plexus just as in the drawings and photo's of LAWRENTJEW and GRIGORIEFF of the syncytial plexus of ganglioncells in their tissue cultures. No capsule cells could be distinguished, the ganglioncells lying freely between the connective-tissue elements. The processes of the cells, the nerve fibers, often run in small bundles, and in this way a very regular plexus is formed. On the wall of the liver thicker bundles are seen running mostly in an oblique direction towards the beginning of the small intestine where the liver is connected with the intestine. From my sections I got the impression, that there where the liver communicates with the intestine, the nerve plexus covering the wall of the liver, is connected with the visceral nerves. This would be the natural connection, because the liver is formed as an outgrowth from the intestinal wall, growing out in a cranial direction. It seems to me, that the nervous plexus covering the wall of the liver, has grown out from the point of origin of the liver-outgrowth together with this formation. Thus we find the thicker bundles of the nervous plexus on the liver running in an oblique ventrodorsal direction towards this point of connection with the intestine; on the wall of the postbranchial intestine we find the same disposition of the nervous plexus and of the coarser connecting bundles. Here too they are found running in an oblique dorsoventral direction, and in some cases I could follow them further on, until they were passing into the visceral nerve bundle going to the dorsal segmental nerves. When we may compare the ganglionic plexus described here with the sympathetic system of the higher vertebrates, perhaps we are entiled to compare the visceral nerves with at least a part of the rami communicantes and of the splanchnic nerves. But of a formation which could be compared with the sympathetic trunks running along the ventral side of the vertebral column, no trace was to be found in my material.

Fig. 1.

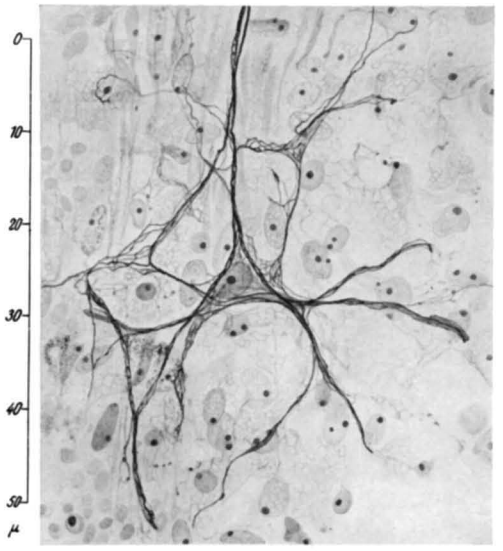

Fig. 2.

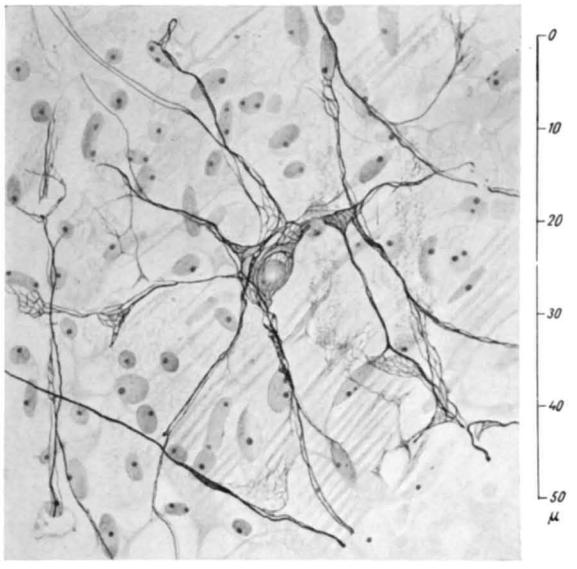

Fig. 3.

But when we compare the enteric plexus with the sympathetic system of the higher vertebrates, viz. with the plexus of AUERBACH and MEISSNER, what are the elements, with which the ganglioncells and their processes are connected?

According to VAN WIJHE the stellate ganglioncells, which he saw on the wall of the liver and the intestine, could not be motoric, because there are no muscle fibers in the neighbourhood.

On this point I cannot agree with him. On the wall of the liver and of the oesophagus and intestine there is to be found a very conspicuous and regular layer of spindle-shaped cells, running parellel to each other, and chiefly in a circular direction around the gut. Often the bundles of these cells run in peculiar vortices, which are difficult to describe without a number of accurate drawings; the whole formation will be described at full length in a better illustrated paper, here I must confine myself to a general description. These spindle-shaped cells are well-known, and have been described as fusiform connective tissue cells (FRANZ, 1927) although the van Gieson stain was not very strong. In my opinion these elongated cells or fibers are typical smooth muscle fibers; they have exactly the same form and structure as the smooth muscle fibers of the higher vertebrates. In the wall of the liver and of the intestine (including the „oesophagus") they form a layer just outside of the intestinal epithelium, with only a very small amount of connective tissue cells between them, and they are covered by the nervous plexus mentioned above. In the figures these spindle-shaped cells or fibers are very conspicuous, just as they appear in the preparations, where they are to be studied with the utmost clearness. The whole layer of these fibers may be compared with the muscular coat of the intestinal wall. And in this connection it is extremely interesting, that the nervous plexus described above seems to be connected everywhere with these elements. When studied with the highest power and strong light we see very delicate neurofibrillae branching off from the nervous strands and ending in fine nets on the spindle-shaped fibers. In some cases (not figured here) I could distinguish extremely small but very sharply defined endrings, lying close to the spindle-shaped fibers, in other cases no endrings could be distinguished but very delicate reticular formations were to be seen; in all cases there seems to exist a distinct terminal connection of the nervous strands with the elongated spindle-shaped cells, viz. the smooth muscle fibers.

There is still more. When we study these formations in longitudinal sections, there appears, as I mentioned before, a small amount of connective tissue between the layer of spindle-shaped fibers and the intestinal epithelium. In this tissue we may distinguish a still more delicate network of neurofibrillae, given off by the nervous strands of the denser plexus, and lying between the connective tissue cells. The details of this formation will be described at full length elsewhere; here I will confine

myself to the suggestion that this formation perhaps might be compared with the plexus of MEISSNER, so much finer than the plexus of AUERBACH.

Finally I may mention, that also on the walls of the blood-vessels, running along the dorsal wall of the liver, a network of very delicate nervous fibers was to be found, connected with the plexus mentioned above.

Thus it seems to me, that we have to alter the statements mentioned above, and that amphioxus possesses, in a very primitive form, a sympathetic system, which may be compared with the enteric plexus of the higher vertebrates.

Utrecht, December 1933.

EXPLANATION OF FIGURES.

Fig. 1. Microphotograph by VAN WIJHE, showing the stellate ganglioncells on the wall of the intestine, after a BIELSCHOWSKY-preparation.

Fig. 2—5. Camera lucida drawings of the elements of the nervous plexus covering the wall of the liver and of the intestine, after silver-preparations. In figg. 3 and 5 the spindle-shaped smooth muscle cells are clearly to be seen. Full-grown amphioxus lanceolatus.
BIELSCHOWSKY-method, sections treated with chloride of gold and afterwards stained weakly with haematoxylin.

Of the literature cited I will only mention the paper by VAN WIJHE, Proceedings of the meeting of the mathem.-phys. Class of the Royal Academy of Science, Amsterdam, Meeting of April 1913, BOEKE, ibidem, April 1902, FRANZ, V., Ergebnisse Anat. und Entwickel. Gesch. 27. Bd., 1927, and YOUNG, J. Z., Quarterly Journ. of Micr. Science, Vol. 75, Part IV, 1933.

Mineralogy. — *Reacting for Tungsten in Minerals.* By J. VERSLUYS and H. L. J. ZERMATTEN.

(Communicated at the meeting of October 28, 1933.)

When a mineral is identified it may be desirable to ascertain whether it contains *Ti, Mo, W* or *Nb*. The method mostly recommended for this purpose in the handbooks and described for instance in the latest editions of the works of PLATTNER [1]) and KRUG [2]), is the following.

The mineral under examination is powdered and broken down with salt of phosphorus or carbonate of soda and saltpeter, heated till all nitrates have been decomposed, after that dissolved in diluted muriatic or sulphuric

[1]) F. KOLBECK: "Plattner's Probirkunst mit dem Lötrohre". 8th ed. Leipzig, 1927.
[2]) CARL KRUG: "Lötrohrprobirkunde". 2nd ed. Berlin, 1925.