$$x_{T_r} - x_{Tpl} = \frac{3}{2} \frac{\left(\frac{\partial p}{\partial x}\right)^2_{vT}}{(MRT_k)^3 \cdot \frac{\partial^3 p}{\partial v^3}} \left\{ \left(\frac{\partial p}{\partial x}\right)^2_{vT} + MRT_k \left(\frac{\partial^2 p}{\partial x \partial v}\right) \right\} x^2_{Tpl} .$$

So that for $x = 0$: $\frac{dx_{Tr}}{dT} = \frac{dx_{Tpl}}{dT}$, or $\frac{dT_{xr}}{dx} = \frac{dT_{xpl}}{dx}$, from which we easily derive that also $\frac{dp_{xr}}{dx} = \frac{dp_{xpl}}{dx}$ so that in the pT-diagram the point of contact curve and the plaitpoint curve touch at the ends. We find further that with the same x:

$$T_{xr} - T_{xpl} = \frac{3}{2} \frac{\left(\frac{\partial p}{\partial x}\right)^2_{vT}}{(MRT_k)^4 \cdot \left(\frac{\partial^3 p}{\partial v^3}\right)\left(\frac{\partial^2 p}{\partial v \partial T}\right)} \left\{ \left(\frac{\partial p}{\partial x}\right)^2_{vT} + MRT_k \left(\frac{\partial^2 p}{\partial x \partial v}\right)_T \right\}^2 x^2 .$$

from which $p_{xr} - p_{xpl}$ can be easily found.

If, as in Communication N°. 75 (Proceedings Nov. 1901), we introduce the law of corresponding states, we find:

$$v_{Tr} - v_{Tpl} = 3\, C_4\, v_k \frac{\beta - \alpha \left(\frac{\partial \pi}{\partial \tau}\right)}{C^3_4 \left(\frac{\partial^3 \pi}{\partial \omega^3}\right)} \left[\left\{ \beta - \alpha \left(\frac{\partial \pi}{\partial \tau}\right) \right\}^2 - C_4\, \alpha \left(\frac{\partial^2 \pi}{\partial \omega \partial \tau}\right) \right] x$$

$$T_{xr} - T_{xpl} = \frac{3}{2} \frac{\left\{ \beta - \alpha \left(\frac{\partial \pi}{\partial \tau}\right) \right\}^2}{C^3_4 \left(\frac{\partial^3 \pi}{\partial \omega^3}\right) \cdot C_4 \left(\frac{\partial^2 \pi}{\partial \omega \partial \tau}\right)} \left[\left\{ \beta - \alpha \left(\frac{\partial \pi}{\partial \tau}\right) \right\}^2 - C_4 \alpha \left(\frac{\partial^2 \pi}{\partial \omega \partial \tau}\right) \right]^2 x^2 .$$

Physiology. — "*On the structure of the light-percepting cells in the spinal cord, on the neurofibrillae in the ganglioncells and on the innervation of the striped muscles in amphioxus lanceolatus.*" By Dr. J. Boeke. (Communicated by Prof. T. Place).

In connection with a former note [1]) I mean to describe here some points of the histology of the central and peripheral nervous system of amphioxus lanceolatus, especially to follow the neurofibrillae in their arrangement and distribution in the cells and in the muscle-plates.

This paper is the outcome of observations begun in 1900 in the Stazione Zoologica at Naples, but then not carried any farther, to study the structure of the pigmented cells of the spinal cord. During

[1]) Proceedings of the Royal Academy of Amsterdam. Meeting of April 19, 1902.

a stay at the Zoological Laboratory of Prof. ST. APÁTHY in Kolozsvár once again I took up the theme, with some excellently fixed material I got through Prof. APÁTHY's kindness. Finally the researches were carried on in the Histological Laboratory of Amsterdam.

A. The structure of the light-percepting cells (eye-cells).

In 1898 HESSE [1]) showed, that the peculiar pigmented cells, which are found in the spinal cord grouped round the ventral wall of the central canal, and which after beginning at the third segment, are arranged segmentally through the whole medulla, are each of them composed of two cells, one of them a big ganglioncell without pigment, the other a cupshaped cell, filled up entirely with dark brown pigmentgrains; the last cell covering the greater part of the firstnamed cell and hiding it from view.

The big unpigmented cells HESSE called eye-cells, light-percepting cells, the cupshaped pigmented cells he called the pigmentcup (Pigmentbecher), and the whole complex he compared with the cupshaped eyes of the Planarians, that are equally composed of two cells, and attributed to it the function of light-perception.

The arrangement of these two-celled eyes in the spinal cord is strictly segmental. They begin in the fourth segment, with two eyes; from there each segment is furnished with about 25 eyes. In the region of the tail the number lessens, until a segment has only one eye or none at all.

The eyes lying ventrally of the central canal are always looking down, their line of vision, if we may call it so, being directed to the ventral side of the animal, those at the left side of the central canal look upward and to the right, those at the right side look down and to the right.

The pigmentcup consists of one cell, the nucleus, when distinguishable, lying at the concave side of the cup.

The eye-cell is coneshaped, the base being covered by the pigmentcup, the top being drawn out into a thin process. At the basal side (turned towards the pigmentcup) the protoplasm is differentiated into a layer of fine small rods, placed at right angles to the cell-periphery, and continuing in the protoplasm as a network of very thin fibres. Another layer of minute rods may be seen close against the pigmentcup. Between those two layers a clear space is formed, which is not caused by a shrinking of the cell.

W. KRAUSE [1]) did not agree with the results of HESSE. He still maintained that the pigmented cells in the spinal cord consisted

[1]) Zeitschr. f. Wiss. Zoologie. Bd. 63. 1898. p. 456.
[1]) Anat. Anzeiger. Bd. 14. Pag. 470. Zoöl. Anzeiger. Bd. 21. p. 481.

each of them of only one cell with the pigmentgrains lying only at the periphery, just as HEIJMANS and v. D. STRICHT [1]) had said in 1898; by BEER [2]) and SCHNEIDER [3]) the description given by HESSE was taken for right and confirmed.

As to the arrangement of the pigmentcells in the spinal cord in the first place, the observations of HESSE are not complete. They do not simply lessen in number going from before backwards. In young pelagic larvae there are to be seen very distinctly two groups of pigmentcells, one in the cranial part of the body, the other in the caudal half. Between those groups there are much less pigment-cells in each segment. In later stages however these two groups become fused, and then the arrangement is in the main as it is described by HESSE.

As regards the position occupied by the pigmentcap on the eye-cell, I can in the main confirm the observations of HESSE. The eyes at the ventral side of the central canal are always looking down, those at the left are mostly looking upwards and to the right, those at the right mostly downwards and to the right.

The histological structure of the eyes seems to me to be slightly different from the one described by HESSE and SCHNEIDER.

The nucleus of the pigment-cell is never lying at the concave, but mostly at the convex side of the cupshaped cell; sometimes the nucleus is found in the middle of the pigment-cell, where often a clear pigmentfree zone of protoplasm may be distinguished. According to HESSE the pigment-cup consists always of only one cell. Now sometimes in young animals, where the pigment is of a light-brown colour and the nucleus may therefore be seen very clearly, I found two nuclei in the pigment-cap, and so it seems to me that there are sometimes two pigment-cells with one eye-cell. So the form of the pigment-cap in fig. 3 seems also to point to the pigment-cap being composed of two cells. As a rule, however, there is only one pigment-cell in each eye.

In the eye-cell, lying under the pigment-cell, HESSE describes a double row of small rods, lying close to the pigment-cell. This double row of small rods exists, but the two parts of it are not separate, but continuous at both ends, in whatever direction the cell is cut through. So a flat oval body is formed, with a striated wall, lying close to the pigment-cap (fig. 1. *a*), and following in its shape the form of the cap. This body seems to me to be homologous with

[1]) Mém. couronn. de l'Acad. roy. de Belgique T. LVI 1898.
[2]) Wiener med. Wochenschrift 1900.
[3]) Lehrb. der vergl. Histologie der Tiere 1902.

the "Glaskorper" with a striated wall, as it is found in the eye-cells of the Hirudines. As is the case with those eye-cells, here in Amphioxus too the vitreous body seems to be filled with a granular-looking substance (coagulation?) but this was not always clearly to be seen.

Between this body (*a*), that lies close to the pigmented cap, and the nucleus (fig. 1 *k*) lying at the other side of the eye-cell, there is in most cases to be seen another beanshaped body, that does not possess a striated wall, but by a clearer tinction with protoplasmic dyes and a more homogeneous substance may be distinguished from the darker and more granular-looking protoplasm of the cell (fig. 1*b*).

This body seems to be connected with the perception of light by the eye-cell in the same way as the vitreous body described above. The arrangement of the neurofibrillae in the eye-cells seems to point to this conclusion. Entering the cell at the ventral side of the nucleus, there, where according to HESSE the eye-cell is drawn out to a point, the neurofibril forms a loosely built network round the nucleus. From this network large neurofibrils ascend through the cell and take the beanshaped body (*b*) between them (fig. 2, fig. 3). Between this body and the pigment-cap these neurofibrils anastomose again and form a second net, which seems to enclose the vitreous body (*a*) with the striated wall. How the further course of these fibrils is between the vitreous body (*a*) and the pigment-cap I could not determine with any accuracy.

To obtain good results with the chlorid of gold-method of APÁTHY the sections may not be very much thinner than 10 μ. Now for the study of the eye-cells it is necessary to make sections of about 6 to 7 μ, because in thicker sections the black pigment of the capshaped cell embraces the greater part of the eye-cell and shuts it out from view. It is therefore not possible to get those deep black neurofibrillae, which may be seen so distinctly in the preparations of APÁTHY (the more so as the neurofibrillae of Amphioxus are thinner than those of Hirudines); and even in sections of 6 to 7 μ that part of the neurofibrillae-network, which is lying beneath the pigment-cap is entirely concealed by the pigment-grains. Probably the network is continuous and anastomoses at the other side with the more ventrally lying network.

In what manner the neurofibrillae leave the eye-cell I could see only in a few cases. The fibril seemed then to proceed horizontally for some time but could not be followed any farther.

B. The neurofibrillae in the ganglion-cells.

On the neurofibrillae in the ganglioncells I'll say only a few words.

It would lead us too far to go into details about the arrangement of the neurofibrillae in all the different types of ganglioncells, and besides, that would not be possible without many plates and drawings. I will therefore confine myself here to the following statements:

According to BETHE[1]) in most of the ganglioncells of the vertebrates the neurofibrillae pass through the cellbody without branching or breaking up into a network. Only in the spinal-ganglioncells and in the cells of the lobus electricus of Torpedo marmorata BETHE observed networks of the neurofibrillae, and according to this author networks possibly occur in the basal part of the cells of PURKINJE in the cerebellum and in the cells of the cornu Ammonis.

According to BOCHENEK[2]) it is on the other hand probable, that in the vertebrate ganglioncells the neurofibrillae form a very fine network with small meshes. The very dense reticulum of neurofibrillae, he was able to demonstrate in the ganglioncells of HELIX, forms according to BOCHENEK an intermediate stage between the coarse network in the cells of Hirudines and Lumbricus, and the very fine network in the vertebrate ganglioncells.

In accordance with the statements by these two authors, we should expect to find in the ganglioncells of Amphioxus either a dense reticulum or a mass of disconnected interwoven very fine threads, passing from one process through the cell-body into another process without branching. This is not the case. In most of the ganglioncells the arrangement and distribution of the neurofibrillae in the ganglioncells resembles very much that which is described by APÁTHY in the ganglioncells of Hirudines and Lumbricus.

Sometimes we find cells as the one shown in fig. 4, where the neurofibrillae pass through the cell-body without interruption, but this is only to be found in a few cases.

In the bigger ganglioncells, which are lying in the dorsal part of the spinal cord and in the dorsal group of ganglioncells behind the brain-ventricle, there is always a network of neurofibrillae branching and anastomosing with each other. After having entered the cell in most cases the neurofibrillae form a network round the nucleus (partially distinguishable in fig. 5). From out this reticulum radial fibrillae go through the cell body to the periphery (often branching on their way) where they form a second network. With this network are connected fibrillae, which pass through one of the processes of the cell (out of the cell or into it?) — in short, a distribution of

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1) Arch. f. Mikrosk. Anatomie, Bd. 55. 1900. P. 513.

2) Le Névraxe. Vol. III. Fasc. I, 1901. P. 85.

the neurofibrillae very much like that described by APÁTHY in the smaller ganglioncells of Lumbricus. The fibrillae however in Amphioxus are thinner, and the reticulum finer.

In other ganglioncells there are not two networks (one round the nucleus and one more at the periphery), connected with each other by means of the radial fibrillae, but the neurofibrillae enter the cell, form a network round the nucleus and leave the cell at the other side, without there being any trace of a more peripheral network to be seen.

A connection between different ganglioncells by means of the neurofibrillae, I could not yet state with a sufficient amount of certainty.

In the colossal ganglioncells the "Kolossalzellen", lying just in the middle of the spinal cord, the arrangement of the neurofibrillae is very peculiar. From out the colossal nerve-fibres, the axons of these cells, a thick bundle of very thin neurofibrillae, arranged very regularly and equally in the whole axis cylinder, enter the ganglioncell; in the cell-body they pursue their way as a thick bundle that passes round the nucleus, turns upon itself, forms a sort of vortex and then seems to condense itself into a few thick (composed of a great number of elementary fibrillae) fibrillae. Where these fibrils go to, I could not state accurately. In the axons the extremely thin neurofibrillae are closely set and parallel to each other, and so a striking resemblance is formed with the "sensorische Schlauche" of Hirudines and Astacus. During the course of these nerve-fibres through the spinal cord the neurofibrillae are seen to pass one by one every now and then in an oblique direction through the wall of these nerve-fibres; then they are lost in the nervous network without, and could not be followed any farther. Perhaps they are connected there with other ganglioncells, which should be in concordance with the character of the colossal ganglioncells as connecting cells ("Schaltzellen").

C. The innervation of the striped muscular tissue.

According to ROHDE [1]) the motor nerves simply enter the muscle-plates there where these end, and there is no trace of a motor nerve endplate; according to HEYMANS and VAN DER STRICHT [2]) however the motor nerves of Amphioxus terminate each in a shovelshaped endplate, that lies itself against the muscle-plate just as the motor nerve endplates of the higher vertebrates do. According to their descriptions and drawings the endplates of Amphioxus are thick shovel-shaped plates without branchings, without further differentiations (Golgi method).

1) SCHNEIDER's Zoologische Beiträge. Bd. II, 1888.
2) Mém. couronn. par l'Acad. roy. de Belgique 1898.

Now APÁTHY and RUFFINI [1]) were able to demonstrate in homo the existance of "ultraterminal" nerve-fibres, that is to say nerve-fibres which grow out from the branching and thickening of the motor nerve known as "endplate", and enter the muscle-fibre (this could not be made out with absolute certainty) pass through it and in many cases are connected with other endplates. Only a few cases are described but they are sufficient to show that the so-called nerve end-plate is not always to be considered as the real termination of the motor nerves.

The following observations seem to point to the same conclusion.

The thin muscle-plates of Amphioxus (fig. 6*a*) present in longitudinal sections a beautiful cross striation. Each isotropous disc (*i*) is divided into two discs by a delicate, but distinct membrane of KRAUSE; each anisotropous disc (*q*) is composed of two discs, separated by a thin layer, that takes but a faint stain with chloride of gold, the median disc of HENSEN. In the middle of this transparent portion there is sometimes to be seen an extremely delicate line, the membrane of HENSEN.

The membranes of KRAUSE form, as is known, crossnets, which bring the fibrillae of the entire muscle-plate in connection with each other. In the adult animal real muscle-cells are not to be distinguished, there are only the thin flattened muscle-plates to be found, which however in hardened specimens sometime appear to be broken up into rows of flat bundles of fibrillae. This is nothing but an artefact.

In longitudinal sections of Amphioxus in which therefore the muscle-plates are cut in the same direction, but mostly appear not as plates but cut obliquely as bundles of muscle-fibres (fig. 6*a*), there are to be found, in case the sections are coloured after the chloride of gold method, in many places just there were the anisotropous and isotropous discs meet, minute black dots, or small corpuscles; seen under a microscope of the highest magnifying power these dots appear as very delicate cross lines, thickened in the middle, running just between *q* and *i*. In these discs belonging to the same muscle-plate these dots are lying in adjoining discs one just beneath the other, so that rows of black dots running parallel to the myofibrillae are formed. In each muscle-plate such longitudinal rows seem to be distributed with some regularity. These black dots were always found only at one side of the anisotropous disc, and, so it seems, always at the same side of *q*, viz. at that turned caudal. The black dots lying in the same muscle-plate in the same longitudinal row, are often

[1]) Rivista di Patologia nervosa e mentale. Vol. V fasc. 10, 1900.

found to be connected with each other by means of very delicate fibrillae, which are running parallel to the myofibrillae. This could be stated in many cases with great clearness. In some cases these fibrillae were straight, in other cases more or less undulating. In fig. 6 *a* a longitudinal section through the muscular plates (cut obliquely) is drawn greatly enlarged. The small dots and fibrillae are easily to be seen.

In transverse sections the same rows of fibrillae and black dots were also to be seen, and here they are seen to be distributed more or less regularly on the muscle-plates (fig. 6*b*). At both ends of the black dot here too a delicate black line may be seen, extending for some way along the muscle-plates but then being lost to view. By playing up and down by means of the micrometer screw of the microscope in cross sections too a longitudinal fibril may be made out extending upwards and downwards from the black corpuscle; this fibril is identical with that, which in longitudinal sections was seen to run parallel to the myofibrillae and to connect the black dots of a longitudinal row with each other.

So we find here in the muscle-plates of Amphioxus an apparatus, which brings the anisotropous discs of the same muscle-plate in connection with each other, which seems to be distributed with some regularity over the whole muscle-plate, and which gives the staining reaction of the neurofibrillae. Although I could not find the connection of these fibrillae with the motor nerves, still these facts seem to point to the conclusion, that we may regard these fibrillae and their knobshaped thickenings at one side of the anisotropous discs as representing the real innervation-apparatus of the striped muscle-fibres. Sometimes I saw one of the longitudinal fibrillae near the place of attachment of the myofibrillae to the myosepts bend off from the muscle-plate; but it was lost almost immediately between the myofibrillae in the neighbourhood and could not be traced any farther. When we consider however the constant position of the small knob-shaped thickenings at one side of the anisotropous disc, the fine often undulated connecting fibrillae, the dark-purple tinction with chloride of gold (Nachvergoldung Apáthy) so characteristic for neurofibrillae, then, I think, it is difficult to avoid the conclusion that they are neurofibrillae.

This seems to me to be important from a general point of view. Although the structure of the striped muscular tissue of Amphioxus differs largely from that of the higher Vertebrates, yet the same type of cross striation, that is, the same structure of the myofibrillae, is present in all.

Where now HEYMANS and VAN DER STRICHT found a motor nerve endplate identical with those of the higher Vertebrates and at the side of this structure can be seen an innervation of each anisotropous disc, as I have attempted to show, there is room for the conclusion that in other vertebrates too the so-called motor nerve endplate is not the ending of the motor nerve, but that from here neurofibrillae enter the muscle-fibre, and that every anisotropous disc is innervated. The truth of this surmise, however, must be tested by further study.

Amsterdam, October 1902.

(November 20, 1902).

J. BOEKE. On the structure of the light percepting cells in the spinal cord, on the neurofibrillae in the ganglioncells and on the innervation of the striped muscles in amphioxus lanceolatus.

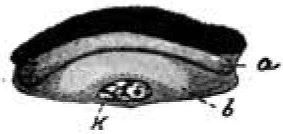


Fig. 1.
Pigment-cup and eye-cel with vitreous bodies.

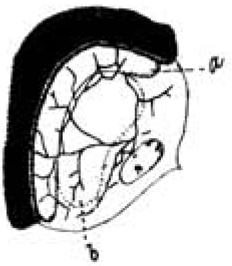


Fig. 2.
Pigment-cup and eye-cell with the network of neurofibrillae
Enlarg : 800.

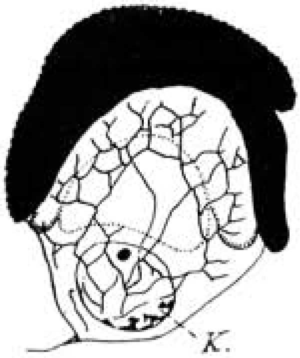


Fig. 3.
Pigment-cup and eye-cell with network of neurofibrillae.
Enlarg : 1200.

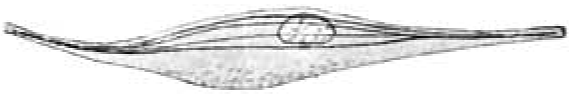


Fig. 4.

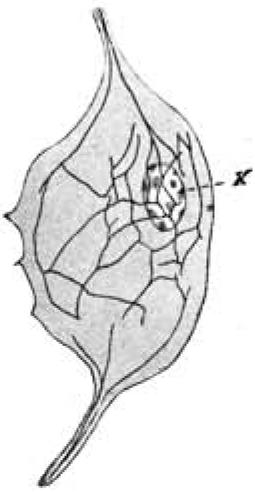
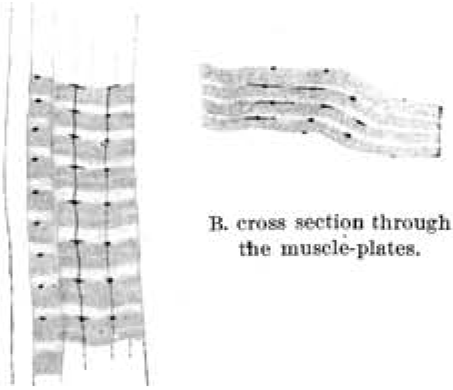


Fig. 5. Arrangement of the neurofibrillae in a section through a ganglioncell from the dorsal part of the medulla. Fibrillae drawn with the drawing apparatus in different optical sections.



B. cross section through the muscle-plates.

Fig. 6.
A. longitudinal section.