Zoology. — *"On the structure of the nerve-cells in the central nervous system of Branchiostoma lanceolatum."* (First comm.) By Dr. J. BOEKE. (Communicated by Prof. G. C. J. VOSMAER).

(Communicated in the meeting of April 26, 1907).

The methods of staining the elements of the nervous system, published in recent years by RAMON Y CAJAL, DONAGGIO, and especially by BIELSCHOWSKY, have enabled us to study the minute structure of the ganglion-cells not only of the lower animals but also of the vertebrates with more success than before. After having published in These Proceedings, some years ago [1]), the results of my former investigations on the structure of the nerve-cells of Branchiostoma, then studied by means of the goldmethod of APATHY, it seemed advisable to describe here too the results of my recent investigations on the same subject by means of the methods mentioned above, because they extend and complete my former results in several directions.

Contradictory to the results of EDINGER [2]), the only author who studied the central nervous system of amphioxus by means of the method of BIELSCHOWSKY, viz. that the method gave only scanty results for the neurofibrillae in the cells, in my preparations, stained after the method of BIELSCHOWSKY—POLLACK, in a great number of nerve-cells of several specimens of Branchiostoma a very clear and distinct picture was obtained of the neurofibrillae, not only in the nerve-fibres, but also in the body of the nerve-cells.

Preparations of material preserved in a mixture of platinum chloride-osmic acid-acetic acid and corrosive sublimate [3]), and stained in thin sections with iron-haematoxylin after HEIDENHAIN, were used as control and for the study of the protoplasmic structures between the neurofibrillae.

The different cell-forms of the central nervous system gave there, where they were satisfactorily stained, as a rule the same mode of arrangement of the neurofibrillae in the cell-body; therefore I will restrict myself to describe here only some cell-forms at length and only refer briefly to the structure of other cells. At another place I hope soon to give more and fuller details.

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1) Proceedings Roy. Akad. of Sc. Amsterdam, of the meeting of Oct. 25, 1902.

2) Anat. Anzeiger, Bd. 28, No. 17, 18, 24 April 1905.

3) According to Dr. LEGROS the best method for the preservation of the nervous system of Branchiostoma. I can fully agree with him in this statement. This mixture gives better results than all the others I tried.

1. As is well known, the very large nerve-cells ("Kolossalzellen") lying at about equal distances from each other in the axis of the spinal cord, possess a thick axonic fibre, that after leaving the cell-body describes a characteristic curve and passes into one of the colossal nerve-fibres that run in a longitudinal direction through the spinal cord, and a number of dendrites, springing from the cell-body at different points.

Sections of these cells, stained after the method of BIELSCHOWSKY, give a very clear picture of the neurofibrillar structure. In a section in which only some of the dendrites are to be seen, and not the axonic fibre with its "cône d'entrance" (of which more later on) these cells show an arrangement of the neurofibrillae as shown in fig. 1.

The cell is surrounded by a glious capsule, composed of fine interwoven fibrillae. The preservation of the nervous system in formol, necessary for the BIELSCHOWSKY-reaction, causes the cells to shrink a little, so that the pericellular cavity is larger than it is in normal life and in well-preserved specimens. Within the cell-body the neurofibrillae form a very distinct and regular network. Everywhere they anastomose with each other, nowhere I could discover free running fibres. The meshes are regular, round or manysided, and nearly all of about the same size. A subperipheral zone is formed, where the meshes are somewhat smaller and the composing neurofibrillae a little coarser. From this zone a few coarse neurofibrillae may be followed in the network radiating to the central zone around the nucleus. The nucleus itself is not coloured in these preparations, but is only to be seen as a clear round or oval spot in the midst of the darkly stained network of the neurofibrillae. There where a dendrite leaves the cell-body, the meshes of the network are elongated in the direction of the processus (fig. 1, 4*b*, 6). In the dendrites themselves, at least in the coarser ones, the anastomosing of the neurofibrillae is to be seen still at some distance from the cell-body. In fig. 1 is drawn a section of 7 μ thick. In three of the following sections, passing through the dendrites, whose origin is shown in fig. 1, I could still see the anastomosing of the composing neurofibrillae. In fig. 2 is drawn one of the large dendrites of a similar colossal cell there where it branches into two. The network of the neurofibrillae, several coarser (and more darkly stained) fibrillae, and the continuity of the network in both branches is clearly to be seen. In the finer dendrites the neurofibrillae seem to become isolated sooner after having left the cell-body (fig. 1 at *b*). The same is to be seen in the smaller nerve-cells of the spinal cord (figs. 4*b*, 6).

The axon of the colossal nerve-cells has a somewhat different structure. As I described in my former paper [1]), the colossal nerve-fibres contain a great number of closely set exceedingly fine separate fibrillae, which in well-preserved preparations are distributed regularly through the whole extent of the fibre. There where the axon enters the cell, this bundle of neurofibrillae may be followed some way into the cell-body; we see the fibres describe a curve or vortex around the nucleus, and then the thin fibres melt into the somewhat coarser network of the neurofibrillae described above.

The smaller, mediumsized and smallest nerve-cells of Branchiostoma, such as those that are drawn in figg. 4, 5 and 6, at the same scale as the cell figured in fig. 1, show the same arrangement of the neurofibrillae as the colossal nerve-cells, viz. a regular network, the meshes elongated there where a dendrite or axon leaves the cell, more or less rounded in the centre of the cell-body. The subperipheral zone with finer meshes and coarser fibrillae I could not find here; the network seemed everywhere to be regular throughout the cell-body. In fig. 4*a* and 4*b* two sections through the same mediumsized nerve-cell are drawn. In fig. 4*a* the nucleus is to be seen, and on it a very regular network of neurofibrillae, with only one layer of meshes, and therefore giving a very clear idea of the regularity of the network. This section passes through the centre of the cell-body. Fig. 4*b* shows the peripheral part of the same cell. The meshes are here more elongated in the direction of the processus, and in the network some fibrillae are coarser and more darkly stained; all of these run in the direction of the dendrite and leave the cell-body there; inside the cell they form part of the general network; in the dendrite they run more or less parallel to each other and do not anastomose any more (see page 2). The same features are to be seen very clearly in fig. 6, showing the neurofibrillar structure of another mediumsized nerve-cell lying somewhat more cephalad in the spinal cord.

In fig. 5 is drawn a very small ganglion cell (magnified to the same scale as the foregoing figures). Here too the network of the neurofibrillae is easily to be seen and the meshes are of about the same size as in the mediumsized nerve-cells described above, though smaller than in the colossal ganglion cells.

Fusiform cells, in which the neurofibrillae simply pass through the cell-body from one processus to the other without interruption, as I described them in my former paper, I was not able to find in

[1]) These proceedings. Meeting of Oct. 25, 1902.

the preparations stained after BIELSCHOWSKY. Where the neurofibrillae were visible, they formed a network. In my preparations stained with chloride of gold after APATHY, which I looked over for these cells I however found them again. The uninterrupted course of the neurofibrillae was clearly to be seen. They are however only very rarely met with.

So we find in nearly all the cells a network of neurofibrillae with regular meshes. In full-grown animals the meshes in different cells are of about the same size. But when we examine the same kind of cells (for example the colossal ganglion cells) in very small animals, we find a neurofibrillar network of the same regularity but with much smaller meshes. So when we compare fig. 1, a colossal ganglion cell of a fullgrown Branchiostoma of 48 m.m. in length, with fig. 3, an analogue cell of an animal of 6 m.m. in length, we find a much smaller-meshed network. Those small animals have finished their metamorphosis already, and present nearly the same organisation as the adult animal. The nerve-cells therefore seem to have assumed already the definite arrangement of their neurofibrillar structure, but the meshes are much smaller. During the following growth of the nerve-cells the reticulum grows, but the structure remains the same. In different adult specimens the size of the meshes seemed always to be of the same order, and only to present the slight differences mentioned above.

When we compare this with the neurofibrillar structure, described for the ganglion cells of other animals, I will here especially call attention to the description of APATHY for Hirudineae and Vermes, of BOCHENEK for Helix, of DONAGGIO, CAJAL, MICHOTTE, LEGENDRE and the many authors, who have studied the ganglioncells of the higher vertebrates by means of the new elective histological methods. Among the descriptions by these authors of the neurofibrillar structure in the nerve-cells of the representatives of different classes of the animal kingdom, that of Branchiostoma takes just the place, we generally give to that animal in the animal series. Is fig. 7 is drawn a sensory cell of a Pontobdella, with the neurofibrillar structure stained after APATHY. We see a very coarse network around the nucleus, with fibrillae radiating to the periphery and forming there a second network. The ganglion cells of Helix give according to BOCHENEK[1]) a much finer network. The meshes of this network are still much larger than those of the nerve-cells of Branchiostoma; these in their turn are larger and the fibrillae coarser than the neurofibrillar struc-

[1]) Le Nevraxe, Vol. III, Fasc. 1. 1901. page 85.

ture, as it presents itself in well-stained preparations of the nerve-cells of the higher vertebrates (as for example in the splendid figures of DONAGGIO). It seems that the higher is the organisation of the animal, and in consequence that of the nerve-cells, the finer and more regular is the network of the neurofibrillae in the nerve-cells. (cf. BOCHENEK).

The network of the neurofibrillae has no definite connection with the protoplasma-reticulum. In preparations, preserved in a mixture of HERMANN's fluid and corrosive sublimate, and stained with iron-haematoxylin, the protoplasma has a very fine granular or fibrillar structure, and in the centre of many cells are shown curious diversely-shaped differentiations that remind us of the pseudochromosomes described by HEIDENHAIN, and of the rings, described in the ganglion cells of vertebrates (Teleostei, Rana). But it would take us too far, to describe these details here at some length.

2. An entirely different type of cells we find in the nerve-cells which form the large group of ganglion cells lying dorsally in the foremost part of the spinal cord just behind the brain ventricle, the so-called oblongata, extending from the niveau of the infundibular organ till beyond the first pigmented eye-cells. It is characteristic of the peculiar difficulties, with which the investigation of the histology of the nervous system of Branchiostoma is encumbered, that of the large number of authors, who have studied the subject, only JOSEPH[1]) two years ago gave a nearly accurate account of the structure of these cells. Even HEYMANS and VAN DER STRICHT in their very elaborate study of the histology of the nervous system of Branchiostoma, published in 1898, do not say a word about it, and only in one of the many beautiful drawings, with which their paper is illustrated, in two cells a slight indication of it is to be seen. JOSEPH says of these cells, that they present at the surface a finely striated border of minute rods, only at the side of the cell turned towards the surface of the animal, and underneath this striated border a coarsely granular darkly staining protoplasm. The same structure JOSEPH described in the cells lying close to the central canal in the spinal cord, covered by a pigment-cap, and being supposed to be light-percepting cells. On these grounds JOSEPH put forward the suggestion, that the dorsal group of cells too consists of eye-cells, light-percepting cells, differing only from the cells of HESSE by the absence of a pigmented cap-shaped cell.

This far-reaching suggestion is, I think, not proved, nor even made probable, by the facts. Even in the most carefully prepared sections

[1]) H. JOSEPH: Ueber einige Zellstructuren im Zentralnervensystem von Amphioxus Verh. d. Anatom. Gesellschaft. Jena 1904. p. 16—26.

in which the structure of both cells was very clearly to be seen, the two types still present some marked differences, both in the nuclei and in the structure of the protoplasm and the differentiations on the surface of the cells. According to JOSEPH the nuclei of the dorsal cells and of the ventral eye-cells possessed a similar granular structure, differing from that of the other nerve-cells. In some cases this is true, but in other cases the same structure is found in the nuclei of other cells, and, when we examine a number of preparations, the structure of the nuclei both of the dorsal cells, of the ventral light-percepting cells and of the other nerve-cells presents so many differences and varieties, that there cannot be drawn any conclusion out of that. But the capital difference between the two cell-forms lies in the absence of a pigmented cap-cell in the dorsal cells and the totally different form and structure of the two types.

The light-percepting cells of the spinal cord possess a border of short minute rods, lying close against the cap-shaped pigment cell. The processus of the dorsal cells are much longer, and not rods, but exactly shaped like hairs, or cilia. These hairs (fig. 8– 11) are rather long, slender and thickly set and their course is often more or less wavy. On the same cell they seem to be all of about the same length; the hairs on different cells do not vary much in length.

The ventral light-percepting cells are all of the same regular form. The dorsal cells however present the most different forms. [1]) Some are rather regular (fig. 8), some are long and slender (fig. 9), some are of a very irregular shape, but in most cases these cells, when we reconstruct them from the thin sections, appear to have a very typical cup-shape. In fig. 10 I have drawn the median section through one of these cups, in which the central hole in the cell is figured, in fig. 11 such a cup-shaped cell is cut vertically to the axis of the cup.

These cells are surrounded by a glious basket of closely interwoven fibres (in the figures this network is represented by a dark colour) and the cells seem to fill up the room left by this basket so that between the surface of the cell and the inside of the basket there remains an open space, in which the hair-like processes of the cell-surface are seen. In well-preserved sections this space has the same width on all sides of the cell, where the surface carries the hair-like structures. The hairs reach from the surface of the cell nearly to

[1]) Only such cells are described here, which seemed to be perfectly preserved. All those cells, of which the irregular form seemed to be caused by bad preservation, are left out of the discussion.

the inside of the basket, as may be seen in the figures. There where no hairs are developed, the glious fibres lie close against the surface of the cell-protoplasm (fig. 8, 10, 11).

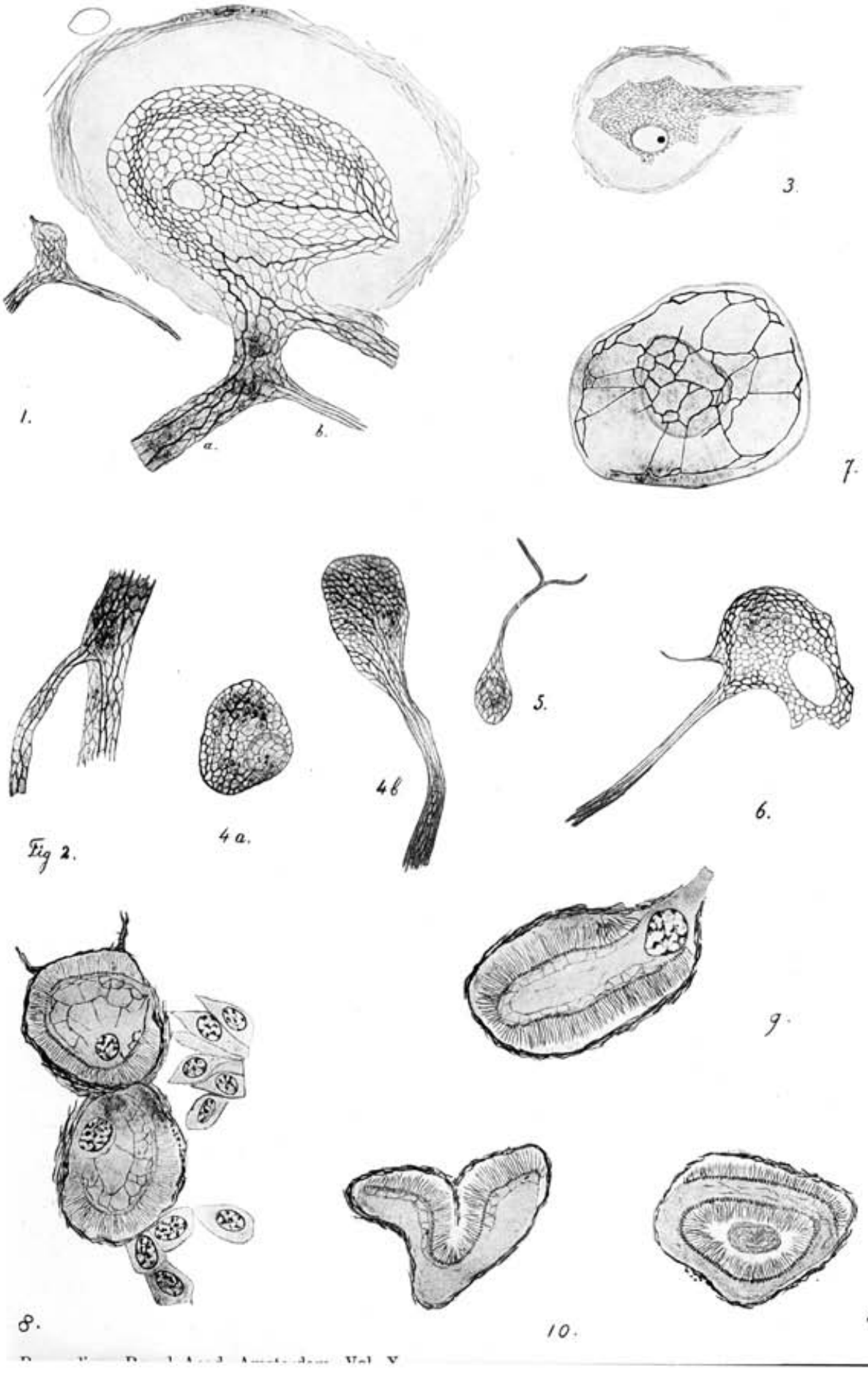
Where JOSEPH considers the hair-like processes to be only present at that side of the cell which is turned towards the surface of the animal, I cannot agree with him. When we compare horizontal and transverse sections carefully with each other, we must draw the conclusion, that the hairs may be developed on all sides of the cell, except there where the cell-body sends a dendritical process through the glious basket. Even there where the cell is shaped like a cup or calix, at both sides of the cup the cilia may be present (figg. 10, 11). The cilia at the inside of the cup are separated from each other by an ingrowth of the fibres of the glious basket (fig. 10). When the cell is cut at right angles to the axis of the calix, this may lead to the appearance of a ring of protoplasm, at both sides covered with the hairs, and surrounding a mass of coiled-up fibres, the ingrowing fibres of the glious basket (fig. 11).

The protoplasm of these cells shows a regular network of neurofibrillae, which differs from the network of the other cells of the spinal cord by its much larger meshes (compare figg. 8—10 with figg. 1—6); only at the periphery of the cell, under the hair-like processes, a finer network of neurofibrillae is to be seen (fig. 8). The hairs themselves seem to be implanted on a layer of small darkly staining granules or small rods, of which the definite structure is difficult to be seen. In many cases it is only represented by a somewhat more coarsely granular layer of protoplasm there where the cell-body is covered with the hairs.

All these things seem to point to the conclusion that these cells do not possess a light-percepting function, as suggested by JOSEPH. The shape of the cells and the peculiar structure at least are not favourable to the hypothesis. But it is sure, that this group of cells, all presenting the same peculiar structure, has a distinct and peculiar function. The structure of the cells reminds us in the first place of a static organ, and especially cells as drawn in fig. 9 and fig. 11, seem to suggest such a function. The peculiar baskets of fibres surrounding the cells remind us of the cells of PURKINJE of the brain of the craniotes, but bearing in mind the very little we know about these cells and about the cells just described, it is more advisable to stop at these general suggestions and not to try to go more into details. The suggestion of JOSEPH at all events seems to me to be untenable.

A third type of cells differing from those which I described here, is that of the cells of the so-called infundibular organ in the ventral

J. BOEKE. On the structure of the nerve-cells in the central nervous system of Branchiostoma lanceolatum. (first communication).



wall of the brain-vesicle. These cells I mean to describe in my second paper.

Leiden, 25 April '07.

DESCRIPTION OF FIGURES ON THE PLATE.

All the figures are magnified 1600 times, and are drawn with a camera lucida of ABBE directly after the preparations. Apochrom. oil-immersion lens of ZEISS and compens-ocular No. 8.

Fig. 1. Colossal nerve-cell with neurofibrillar network, of a Branchiostoma of 4.8 cM. in length (BIELSCHOWSKY—POLLACK's method).

„ 2. Dendrites of a similar cell of an animal of 5 cM. in length (same method).

„ 3. Neurofibrillar network of a colossal nerve-cell of a Branchiostoma of 6 mM. in length.

„ 4 *a* and *b*. Sections of a medium-sized nerve-cell of the same spinal cord as fig. 2.

„ 5. Section of a very small nerve-cell, with neurofibrillar network.

„ 6. The same as in fig. 4.

„ 7. Section of a sensory cell of Pontobdella, of 10μ, treated after the gold-method of APATHY.

„ 8—11. Sections through different cells of the dorsal group of cells lying behind the brain-vesicle, taken from preparations of several adult specimens of Branchiostoma. In fig. 8 some of the adjoining cells are drawn, to demonstrate the similarity of structure of the nuclei in the two cell types.

In fig. 10 and fig. 11 are drawn two typical sections through cup-shaped cells of the dorsal group of cells. The body contained in the centre of the cell of fig. 11 is the prolongation of the glious basket surrounding the cell. Compare fig. 10.

Physiology. — *"On a third heart sound"*. By W. EINTHOVEN, in collaboration with Messrs. J. H. WIERINGA and E. P. SNIJDERS, assistents at the physiological laboratory at Leyden.

When continuing the investigation of the heart sounds by means of the string galvanometer [1]), we noticed that in some cardiophonograms, especially with the apex sounds of W i, recorded in February last, shortly after the vibrations of the second sound still another vibration was present, which admitted of no other interpretation than by regarding it as a third heart sound.

We could not at once explain how this third sound was produced, and we put off the closer investigation of this phenomenon, however

[1]) See: Die Registrirung der menschlichen Herztöne mittels des Saitengalvanometers. PFLÜGER's Arch. f. d. gesammte Physiol. Vol. 117, p. 461, 1907.