Histology. — *Nerve-endings in the muscles of the arm of Sepia officinalis.* By H. BERKELBACH VAN DER SPRENKEL. From the Department of Embryology & Histology of the State-University of Utrecht (Holland). (Director Professor J. BOEKE. M.D.). (Communicated by Prof. J. BOEKE.)

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Whereas during the last ten years the nerve-endings in vertebrate muscles have been the subject of rather intense investigation, it is surprising that such little work has been done in the field of invertebrates, although it does not lie quite fallow. Most probable the reason for this is that the (medical) histologist is, consciously or unconsciously, inclined to remain as near as possible to the form around which the whole of his scientific effort is constructed, viz.: Homo.

But from a wider scientific point of view, it is most desirable, and perhaps probably necessary in order to gain a clear insight, to make the group of the invertebrates, which contains such a great number of forms, object of modern histological investigation.

A few investigations on nerve-endings in the muscles of invertebrates have certainly been published, but the majority of them is based either on the older gold method (APATHY [1])) or on the methylen-blue technique [2]) so that it appears that the advice of GUÉRIN [3]) (p. 122): il est nécessaire d'étudier les connexions du système nerveux des Céphalopodes dans les matériaux impregnés par les sels d'argent" has not been followed by anybody.

For that reason it was decided to undertake an investigation into the course and endings of nerves in the arm-musculature of Cephalopodes using the BIELSCHOWSKY technique modified by GROS.

The material used was a piece cut transversally from one of the eight ordinary arms of sepia officinalis, which had been preserved in neutral formalin for a number of years. Frozen sections (15—20 μ) are kept in aq. dest. and are passed straight on to 20 % $AgNO_3$. The sections are manipulated with a glass rod around which they can lie easily without overlapping or wrinkling, for wrinkling invariably leads to irregular

[1]) APATHY, S. Das leitende Element des Nervensystems und seine topographischen Beziehungen zu den Zellen. Mitt. Zool. Station Neapel., Bnd. **12**. 1897. p. 495—750.

[2]) MICHAILOFF. Système nerv. périph. d. Céphalopodes. Bull. Instit. océanographique Monaco. N°. 402. 25 Oct, 1921.

[3]) GUÉRIN. Syst. nerv. et musc. d. l'appareil tenticulaire d. Céphalopodes. Arch. Zool. exp. et générale, IVe Série. T. VIII. p. 1—178. 1908.

impregnation which must be avoided. If however wrinkling does occur, the section should be discarded at once to avoid unnecessary waste of time.

Although the time prescribed for the first impregnation in $AgNO_3$ is from 5—30 min., it was found for the material used in this investigation a longer stay for one to two hours gave the best result. From this silver bath the sections are transferred into a dish containing at least 75 cc. of 20 % formalin made by diluting formalin by tap-water. The section is then passed through 2 further similar formalin baths. Itwas observed that if the sections remained longer than 2 à 4 minutes in contact with the formalin a brown rather than a black impregnation was obtained.

With regard to the other steps in the technique, LAWRENTJEW [1]) has already pointed out (p. 469) that the length of the stay in the ammoniacal silver is of the greatest importance in order to get the right impregnation. After a long stay in the 20 % silver-nitrate, one must be able to interrupt the action of the NH_3-silvernitrate abruptly, for it may be then a question of a second longer whether the section will be an excellent one or will be spoiled. The best way to do, I think, is to collect sections treated according to different long stays in the different solutions, for then it is possible to have the advantages of all shades of impregnation side to side and one is able to construct an image of reality out of different aspects.

After the impregnation the sections were toned in the usual way, counterstained with haematoxylin and mounted in laevulose-gelatine according to the prescription of HERINGA (Ned. Tijdschr. Geneesk. 1923, 2nd half. p. 448).

From the mass of nervous tissue forming the axis of the arm, nerves appear following the stripes of tissue which subdivide the longitudinal muscle-fibers (cross-sectioned) (conf. figg. 30 and 33 Pl. IV. GUÉRIN). Such a heavy nerve is drawn in fig. 3 (*z*). Looking for the endings of these nerve-threads in the muscle-mass, one finds images as reproduced in the accompanying figures. Fiber-bundles (imbedded in protoplasm) give off delicate branches which often run over great distances amongst the muscle-fibers.

In figure 1 two heavy bundles cross a number of muscle-cells, the protoplasm of the left nerve is distinctly impregnated. Between the 2 bundles runs a very delicate branch which ends in a point situated on (or within) a muscle-cell. Apart from that lies a group of fibrils which shows at least 4 endings: the right and left upper-ones are point-shaped, close to it is a well-formed endring which, except for its minuteness and proportions, is not to be discerned from the "endöse" of the plain muscle-cell in Vertebrates (BOEKE [2]), LAWRENTJEW [3])); the right branch however curls around the muscle-fiber and ends in an endring underneath the muscle-cell. So we learn that one nerve-fiber is able to form as well ring-shaped as point-shaped endrings which must prevent us from estimating the difference in form too high in importance. How these endings lie in relation to the muscle-cell, whether within or without, is indiscriminable here.

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[1]) LAWRENTJEW. Verbreitung d. nerv. Elemente in d. glatten Muskulatur. Zeitschr. mikr.-anat. Forsch., Bnd. **6**. '26. p. 467.

[2]) BOEKE, J. Samenhang tusschen zenuweindiging en gladde spiercel. Versl. Kon. Akad. v. Wet. A'dam, 18 Jan. 1915; Noch einmal d. peritermin. Netzwerk u.s.w. Zeitschr. mikr.-anat. Forsch., Bnd. **7**. 1926. p. 95. fig. 10.

[3]) LAWRENTJEW. l.c. fig. 14.

Another image of endings is presented to us by figure 2, where 2 heavy bundles run longitudinally over muscle-cells breaking up in a number of thin fibers, many of which have endings, often crowded together. As the right half of the drawing shows us, a delicate fiber may leave a heavy bundle, ending after an elaborate course against a remote muscle-fiber. A remarkable ending is formed by the thread at the left side, an ending which has the shape of a network extending half-cylindrically along the muscle-fiber. Even the highest magnification did not allow me to state the existence of a periterminal network on this spot, although I believe, considering other images, that it is doubtlessly present. The same as is stated by LAWRENTJEW (l.c. p. 478) viz. that ultra-terminal fibers are present, I could plainly see at numerous places, where from an ending a fiber branches off ending on another muscle-fiber. In this drawing impregnation of the protoplasm, in which the nerve-fibrils run as distinct black lines, is very clear.

In addition to the form of the ending the main points of interest are *a.* its situation in relation to surrounding cells and *b.* its physiological task. In other words *a.*: Is the ending intra- or extracellular; *b.*: Is the ending motor or sensory?

Commencing with the latter point, I cannot state with surety of what character these endings are, but where I have found always the same type, I think it quite reasonable (and especially quite simple) to see in them motor endings, the more so as sensory endings in non-striated muscle-fibers are hardly known with certainty. The motor function of an ending is after my opinion only guaranteed by images as given by BOEKE [1]) where the periterminal network continually extends from the ending to the myofibrils. On what base MICHAILOFF calls his ending in l.c. fig. 6 sensory, remains unclear to me, we'll have to wait for his description of a *motor* ending (as he promises us). Upto the moment convincing results compel us to change our opinion, the most cautious thing to do seems to interpret the above described endings as motor.

The question of the intra- or extracellular position of the endings is another-one. The best way to solve this point is to investigate cross-sections, but figure 3 demonstrates how longitudinal sections can inform us just as well. At the left side of the heavy bundle *z*, a delicate thread divides up in several twigs. One of these branches forms an ending (*b*) which carries at an ultraterminal thread a distinct ring-shaped ending (*a*) the latter lying so close to the nucleus of the muscle-cell, that it is unimaginable that the ending should be situated outside of the cell and that the myofibrils, groupwise (see below), should run in between nucleus and ending. A situation, as described above, very probably excludes an extracellular position. By careful focussing the nucleus appears to be bent around the nerve-thread, what indicates a narrow relation between ending and nucleus. The other thread continues its original course and also ends close to the

[1]) BOEKE, l.c. '26. fig. 3. p. 100.

nucleus of a muscle-cell (c). The same drawing shows us a heavy thread that, crossing a number of muscle-fibers, gives off very delicate fibril-bundles forming on the muscle-fibers minute endrings.

The cross-sectioned muscle-fibers show a peculiar order of the fibrils which are radially arranged, so that the cross-section of the muscle-cell shows a clear protoplasmic centre in which the rather large muscle nucleus may be cut (figg. 4 & 5) and around which radially directed lines represent the flat groups of fibrils (c.f. some muscle-cells of fig. 4). It is not at all difficult by scrutinizing to find a great number of endings in a cross-sectioned muscle-group, it is however rare that one of these endings can be used to demonstrate intra- or extracellular position. I believe, however, to have drawn in figure 4 a case where distinctly an ending is so situated in a curve of a nucleus of the muscle-cell, that myofibrils lying between nucleus and ending are to be excluded, this ending has doubtless to be interpreted as intra-cellular.

It strikes the investigator how on the cross-section of a muscle a great number of very delicate threads runs in amongst the muscle-fibers (fig. 4); in order to prevent mistakes, I did not assume such a thread as a nerve thread unless it could be followed up to an unquestionable nervestem.

A last point of importance in the innervation of non-crossstriped muscle-cells are the interstitial cells or lemmoblasts. I am not entitled to assume without further considerations that in this non-vertebrate tissue interstitial cells are present, but the branches of the nerve-fibers show here images which agree completely with those LAWRENTJEW (l.c.) [1]) has published.

It is conspicuous how the slightly thicker fiber-bundles are embedded in protoplasm (which sometimes is impregnated just as well), in which nuclei lie scattered. The fibrils lie close to the nuclei and form an intricate network around it, the nuclei are less regularly shaped and darker impregnated than the nuclei of the plain muscle-cells. So it appears appropriate to interpret cell "a" of fig. 5 as a cell comparable to the interstitial cells of the vertebrates: a kind of lemmoblast. Cell "a" of fig. 4 shows us a lemmoblast situated among the cross-sectioned muscle-fibers. The same figure 5 shows us clearly the inclination of the endings to seek the vicinity of the nuclei; especially the 3 endings lying at the upper part of the figure are situated close to the nuclei of the cells which they innervate.

The results stated here agree in many points of view with those APATHY communicated in his classic article of 1897. Images as he gives in his figures 1 and 2 of Pl. 32 (Pontobdella) I have got essentially the same: endings lying within the muscle-cell (in Sepia one ending was found which was surrounded by myofibrils just as is indicated in APATHY's double-innervated muscle-cell); as APATHY states for Hirudo and Pontobdella, in Sepia often was found that over great distances no ending was to be detected, on another spot many crowded together. Where APATHY however believes

[1]) LAWRENTJEW. l.c. '26. p. 474, fig. 6.; conf. LAWRENTJEW. Proc. Kon. Akad. v Wet. A'dam. Vol. 28. p. 977.

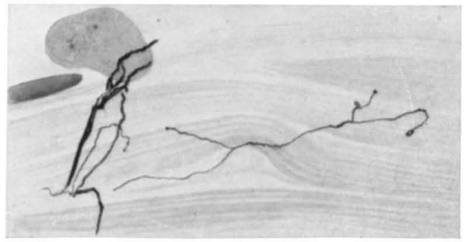

Fig. 1. Nerve-endings on (or within?) muscle-fibers. Ringshaped and other endings formed by one thread. Magn. 1400 times. Zeiss 1.5 mm. Apochrom. Leitz, Periplan. 8 ×

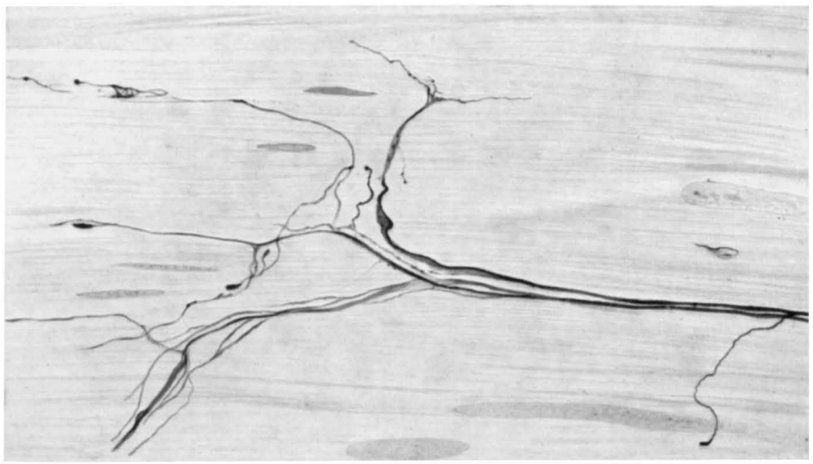

Fig. 2. Nerve-endings on (or within) muscle-cells. At the left side a remarkable form. Magn. 1800 ×. Zeiss. Apochrom. 1.5 mm. Ocul. IV.

Fig. 3. Nerve-endings in muscle-cells, network on muscle-cells. Ring-shaped ending close to the nucleus. Magn. 1800 ×. Zeiss. Apochrom, 1.5 mm. Eyep. IV.

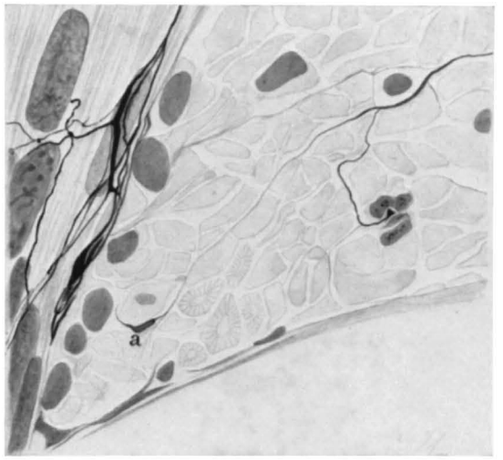

Fig. 4. Ending within a muscle-cell. (cross-sectioned). Nucleus curved. *a*. Interstitial cell. Endings at the left side in the vicinity of the nuclei. Magn. 1375 ×. Leitz. Oil-imm. $^1/_{12}$. Periplan Eyep. Leitz. 8 ×.

Fig. 5. Nerve on muscle-cells. *a*. Interstitial cell?. Endings in the neighbourhood of the nuclei. Magn. 1000 ×. Leitz. $^1/_{12}$. Peripl. eyepiece. Leitz 8 ×.

(p. 692) that ending and nucleus have no relations whatever to each other, I must disagree with him on this point for there are too many spots where the ending apparently seeks the nucleus than that I can assume the idea that between nucleus ending there would be no more than a casual relation. I, therefore, assume that in Sepia the state of affairs is the same as in Vertebrates where BOEKE (1915, 1926) and LAWRENTJEW have shown that ending and nucleus tend to come in contact as close as possible.

APATHY tells (p. 693) that he has come across many motor threads, which run for a great distance within the muscle-cell in order to leave the cell and to innervate quite another fiber ; although I have given much attention to this point, I have not seen one case of such a relation in the arm of the Cephalopodes.

I believe that this short note is justified as it has been possible to state some points concerning the innervation of the muscle of the arm of the Cephalopodes, viz. :

1°. The nerves end in the muscle-cells in ring-shaped or point-shaped endings.

2°. Very probably the position of the endings is intra-cellular, for preference in the vicinity of the nucleus, which may show a change of form (impressed by the ending).

3°. Cells are present in the course of the fiber-bundles which are comparable to the interstitial cells of the sympathetic innervation system in vertebrates.

Herewith I express my thanks to Professor BOEKE for his continuous interest in every step of this work and for his patience in discussing and criticizing of what I thought to have stated.