Physiology. — *The significance of the interstitial cells in the development of the motor end-plates in mammals (talpa, mus, homo, lepus).* By J. BOEKE, LL.D., M.D., Utrecht.

(Communicated at the meeting of April 25, 1942.)

In a former communication (Proceedings of the meeting of February 28, 1942) I discussed the problem of the interstitial cells (neurones interstitiels of CAJAL) and demonstrated that they are not only found lying everywhere at the end of the sympathetic plexuses [1]), but that homologous elements may be found also in the endformation of the spino-cerebral nerves.

As it was mentioned allready in a lecture, delivered in England (Universities of London and Oxford) in 1937, a lecture which was published by the Oxford University Press in 1940, it was suggested in the communication mentioned above, that the cells of the core of the sensory corpuscles, which are in a syncytial connexion with the neurofibrillar ending and its surrounding neuroplasma, and even part of the constituents of the motor end-plates of the cross-striated muscle-fibres might be homologized with the interstitial cells of the sympathetic endformation. It was suggested in 1937 that these interstitial elements formed the "neurohumoral region" of these afferent and efferent nerve-endings (as of the sympathetic endformation), in which the humoral energy, necessary to transform the nervous stimulus, is produced.

In the communication in the meeting of February the development of the motor end-plates was sketched and the derivation of the nuclei of the soleplate known as the nuclei of the arborisation (noyaux de l'arborisation de RANVIER) from ingrowing elements of the nerve-fibres was described. Here I may be allowed to describe this development more fully and in more details.

The development of the motor end-plate with special reference to the part played by the different nuclei in the formation of the sole-plate and of the establishment of the connexion of the nerve-endings and the protoplasm of the muscle-fibre during this development was studied especially in the tongue of embryos of the mole and of human embryos of 4½ and 5½ months, the developing motor end-plates of young mice and of rabbit embryos, which showed the same details, being only used to confirm the conclusions drawn from the study of embryos of the mole and human embryos.

The embryos studied were fixated in a mixture of formalin and alcohol or in neutralized formalin (10 %). They were impregnated after the method of BIELSCHOWSKY and afterwards treated with chloride of gold. In this way we get not only a splendid black

[1]) Most modern writers, as far back as LA VILLA in 1898, BETHE in 1903, MUENCH and SCHOCK 1910, LEONTOWITCH and ERIK MÜLLER in 1921, have accepted the view of CAJAL, that they are of nervous origin and nature, and in 1926 and 1928 LAWRENTJEW and VAN ESVELD showed that they are the normal endings of the plexus-elements. This view was accepted for instance by SCHABADASCH in 1934, BOEKE 1933, 1935, LEEUWE 1937, MEYLING 1938, TINEL 1938, PEY-LIN LI, 1940, a.o.. Even STOEHR has been compelled in 1941 to make room for the interstitial cells in his conception of the "terminal reticulum" (which in its original conception was entirely devoid of nuclei and cellular structures), be it together with the other small cells of the sympathetic ganglia, the "Nebenzellen" of KOHN etc.; but in my opinion these small cells are of an entirely different nature and have nothing to do with the interstitial elements lying at the end of the sympathetic plexus (see the paper in the Acta Morphologiae Neerlandica).

impregnation of the neurofibrillar structures but also an excellent colouring of the nuclei and the protoplasma of the nervous elements and of the surrounding elements of the different tissues. This enables us to study not only the development of the neurofibrillar structure of the motor nerves and their endings but also the structure of the muscle-fibres and the development of the sole-plate, the different nuclei and their movements, and the form and extension of the protoplasma of the interstitial elements during the formation of the motor endings. The importance of this for the study of these elements needs no demonstration. Without it a study of these elements is impossible. In the excellent and exhaustive description of the development of the motor endings by TELLO (1917), in which he studied and pictured preparations made by the method of CAJAL, he only describes the development of the motor nerves and the neurofibrillar structures of the outgrowing nerve-fibres, because according to his own statement "the protoplasma was not stained in his preparations and therefore could not be studied." (l.c. page 172). However excellent therefore the observations recorded in his paper might be, they refer unevitably only to one small part of the question. For when we study closely the beautiful drawings which are reproduced in his paper, we get the impression that TELLO has seen exactly what we are going to describe here, only he did not pay any attention to it because he could not trace the different elements without the protoplasm being stained and visible, and because as a true neuronist he was convinced that the nerve-fibres develop and grow as free independent neurofibrillar structures without any connexion with the surrounding neighbouring elements; for him therefore these surrounding elements were not of any importance, and he simply mentions the position of the nuclei at the end of the nerve-terminations at the time that these are seen to push their way into the sarcoplasma of the muscle-fibres (that is at the time the "miofibras" develop by longitudinal fissure of the primary "miotubos", and before a membranous sarcolemma was formed, by which the growing muscle-fibre gets its individuality and its independency), without paying any further attention to them.

According to his description the muscle-fibres develop from the primary muscle-tubes (miotubos) by a longitudinal fissure, the nerve-fibres grow into the connective tissue of the muscular masses, they divide and form at their end the bulbous swellings known from the regeneration-process of the nerve-fibres and so characteristic for the growing fibres of the embryonic nerves (c.f. SPEIDEL, 1936). These terminal swellings (cônes de croissance, which after the description of CAJAL "act like battering-rams against the surrounding elements") lie free in the connective tissue between the developing muscle-fibres; before a sarcolemma is formed (c.f. BARDEEN, 1907) they grow into the developing muscle-fibres there where a multiplication of the nuclei of the muscle-fibre indicates the place of the future sole-plate of the fibre. According to TELLO the growing nerve-fibres are attracted by these sole-plate-formations (by neurotropism), enter them and form in this sarcoplasma of the sole-plate their terminal ramifications (by neurocladism).

According to this description the developing nerve-fibres act entirely by their own force. They may be followed in their course by the elements of the nerve-sheath, the lemmoblasts, which envelop them later on, but they sprout and push their way independently of the surrounding elements. According to TELLO there is no trace of any conducting elements. Here he follows the line of most authors.

It is to be regretted that the beautiful drawings by which the paper of TELLO is illustrated, are for the greater part sketched under a low power and are meant only as pictures illustrating the general mode of development. Only in some cases they give minor details, and then we see (for instance in fig. 15 and 33) that there where the terminal bulbs of the ingrowing nerve-fibres touch the muscle-fibres a curious-shaped nucleus is found lying close to the nerve-ending, which nucleus according to TELLO belongs to the muscle-fibre itself and simply indicates the beginning of the process of formation of the nuclei of the sole-plate and of their ultimate arrangement (TELLO, page 172).

As I mentioned allready, TELLO is not the only author, who mentions the presence of nuclei in the region of the muscle-fibre where the motor end-plate is going to be formed. According to nearly all the writers on the development of the motor nerve-endings on striated muscle-fibres (for instance KÜHNE, KRAUSE, TRINCHESE, LONDON & PESKER, MAYS a.o.) the developing motor nerves come into contact with the muscle-fibres at a spot, where one or more nuclei are found lying under the sarcolemma ("nuclear region", "Kerngebiet", "besonders dichte Anhäufung von Kernen" etc.). They are convinced that these nuclei belong to the muscle-fibre and not to the ingrowing nerve-fibres. Some of them however (f.i. TRINCHESE) go more into details and describe the nuclei present as belonging to the fundamental nuclei of the sole-plate (Grundkern, TRINCHESE) while according to others (f.i. MAYS) the nucleus lying at the spot, where the nerve-ending is going to develop, is a nucleus of the arborisation ("Geästkern"), which disappears however after the motor end-plate is fully developed. But after all they are convinced that the ingrowing nerves are devoid of nuclei, and the nuclei they describe belong to the muscle-fibres; TELLO however pictures in his figures of the ingrowing nerves distinct elongated nuclei between the bundles of nerve-fibres, without paying any attention to them.

As TELLO is the author, who describes the development of the motor nerve-endings with the greatest accuracy, I may be allowed to restrict myself to discuss here only his description and figures.

As it was mentioned in my former communication (Febr. 1942, page 213) this assertion that the nuclei visible around the ingrowing nerve-endings belong undeniably to the muscle-fibre, is liable to be critisized, and it is still an open question, whether the ingrowing nerve-fibres are in reality devoid of accompanying cellular elements, as it was described so convincingly by HARRISON (R. G. HARRISON, Neuroblast versus sheath-cell, Journ. of comp. Neurology, Vol. **37**, 1923). Where the sympathetic nerve elements in reality come from, is still unknown. As it was demonstrated by LEEUWE (see my XII. Innervationsstudie, Acta Morph. Neerl. 1942), the interstitial cells are derived from the ganglia of the sympathetic chain and plexus and grow out from them as distinct cellular elements, which remain in a syncytial arrangement and in connexion with the true ganglion cells. They swerve out into the surrounding tissues and accompany the outgrowing nerve-fibres. Where even their existence has been denied and in embryonic tissues most writers could not find them, allthough they must have been there, it is premature to deny the presence of cellular elements accompanying the embryonic nerve-fibres on their way to their ultimate destination, simply because we could not find them. In my opinion the nuclei (one or two in number) figured by TELLO as lying at the top of the ingrowing nerves, do *not* belong to the muscle-fibre but to the ingrowing nerve-fibres themselves, as they clearly surpass the outlines of the growing muscle-fibres. In my BIELSCHOWSKY-preparations (talpa and human embryos of 4½ and 5½ months and new-born mice) not only these nuclei but also the surrounding protoplasma was stained and therefore clearly visible; the nuclei with their surrounding protoplasma belong undoubtedly to the ingrowing nerve-fibres. They surround the nervous terminal bulbs and are lying outside the muscle-fibres as distinct conducting elements, constituting, as far as could be studied in the preparations, the syncytial terminal elements of the ingrowing nerve-fibres. In longitudinal and cross-sections of developing muscle-fibres I could study these details with the greatest accuracy.

These nuclei are allways lying at the end of the growing nerve-fibres, and when these nerve-fibres reach their destination, the muscle-fibres, the nuclei are allways lying at the side of the terminal bulbs or terminal rings of the nerve-fibre which is turned away from the muscle-fibre and never between the bulbs and the sarcoplasma of the muscle-fibre itself; they cover the terminal arborisations which are developing now. These terminal ramifications of the growing motor nerve-endings are formed inside the protoplasma of these terminal conductive elements. The conducting elements flatten themselves against the surface of the muscle-fibre, their protoplasma fuses with the sarcoplasma of the muscle-fibre, becomes a part of the sarcoplasmic sole-plate, and

only then a distinct sarcolemma is formed, which envelops both the flattened conductive element and the sarcoplasma of the muscle-fibre with which it was fused, and in this way the definite sole-plate and the motor nerve-ending is formed.

In the sections of talpa-embryos of different sizes (18—31 m.M.) and of the developing muscle-fibres of human embryos of 4½ and 5½ months I could follow the different phases of this process of fusion with great clearness. In cross-sections through the muscle-fibres and in longitudinal sections there where the terminal ramifications of the nerve-fibres and their conducting elements were lying "en profil" at the side of the muscle-fibre, this process of fusion and of the formation of the terminal ramifications of the nerve-ending inside the protoplasma of these conducting elements was to be followed with the utmost clearness; after the fusion of the two elements and the formation of the definite sole-plate with its accumulation of nuclei in the sarcoplasma of the muscle-fibre the terminal ramifications of the nerve-ending seem to extend throughout the whole mass of protoplasma (sarcoplasma); at least I could not find a separation or demarcation-line between the sarcoplasma of the original muscle-fibre and the protoplasma of the conducting element fused with it. But here I could call attention to the curious fact, described allready by KÜHNE and by the other histologists of the former century and affirmed by nearly every writer on the subject, that the terminal arborescence of the motor end-plate *never* comes into contact with the myofibrillar structure itself, but that in every case it remains separated from the contractile myofibrillar structure itself by a thin but distinct layer of sarcoplasma, in which the periterminal network (the receptive substance of LANGLEY) furnishes an intermediate substance. Perhaps this curious fact finds a solution in the observations just recorded.

That the protoplasm of two different elements fuses to form a living and functional unity, is not rare or uncommon. We see it everywhere in the organism. The free cells of the embryonic mesenchym fuse to form a syncytium; the cells of the developing heart-muscle fuse in the same way (GODLEWSKI). SPEIDEL for instance (1932) describes how during the development of the nerves in the growing *living* organism "sheath cells may transfer readily from one nerve to another after temporary or permanent anastomoses. They may also bridge the gap and effect transfer between two nerves merely placed in close proximity without any anastomosis," (SPEIDEL, 1932, page 306). The interstitial elements of he sympathetic endformation are everywhere in a syncytial connexion, the elements of the core of the sensory corpuscles exhibit the same syncytial arrangement, and in their protoplasm the conducting periterminal network is formed. Every one who has seen the splendid films of growing tissue-cultures by CANTI, remembers how in these cultures of fibroblasts forming a syncytial reticulum distinct cells which for a long time creep about as distinct independent elements, all at once wriggle themselves into the reticulum formed by the fibroblasts and become part of it. We could multiply these comparable cases, but I may leave it at these few examples.

Whenever a terminal nervous arborisation on the muscle-fibre is formed, these nuclei are allways present, and they may be readily distinguished from the pale elongated nuclei of the muscle-fibre itself by their form, their size and their structure [1]). In older stages of the embryonic development, when the accumulation of the nuclei of the sole-plate begins to show itself and a distinct sarcolemma is formed, the difference between the nuclei of the arborisation and the fundamental nuclei belonging to the muscle-fibre itself often becomes still more prominent, and even in young mice (one to three days old) the two sorts of nuclei are readily to be distinguished, as it was pictured and described in my former papers. These details however will be described in a separate and extensive paper; here only the outlines of the problem investigated can be recorded.

The elements just described belong to the nerve-fibres and are of nervous origin;

1) In a paper appearing in the Acta Morphologiae Neerlandica these details will be described more fully and with ample illustrations. Here of course only the outlines of my investigations can be recorded.

apparently they swerve out at the end of the outgrowing nerve-fibres until they reach the developing muscle-fibres. This reminds us of the observation recorded in my former paper (XII. Innervationsstudy in the Acta Morphologiae Neerlandica), that according to the investigation of LEEUWE the interstitial elements swerve out from the growing accumulations of the ganglion cells of the sympathetic plexus and become connected with them later on by their neurofibrillar structure. It seems to me that we are entirely justified to regard the elements described as the homologa of the interstitial syncytial elements of the sympathetic endformation.

But after all we need to be very careful with regard to their origin. As it was discussed briefly on page 5 of this communication we still know very little about the cells accompanying the outgrowing nerve-fibres in the living organism, and the question whether the interstitial cells and the other small elements of the ganglia (especially of the sympathetic system) are all of them of nervous origin is still under discussion; we only need to point to the critique of HERZOG and GÜNTHER of the work on these elements of STÖHR (1941). To follow the elements just described through all the phases of their development and wanderings will have to be done still. And the way of these investigations is full of pitfalls. For instance in several sections of the growing musculature, especially in the human embryos which I could study, I often encountered between the developing muscle-fibres rather large cells with branching processes and a granular protoplasm full of small elongated and oval granules, which are stained black or dark brown with silver (BIELSCHOWSKY-preparations). These cells were not only seen lying close to the muscle-fibres, but they seem to fuse with the sarcolemma by means of their cell-processes. They are seemingly of mesodermal origin and belong to the different elements of the connective tissue surrounding the growing muscle-fibres; they look like cells capable of ameboid motion, have large and often long processes and a granular protoplasma, and resemble most the resting wandering cells of MAXIMOW, the histiocytes or macrophages of the connective tissue, but in a peculiar form. I mention them here, because at first sight one might regard them perhaps as interstitial cells because of their form and the granular structure of their protoplasm. Closer study however reveals them as connective tissue elements, never in connexion with nervous elements, but often in connexion with the wall of the growing blood capillaries; in the more elaborate paper I hope to describe them more fully and to show the details of their protoplasmic structure in accurate figures. In the same paper the origin of the interstitial elements described here will be discussed more fully, together with the question, whether they belong to the sheath cells or to the neuroblasts, a question which was discussed allready in my former paper, in a discussion which led to the conclusion, that a sharp line of demarcation between neuroblasts and sheath cells is not to be drawn and that the interstitial cells form a distinct intermediate structure between them which has a sharply defined chemical function (the neuro-humoral region). There they will be compared with the interstitial elements in the connective tissue of the iris and of the cornea, and in this way their full value in the organism will be reviewed.

Geophysics. — *On Surface Waves in a Stratisfied Medium.* II. By J. G. SCHOLTE.
(Communicated by Prof. J. D. VAN DER WAALS.)

(Communicated at the meeting of March 28, 1942.)

§ 4. *The waves in an isolated layer.*

The wave system possible in an isolated layer consists of the waves existing in a superficial layer (fig. 2). As the tension at the free surfaces is equal to zero we have

$$\text{at } z=d \begin{cases} n e^{-i\alpha} \cos 2r \,.\, A_e - e^{-i\beta} \sin 2r \,.\, \mathfrak{A}_e + n e^{i\alpha} \cos 2r \,.\, A_r - e^{i\beta} \sin 2r \,.\, \mathfrak{A}_r = 0 \\ e^{-i\alpha} \sin 2i \,.\, A_e + n e^{-i\beta} \cos 2r \,.\, \mathfrak{A}_e - e^{i\alpha} \sin 2i \,.\, A_e - n e^{i\beta} \cos 2r \,.\, \mathfrak{A}_r = 0 \end{cases}$$

$$\text{at } z=0 \begin{cases} n \cos 2r \,.\, A_e - \sin 2r \,.\, \mathfrak{A}_r + n \cos 2r \,.\, A_r - \sin 2r \,.\, \mathfrak{A}_r = 0 \\ \sin 2i \,.\, A_e + n \cos 2r \,.\, \mathfrak{A}_r - \sin 2i \,.\, A_e - n \cos 2r \,.\, \mathfrak{A}_r = 0 \end{cases}$$

where $\alpha = h\,d \cos i$ and $\beta = k\,d \cos r$.

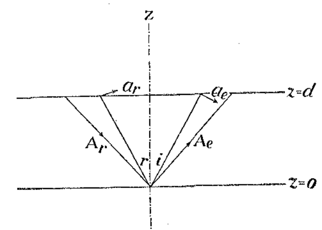

Fig. 3

The wave system $\{A_e\, A_r\, \mathfrak{A}_e\, \mathfrak{A}_r\}$ is therefore only then possible if

$$\begin{vmatrix} n \cos 2r \,.\, e^{-i\alpha} & -\sin 2r \,.\, e^{-i\beta} & +n \cos 2r \,.\, e^{i\alpha} & -\sin 2r \,.\, e^{i\beta} \\ \sin 2i \,.\, e^{-i\alpha} & +n \cos 2r \,.\, e^{-i\beta} & -\sin 2i \,.\, e^{i\alpha} & -n \cos 2r \,.\, e^{i\beta} \\ n \cos 2r & -\sin 2r & n \cos 2r & -\sin 2r \\ \sin 2i & +n \cos 2r & -\sin 2i & -n \cos 2r \end{vmatrix} = 0,$$

or

$$(\sin^2 2i \sin^2 2r + n^4 \cos^4 2r) \sin\alpha \sin\beta + 2 \sin 2i \sin 2r \,.\, n^2 \cos^2 2r (1 - \cos\alpha \cos\beta) = 0.$$

or

$$\left(\sin 2i \sin 2r + \frac{tg\,{}^1/_2\,\alpha}{tg\,{}^1/_2\,\beta} \,.\, n^2 \cos^2 2r\right)\left(\sin 2i \sin 2r + \frac{tg\,{}^1/_2\,\beta}{tg\,{}^1/_2\,\alpha} \,.\, n^2 \cos^2 2r\right) = 0.$$

Hence the equation of the elastic vibrations possible in an isolated layer is

$$\frac{\sin 2i \sin 2r}{n^2 \cos^2 2r} = -\frac{tg\,{}^1/_2\,\alpha}{tg\,{}^1/_2\,\beta} \quad \text{and} \quad \frac{\sin 2i \sin 2r}{n^2 \cos^2 2r} = -\frac{tg\,{}^1/_2\,\beta}{tg\,{}^1/_2\,\alpha}.$$