Histology. — *The nature of the interneuronal connections (Synapses).* By Prof. Dr. J. BOEKE.

(Communicated at the meeting of June 29, 1929).

The term "synapse" was introduced by SHERRINGTON and FOSTER in 1897 to denote the nexus between neurone and neurone in the central nervous system, especially in the reflex-arc, and has been extended to the connection between the nerve-endings and the elements innervated by them, the contractile muscular elements and the sensory elements, tactile cells, etc. The term has in the first place a physiological meaning. In the synapse the stimulus is altered, there is a delay in the transmission of the stimulus in the reflex-arc, which is referable to the transmission in the synapse, several drugs may act especially on the synapse, as curari, nicotine a.o., in the synapse the conduction of the stimulus is polarised, made irreversible, and thus the physiological meaning of the synapse is quite clear, and its nature points undoubtedly to an independant nature of the different neurones. To the physiologist the independance of the neurones is a definite and undisputed reality.

This physiological conception of the synapse must of course have a sound histological basis, and SHERRINGTON describes it in the following manner, which I may be allowed to quote somewhat extensively, as it gives such a clear expression to his opinion and to the current opinion amongst histologists. "As to the existence or the non-existence of a surface of separation or membrane between neurone and neurone, that is a structural question on which histology might be competent to give valuable information. In certain cases, especially in Invertebrata, observation indicates that many nerve-cells are actually continuous one with another It is noteworthy that in several of these cases the irreversibility of direction of conduction which is characteristic of spinal reflex-arcs is not demonstrable; thus the nerve-net in some cases e.g. Medusa, exhibits reversible conduction. But in the neurone-chains of the gray-centred system of vertebrates histology on the whole furnishes evidence that a surface of separation does exist between neurone and neurone. And the evidence of Wallerian secondary degeneration is clear in showing that that process observes strictly a boundary between neurone and neurone and does not transgress it. It seems therefore likely that the nexus between neurone and neurone in the reflex-arc, at least in the spinal arc of the vertebrate, involves a surface of separation between neurone and neurone; and this as a transverse membrane across the conductor must be an important element in intercellular conduction. The characters distinguishing reflex-arc conduction from nerve-trunk conduction may therefore be

largely due to intercellular barriers, delicate transverse membranes, in the former." (SHERRINGTON, The integrative action of the Nervous System, p. 18).

As there is evidence that similar features, though not usually in such marked extent, characterize conduction from efferent nerve-fibre to efferent organ, e.g., in nerve-muscle preparation, in nerve-electric-organ preparation, here too the change may well be referable to the surface of separation admittedly existent between efferent neurone and effector cell. (l.c. p. 16). "Even should a membrane visible to the microscope not appear, the mere fact of non-confluence of the conductive element of one cell with the conductive part of the other implies the existence of a surface of separation. Such a membrane would be a mechanism where nervous conduction, especially if predominantly physical in nature, might have grafted upon it characters just such as those differentiating reflex-arc conduction from nerve-trunk conduction. For instance, change from reversibility of direction of conduction to irreversibility might be referable to the membrane possessing irreciprocal permeability." (l.c. p. 17.)

So the physiological conception of a synapse separating the neurones is sound, and if the conductive element of the neurone be fluid, there must be a membranous surface of separation. For physiologists this surface of separation is and remains living substance, but the morphologists looking at the problem from the standpoint of the morphological independance of the neurones have proclaimed their nexus to be consisting of dead matter, of cement substance. According to the theory of independant neurones, expressed very clearly by CAJAL, the end-knobs of the afferent cell-processes are connected with the perikaryon by means of a "ciment unitif", "une substance granuleuse ou vacuolée", which separates entirely the neurofibrillar endloops from the neurofibrillar network inside the cell. Now to my mind this cannot be true. It seems impossible to conceive a stimulus passing from one neurone to another through a non-living membrane without disintegrating. There must be living substance between. On the other hand this separating substance must differ from the conducting apparatus of the nervous elements connected by it. If the conducting element is fluid, it must be a membrane, so it all depends on the nature of the *conducting element* inside the neurones.

Are nerve impulses transmitted by the neurofibrils, those delicate fibrillae, we can detect everywhere in the nerve-cells and their processes, or was VON LENHOSSEK correct in declaring that no specific part of such a cell can be singled out for this special activity? Is the nerve-cell and especially the nerve-fibre of a viscous semi-fluid or fluid nature, as some micrurgists (LEWIS, PÉTERFI, DE RÉNYI) maintain, with no neurofibrils at all, these being only artefacts caused by the fixation reagents?

In the nerve-endings the neurofibrillar structure of the end-ramification of the nerve-fibre is seen to be prolonged into the lamellae of a distinct alveolar or netlike structure lying inside the protoplasm of the sole-plate

of the motor end-plates (connecting the nervous structure with the contractile substance), or inside the protoplasm of the tactile cells of the sensory endcorpuscles. No traces of a membrane are to be seen, as many histologists think probable for every synapse under the influence of the physiological conception of SHERRINGTON, mentioned above. This "periterminal network", which I suggested to be identical with the "receptive substance" of LANGLEY, about which more later on, must be regarded as an artefact as soon as we regard the neurofibrillae, into which it passes as an artefact. So we can discuss both at the same time.

If the neurofibrillae are present in the living nervous elements, but have nothing to do with the transmission of the nervous impulses but only with metabolic processes as was suggested by PARKER, this periterminal network too loses its significance as a means of transmissing the stimulus in the synapse, and we have to return to the synaptic-membrane-hypothesis of SHERRINGTON. So the whole conception depends on the answer to the question, whether the neurofibrillae are present in the living cells, and if so, whether they have anything to do with the transmission of the nervous stimulus or not. As I have discussed this question at some length in a paper published in 1926 [1]), and the reader finds a very thorough discussion of it in the chapter on the conducting element, written by PÉTERFI in BETHE's Handbook of Physiology in 1929, I will here give only a few outlines.

Allthough only in a few cases the neurofibrillae have been seen in the living tissue (SCHULTZE, HESSE 1895, BOZLER 1927), their arrangement in different cells, their ubiquity in nervous tissue, their staining capacity with methylene blue in living cells, the alterations they present in certain diseases (rabies f.i.) and in hibernating animals, the way in which they always appear in exactly the same form and arrangement in the same cells (f.i. the nerve cells of Hirudo or Pontobdella), when treated with different staining methods (methylene blue, chloride of gold after Apathy and silver), all these phenomena, to which others easily may be added, build up such a series of arguments, that it is absolutely impossible to regard the neurofibrillae as mere artefacts or to deny their existence in the living cell (BOEKE, 1926). The same conclusion was drawn in the latest contribution to the subject by PARKER [2]) in 1929.

Even when the living protoplasm of the nerve-fibre as seen under the microscope, with ordinary or with dark-field illumination, appears to be of a homogeneous nature (MATSUMOTO 1920, LEWIS 1924, DE RÉNYI 1929), we are not allowed to deny the existence of differentiations in this seemingly homogeneous mass, *just because it is living protoplasm*. When we draw from what we see (or better from what we do not see) under the microscope the conclusion, that the protoplasm of the nerve-fibre is of

---

[1]) BOEKE, Zeitschrift f. Mikrosk.-Anatom. Forschung. VII. Bd. 1. Heft, 1926.

[2]) PARKER, American Naturalist. Vol. **53**, March-April 1929.

a homogeneous semi-fluid nature (General Cytology, 1924, p. 403) and that the neurofibrillae are probably due to the peculiar manner in which the apparently homogeneous protoplasm coagulates, we overlook the fact that we have to do with living protoplasm, with a mechanism, not with a chemical compound, and secondly we must never forget, that our micrurgical methods, for all their wonderful and delicate technique, are still as if we would try to dissect a watch with a knife and fork. The more we study the neurofibrillae with every histological method available, in living and in fixed conditions, the more we are convinced of their reality, even if we admit that in the living nerve-cells the protoplasm, as far as we can see it, may exhibit the characteristics of a highly viscous fluid (CHAMBERS, PÉTERFI).

Only this reality does not necessarily include, that the neurofibrillae are present as tough filaments, as VON LENHOSSEK thought they were. To my opinion the neurofibrillae are present as differentiations of the living protoplasm with a linear arrangement of the living units (micellae or neurotagms or neurobionts or whatever we may call them). This differentiation may of course have a texture not much firmer than the common protoplasm. Even there where the neurofibrils could be seen in living cells and fibres, BOZLER [1]) points out, that they appear as strands relatively resistant, but that this extra firmness is so slight, as to be of no real value in forming a skeletal organelle, and about the same conclusion was drawn by PÉTERFI from his micrurgical experiments. We certainly may not regard them as tonofibrillae, in this sense, that they have exclusively the function of supporting elements.

In some cases this linear differentiation perhaps may be transient, but it tends to become a fixed structure though variable in form, as we learn from the study of the regeneration phenomena. That linear protoplasmic structures may be transient DOEFLEIN showed us, that they may easily be repaired the beautiful micrurgical experiments of CHAMBERS (1924) on living cells in mitotic division have shown. That they may alter their form and arrangement even after they have developed into a regular network is shown us by their behaviour during the period of transition of the spinal ganglion cells of the embryo from bipolar to pseudo-unipolar cells (CAJAL). That they must be regarded as a structure of fundamental importance for the nerve-cell is shown by their ubiquity in nervous tissue, by their arrangement and by the fact that they tend to remain intact as long as the cell shows any signs of vitality. So VAN ESVELD (1928) was able to show, that they are still present and stainible in sympathetic cells of the plexus of AUERBACH and in the nervous strands of the plexus themselves after the piece of intestinal wall containing them had been kept for 9 days after the death of the animal at a temperature of 0° C. That they may be concerned with metabolic influences in the nervous

[1]) BOZLER, Zeitschr. f. Zellforschung u. Mikrosk. Anatomie. 5. Bd. 1927.

elements, as is supposed by PARKER (1929), at least in the form of "chains of ionic readjustment" (PARKER), may be quite true, but they certainly have to do with the conduction of the nervous impulses. The evidence on which this could be denied seems to me to be very slight indeed. On the contrary there is every reason to suppose they are.

The curious phenomenon, that in the nerve-endings the endloops and endrings of the neurofibrillar structure often lie quite close to the nucleus, and that in the tactile cells the nucleus is pressed against the neurofibrillar expansion of the nervous disc, may have something to do with metabolic influences, but can never be used as an argument against the conducting function of the neurofibrillar structure. Perhaps the nucleus might heighten the conductability or the excitability of the conducting structure, or at least have a metabolic influence upon it by which the special nervous function is facilitated. There is sufficient room for hypothetical conjectures here.

The nature of the nerve impulse may be that it is a progressive wave of ionic readjustment in some membranous layer of the neurone (R. LILLIE). This may be present on the surface of the cells and fibres, but also on the surface of the linear differentiations we see as neurofibrillae. They may be a two-phasic system. Here perhaps the still hypothetical "argentophile substance" of the neurofibrillae may be brought into the discussion.

The "argentophile substance", which is the reason of the staining capacity of the neurofibrils with silver salts (a substance of which we know nothing more, except than that LEVI saw it oozing out from the cut ends of the neuroblast-processes in his cultures in vitro) may be present as a surface-film covering the linear units of the protoplasmic structure which we see in our preparations as the neurofibrillae. Biphasic protoplasmic structures have often been held responsible for conductability of stimuli. So R. LILLIE showed in a series of important papers, that the property of living protoplasm for conducting stimuli in a distinct direction always depends on the presence of surface-films of lipoid substances on the cell or on the protoplasmic units, i.e. in this case on the surface of the protoplasmic structure we see as neurofibrillae. This may be the cause of their staining capacity. In the periterminal network we may see a protoplasmic alveolar structure into which is prolonged the linear arrangement of the protoplasmic units perhaps without this lipoid surface-film, and so the staining capacity of the periterminal network is far less than of the neurofibrillar structure.

This argentophile substance (the name was given to it by CAJAL) is obviously very labile (LEVI, 1925). It may be present in other cells too (TELLO, 1926).

It is the first thing to degenerate and to break down after the section of the nerve. Afterwards the protoplasmic structure itself is altered. The old conception, that when a nerve is cut or crushed so as to sever the continuity of its fibres the distal part degenerates and disappears, a fact in

itself not easily to be understood, but always accepted as a dogma, cannot be true. It is only the conducting structure (differentiation) which disappears, the protoplasm itself remaining present and forming together with that of the cell of SCHWANN the protoplasmic band of BUENGNER, which fills up the space inside the neurilemmal tube, and conducts the outgrowing regenerating conducting structure to the end-organs. In the corpuscles of GRANDRY for instance the axis-cylindre of the nerve-fibre entering the corpuscle loses its neurilemma and its myelin sheath and is broadened into the nervous disc lying between the two tactile cells. This nervous disc is according to all observers a flattened-out axis-cylindre, in which the neurofibrillar structure appears as a disc-like network. Now when we section the trigeminal nerve, the neurofibrillar apparatus of the nervous disc degenerates and disappears. But the nervous disc itself remains, often shrunk and adhering to the tactile cells, but distinctly visible (BOEKE, 1926), and when regeneration sets in, a new neurofibrillar apparatus is formed by the ingrowing (or differentiating) neurofibrillar strands in this protoplasmic disc, reestablishing in this way the conducting apparatus for the stimuli coming from the tactile cells covering the disc, in which cells then a new periterminal network is differentiated too. So in this case too the neurofibrillar apparatus appears as a differentiation of the protoplasm, which disappears as soon as the connection with the trophic centrum is severed, and reappears when protoplasmic reunion has been reestablished and conductibility of stimuli in a distinct praeformated direction is needed again in the organisation of the organism. When the neurofibrillae in the neurones are connected specially with the distribution of the metabolic influences and not with the conduction of the nerve impulses, as PARKER supposes, it would not be clear, why this apparatus would disappear after the cutting of the nerve and leave the protoplasm living as before, but without the faculty of conducting the impulse in a distinct direction, and why this faculty is reestablished again as soon as the neurofibrillar apparatus is differentiated again.

In the nerve-endings the neurofibrillar linear structure is, as I said before, prolonged into the lamellae of a distinct alveolar (or netlike) structure, connecting the neurofibrillar endings and end-ramifications with the elements into which the stimulus has to pass or from which it has to be received, and being comparable with the "receptive substance" of LANGLEY.

Of course it is here not the proper place to review all the literature on the subject of this receptive substance. I will here only quote some statements by LANGLEY himself, to make his meaning clear.

LANGLEY in his well-known Croonian Lecture formulated his conception of this receptive substance in the following way: "as none of the phenomena of nerve and muscle stimulation are due to a chemical difference between the axis-cylindre and the nerve-endings, it follows not only that the poisoning phenomena of a large number of drugs are due to changes

brought about directly in some constituent of the muscle, but also that the peripheral fatigue usually attributed to changes in the nerve-endings is really due to fatigue of a special constituent of the muscle. Since neither curari nor nicotine even in large doses, prevents direct stimulation of muscle from causing contraction, it is obvious that the muscle substance which combines with nicotine or curari is not identical with the substance which contracts. It is convenient to have a term for the specially excitable constituent, and I have called it the receptive substance. It receives the stimulus and, by transmitting it, causes contraction." (l.c. page 182.)

The receptive substance may be a part of the sarcoplasm or it may be a radicle of the contractile molecule. It might be urged in favour of this former view, that in most cases the nerve-endings inside the sole-plate are completely separated from the contracting myofibrillae by sarcoplasm.

A little farther on LANGLEY admits the possibility, that a certain region of the junction should belong to both nerve and muscle; the special properties attributed to the nerve-ending might then be attributed to the junctional region. Here we have the physiological conception of the "synapse".

So it seems to me, that when we see the endramifications of the neurofibrillar structure of the motor nerve lying inside the protoplasm of the sole without a trace of an intervening membrane between, and when we find a distinct histological differentiation, inside that sarcoplasm, connecting the neurofibrillar structure with the contractile substance, we are entitled to see in it the material basis for the receptive substance of LANGLEY. And perhaps it may be present in every synapse. According to a number of authors the endrings and endloops ("Endfüsschen" of AUERBACH) of the afferent nerve-fibres are connected with the internal neurofibrillar structure of the nerve cells themselves by means of very delicate fibrillae. which by them are identified with the neurofibrillae and are demonstrated as a token of the continuity of the nervous elements (HELD, HOLMGREN, OUDENDAL, TIEGS, a.o.). The exactness of a number of these morphological observations cannot be denied, and when we see in the neurofibrillar structure at least a part of the conducting of the nervous elements, and when we have to admit that in the synaptic region the stimulus is not stopped but simply altered, polarised a.s.o., a separation by a membrane in the sense of SHERRINGTON seems to be out of the question. The living substance which must connect the neuronic elements, must be able to conduct the stimulus, and therefore must connect in a certain way the neurofibrillar structures of the two elements connected. May we regard this fibrillar connection, a differentiation inside the living substance of the synapse, as neurofibrillae of the same nature as the neurofibrillar structure of the elements connected? We certainly have to account for the physiological peculiarities of the synapse. So it seems to me, that we may regard these fine and delicate fibrillae which according to a number of authors connect the endrings and endloops of the terminal branches of the nerve-

fibres with the internal neurofibrillar structure of the nerve cells themselves (the interneuronal synapses), as being of the same nature as the periterminal network of the peripheral synapses. This would account for their weak staining capacity and the difficulty of demonstrating them in a satisfactory manner. If we could regard these interneuronal junctions as of the same nature as the periterminal network of the different peripheral nerve-endings, the physiological independance of the neurones in relation to drug-action and function, and the peculiar way in which the synapse differs from both the nerve cell and the terminal branches of the nerve-fibres, would be satisfactorily accounted for, together with the anatomical continuity of structure (and connection by living substance) between them which we have to acknowledge in the light of modern histology.

About the same suggestion was made by PÉTERFI (l.c. page 115).

In the motor endplates we often find in silver-preparations a very black impregnation of the neurofibrillar structure of the nerve-ending together with a weak staining of the periterminal network or no staining at all. And in the synapses inside the central nervous system we often see a very strong impregnation of the extracellular endfibres together with a weaker staining of the neurofibrillae of the other neuron and a very weak staining of the synaptic connection or no staining at all. This is always held a very strong argument for the discontinuity of the different neurones, but it can be only a strong argument for a difference between the synaptic connection and the neurofibrillar structures connected by it. In motor endplates we are absolutely sure of the hypolemmal position of the nerve-ending and therefore of a *protoplasmic* connection of the neurofibrillar structure with the contractile substance. And yet we may examine a number of preparations with a very strong impregnation of the neurofibrillae of the ending without a trace of staining of the periterminal network or of any other protoplasmic conjunction. In the same way in the synapse there must be a connection of the two neuronic parts by *living* substance. In my opinion this is the only way to account for the transmission of the stimulus. Even the hypothetical synaptic membrane of SHERRINGTON must be an arrangement of units of the *living* substance. and this arrangement may be present in the periterminal network, not as a real visible membrane, but as a biphasic condition of the living substance itself.

But the physiological independance of the neurones and the anatomical character of the synapse as a secondary connection, and as a connection differing in character from both the connected parts must be accounted for too. So it seems to me that the only way to bring together the opposite views and to give a firm basis for further work is to regard the intervening substance not as a sort of non-living cement substance, sometimes faintly striated, as CAJAL does, a connection in which no alteration is possible, but as a living substance, in which there is present a structure akin to the periterminal network of the peripheral junctions, and connecting the

neurofibrillar endloops with the neurofibrillar network inside the cell. As a living substance the synapse may alter its structure, may obtain a more definite linear arrangement of the units composing its connecting fibrils, inside its protoplasmic substance there may be formed a more complex organisation of its linear (fibrillar) structure, a transient part of this linear structure may become fixed, in short, inside the synaptic *living* substance the conducting apparatus may become more and better organised. In this way it might be possible to explain those alterations in the synaptic connections and in the transmission of the stimuli ("Bahnung", memory, etc.), which are entirely unexplained when we have to regard the synaptic substance simply as a cement, a non-living matter, in which no alteration is ever possible. For we must not forget, that those alterations of the transmission of the nervous stimuli, by which stimuli, coming from particular cells, are enabled to follow a particular path of transmission quicker and easier than another path, must be localized in the synapses between the different neurones, and that these alterations are only possible in a living substance, in which a structure for the transmission of those stimuli may be developed.

Utrecht, June 1929.