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Study of Neuron Processes and Terminals by Electron Microscopic Method Review

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ABSTRACT

The study of the processes of neurons and terminals using the electron microscopic method is a necessary link in the study of the morphofunctional state of nerve cells. It is through the processes and synapses that important functions of the central and peripheral nervous system are carried out. The data presented in this review will serve as a fundamental basis for the study of neurons in normal conditions and in experimental pathology, which is an integral basis for clinical research.

Keywords: Neuron; Terminal; Electron Microscopic Method

Abbreviations: FC: Fibroblast Shell; SC: Schwann Cells, M: Mesaxons, E: Extension

Introduction

The first electron micrographs of limp nerve fibers were presented by Gasser [1], who showed that although the Schwann cell surrounds the axon, these are two completely independent formations separated by their own plasma membranes. The axons are embedded in deep grooves in the surface of the Schwann cell. The edges of the Schwann cytoplasm and its plasma membrane, covering the axon e on both sides, close over it, and a paired membrane structure is formed, which Gasser called a "mesaxon". Such relationships are a general rule, and very few exceptions have been found in the study of a wide variety of peripheral nerves. With the exception of the endings and end sections, the limp fibers are enclosed throughout their entire length in the shells of Schwann cells and therefore do not come into direct contact with the extracellular environment. Although it has not yet been clarified how the situation is at the border of Schwann cells located one after another along the fiber, it is likely that the same close connection takes place here [2]. The degree of complexity of the relationship between the axon and the Schwann cell enveloping it (photo 197-199) can be very different. In some nerves, especially skin nerves (photo 197), only a few axons may be connected to each Schwann cell. In these cases, the mesaxons (M) can be short and straight or strongly twisted and sinuous." In other places, for example, in the posterior roots and olfactory nerves, sometimes there are large bundles of axons and a separate branch of the mesaxon goes to each such bundle.

Each of the main mesaxons branches many times, covering a large number of such bundles. In the intramural plexuses of the intestine (photos 198, 199), Schwann cells envelop many such axons, and each mesaxon (photo 199, shown by an arrow), repeatedly branching, surrounds small groups of axons [3]. The axolemma of an unmyelinated axon, as well as the plasma membrane of a Schwann cell, is an elementary membrane 75 A thick. These membranes are separated by a slit with a width of 100-150 A (photos 197, 199). The accuracy of determining these sizes is essential for the problem of myelin formation and for understanding the nature of the myelin sheath [4].

Formation of Myelin

After clarifying the fundamental nature of the relationship between the Schwann cell and the unmyelinated axon, the problem of myelin sheath formation remained unresolved. Her decision was led by Guerin's observations regarding nerve myelination in a chicken embryo, confirmed by Robertson in other animal species. During the formation of myelin, the following process occurs in general terms .

At an early stage of its differentiation, the future myelinated axon is located in the recess of the surface of the Schwann cell. The process of its "wrapping" by a Schwann cell begins, and eventually the axon turns out to be enveloped by this cell and connected to its surface by a mesaxone. Thus, at this stage we see the same picture as was described for a limp fiber, but with the significant difference that each Schwann cell usually covers only one axon here. The extracellular space between the two sheets of the plasma membrane forming the mesaxone still retains a width of about 100-150 A in the early stages. Soon this gap closes, and the outer surfaces of the Schwann cell plasmalemma come into contact with each other, forming a five—layer structure like a double elementary membrane with a thickness of about 150 A - the so-called outer complex membrane. Next stages of this process include the growth and elongation of the mesacon, which spirals around the axon, forming many layers like a roll. Initially, the mesaxone coils are separated from each other by the cytoplasm of the Schwann cell, which creates another gap, this time cytoplasmic. Soon this gap closes and successive turns come into close contact with each other.

The layer formed in this case consists of tightly adjacent cytoplasmic surfaces of Schwann cell membranes and forms the "main dense line" of the mature myelin sheath. The layer formed as a result of the contact of the outer surfaces of these membranes forms an "intermediate" line. Thus, compact myelin consists of spirally stacked plates forming a repeating structure in the radial direction with a period of about 120 A (the distance between the axes of the main dense lines). This period is divided into two equal parts by an intermediate line. The details of the structure of myelin will be discussed in the next section. The external mesaxone is preserved, so that the myelin plates continue to pass into the plasma membrane of the Schwann cell without interruption. One of the most important conclusions from these observations is that the myelin sheath is formed directly from the plasma membrane of the Schwann cell. As we will see now, this is of great importance in connection with the problem of the structure of the plasma membrane [3,5,6]. There are still a number of questions concerning the specific nature of the mechanisms of myelin formation. First of all, the question arises about how the mesaxone coils of the Schwann cell are formed around the axon. The true rotation of this cell around the axon is highly unlikely, especially since there may be several myelinated axons in one Schwann cell. The most plausible explanation is that the mesaxone itself grows and this leads to its introduction into the cytoplasm of the Schwann cell along a spiral path and, thus, to winding it onto the axon. The location and mechanism of this membrane growth remain the subject of numerous assumptions, and the lack of a complete solution to this issue has led to a difference of opinion regarding the structure of the plasma membrane.

The Myelin Sheath of the Peripheral Nerve

With this information about the formation of the myelin sheath, we can now better understand the structure of the fully formed myelin sheath, as well as draw some conclusions about the structure of the elementary membrane. Below we give a brief overview of the current state of this rapidly developing field of knowledge. The reader will find more specific details in a number of recently published reviews and in the original works to which we give references [5]. The optical properties of the myelin sheath in polarized light have been known for more than 100 years. In a series of detailed studies, Schmidt put forward the idea that the myelin sheath consists of thin layers of lipid molecules, the long axes of which are oriented radially with respect to the axon. Between these lipid molecules, according to Schmidt, there are protein molecules with long axes directed tangentially with respect to the axon. Quantitative studies conducted later in polarized light mostly confirmed these assumptions [6,7]. Based on the analysis of X-ray diffraction at small angles, it was possible to develop this concept and determine the exact dimensions of the radial repeating unit. For a fresh peripheral nerve of a mammal, a period value of about 180 A. In the dried nerve, this period is about 20-30 A less. As a result of optical studies, a repeating period model was proposed — a structure of two bimolecular lipid layers separated by protein monolayers. Finean put forward another similar idea of the repetitive structural unit of myelin; according to this view, there are two bimolecular*. lipid layers, the polar surfaces of which are covered with protein monolayers.

To explain the measured value — 171 A (peripheral nerve of a frog) - Finean included an unknown "difference factor" in a structure that would otherwise be symmetrical and thus create a period equal to half of the actually measured value. As we will see in the next section, the inclusion of this factor is important to explain the asymmetry of the plasma membrane of a Schwann cell. The reader will find details about X-ray diffraction studies in other works [8,9]. The first electron microscopic studies of the myelin sheath were carried out by Fernandez-Moran [10] and Shestrand. As a result of a number of subsequent works by these and other authors, the now generally accepted idea of the structure of the fully formed myelin sheath of the peripheral nerve has developed. This representation is based on the study of drugs fixed with both 0z04 and permanganate; in both cases, very similar images are obtained. The strikingly regular structure of the myelin sheath (photos 200, 201) consists of a series of dense lines about 30 A thick; the distance between their axes, so the value of the repeating period, is about 120 A (up to 150 A in some preparations) [11]. These are the main dense lines. In favorable cases, especially after fixation with permanganate (photo 202), it can be seen that this main period is subdivided by a less dense intermediate line with a thickness of about 30 A. As noted in the previous section, the main dense line is formed as a result of the closure of the inner surfaces of the plasma membrane of the Schwann cell, and the intermediate line is formed as a result of the closure of its outer surfaces.

This is where the discrepancy is observed; the addition of two membranes with a thickness of 75 A should create a repeating period of not 120 A, as determined by measurements, but 150 A. Although there is no definite solution to this problem yet, it has been

suggested that the plasmalemmas partially merge and this leads to a decrease in the total thickness. Other explanations have been put forward [12]. Another discrepancy is found when comparing the period found according to X-ray diffraction analysis (180 A) with an electron microscopic image (120 A). This difference was explained by compression occurring during fixation, dehydration, pouring and preparation of slices. Since myelin is formed from the plasma membrane of a Schwann cell, the assumed molecular structure of the myelin sheath has been extrapolated back to the corresponding layers of the plasma membrane. The models proposed for the structure of the elementary membrane basically consist of a bimolecular layer of lipids [13], the polar groups of which are adjacent to protein layers or one layer of protein and one layer of polysaccharide. This latter is probably due to Finean's "difference factor".; but instead, the model can be made more asymmetric by adding a third layer of protein at the cytoplasmic surface. When evaluating hypothetical representations of the elementary membrane based on the data on the structure of the myelin sheath, some caution is necessary, as indicated by Fawcett. It is possible that during the formation of a new membrane material during myelination, some components of the ordinary plasma membrane are lost or, conversely, something is added.

The structure of the Ranvier interception quite logically follows from the method of myelin sheath formation [14]. To understand the structure of the interception, you need to understand that the length of the myelin spiral (along the fiber axis) varies from one turn to another. The coil adjacent to the axon is the shortest, and as it approaches the surface of the myelin sheath, the length of the coils gradually increases. Thus, near the intercept, the myelin plates sequentially peel off from the compact mass of myelin, starting from the innermost of them (photo 202). After the interception, the outermost layer ends, and here only the cytoplasm of the Schwann cell remains above the axon. In each of those areas where the plates bend away towards the axon, the main dense line splits (photo 202, shown by the arrow) and the cytoplasm of the Schwann cell (SC) appears in the gap. Here, the relationships that took place in the development process after the formation of mesaxone before the closure of the cytoplasmic gap are essentially preserved. There is not much I in this gap of the Schwann cytoplasm. It often contains small dense granules of about 100-150 A in size. There is a small gap less than 100 A wide between the Schwann llasmalemma and the axolemma, and in places one or two light lines can be seen here, as in a "dense junction" [15]. In the peripheral nerve in the interception area, the surface of the axon is usually not bare, since adjacent Schwann cells, linked by their processes, form a continuous shell around it. In smaller—caliber fibers, this shell is very thin - the axolemma is almost directly in contact with the extracellular space.

In addition, in thicker fibers, the area not covered with myelin in the intercept region may have a thickness of about 0.5 mk, whereas in thinner axons its length may reach 2-3 mk. Thus, in Ranvier interceptions, the axolemma is either separated from the extracellular space only by a plexus of Schwann cell processes, or — in the case of thinner axons — is in almost direct contact with it. The significance of these morphological facts for ideas about the mechanism of action potential is obvious (Bertson discusses this issue in detail). Near the place where the myelin ends, the thickness of the axon usually decreases slightly, whereas in the area of the interception itself, the axon may be thickened. In the axoplasm, clusters of small mitochondria, neurofilaments, small vesicles, and elements of the agranular reticulum of small granules are visible here. The Schmidt—Lanterman notches, which have long been controversial, are now recognized as real structures; in fact, they are funnel-shaped ruptures in the myelin sheath [Photo 203 shows an oblique section of the myelin fiber of the cutaneous nerve passing through the notch [16]. The axon is enclosed in a shell of Schwann cells (SC). The section was "stained" with phosphovolframic acid; therefore, the collagen fibrils of the endoneurium look very dense. The endoneurium is enveloped by the bodies and flattened processes of fibroblasts [17]. Such a fibroblast shell (FC) is also visible in photo 200. In these structures, the myelin plates are stratified along the main dense line, and the cytoplasm of the Schwann cell appears in the gaps.

Thus, although ruptures are possible in the myelin, the plasmalemma and cytoplasm of the Schwann cell retain their continuity. It is impossible to establish an obvious functional significance for this structure, and it is inclined to be considered a defect that occurs during development due to mechanical stresses experienced by a nerve fiber. Another type of myelin sheath should be mentioned here — the myelin sheaths of the neuronal bodies in the nucleus of the VIII cranial nerve [18]. This shell, formed by satellite cells, differs in some respects from the myelin of nerve fibers. First of all, it has a very irregular structure — typical myelin plates are interspersed here with thin layers of cytoplasm. The myelin plates split in places, suddenly end in blind loops or turn in the opposite direction. They can be compact or loose. In addition, the myelin layers are formed by more than one satellite cell. These facts seem to indicate that myelin of this type is not formed from a single satellite cell in an orderly manner, as it occurs in internodes. Although the method of its formation is not exactly known, there is no doubt that this process is associated with a complex irregular intertwining of several satellite cells and with incomplete fusion of their membranes into myelin plates.

Peripheral Nerve Endings

Synapses

The detailed structure, varieties and functional significance of synapses and synaptic structures will be discussed more fully in the next chapter. In the peripheral nervous system, the structural elements of synapses have been studied in sympathetic ganglia [19], ciliary ganglia [20], intramural plexuses of the intestine and other places. In all these cases, whether it is the axon ending on the soma or dendrite, or the postganglionic ending, there is a striking uniformi-

ty of structure. The two plasma membranes in the synapse or in the nerve terminal are separated by a gap of width from 60 to 200 A or more, depending on the localization. Occasionally, indistinct seals can be seen in this interval. One or both adjacent membranes may have an increased density. Usually, the terminal section of the axon expands and contains a group of small bubbles 300-500 A in diameter (photo 204, shown by an arrow) — the so-called synaptic bubbles. In addition, in many places, but in a smaller number, there are larger bubbles (about. 1000 A in diameter), containing a dense central mass. These bubbles with a dense "core" especially attract attention in the postganglionic endings [21] it is believed that they have something to do with catecholamines. Small mitochondria, although usually present, are not as widespread as vesicular elements of the cytoplasm. The axons are covered with Schwann sheaths up to the ends [22].

General Sensitivity Receptors

Receptors of general sensitivity have endings of a peculiar type. Although it cannot be said that true synapses exist here, the structure of the nerve endings shows great similarity to what we see in synapses. Typically, these receptors are characterized by the presence of one or more support or receptor cells. Nerve fibers, losing their myelin sheath (if there was one), enter the receptor and form terminal extensions on the receptor cells present here. These extensions contain many small bubbles and small dense mitochondria. As we saw in the previous section, these are two distinctive features of a synapse. An example of such a structure is the taste bud [23]. It is formed by two types of cells. Cells of one type are support cells, so named because they envelop nerve fibers from where they enter the kidney to their termination. In this respect, the support cell is completely analogous to the Schwann cell in its function. It is possible that it even represents a derivative of the Schwann cell. The second type of cells are taste receptor cells equipped with apical microvilli and cytoplasm with dense granularity [24]. The trigeminal nerve fiber ends at this cell in the form of an extension containing many small (300-600 A) vesicles and mitochondria. Based on its ultramicroscopic morphology, this area is considered a synapse, although this does not agree with the strict physiological definition of the concept of "synapse", since there is no pulse transmission here. It is interesting, however, to note that the characteristic synaptic structures are located in that of the contacting formations, which would be a postsynaptic element.

The structure of the olfactory epithelium [25] is in many ways much simpler than the structure of the taste bud, since the primary neuron — the olfactory receptor cell — lies within the mucous membrane. The dendritic section of the receptor cell, heading towards the surface of the epithelium, ends in the form of a rod covered with cilia. The proximal processes of these cells are axons that form olfactory filaments (Sha on Dopa). Inside the epithelium, these axons are enclosed in a shell of two other types of cells — supporting and basal. In the basal layer of the epithelium, axon bundles are surrounded by Schwann cells forming a typical structure with a mesaxone. We find great similarity with the taste bud in Pacini corpuscles [26]. These bodies consist of numerous cytoplasmic plates arranged concentrically in the outer zone and bilaterally in the inner zone. It is assumed that these lamellar structures originate from fibroblasts, and not from Schwann cells. The myelin nerve fiber, approaching the Pacini body, first loses its myelin sheath, and then the sheath of Schwann cells, so that its expanded end is in direct contact with the most centrally located plate of the inner bulb of the taurus. The axoplasmic components of these endings also resemble the structures contained in a true presynaptic element. Along the entire perimeter of the nerve fiber there are numerous small mitochondria and many vesicles 500 A across. Thus, we find here morphological signs of a synapse, with both characteristic components (mitochondria and vesicles) contained in the supposed postsynaptic element. Of course, due to the lack of decisive physiological data, the relationship between the unmyelinated nerve ending and the lamellar cell in the Pacini body cannot be called a synapse.

Meissner corpuscles consist of a "bundle" of flattened tactile (lamellar) cells (photo 205) forming a series of transverse layers [27]. Nerve fibers, losing their myelin sheaths upon entering the body, pass through winding paths between flat cells (photo 205, PH). The nerve endings are surrounded by a complex interweaving of processes of tactile cells. The terminal nerve fiber can form several successive extensions (photo 206, E, H). The expanded end sections of nerve fibers contain many small dense mitochondria and small vesicles (400-500 A). Photo 207 shows one such extension (E) surrounded by several appendages of tactile cells. It contains a large number of small mitochondria, some of which exhibit a concentric lamellar structure. Groups of small bubbles are scattered inside the nerve end, and in the tactile cell, some bubbles are located along the plasma membrane. The axolemmas of these nerve endings seem to be closely adjacent to the plasma membranes of the tactile cells. In some cases, both adjacent membranes are thickened and near these thickenings there is a concentration of small bubbles — both in the tactile cell and in the nerve terminal. This pattern, as in the previously described receptors, has morphological features of a synapse. However, we do not have data on the transfer of membrane potential through this compound. An interesting fact is that the distribution of small "synaptic" bubbles on both sides of the putative synapse does not reveal a mutual correspondence. The sensitive endings of the muscular spindle [28,29] are also characterized by a terminal expansion filled with small mitochondria and many small vesicles. The axolemma comes into close contact with the sarcolemma [30]. As in the previous cases, if we can talk about a "synapse" here, then the characteristic structures are in the postsynaptic element. Thus, the morphological polarity is reversed. The current level of our knowledge about general sensitivity receptors does not allow us to more closely link the available morphological, physiological and pharmacological data. However, due to their size and accessibility, these nerve formations can apparently serve as a convenient object for appropriate research.

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