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FINE STRUCTURAL ANALYSIS OF THE NEURONS OF *ANODONTA CYGNEA L.* (PELECYPODA)

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Only little information is available on the ultrastructure of the neurons of Pelecypoda. Electron microscopic studies on the neurosecretion of *Unio tumidus* have been performed by FÄHRMAN (1961), he has given, however, only a general description of the ultrastructural features of the nerve cells. In earlier investigations on the cerebral ganglia of *Anodonta cygnea* (ZS.-NAGY 1964) we examined the fundamental structure of the cytoplasm, the vesicular components of the axons and further the special glial structures. In some previous papers of ours some submicroscopic data (ZS.-NAGY 1967, 1968, ZS.-NAGY et al. 1965) on these structures have been presented, a comprehensive description of results obtained during these electron microscopic investigations, however, has not been given. In the course of examinations performed lately on the visceral and pedal ganglia, numerous newer details that may command interest were revealed; on the other hand, the interpretation of certain structural elements described formerly changed in the light of recent literature, thus it became actual to touch upon these questions anew.

The objective of the present study was to describe by electron microscopic methods the structure of the neurons in all three ganglia of *Anodonta cygnea* and to form a basis on cytological level for examining the functional organization of the ganglia.

Material and method

For experimental purpose 12—18 cm long specimens of *Anodonta cygnea* L. were used. The animals were obtained from fish ponds, and were kept previous to experiment in aquaria containing Balaton-lake water. The preparations of the cerebral, visceral and pedal ganglia were made as follows:

1.5 percent OsO_4 solution buffered with *s*-collidine (PEASE 1964) was used as fixative. Fixation was made at first at 0 °C for 0.5—1 hours, and subsequently at room temperature for 10—15 mins. Dehydration in graded series of alcohol solutions and later with propylene oxide. Following this the ganglia were embedded into araldite (Durcupan ACM, Fluka). Serial sections were made on LKB Ultrotome III, and the micrographs were taken with TESLA BS413A electron microscope. Contrasting with saturated

uranyl acetate solution in 70 percent alcohol during dehydration (PEASE 1964). Lead citrate staining according to REYNOLDS (1963).

Experimental results

1. *Nerve cells*

Nerve cells though different in size, bear resemblance to each other as regards their submicroscopic constituents. Structural elements characterizing explicitly either large or small sized cells were not demonstrable. The larger cells, of course, exhibit a more rich structure than the smaller ones, and the single cell constituents also show a much greater variety. In the following a survey on the most important ultrastructural features of the single cell components observed in the three ganglia examined will be given.

a. Nucleus

The nuclei are either round or ovoid. Their location inside the cytoplasm is usually excentric. On occasion this eccentricity may be of such an extent that one side of the nucleus is bordered only by a very narrow rim of cytoplasm. The nucleus has an even surface and on it regular nuclear pores are observable. Occasionally nuclei with lobulated, uneven surface were also

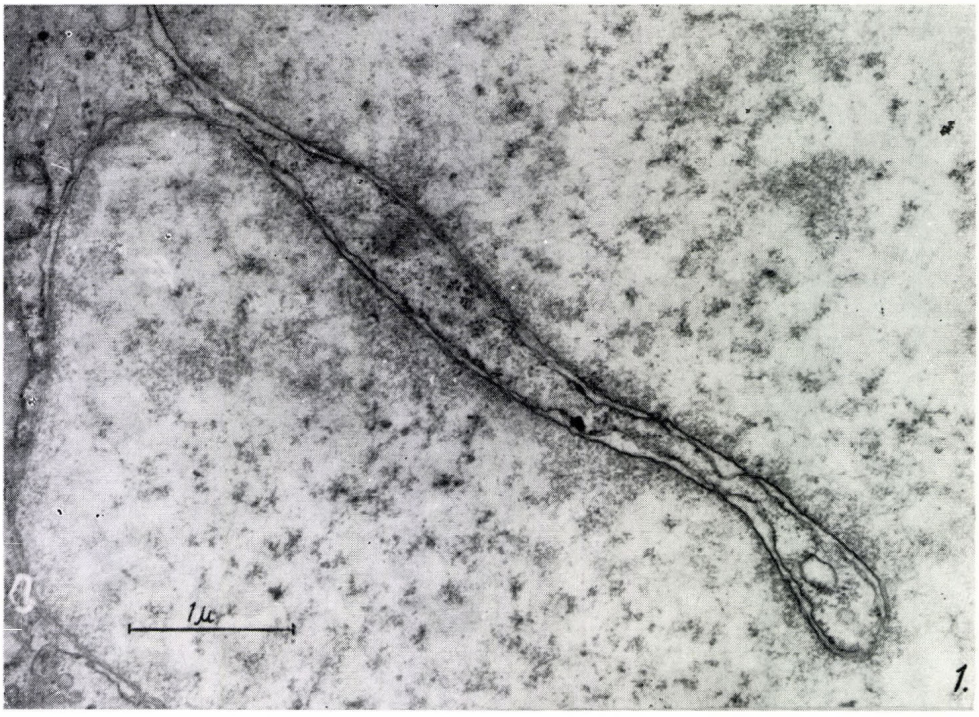


Fig. 1. Invagination of the nuclear membrane. Cerebral ganglion. ×22 500

1. ábra. Maghártyabetüremkedés képe. Cerebrális ganglion. Nagyítás: 22 500×

observable, and in some instances there were also membrane invaginations seen almost all around the whole nucleus *(Fig. 1)*, and dividing it into partitions. Images illustrating two completely isolated nuclei inside one single nerve

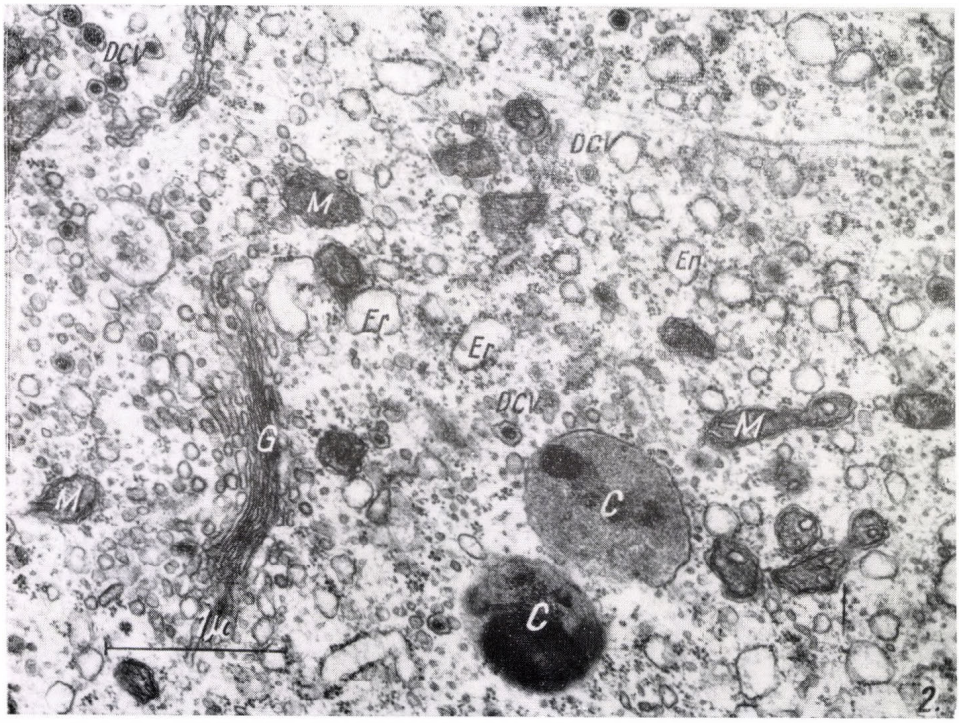


Fig. 2. A section of the cytoplasm. Cerebral ganglion
Er — endoplasmic reticulum; G — Golgi apparatus, C — cytosomes; M — mitochondria; DCV — dense-core vesicles. (Abbreviations are the same in every figure). ×25 000

2. ábra. Citoplazmarészlet. Cerebrális ganglion. Er — endoplazmás reticulum; G — Golgi apparátus, C— cytosomák; *M* — mitochondriumok; DCV — dense-core vezikulumok. (A rövidítések a további ábrákon is ugyanazt jelentik.) Nagyítás: 25 000×

cell originate, most probably, from transversal sectioning of such invaginations. The nuclear membran is mostly complete on the whole surface of the nucleus but sometimes a certain loosening up is observable on it. The nuclear substance is of varying density. Beside one or more nucleoli a narrow rim of granular substance of higher electron density than the nucleus itself is seen attached to the internal surface of the nuclear membrane, whilst on other occasion, and this is more frequent, the nuclear substance is homogeneous.

b. Cytoplasm

The endoplasmic reticulum of vesicular type forms the ground substance of the cytoplasm *(Fig. 2)*. The external wall of the vesicles is smooth-surfaced,

very often, however, ribosomes, too, are observable on them. Their internal surface is always smooth. The vesicles are either translucent or contain a substance of very fine distribution and of low electron density. Their diameter is of varying length, and they appear occasionally, especially in the large cells as 1 μ great, wide cysternae, and on other occasions as flattened or bifur-

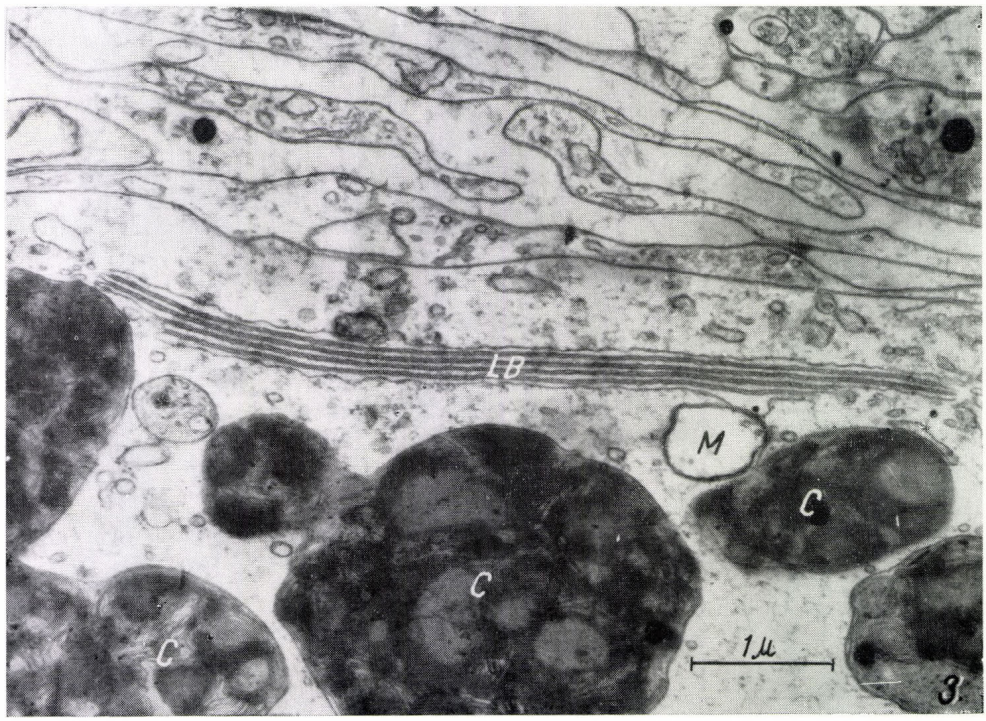


Fig. 3. Lamellar formation consisting of 5 cysternae (LB). Visceral ganglion. ×20 000

3. ábra. 5 ciszternából álló lemezes képlet (LB). Viscerális ganglion. Nagyítás: 20 000×

cating vesicular elements. They are to be found everywhere inside the cytoplasm amidst the other cell constituents.

There is in the endoplasmic reticulum a peculiar formation which is constituted of two or more flattened, parallelly arranged cysternae *(Fig. 3)*. The gap between the opposite surfaces of the cysternae is 100—150 Å wide, whilst the width of the cysternae proper is about 2—3 times as great. The inner part of the cysternae is less dense than the zone between them, and this produces a pattern of alternate dark and light lamellae. The fact, that ribosomes are frequently observed on the outer membrane of cysternae indicates that the cysternae belong to the endoplasmic reticulum. Cysternae are arranged generally side by side 2—8 in number, resulting in 1—7 dark bands. These formations might extend in the plain with a maximum length of 6 μ, and maximum thickness of 0.4 μ depending on the number of lamellae. The fre-

quency of their occurrence suggests that they are most possibly present in many cells, only they may be absent from the plain of section. Occasionally even 2 or 3 such formations were observable within one single cell.

The Golgi apparatus is located in general, but not explicitly, near the nucleus *(Fig. 4)*. It appears in the form of kidney-shaped formations, the

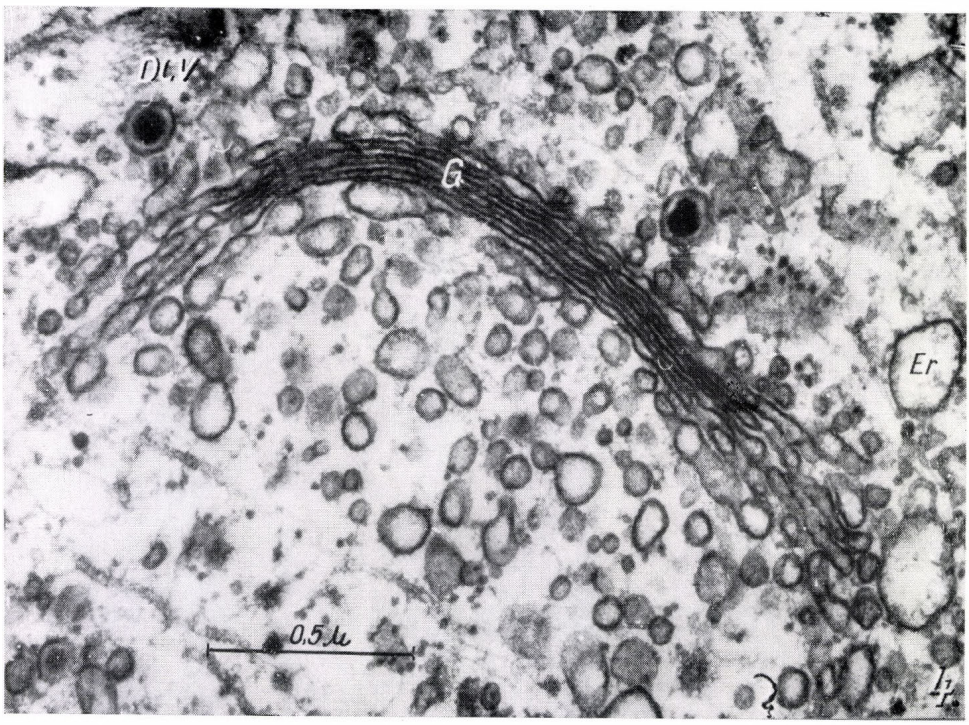


Fig. 4. Golgi apparatus and its surroundings. Cerebral ganglion. ×58 500

4. ábra. Golgi apparatus és környezetének képe. Cerebrális ganglion. Nagyítás: 58 500×

middle part of which is made up by several parallelly arranged lamellae (Golgi cysternae), and at its two poles there are several translucent vesicles mostly about 1000 Å or sometimes even much greater in diameter. There is a substance of high electron density inside certain vesicles which are very similar to dense-core vesicles (DCV). Golgi areas in which the lamellae were not parallelly but irregularly arranged were also noticed, and in such cases the whole lamellar system much rather resembled a pile of vesicles.

Obligate constituents of the ctyoplasm are the microtubuli *(Fig. 5)*. These generally range from 100 to 150 Å in thickness, and the average thickness of their wall ranges from 25 to 30 Å. Inside they seem to be translucent. It is not possible to determine their length exactly; sometimes, namely, they may be trailed in some microns length, another time, however, they are leaving soon, after a short run, the plain of section. A characteristic feature of their

location inside the cytoplasm is that they show a tendency to converge towards the area where the axons are originating from.

As already reported in our previous publication (Zs.-Nagy 1967) there are to be found in the cytoplasm two different types of mitochondria (mitochondria with dark or light matrix). Also certain ultrastructural features of the

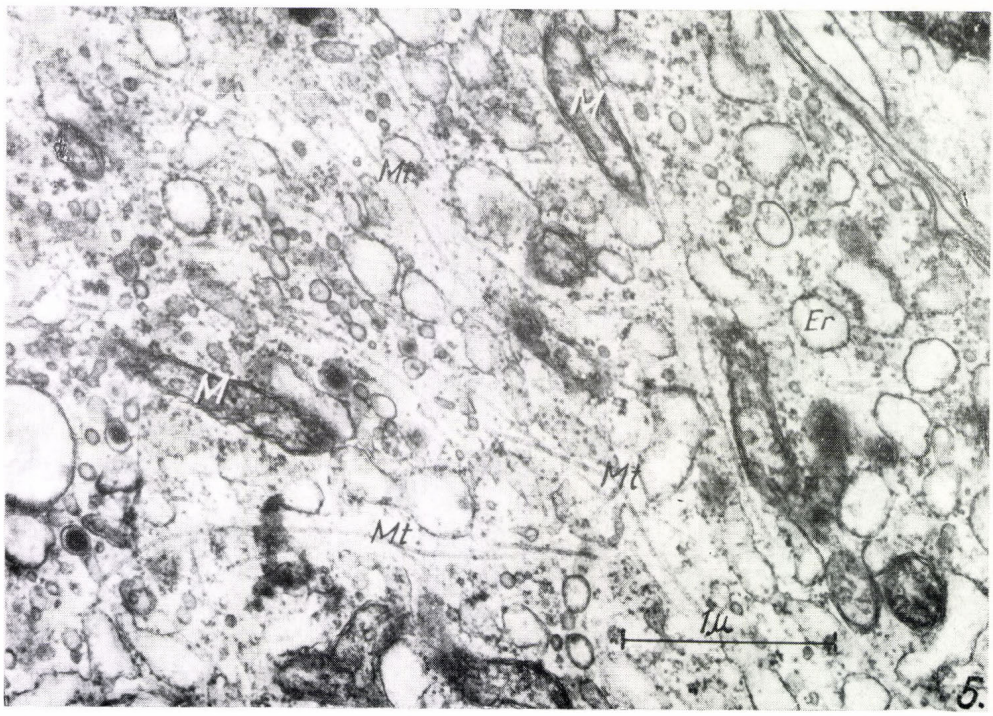


Fig. 5. Section of the cytoplasm with numerous microtubuli (Mt). Cerebral ganglion. ×30 000

5. ábra. Sok mikrotubulust (Mt) tartalmazó citoplazmarészlet. Cerebrális ganglion. Nagyítás: 30 000×

cytosomes were described in the same paper. These, however, must be complemented by the following data: the mitochondria are most variable components of the cytoplasm. They are generally 0.3—1.0 μ in length. Greater ones are very seldom found. They exhibit a large variety of shape from simple ovoid or rod shape to bizarre, bended forms with invaginations and protrusions on their surface. Mitochondria with typical cristae were not observed, their majority being much rather of tubular character.

The cytosomes *(Fig. 6)* are round-shaped or ovoid formations, their size ranging from 0.3 to 12 μ. Those of 1—3 μ in size are of more frequent occurrence. They are identical with the yellow pigment granules observed in the cells in native condition. They are „unite membrane" bound which at certain places is not continuous. Their internal substance exhibits great variety.

Very dark homogeneous areas are alternating with similarly homogeneous spots of less electron density located inside them. Besides these a lamellar system similarly of „unite membrane" construction and resembling very fine myelin figures, further a granular substance and vesicles too might also be located inside the cytosomes. The varying ratio of occurrence of these con-

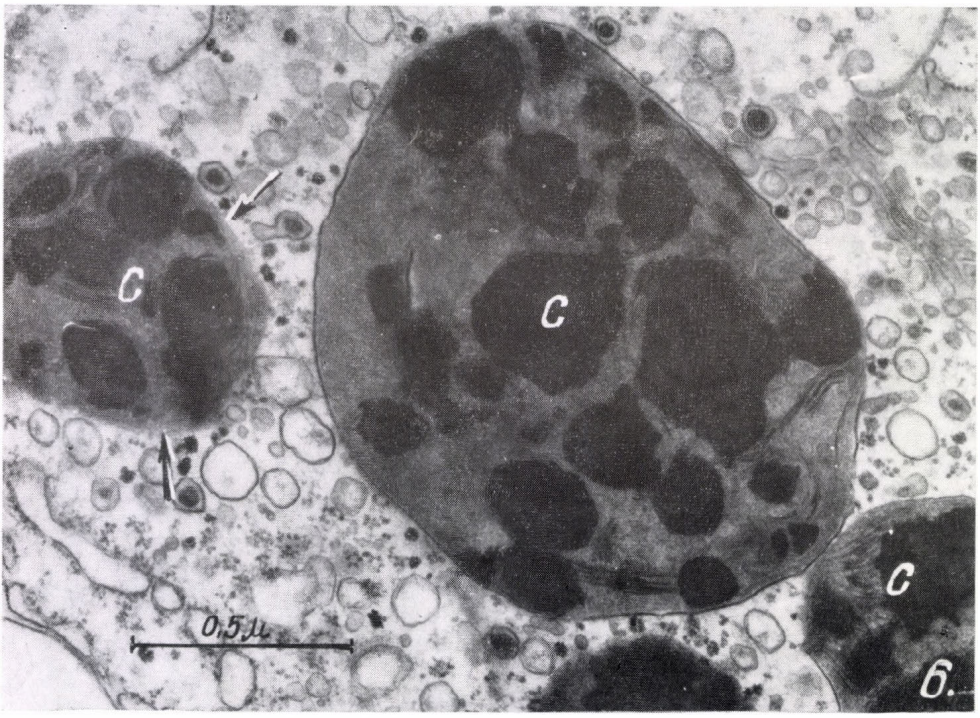


Fig. 6. Typical cytosome. Arrow indicating areas where cytosome-membrane cannot be recognized. Cerebral ganglion. ×62 400

6. ábra. Típusos cytosoma képe. A nyíl olyan helyekre mutat, ahol a citoszoma-membrán nem ismerhető fel. Cerebrális ganglion. Nagyítás: 62 400×

stituents results in an almost untipifiable richness of forms. Great sized cytosomes very often exhibit irregular, complex forms. They are demonstrable usually arranged in groups in most diverse areas of the cytoplasm even in the place of origin of the axons.

Formations also occur which so to say represent transitions between mitochondria and cytosomes. On basis of their outer membrane and tubuli they might be taken for mitochondria, their internal substance, however, exhibits the characteristics of cytosomes: they contain substance of granular and lamellar structure *(Fig. 7)*.

Permanent constituents of the cytoplasm *(Fig. 8)* are also the multivesicular bodies (Sotelo and Porter 1959). These are 0.2—0.5 μ sized membrane bound formations with numerous vesicles ranging from 200 to 400 Å in diameter inside them.

Besides the above organella the cytoplasm contains numerous ribosomes, vesicles and other granules too, the amount of which varies in nearly every single cell. These organella as a whole can be ranged into the group of microsomes. Large masses of ribosomes occur free in the cytoplasm, a number of them, however, is connected to the vesicles of the endoplasmic reticulum.

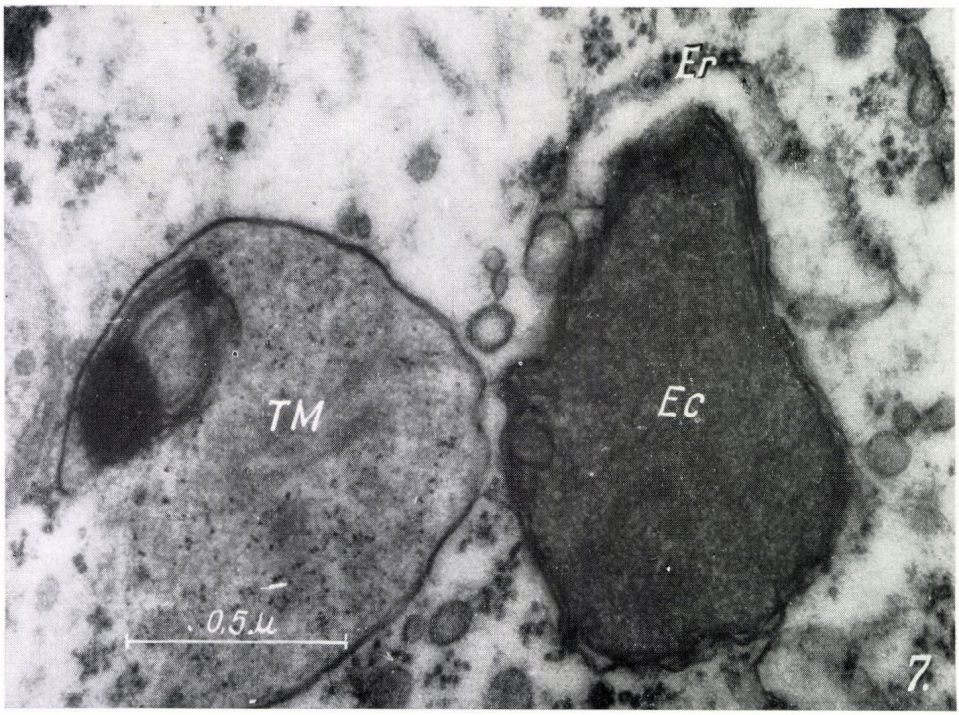


Fig. 7. Mitochondrium in the condition of transformation, in its inner part accumulation of granular substance (EC). Beside it an „emptying" cytosome (TM). Cerebral ganglion. × 62.400

7. ábra. Átalakulóban levő mitochondrium, amelynek belsejében szemcsés állomány halmozódott fel (EC). Mellette kiürülőben levő citoszoma (TM). Cerebrális ganglion. Nagyítás: 62 400 ×

There are also polyribosomes observable in the cytoplasm. Special granules *(Fig. 9)* may also occur in certain cells. They are of high electron density and range from 200 to 500 Å in size, and their surface is irregular. They appear in certain cells only following contrasting with uranyl acetate and lead citrate. It is most practical to call them simply dark granules. They are usually located in areas in which the cytosomes also occur. On occasions they might be observed arranged in regular rows around the cytosomes.

In some rather rarely occurring cells DCVs are also to be found in large numbers. Their sporadic occurrence in few numbers in the cytoplasm is not uncommon *(Figs 2, 4)*, their mass appearance, however, was noted only in few

cells. They are membrane bound with a substance of high electron density in their middle part, and range from 1000 to 2000 Å in size. Morphologically they appear to be identical to the DCVs seen in the axons, but in the cytoplasm their average size is greater. A more detailed description of them will be given in connection with the axons, it is to be remarked, however, right here that the DCVs of the cytoplasm do not exhibit depletion phenomena.

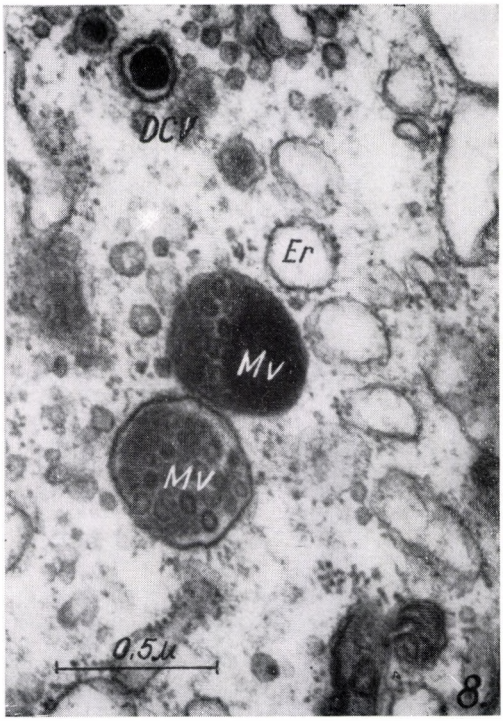


Fig. 8. Multivesicular bodies (MV). Cerebral ganglion. ×45 000

8. ábra. Multivezikuláris testek (MV). Cerebrális ganglion. Nagyítás: 45 000×

2. *Processes*

a. Axons

The axons constitute the bulk of the ganglia. The whole neuropil is made up of numbers of axons and glial elements, lots of axons were, however, found also among the nerve cells in the cortical area. The axons are naked, in general, and unsheathed. The free surfaces of the parallelly running axons are contiguous with each other, and the processes of glial cells are located only around bundles of several hundred or thousand axons, and are forming there a thin layer. Regular round cross sections of axons are seldom observable, because the axons passing side by side in large numbers completely fill the area at their disposal, and this results in a great variety of cross section images *(Fig. 10)*.

The axons are of most varying thickness. The thinner ones are 0.3 μ in diameter, but every size up to 4 μ might occur, but axons greater than 2 μ in thickness are relatively rare. With the rise in diameter certain structural differences appear in the substance of axo-plasm. It is most practical, therefore, to divide the axons into two groups: those below 1 μ in thickness are termed thin *(Fig. 11)*, those of greater diameter are called thick *(Fig. 12)*.

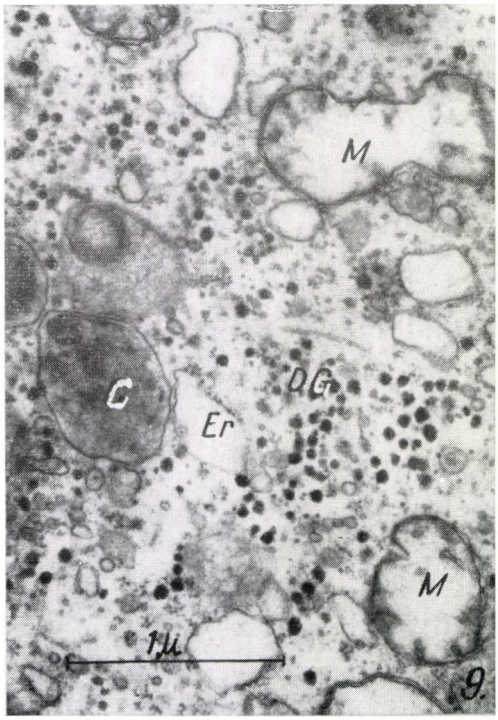


Fig. 9. Dark granules (DG). Pedal ganglion. ×30 000

9. ábra. A sötét granulumok képe (DG). Pedális ganglion. Nagyítás: 30 000×

The thick axons are originating from large-sized cells. The axolemma is a direct continuation of the cell-membrane. The axons are considered to originate in that place where the normal cytoplasmic structure terminates and continues in the axoplasm of more pure content. This transition is more distal in the initial part of the process.

The thin axons are partly the processes of smaller nerve cells, or partly afferent fibres originating obviously from other ganglia or from the periphery. Thin fibres originating from the branching-off of thick axons were also observable.

Microtubuli that might sometimes be thicker than those of the cytoplasm are constant components of the axoplasm. They are occurring generally in masses in parallel orientation to the longitudinal axis of axons *(Fig. 11)*.

The measurements show that they may range up to 6 μ in length, but, obviously, there may be longer ones, too. There are places where microtubuli are not observable, instead of them, however, a very fine network consisting of fibres much thinner than the tubuli were demonstrable. This is mainly characteristic of thick axons *(Fig. 12)*.

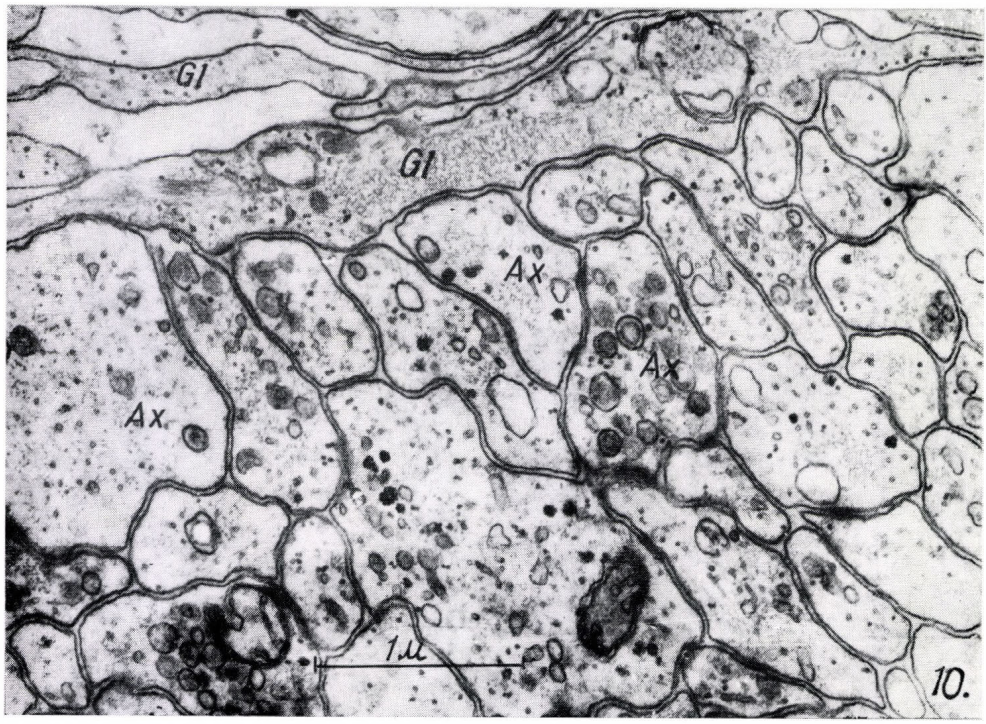


Fig. 10. Transverse section of grouped axons. Ax — axons; Gl — processes of glial cells. Pedal ganglion. ×30 000

10. ábra. Axoncsoport keresztmetszeti képe. Ax — axonok; Gl — gliasejtnyúlvány. Pedális ganglion. Nagyítás: 30 000 ×

In the axoplasm there are very tiny vesicle-like formations too, occurring sporadically in the cytoplasm. They bear resemblance to the endoplasmic reticulum, on basis of the irregularity of their shape, however, they may be definitely distinguished from other vesicular components of the cytoplasm, which will be discussed in the following.

The mitochondria of the axons exhibit a tubular character, their matrix is always dense and they contain tubuli in relatively large numbers. They are small in general, they may, however, range up to 2 μ in length in certain thick axons. There are relatively few mitochondria present in the axons.

The most conspicuous structural element of the axons is constituted by the vesicles. They range from 700 to 1400 Å in diameter, the most frequent

are, however, those of 1000 Å in size. Exceptionally forms 2000 Å in diameter might also be found among smaller ones; this occurs, however, very seldom, in about 0.25 percent of the cases. 26 percent of them are empty inside, and in the others a ,,nucleus'' of varying diameter and high density is to be found *(Fig. 13)*. Their membrane, which is thinner than the axolemma, has a ,,unite

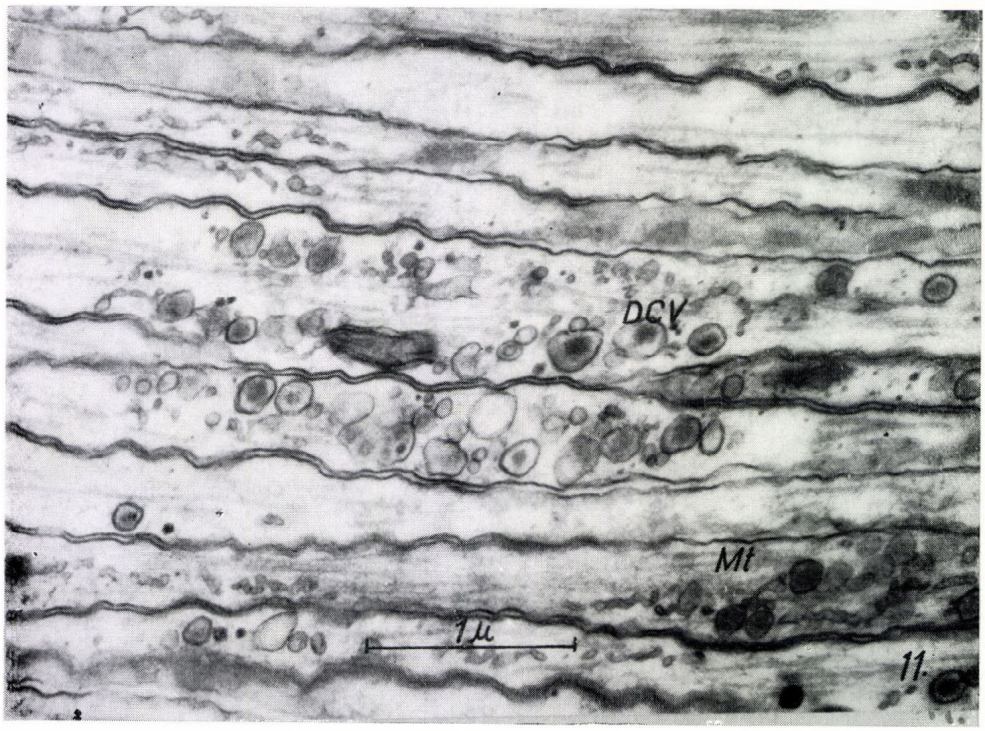


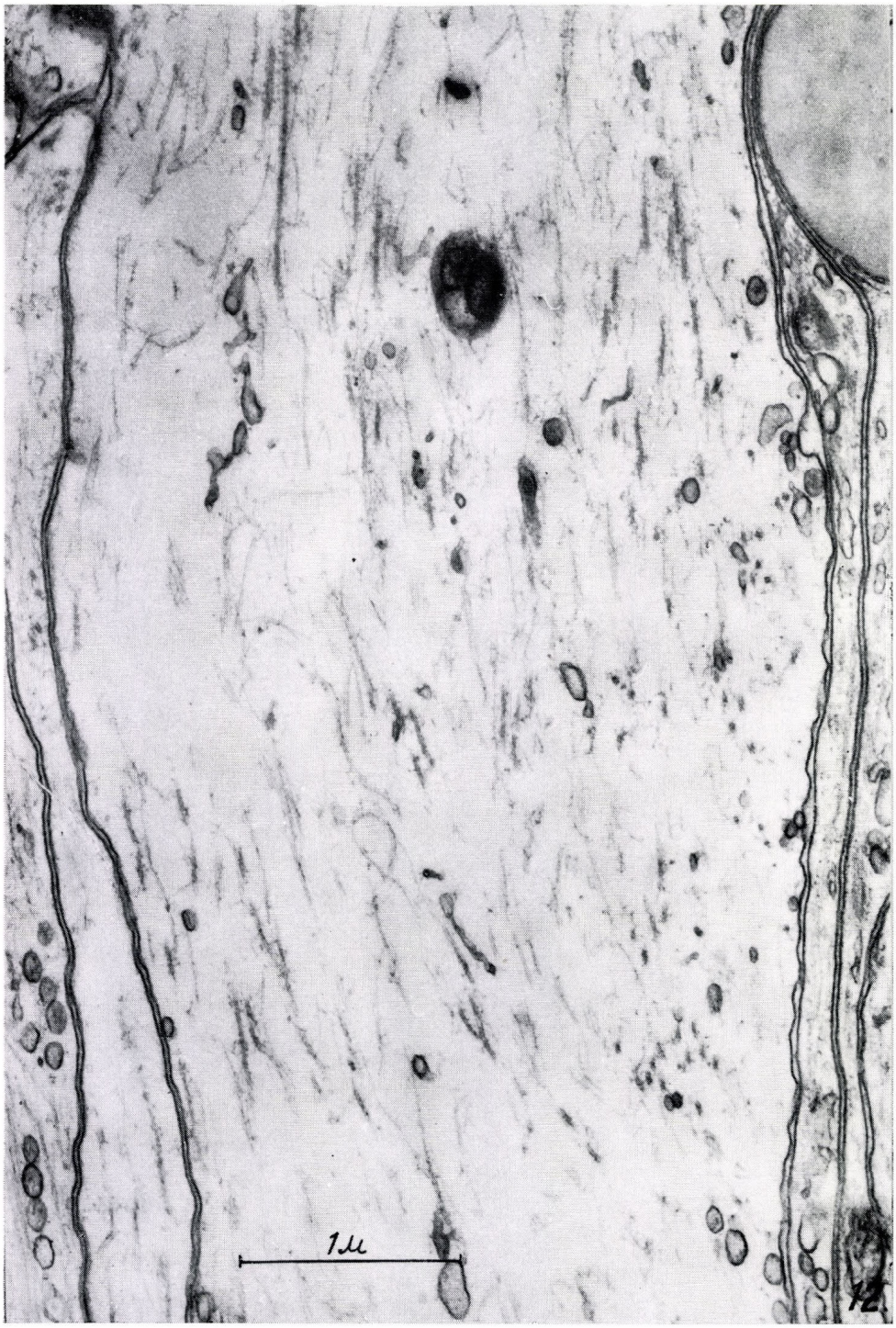
Fig. 11. Longitudinal section of thin axons. Visceral ganglion. ×30 000

11. ábra. Vékony axonok hosszmetszeti képe. Viscerális ganglion. Nagyítás: 30 000×

membrane'' construction. It is mostly continuous, there may be, however, openings visible on it too. A light halo of varying thickness is located beneath the membrane surrounding the dark nucleus. In about one half of cases (46 percent) its greatest diameter comes to two thirds of the outer diameter of the vesicles. In other instances, however, the middle part is smaller (28 percent). Inside one axon several variaties may also occur, but only one form dominates in general, thus resulting in characteristic axon-petterns. In case of some DCVs,

Fig. 12. A detail of a thick axon presented in longitudinal section. Visceral ganglion. ×32 500

12. ábra. Vastag axon részlete hosszmetszetben. Viscerális ganglion. Nagyítás: 32 500×


1μ

a loosening of the dark inner part is observable, which may often become so expressed that the whole vesicle appears to be made up of two concentric rings. It is distinctly to be seen at higher resolution, that the „nuclei" of the DCVs are not homogeneous but have a granular structure. *Fig. 14* illustrates the distribution in size of vesicles in the axons. The DCVs may occur, in general, everywhere along the axons, in more dilated areas, however, they may be observed also in groups of several 100. These places are most obviously formations identical to nerve terminations. In such places mitochondria, microtubuli and more seldom cytosomes too were encountered. These latter do not occur in general in the axoplasm, and are encountered only exceptionally.

In some thick axons a „limiting membrane", consisting of a row of vesicles is observable in general near the contact line between cortex and neuropil, which separates certain areas from one another inside the axons *(Fig. 15)*. This row of vesicles is built up of round or elongated vesicles, and appears to form a porous membrane. It also happens, that this porous membrane proceeds directly as a regular axolemma, and the two parts of axons bound only by a porous membrane are passing on henceforth as two axons separated from each other by a regular axolemma. It has been also observed that on both sides of the porous membrane the axoplasm was of different character.

b. Dendrites

Besides the axons there are also other processes which originate from the surface of the nerve cells. They are of varying thickness, they are generally short, some-times branching off, and inside them a structure similar to the cytoplasm of nerve cells is observable. These processes are identical to the dendrites and, in conformity to the fact that the majority of cells is unipolar, they are rather seldom encountered. They usually have contact with the axons *(Fig. 13)*.

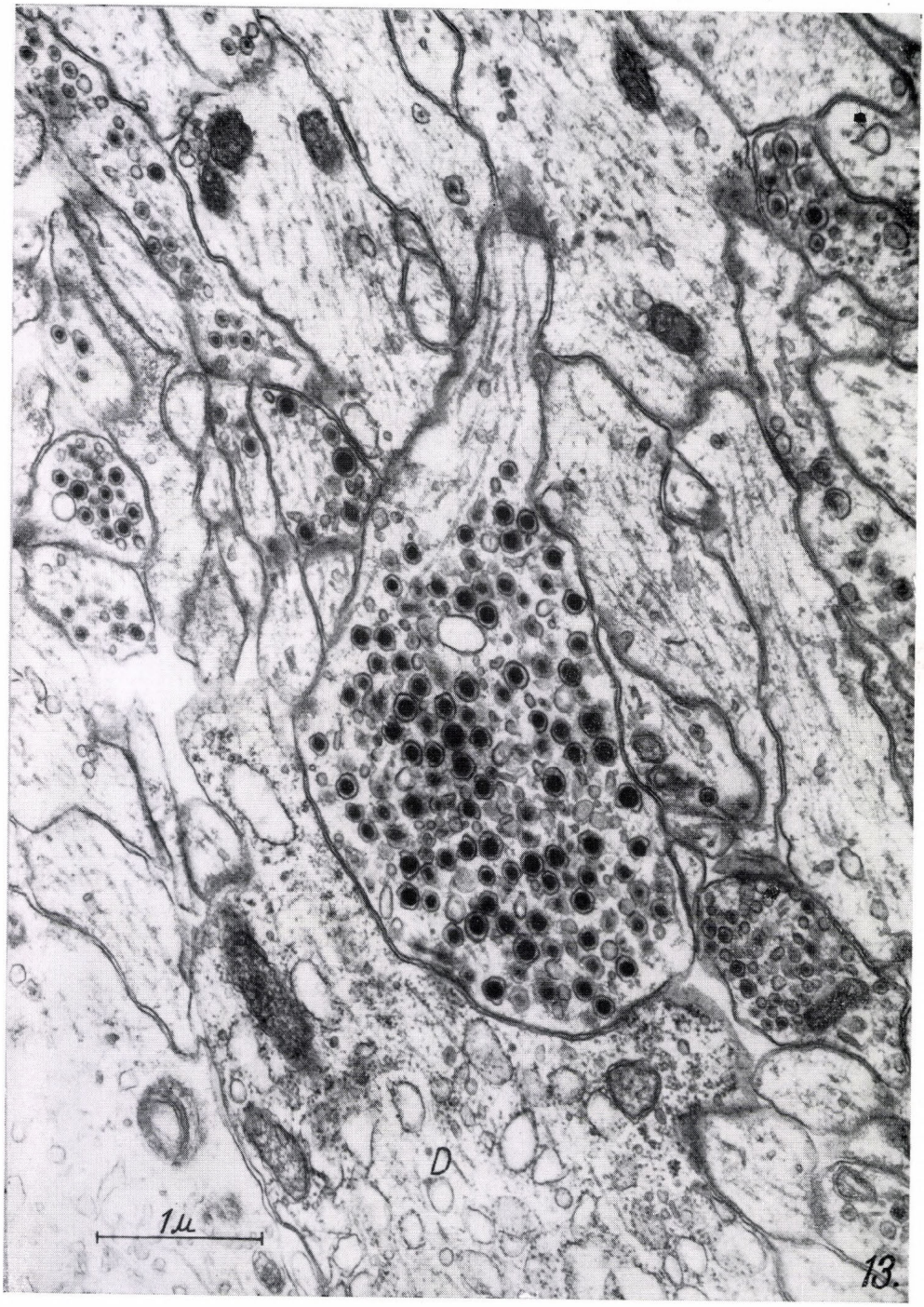
3. *Interneuronal connections*

a. Soma-soma

A close contact may exist between nerve cells lying side by side, when, namely, only a narrow intercellular space exists between the cell membranes. On occasion this space becomes dilated and forms lymphatic channels. Direct cytoplasmic connections were not observed between adjoining cell bodies. In certain places glial cells forming layers of 1—2 are located between the soma of two nerve cells.

Fig. 13. DCVs of different type in the axons of the cerebral ganglion. D — dendrite. ×25 000

13. ábra. DCV-ok különböző típusai a cerebrális ganglion axonjában. D — dendrit. Nagyítás: 25 000×


D
1μ
13.

b. Axon-soma and axon-dendrite

Similar to that described in connection with the cerebral ganglion (Zs.-Nagy 1964), in the visceral and pedal ganglia too, the axons are very often contiguous with the body of the nerve cells. A contact may be formed on the surface of the soma *(Fig. 16)*, when the dilated part, most probably the termination of the axon, which is filled with DCVs, fits closely on the cell

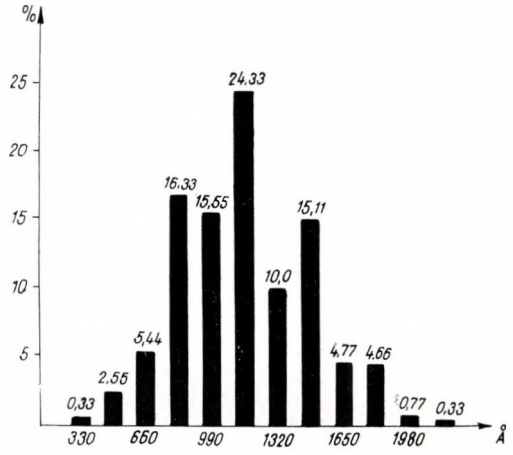


Fig. 14. Distribution according to the size of the vesicular substance of the axons as expressed in the average of measurements on 3000 vesicles

14. ábra. Az axonok vezikuláris állományának nagyság szerinti megoszlása mintegy 3000 vezikulum mérésének átlagában

membrane. On the surface of contact the membranes may be thickened and sometimes blurred. The massing of DCVs in the axons is observable near the place of contact, and close to this there are very often DCVs seen, which are either in the process of depletion or empty. The grouping of an electron dense substance on the soma side of the contact may also be observed, this is, however, mostly lacking. A certain type of axo-somatic connection may also come about, when, namely, one or more axons are deeply invaginating into the substance of the soma and are forming there a close contact with the cell membrane.

Axo-somatic connections were demonstrable mostly near the place of origin of the axons; they may, however, occur at any place on the surface of the soma.

The structure of axo-dendritic connections resembles that of the axo-somatic ones. Rather seldom do we find, however, connections that might be identified as such with certainty. This is attributable to the smaller number of dendrites.

c. Axon-axon

Axo-axonic connections represent enormous surfaces, because practically every axon is contiguous in its whole course with the other ones, and the axons are separated only by interaxonal spaces from each other. Areas in

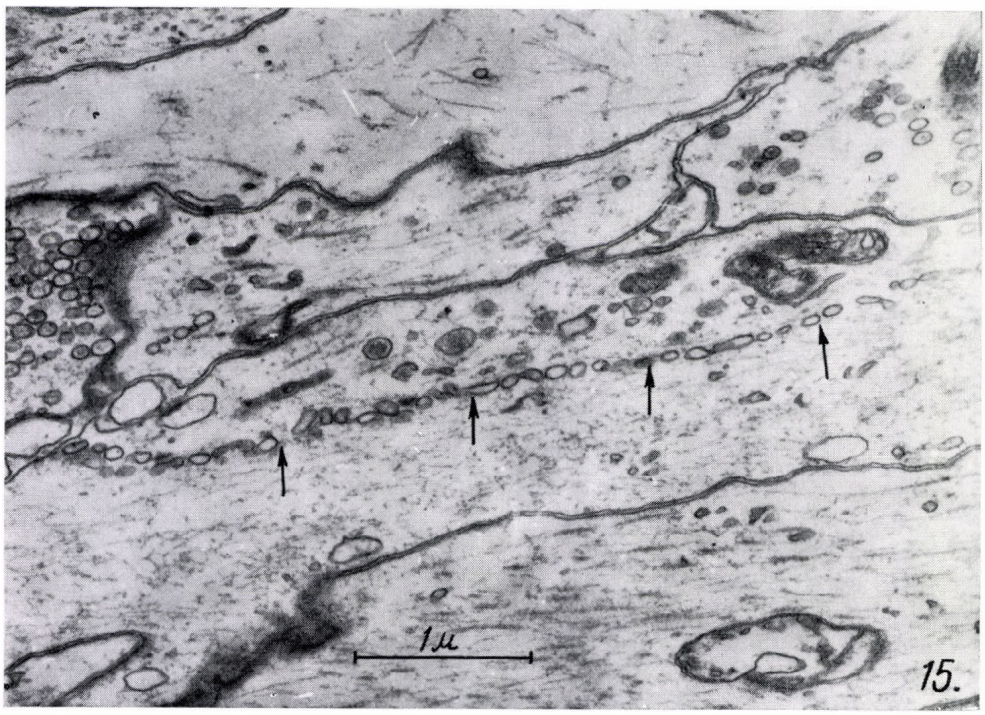


Fig. 15. Limiting membrane-like formation of vesicles in the longitudinal section of axons. Visceral ganglion. ×25 000

15. ábra. Vezikulumokból álló választófal axonok hosszmetszeti képén. Viscerális ganglion. Nagyítás: 25 000×

which the grouping of DCVs occur on the one side (presynapsis) along certain places of the contiguous axolemmae, may most obviously be of distinguished importance. In such places the axolemma is somewhat thickened on both sides, and the interaxonal space is slightly broader than elsewhere, and inside it very thin, fine transverse bridges appear. One axon may form several such contacts with neighbouring axons. DCVs may occur on both sides of such contacts, but on the one side there is always the grouping of DCVs observable and depletion phenomena also frequently occur.

Such contacts may often be found in the initial section of thick axons, where, namely, the thick axons assumably represent the postsynaptic side. Such connections may, nevertheless, exist also between axons running parallel, where that part of axon which might be considered as presynaptic is evidently not a nerve termination *(Fig. 17)*.

In the course of this study we observed only very seldom, not more than only in some instances such axo-axonic connections in which formations closely resembling synaptic vesicles characteristic of nerve terminations of higher animals were seen on the presynaptic side, and a subsynaptic specialization on the other side.

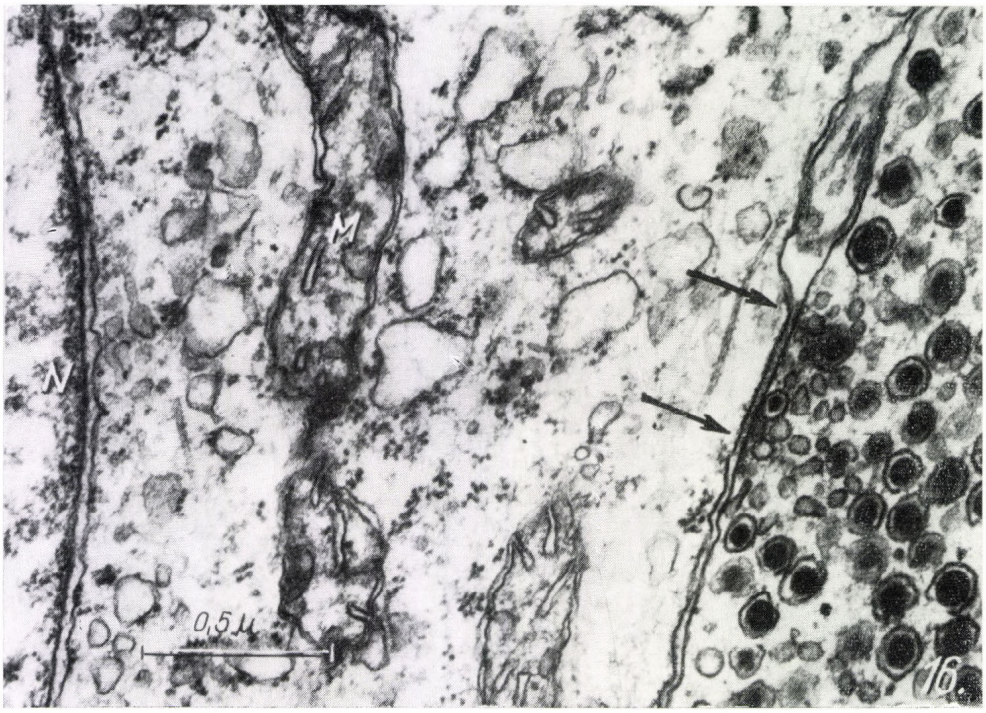


Fig. 16. Structure of axo-somatic synapses (arrow) N — nucleus. Cerebral ganglion. ×48 750

16. ábra. Axon-somatikus synapsis szerkezete (nyíl). N — sejtmag. Cerebrális ganglion. Nagyítás: 48 750×

4. *Glial tissue*

Two forms of glial cells may be distinguished. One is located in the cortical part, and is thus nominated cortical glia as opposed to the medullary glia located in the neuropil. The cortical glial cells which are identical to the lamellar glial cells described previously (Zs.-Nagy 1964) in the cerebral ganglia, are demonstrable in all three pairs of ganglia. Most surprisingly, the DCVs were also found to occur occasionally in the cortical glial cells. Glial cells do not form trophospongium.

The medullary glial cells are of much more complicated structure, as their shape is not flattened but extends in every direction in space. A narrow rim of plasm is seen near their nucleus, and from there processes are passing in every direction into the axons of the neuropil *(Fig. 10)* and appearing to

furnish with a sheath the greater groups of axons. This sheath is, however, not closed in the least. In the medullary glia the structure of the plasm is similar to that observed in the cortical glial cell, inside it, however, DCV was never observed, whereas a very fine fibrillar substance was demonstrable in its plasm, which, in turn, is not the property of the cortical glia.

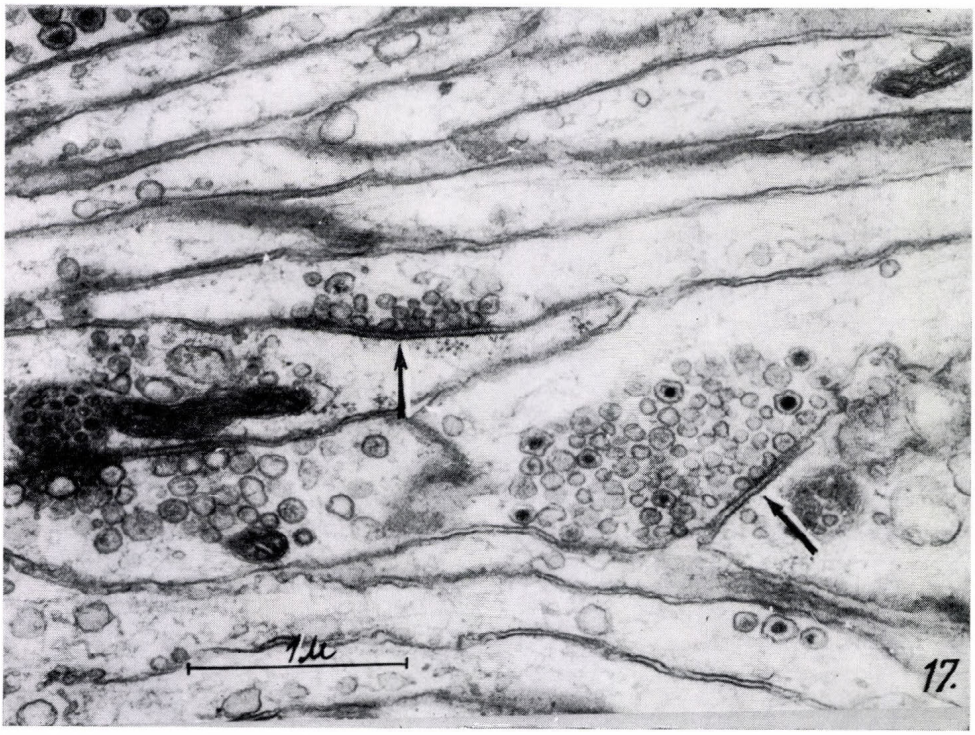


Fig. 17. Axo-axonic synapses between parallelly running axons (arrow). Cerebral ganglion ×30 000

17. ábra. Axo-axonikus synapsisok párhuzamosan futó axonok között (nyilak). Cerebrális ganglion. 30 000× nagyítás

Discussion

The fact that the nuclear membrane might become loosened up in some cases, indicates that under certain conditions the nucleo-cytoplasmic connection might become more intensive. This and the differences observed in the nuclear substance necessitate the supposition of functional changes. The invaginations of the nuclear membrane, which increase the surface considerably for the metabolic activity of the nucleoplasm, might be considered analogous to similar phenomena observed in the nerve cells of higher animals (TSCHENTZOV et al. 1961). As demonstrated by BRODSKY (1961) nuclei of similar morpho-

logical appearance might intensively participate in the protein synthesis of cells. This is in complete accordance with the experimental observation, that in the *Anodonta cygnea*, in the course of restitution processess following the depletion of the monoamines by reserpine, when, namely, there is a very vigorous catecholamine production, and thus obviously an enhanced metabolic activity too in nearly every nerve cell of the CG, the loosening up of the nuclei and their invaginations are more frequently noticed, than under normal conditions, and the nucleus itself also appears to be more active (Zs.-Nagy 1968).

The peculiar lamellar formations of the endoplasmic reticulum may be parallelled to those formations described by Fernandez—Morán (1957) in the Purkinje-cells of the rat, which were further examined by Herndon (1964) and termed by him as „lamellar bodies". Similar formations were described by Kása and Joó (1965) in the Purkinje cells of the guinea-pig. A parallel arrangement similar to that of the endoplasmic reticulum but of different type was observed by Lemos and Pick (1966) in the sympathetic trunk of the rat. To our knowledge, such formations were not described heretofore in the nerve cells of the molluscs. It is suggested that this lamellar formation may be identical with a certain form of the endoplasmic reticulum which differentiated to perform a special function.

The fact that the Golgi apparatus is rich in structural elements, that it has connections with the DCVs, and that its activity increases following reserpine treatment (Zs.-Nagy 1968), suggests that it may have an important role in the formation of DCVs. Similar phenomena were reported by others (Amoroso et al. 1964, Lane 1964, Simpson et al. 1964, Nolte et al. 1965) in the nerve cells of molluscs.

The importance of the microtubules is unknown as yet. As suggested by Tokin (1966) the microtubules may be considered as generally occurring cell organella, which most probably form a cytoskeleton, and this may be of importance not only mechanically in the orientation of the cell organella, but may have an active role as well.

The mitochondria exhibit a characteristic structure. The variety with light matrix is identical to that one described by Nolte et al. (1965) in the ganglion cells of Gastropods, and the variety with dark matrix is completely similar to the mitochondria described by Fährmann (1961) in the nerve cells of *Unio tumidus*. Nevertheless, the latter author refers in the text to mitochondria of the crista type. This appears to be a mistake, because it is not the dark but the light matrix variety that does not show the typical morphological characteristics of the crista type mitochondria, and though they differ in some sense also from tubular mitochondria still they are standing much closer to them.

Considering, that on the effect of reserpine treatment the majority of mitochondria appear in the form of the dark matrix variety (Zs.-Nagy 1968), whilst under normal conditions the light matrix variety prevails, further that both may occur inside the very same cell, it seems to be justified to suggest that the two forms represent varieties with different functions, and that in the course of intracellular changes they may be transformed into each other.

The cytosomes are very conspicuous formations of the cytoplasm. This term was firstly used by Nolte et al. (1965) for designating those special formations in the nerve cells of molluscs, which were already known to previous authors as pigment granules (Schultze 1879, Rawitz 1887, Bochenek 1905)

The progress made in the field of ultrastructural researches has led to the accumulation of data evidencing that formations similar to the cytosomes occur widely in the kingdom of invertebrates, and are not absent from certain tissues of higher animals either. Though the term ,,cytosome'' has not been adopted by every author, the situation remains the same, namely, the formations described are morphologically identical to the cytosomes. The most important works on molluscs were collected and reviewed on by NOLTE et al. (1965). Formations identical to cytosomes were described in the nerve cells of the Annelida by RÖHLICH et al. (1962), COGGESHALL and FAWCETT (1963) and in the corpus cardiacum of the Insects by SCHARRER (1963). Cytosomes occur also in certain nerve cells of the vertebrates and even in other tissues of them (LEMOS and PICK 1966).

Cytosomes are normal constituents of the nerve cells in the molluscs. Their relationship to other well defined cell organella is not clear, it is worth to note, however, that even FÄHRMANN (1961) considered it possible that they might take their origin from mitochondria. Mitochondria that might be taken as transitional forms to the cytosomes were observed by SCHARRER (1963) in the corpus cardiacum of the Insects. A transition of similar nature of the mitochondria was reported by GAUDECKER (1963) in the larvae of *Drosophila*. It is assumed that those forms of mitochondria which we have observed in the nerve cells of *Anodonta cygnea* and in which the appearance of granular or lamellar substance was observable, also speak in favour of the suggestion that the cytosomes may originate from the mitochondria by way of a special transformation. The fact that the membrane of the mitochondria is thinner than that of the cytosomes, and that the latter has the construction of the ,,unite membrane'', seems to contradict, in some respect, to this hypothesis. Considering, however, that the membranes of the cytosomes are most variable, and may open up or disappear even under normal conditions, it is thought not improbable that at a certain stage of the process of cytosome formation the membranes are also rebuilt, and this may be the explanation to the above contradiction.

The lysosomes are in some respect similar to the cytosomes morphologically. Questions pertaining to this and to the relationship between cytosomes and neurosecreta are discussed in our previous paper (ZS.-NAGY 1967).

The origin of the dark granules in the cytoplasm has not been explained as yet. It seems probable that they may originate from the polyribosomes, respecting, however, their close topographic connection with the cytosomes, it is assumable that the substance of the cytosomes is also implicated in their formation. They somewhat resemble morphologically glycogen granules that are observable in other cells; on the basis of the observation, however, that they appear only following contrasting with uranyl acetate and lead citrate, and that subsequent to contrasting with lead citrate, the glycogen granules are of low electron density, whereas the ribosomes of a high one (REYNOLDS 1963), their identity is considered disputable.

A small proportion of nerve cells and an overwhelming majority of the axons contain DCVs. The DCVs appear to be identical morphologically to formations in the sympathetic nervous system of higher animals. At present there are numerous data evidencing that noradrenaline is localized in the DCVs of the vertebrates. The literature on this subject has been thoroughly reviewed by HÖCKFELT (1967).

The DCVs are common in the nerve fibres of other molluscs, too (SCHLOTE 1957, FÄHRMANN 1961, GERSCHENFELD 1963, ROSENBLUTH 1963, BAXTER and NISBET 1963, SCHLOTE and HANNEFORTH 1963, RÖHNISCH 1964, NOLTE 1964, SAKHAROV et al. 1965 etc.). GERSCHENFELD (1963) believes them to be non-cholinergic synaptic vesicles in case of Gastropoda, and suggests that they might contain catecholamines and perhaps serotonin as well. FÄHRMANN (1961), SCHLOTE and HANNEFORTH (1963) are of the opinion that the DCVs of the Gastropoda are comparable to primary neurosecretory granules. Several types of these granules were described on basis of their size and the state of the middle part by the latter authors. GERSCHENFELD (1963) also adopted tipification according to size. We have already called attention (Zs.-NAGY 1964) to the risk involved in tipifying according to size, and our recent experimental evidences speak also in favour of this. A significant decrease in diameter of granules and a considerable loosening up of their inner substance was demonstrated by RINNE and ARSTILA (1966) in the rat following reserpine treatment. Evidences to the same effect were obtained by our own experiments with reserpine (Zs.-NAGY 1968). All these seem to support the suggestion proposed by us that both in the cells and in the axons the DCVs of different size correspond to representants of the same organellum being in different state.

According to the above the empty vesicles may be the representants of completely depleted forms of the DCVs. This suggestion is supported by the fact that the empty vesicles are very often observable close by the synaptic connection, where the consumption of the substance of the DCV may be maximal. It is also likely that the empty vesicles are of other nature than the depleted DCV. Neither can the suggestion be rejected that those empty vesicles which occur seldom and alone without DCVs in the very small-sized nerve terminations, and which never range beyond 4—500 Å in diameter are such that differ from the DCV and perhaps correspond to the synaptic vesicles of the vertebrates.

In another paper of ours (Zs.-NAGY 1968) we deal with the origin of the DCVs and with their relation to the catecholamines.

Besides the presence of DCVs, the axons may be also characterized by the absence of sheath and branching tendency. The conduction of stimuli with high decrement might be explained, at least partly, by these morphological features (LÁBOS et al. 1963).

A most peculiar phenomenon observed is the porous membrane in certain axons. Our present knowledge does not allow to interpret it functionally. Even if it is a fixation by-product, it may indicate a special state in certain places of the axolemma. It is assumable that the separation into two areas of the axon by this porous membrane may be of functional importance; this is indicated, at least, by the fact that there are often observable on both sides of the porous membrane parts of axons which have different structures. The question, however, remains open, i.e. do the two separated areas correspond to processes of two different nerve cells, or do they respresent two parts of the same axon? In view of the branching property of axons it is difficult to decide. It still remains to be answered, whether the porous membrane may be considered an axolemma either in the making or decomposing, or is it always functioning in this porous form.

It has been found, on the one hand, when examining the structure of interneuronal connections that similar to the observation of LAMPARTER (1966)

in the prothoracal ganglia of the ant, in the *Anodonta cygnea* there is no direct cytoplasmic connection between the soma of the nerve cells. On the other hand, a variety of interneuronal connections may occur (Axon-soma, axon-dendrite, axon-axon) in all three ganglia, which constitutes an essential difference as compared to the Gastropoda in which the existence of axo-somatic synapses is disclaimed in general (BULLOCK and HORRIDGE 1965). Naturally, as long as there are no direct physiological data available evidencing that the axo-somatic places of contact observed by us are functioning really as true synapses, we may only hypothetically speak of the existence of an axo-somatic synapsis in fresh water mussel. The probability of the existence of such synapses is, nevertheless, suggested by the structure of axo-somatic contacts observed.

In this respect it is most decisive that depletion phenomena observed in the DCVs are most expressed in the vicinity of such places, and that just the same unloading phenomena occur when the catecholamine content of the axons is depleted with reserpine (ZS.-NAGY 1968), and this is accompanied by a decisive change in the activity of the whole nervous system of the animal, i.e. the animal is incapable of tonic contraction of its adductors (SALÁNKI 1963) and its other muscles are relaxing too. Chemical transfer of stimuli may most obviously be expected to occur in areas of the membrane in which the structural conditions for such a transfer are given. In the light of the above it is thought that the special places of axo-somatic, axo-dendritic and axo-axonic contacts where accumulation of DCV, depletion phenomena, membrane thickening and perhaps also subsynaptic massing, further the filling up of the intermembraneous spaces with special bridges occur, may be considered as synapses with consideration for analogues existing in other animals (LEMOS and PICK 1966, CASTEJON and VILLEGAS 1964).

Summary

Author describes the general submicroscopic structure of the neurons in the three pairs of ganglia of *Anodonta cygnea*. He describes the different forms of appearance of the nuclei. The special lamellar structure of the endoplasmic reticulum is also discussed. He gives a detailed description of the structural characteristics of other cytoplasmic components (Golgi apparatus, microtubuli, cytosomes, dense-core vesicles) and describes the structure of axo-somatic and axo-axonic synapses. Data on the two types of glial cells are also presented.

REFERENCES

AMOROSO, E. C., F. R. S. MARGARET, I. BAXTER, A. D. CHIQUOINE, R. H. NISBET (1964): The fine structure of neurones and other elements in the nervous system of the giant African land snail *Archachatina marginata*. — *Proc. Roy. Soc.* B **160,** 167—181.

BAXTER, M. I., R. H. NISBET (1963): Features of the nervous system and heart of Archachatina revealed by the electron microscope and by electrophysiological recording. — *Proc. Malac. Soc. Lond.* **35,** 167—177.

BOCHENEK, M. A. (1905): Untersuchungen über das zentrale Nervensystem der Wirbellosen. — *Bull. int. Acad. Cracovie, Ser. B.* **45,** 205—220.

BRODSKY, V. J. (1961): Бводский, В. Я.: Некоторые цитологические особенности ядерного типа синтеза клеточного белка на примере исследования ганглизного нейрона сетчатки. Цитология, **3,** 312—326.

Bullock, T. H., G. A. Horridge (1965): Structure and function in the nervous system of Invertebrates. — *Chap.* **24**, P: 1387—1432. Freeman *and Co. San Francisco. London.*

Castejon, O. J., G. M. Villegas (1964): Fine structure of the synaptic contacts in the stellate ganglion of the squide. — *J. Ultrastruct. Res.* **10,** 585—598.

Coggeshall, R. E., D. W. Fawcett (1964): The fine structure of the central nervous system of the leech *Hirudo medicinalis.* — *J. Neurophysiol.* **27,** 229—289.

Fährmann, W. (1961): Licht- und elektronenmikroskopische Untersuchungen des Nervensystems von *Unio tumidus* (Philipsson) unter besonderer Berücksichtigung der Neurosekretion. — *Z. Zellforsch.* **54,** 689—716.

Fernández-Morán, H. (1957): Electron microscopy of nervous tissue. — In: *Metabolism of the nervous system. Pergamon Press, New York.*

Gaudecker, B. (1963): Über den Formwechsel einiger Zellorganelle bei der Bildung der Reservestoffe im Fettkörper von Drosophila-Larven. — *Z. Zellforsch.* **61,** 56—95.

Gerschenfeld, H. M. (1963): Observations on the ultrastructure of synapses in some Pulmonate Molluscs. — *Z. Zellforsch.* **60,** 258—275.

Herndon, R. M. (1964): Lamellar bodies, an unusual arrangement of the granular endoplasmic reticulum. — *J. Cell. Biol.* **20,** 338—342.

Höckfelt, T. (1967): On the ultrastructural localization of noradrenaline in the central nervous system of the rat. — *Z. Zellforsch.* **79,** 110—117.

Kása, P., F. Joó (1965): Lamellar bodies in Purkinje cells of the guinea pig. — *Acta Biol. Hung.* **15,** 459—464.

Lamparter, H. E. (1966): Die strukturelle Organisation des Prothoracalganglions bei der Waldameise *(Formica lugubris* Zett). — *Z. Zellforsch.* **74,** 198—231.

Lane, N. J. (1964): Elementary neurosecretory granules in the neurones of the snail, *Helix aspersa.* — *Quart. J. micr. Sci.* **115,** 31—34.

Lábos, E., I. Zs.-Nagy, K. Benkő, J. Salánki (1963): Electrophysiological and electron microscopic studies on the fibre composition of the cerebrovisceral connective of *Anodonta cygnea* L. — *Annal. Biol. Tihany* **30,** 59—65.

De Lemos, C. J. Pick (1966): The fine structure of thoracic sympathetic neurons in the adult rat. — *Z. Zellforsch.* **71,** 189—206.

Nolte, A. (1964): Ultastruktur des »Neurosekretmantels« des Nervus labialis medius von *Planorbarius corneus* (Basommatophora). — *Naturwissenscahaften* **51,** 148.

Nolte, A., H. Breucker, D. Kuhlmann (1965): Cytosomale Einschlüsse und Neurosekret im Nervengewebe von Gastropoden. — *Z. Zellforsch.* **68,** 1—27.

Pease, D. C. (1964): Histological Techniques for Electron Microscopy. — *Academic Press, New York, London.*

Rawitz, B. (1887): Das zentrale Nervensystem der Acephalen. — *Jena, Z. Naturw.* **20,** 384—460.

Reynolds, E. S. (1964): The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. — *J. Cell. Biol.* **17,** 208—212.

Rinne, U. K., A. U. Arstila (1966): Electron microscopic evidence on the significance of the granular and vesicular inclusions of the neurosecretory nerve endings in median eminence of the rat. — *Med. Pharmacol. exp.* **15,** 357—369.

Rosenbluth, J. (1963): The visceral ganglion of *Aplysia californica* — *Z. Zellforsch.* **60,** 213—236.

Röchlich, P., B. Aros, B. Vigh (1962): Elektronmikroskopische Untersuchung der Neurosekretion im Zerebralganglion des Regenwurms *(Lumbricus terrestris).* — *Z. Zellforsch.* **58,** 524—545.

Röhnisch, S. (1964): Untersuchungen zur Neurosekretion bei *Planorbarius corneus* L. (Basommatophora). — *Z. Zellforsch.* **63,** 767—798.

Sakharov, D. A., V. L. Borovyagin, I. Zs.-Nagy (1965): Light, fluorescence and electron microscopic studies on „neurosecretion" in *Tritonia diomedia* Bergh (Mollusca, Nudibranchia). — *Z. Zellforsch.* **68,** 660—673.

Salánki, J. (1963): The effect of serotonin and catecholamines on the nervous control of periodic activity in fresh-water mussel *(Anodonta cygnea).* — *Comp. Biochem. Physiol.* **8,** 163—171.

Scharrer, B. (1963): Neurosekretion. XIII. The Ultrastructure of the corpus cardiacum of the insect *Leucophaea maderae.* — *Z. Zellforsch.* **60,** 761—796.

Schlote, F. W. (1957): Submikroskopische Morphologie der Gastropodennerven. — *Z. Zellforsch.* **45,** 534—568.

Schlote, F. W., W. Hanneforth (1963): Endoplasmatische Membransysteme und Granatypen in Neuronen und Gliazellen von Gastropodennerven. — *Z. Zellforsch.* **60,** 872—892.
Schultze, H. (1879): Die fibrilläre Struktur der Nervenelemente bei Wirbellosen. — *Arch. Mikr. Anat.* **16,** 57—111.
Simpson, L., H. A. Bern, R. S. Nishioka (1963): Inclusions in the neurons of *Aplysia californica* (Cooper 1863) (Gastropoda Opistobranchiata). — *J. comp. Neurol.* **121,** 237—247.
Sotelo, J. R., K. R. Porter (1959): An electron microscope study of the rat ovum. — *J. Biophys. Biochem. Cytol.* **5,** 327—442.
Токин, I. B. (1966): Токин, И, Б.: Микротрубочки — новый компонент цитоплазмы. Арх. Анатомии, Гистологии и Эмбриологии **51,** 3—22.
Tschentzov, J. S., Brodsky, V. J., Borovyagin V. L. (1961): Ченцов, Ю. С., Бродский, В. Я., Боровягин, В. Л.: Субмикроскопическая морфология ганглиозных нейронов сетчатки, как отражение некоторых особенностей их белкового метаболизма. Биофизика, 6. 590—595
Zs.-Nagy, I. (1964): Electron-microscopic observations on the cerebral ganglion of the fresh-water mussel (*Anodonta cygnea* L.) — *Annal. Biol. Tihany* **31,** 147—152.
Zs.-Nagy, I. (1968): Histological, histochemical and electron microscopical studies on the cytosomes of the nerve cells in *Anodonta cygnea* L. (Mollusca, Lamellibranchiata). — *Annal. Biol. Tihany* **34,** 25—39.
Zs.-Nagy, I. (1967): Histochemical and electron microscopical studies on the relation of dopamine and dense-core vesicles in the neurons of *Anodonta cygnea* L. — *Symp. on Invertebrate Neurobiol. Tihany* 1967. IX. 4—7. (in press)
Zs.-Nagy, I., Katalin S.-Rózsa, J. Salánki, I. Földes, L. Perényi, Magda Demeter (1965): Subcellular localization of 5-hydroxytryptamine in the central nervous system of Lamellibranchiates. — *J. Neurochemistry* **12,** 245—251.

ÚJABB ADATOK AZ *ANODONTA CYGNEA* L. (PELEOCYPODA) NEURONJAINAK SZUBMIKROSZKÓPOS SZERKEZETÉHEZ

Zs.-Nagy Imre

Összefoglalás

Szerző leírja a neuronok általános szubmikroszkópos szerkezetét az *Anodonta cygnea* mindhárom ganglionjában. Ismerteti a sejtmagok különböző megjelenési formáit. Beszámol az endoplazmás retikulum különleges lemezes képződményeiről. Részletesen taglalja az egyéb citoplazmatikus komponensek (Golgi apparátus, mikrotubulusok, cytosomák, dense-core vesiculumok) szerkezeti sajátságait, s ismerteti az axo-somatikus és axo-axonikus synapsisok szerkezetét. Adatokat közöl továbbá a gliasejtek két típusáról.

НОВЫЕ ДАННЫЕ О СУБМИКРОСКОПИЧЕСКОМ СТРОЕНИИ НЕЙРОНОВ БЕЗЗУБКИ

И. Ж.-Надь

Описано субмикроскопическое строение нейронов во всех трех парах ганглиев беззубки. Отмечены существующие различия в форме клеточного ядра. Описаны особые пластинчатообразные структуры эндоплазматической сети. Дается подробное описание других структур цитоплазмы (аппарат Гольджи, микротубулы, цитосомы, везикулы с электронноплотным центральным зерном), а также строения аксо-соматических и аксо-аксонных синапсов. Обнаружены два типа глиальных клеток.