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HISTOLOGICAL EXAMINATION OF THE GANGLIONS IN THE FRESH WATER MUSSEL WITH IMPREGNATION METHODS *ANODONTA CYGNEA* L.

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Introduction

The fresh water mussel takes up the attention both of physiologists (PAWLOW 1885) and morphologists (HANSTRÖM 1928) for a long time past. From more recent works those of ÁBRAHÁM and MINKER (1959), NAGY(1962) BARANYI and SALÁNKI (1963), ZS.-NAGY (1964), SALÁNKI (1965) should be named who investigated morphology and physiology of the nervous system of *Anodonta* from different viewpoints. Histological description of the ganglions in *Anodonta cygnea* however is not sufficiently disclosed neither in foreign nor in Hungarian literature. HANSTRÖM (1928) in his book characterized the nervous system of the Lamellibranchiates on the grounds of the *Unio pictorum*. NAGY (1962) described a gold-yellow pigment in the ganglions but gives only outlines of their general histology. It should be noted that a more exact description of the nervous tissue of the mussel was hindered by the fact that up to now impregnation of the nervous system of lower animals encountered difficulties. The classic methods did not supply satisfactory results to obtain a knowledge of these. In our earlier work (GUBICZA and ZS.-NAGY 1964) we discussed modified methods which are suitable for the impregnation of the neurons and fibres of *Anodonta cygnea*. In the present study the results of the histological examination of the fresh water mussel obtained with these methods are described.

Material and method

Our experimental animal was *Anodonta cygnea* L. originating from a branch of the Danube. Several wall proved methods were employed for the histological examination of the *ganglion cerebrale*, *ggl. pedale* and *ggl. viscerale* of fresh water mussel.

1. Modified CAJAL I. proceeding. This block impregnation method is mainly suited to indicate the cells (GUBICZA—ZS.-NAGY 1964).

2. Impregnation of CAUNA's frozen sections worked well in investigation of the fibres but no serial sections can be produced with this method.

3. The paraffin section impregnation method of ROWELL (1963) has been employed for the demonstration of thin fibres.

Beside the impregnation methods the ganglions embedded in paraffin were also stained with VAN-GIESON. The application of this method became necessary for the examination of the connective tissue surrounding the ganglions.

Results

It is characteristic of the ganglions *(ggl. cerebrale, ggl. pedale* and *ggl. viscerale)* of fresh water mussels that the neurons and the fibre stock readily separate from each other. The cells are located at the edge of the ganglion as if forming a cortex in which a further arrangement, stratification can not be recognized. The thickness of the cell layer is varying but generally about 200 to 250 μ.

The cells are oval or pyriform and rather varying as to their longitudinal dimension, all measures occurring from 5 to 50 μ. Most frequent are the cells of 5 to 15 μ which mostly occur in groups *(Fig. 1)*. The longitudinal dimension of the largest neurons is 3.5 to 40 μ but sometimes 50 to 55 μ are measured. It can be observed that large cells generally occur next to areas where nerve fibres are entering or leaving the ganglion. These large size cells are surrounded by glia cells readily visible also under the light microscope *(Fig. 2)*.

As to their processes the majority of the cells are unipolar. Very seldom also bipolar and multipolar cells occur *(Fig. 3)*. In the evaluation of serial sections in several cases pyramidoidal cells were observed *(Fig. 4)*.

The neurons differ also from the point of view of impregnation. In the large size cells often an area seeming void appears in the cytoplasm *(Fig. 5)*. In other cases the plasma is impregnated uniformly *(Fig. 1—2)*.

In the central part of the ganglions the neuropile is located which consists almost exclusively of fibres. In it, on the strength of preparations obtained with the $FeSO_4$-method, two fibre types can be distinguished which are readily separable from each other. Most frequent are the thin, less impregnated, so-called fine fibres which mostly appear in bundles. The other type is the thick axon impregnated to a dark colour, the diameter of which ranges from 2 to 4 μ. They occur on the pathways entering the ganglion and in the neighbourhood of the large cells. The thick fibres have many forms. Most frequent is the fibre of straight shape which can be also branching *(Fig. 6)*. In the *ggl. viscerale* even threefold branching was found *(Fig. 7)*. Such, however, could be observed only in one case out of several hundred of sections carefully checked. The continuation of this threefold branching could not be followed in the serial sections. The thick fibres are sometimes undulating *(Fig. 8)*. A form of the thick fibre, the varicose fibre, deserves to be especially mentioned. The thickening of these fibres shows the most diversified picture *(Fig. 9)*. In some cases they are even branching *(Fig. 10)*. Origin and ending of the straight shaped and undulating thick fibres and of the varicose fibres could not be demonstrated.

In the neuropile beside fibres of various types the glia nuclei impregnating to a dark colour are frequent *(Fig. 11)*. In the neuropile neurons seldom occur. The medium-size (20 to 25 μ) neurons located here appear, according to our observations, singly or in groups *(Figs. 12* to *13)*. In the nerve branches leaving the ganglions beside the fibres these are glia. The ganglions are covered by 5 to 7 μ thick connective tissue.

Evaluating the general histological picture of the *ggl. cerebrale, ggl. viscerale* and *ggl. pedale* of *Anodonta cygnea* L. as compared with each other certain differences can be observed. These are the following:

The histological picture of the neuropile in the *ggl. viscerale* shows a higher number of more manyfold nerve elements than that of the two other pairs of ganglions. Whereas in the *ggl. cerebrale* and *ggl. pedale* the presence of thick fibres is unfrequent, in the *ggl. viscerale* they often occur. The double and threefold branching of the thick fibres was only found in the *ggl. viscerale*. The same applies to the varicose fibres. Comparative examination of the ganglions of mussels of identical dimensions revealed further differences concerning distribution of the cells of different dimensions. Most so-called large neurons occur in the *ggl. viscerale*. From the three ganglions in *ggl. viscerale* the lowest number of darkly impregnated glia nuclei were found.

Discussion

On the strength of the general histological examination of the ganglions in *Anodonta cygnea* L. it may be established that all three ggl. are divided into a well separated exterior cellular layer and a neuropile consisting of fibres, as in lower animals generally. No such type of layers of the neurons in the mussel's ganglions can be demonstrated as have been described by HANSTRÖM (1928) and M. NAGY (1952). Small and large size cells are mixed together. The nerve fibre bundles leaving the ggl. are the axons of the unipolar and multipolar cells of larger dimensions, presumably centrifugal fibres.

In the cellular layer of the ganglions the fibres running parallel with the surface are probably processes of the associative cells. The location of these axons is such that there is a possibility for the formation of axo-somatic synapses as they could be also demonstrated in the cerebral ggl. with the electron microscope (ZS.-NAGY 1964).

The peculiar structure of glia cells surrounding a single ganglion cell or cell group appears already under the light microscope. Earlier electron microscopic examinations (ZS.-NAGY 1964) revealed that the glia cells of several layers are arranged around the neuron or neurons thus, that there are intercellular gaps among them and the haemolymph may freely circulate. This makes it probable that the glia cells have a trophic importance in connexion with the neurocyte.

In the ganglions of *Anodonta cygnea* L. no synapses are found as recently demonstrated on the large cells and on the bases of their processes in the *Aplysia ggl. viscerale* by A. ÁBRAHÁM (1963).

General histological examination of the ganglia in *Anodonta cygnea* L. revealed that *ggl. viscerale* contains the most complicated and at the same time more diversified nerve elements. From this fact it may be concluded that the basic life-processes of the fresh water mussel are bound to the *ggl. viscerale* so diversified from the morphological point of view.

Summary

1. It is characteristic of the ganglions of fresh water mussels that the neurons and the fibrous stock are separating from each other.

2. No separation into layers according to the dimensions of cells forming a cortex at the edge of the ganglions can be observed.

3. Longitudinal dimension of the cells ranges from 5 to 50 μ.

4. As to their processes the neurons are unipolar. Bi- and multipolar cells seldom occur.

5. Two further types of neurons can be distinguished according to whether areas seeming void appear in the cytoplasm or they are uniformly impregnated.

6. Some large size neurons are surrounded by glia arranged in layers.

7. In the ganglions there are two types of fibres:

a) thin, palely impregnated and

b) thick, darkly impregnated fibres which may be of a straight shape, undulating and varicose.

8. The sraight shaped and varicose fibres show branchings.

9. In the neuropile, glia nuclei and few neurons occur.

10. Out of the ganglions of the fresh water mussels from the morphological point of view *ggl. viscerale* is the most diversified.

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AZ ÉDESVÍZI KAGYLÓ *(ANODONTA CYGNEA* L.) GANGLIONJAINAK SZÖVETTANI VIZSGÁLATA IMPREGNÁCIÓS MÓDSZEREK ALAPJÁN

Összefoglalás

Gubicza András és Zs. Nagy Imre

A szerzők munkájukban az *Anodonta cygnea* L. *ganglion cerebrale, ggl. pedale* és *ggl. viscerale* általános szövettani szerkezetét írják le. Az idegdúcok vizsgálatára a módosított Cajal I., Cauna és Rowell-féle impregnációs módszereket alkalmazták. Eredményeik az alábbiakban foglalhatók össze:

Az édesvízi kagylók idegdúcaira jellemző, hogy a sejtes és rostos állomány egymástól jól elkülönül. A ganglionok széli részén kérget alkotó idegsejtek méretük szerinti rétegződése nem figyelhető meg. A sejtek hosszmérete 5—50 μ között változik. Nyúlványaikat tekintve unipolarisak *(5. kép)*, de kevés bi- és multipoláris sejt *(3—4. kép)* is előfordul. Az idegsejtek két típusát különböztették meg aszerint, hogy a citoplazmában üresnek tűnő területek mutatkoztak *(5. kép)*, avagy azok egyenletesen impregnálódtak *(6. kép)*. Néhány nagyméretű idegsejtet rétegesen elhelyezkedő glia vesz körül *(2. kép)*.

A dúcokban két, egymástól jól elkülöníthető rosttípust találtak. Leggyakoribb a vékony, halványan impregnálódott rost. A másik rosttípus vastag sötét színre impregnálódott. A vastag rostok kettős és hármas elágazást is mutatnak *(7—8. kép)* lefutásuk lehet egyenes vagy hullámos *(9. kép)*, legritkább a varixos rost *(9. kép)*, amelyek szintén elágazhatnak *(10. kép)*. A neuropil rostjai között sok gliamag *(11. kép)* és kevés idegsejt fordul elő *(12—13. kép)*.

Végül megállapították, hogy az édesvízi kagyló idegdúcai közül morfológiai szempontból a *ggl. viscerale* a legváltozatosabb.

ГИСТОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ ГАНГЛИЕВ БЕЗЗУБКИ (Anodonta cygnea L.) ПРИ ПОМОЩИ ИМПРЕГНАЦИИ

А. Губица, И. Ж.-Надь

1. Ганглии беззубки характеризуются тем, что нервные клетки и волокнистый состав отделены друг от друга.

2. Распределение клеток составляющих корковую часть ганглиев, по размеру, не наблюдается.

3. Длина клеток варьирует от 5 до 50 м.

4. Нервные клетки в большинстве случаев являются униполярными. Редко встречаются и би-, и мультиполярные клетки.

5. Можно различать два разных типа нервных клеток в зависимости от того, выявляются ли при инпрегнации в цитоплазме пустые места или же нервная клетка импрегнируется равномерно.

6. Вокруг некоторых нервных клеток большого размера обнаруживается глия, расположенная слоями.

7. В ганглиях наблюдается два типа волокон:

а) тонкие волокна, импрегнированные слабо,

б) толстые волокна, импрегнированные сильно, которые могут быть прямыми, волнообразными или варикозными.

8. Прямые и варикозные волокна разветвляются.

9. В невропиле обнаруживаются ядра глии и небольшого числа нервных клеток.

10. Среди ганглиев беззубки самым разнообразным является висцеральный ганглий по своим морфологическим свойствам.

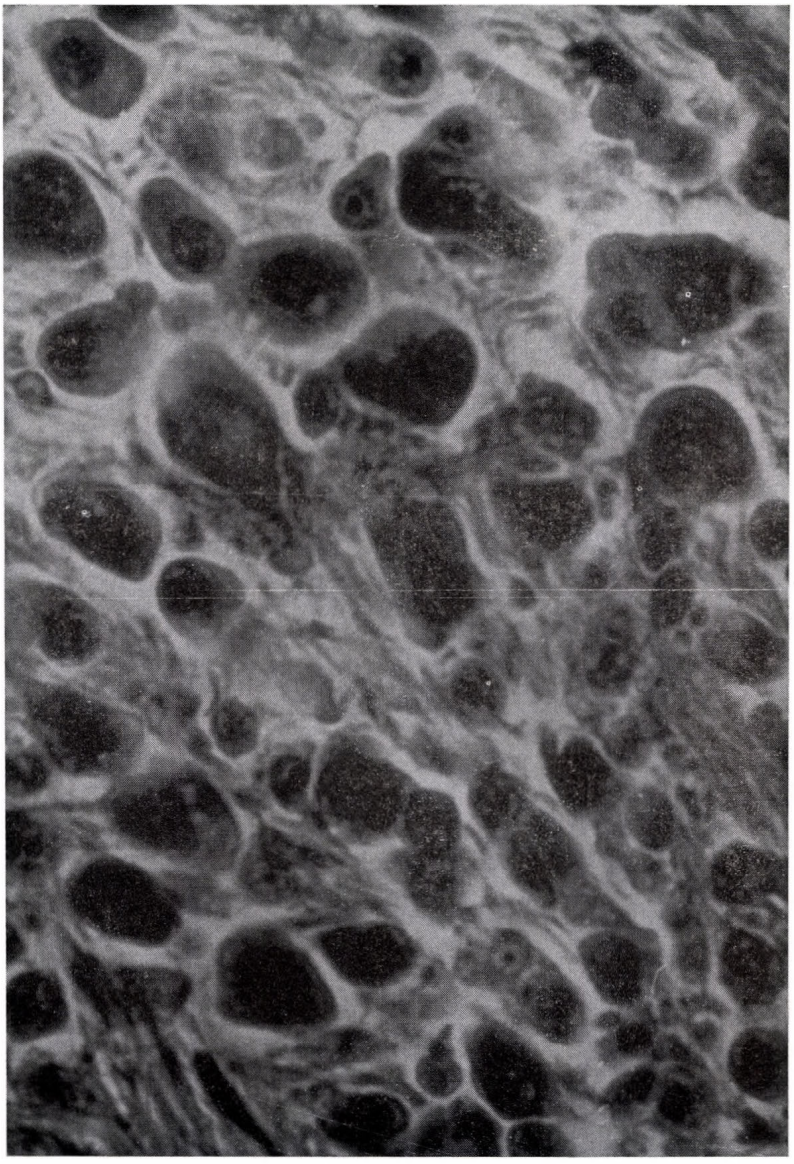

Fig. 1. Small and medium size neurons in the cortical part of ganglion viscerale (820×)

1. ábra. Kis és középméretű idegsejtek a ganglion viscerale kérgi részében (820× nagyítás)

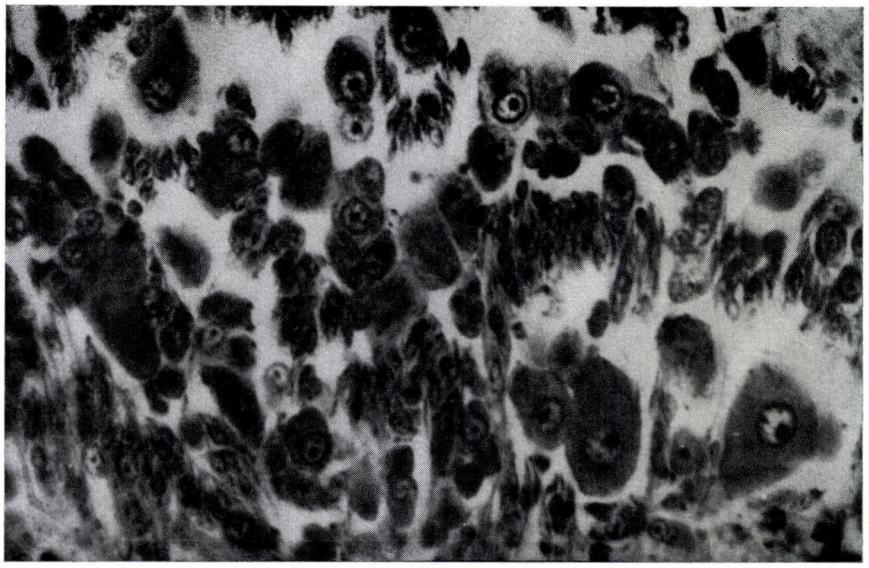

Fig. 2. Large size (35—40 μ) neurons with glia cells arranged around them in layers (500×)

2. ábra. A nagyméretű (35—40 μ) idegsejtek a körölöttük rétegesen elhelyezkedő gliasejtekkel (500× nagyítás)

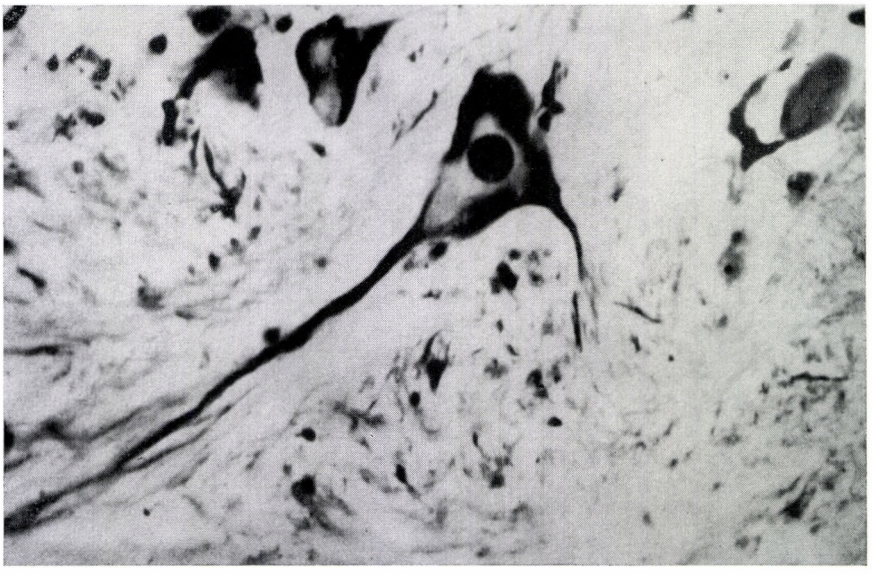

Fig. 3. Multipolar neuron from the cortical part of the *ggl. viscerale* (500×)

3. ábra. Multipoláris idegsejt a *ggl. viscerale* kérgi részéből (500 × nagyítás)

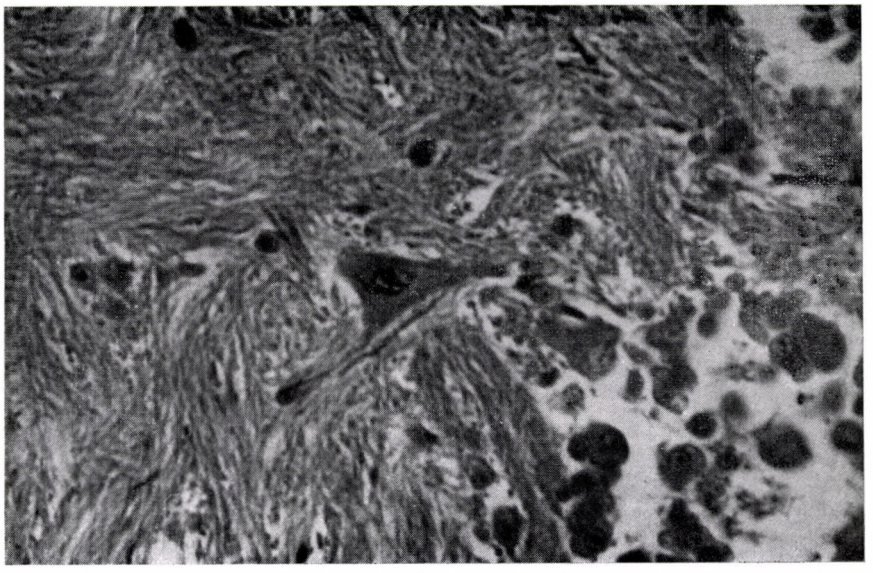

Fig. 4. Pyramidoidal neuron — presumably with several appendages — at the boundary of cortex and neuropile (500×)

4. ábra. Piramis alakú — feltehetően több nyúlványú — idegsejt a kéreg és a neuropil határán (500× nagyítás)

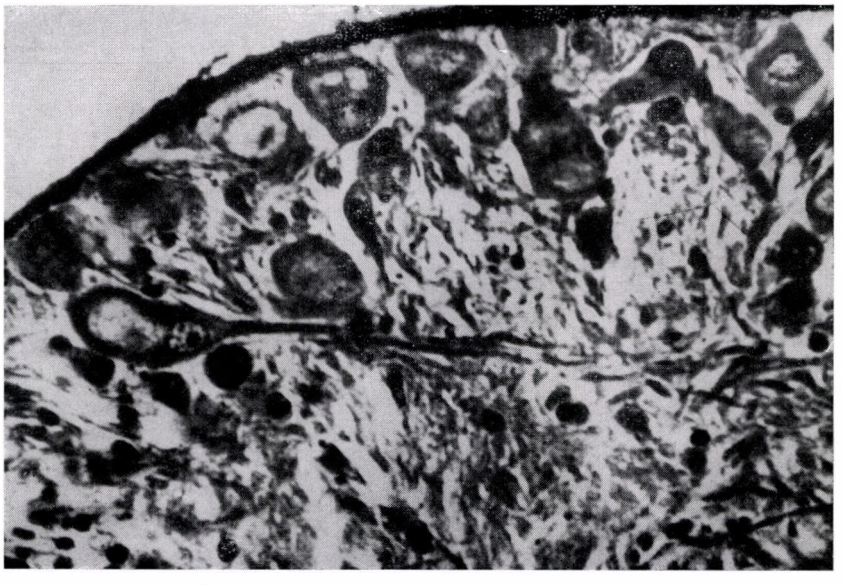

Fig. 5. The cytoplasm of the large size neurons often does not get impregnated uniformly (510×).

5. ábra. A nagyobbméretű idegsejtek citoplazmája gyakran nem egyenletesen impregnálódik (510× nagyítás)

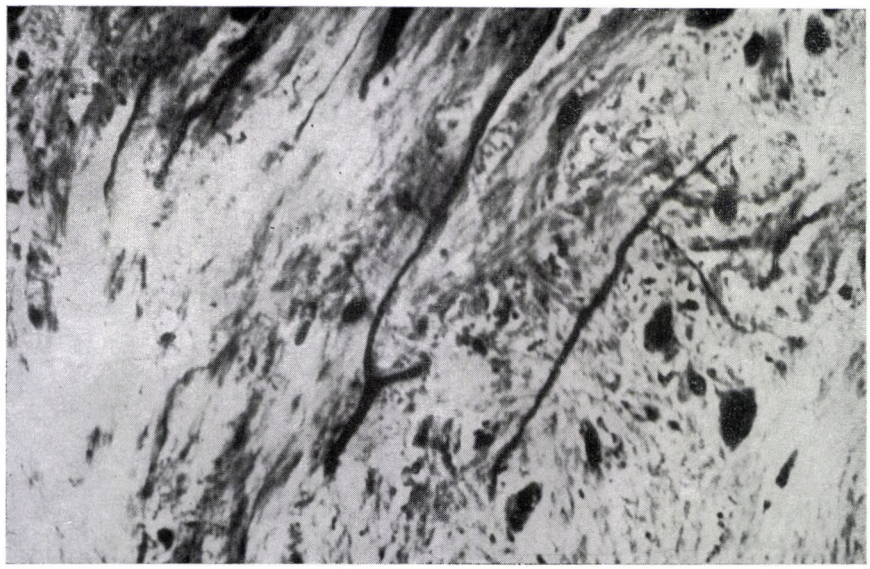

Fig. 6. Straight shaped and branching thick fibre visible in the neuropile of the *ggl. viscerale* (510×)

6. ábra. A *ggl. viscerale* neuropiljében látható egyenes lefutású és elágazó vastag rost (510× nagyítás)

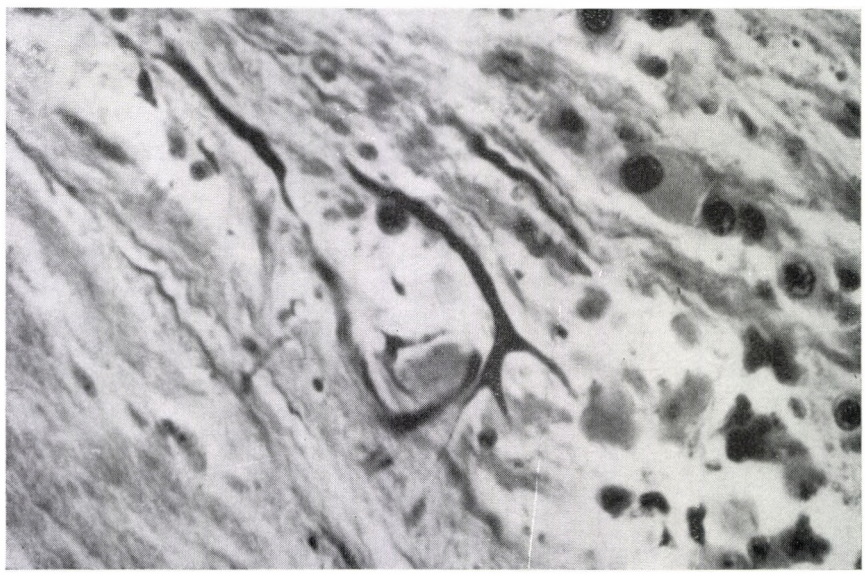

Fig. 7. Threefold branching of the thick fibre of *ggl. viscerale*. The branching fibre is at the boundary of cortex and neuropile (510×)

7. ábra. A *ggl. viscerale* vastag rostjának hármas elágazása. Az elágazó rost a kéreg és a neuropil határán van (510× nagyítás)

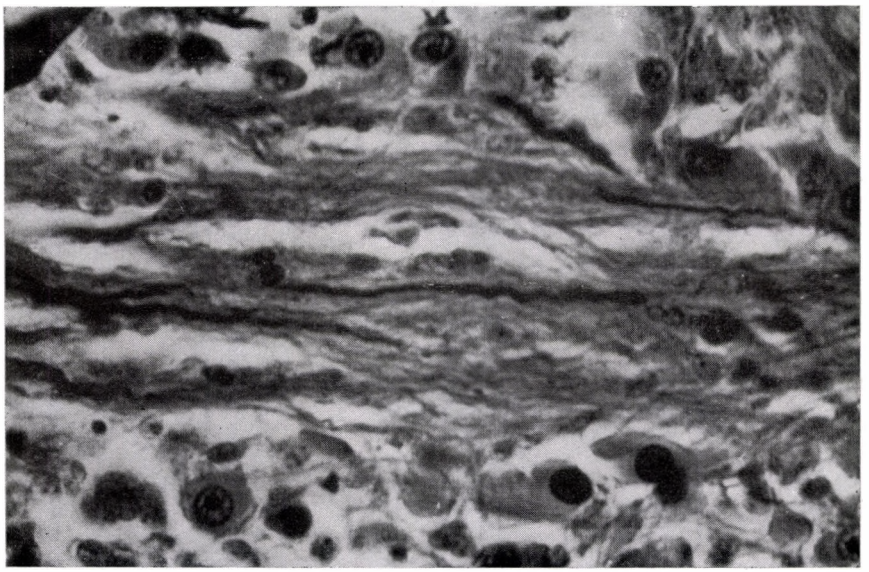

Fig. 8. Undulating fibres from the *ggl. viscerale* (500×)
8. ábra. Hullámos lefutású rostok a *ggl. visceraleből* (500× nagyítás)

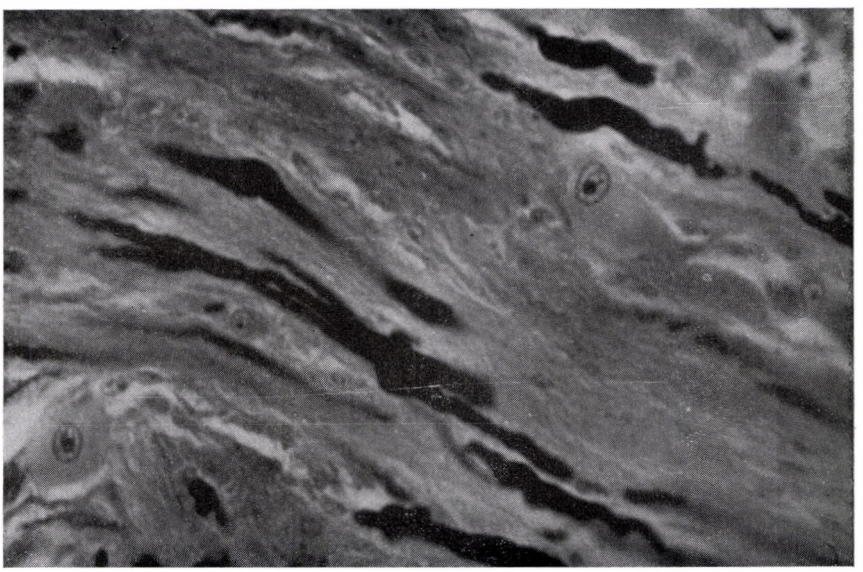

Fig. 9. Varicose fibres in the *ggl. viscerate* (500×)
9. ábra. Varixos rostok a *ggl. visceraleban* (500× nagyítás)

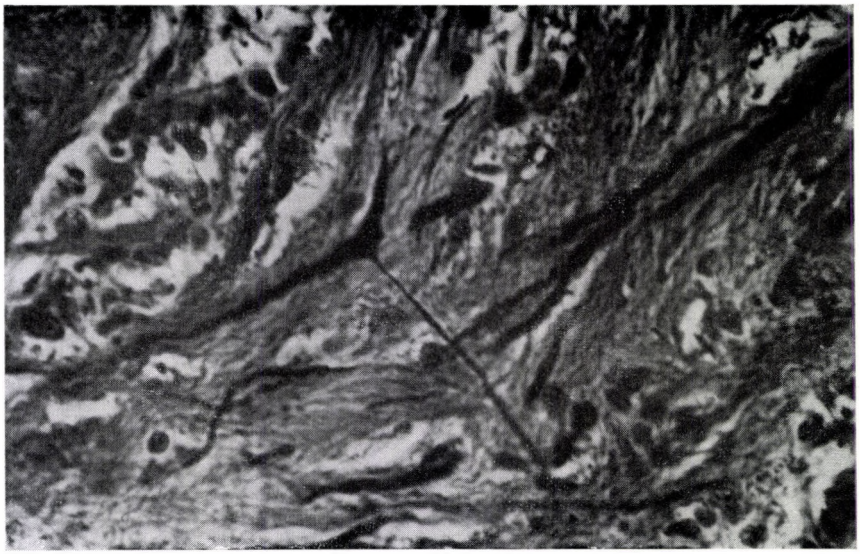

Fig. 10. Branching varicose fibre (500×)
10. ábra. Elágazó varixos rost (500× nagyítás)

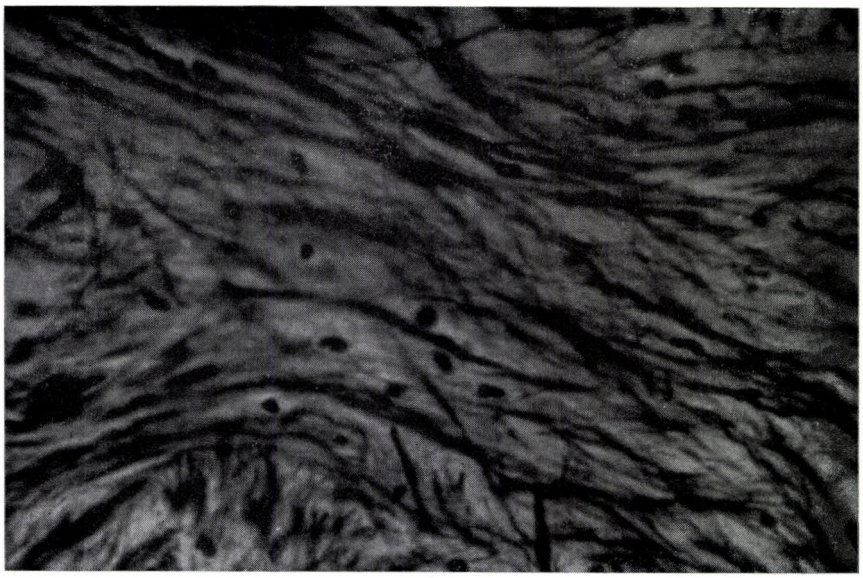

Fig. 11. Detail of the neuropile where only neurons and glia nuclei are found (500×)
11. ábra. A neuropil egy részlete, ahol csak idegrostok és a gliamagvak találhatók (500× nagyítás)

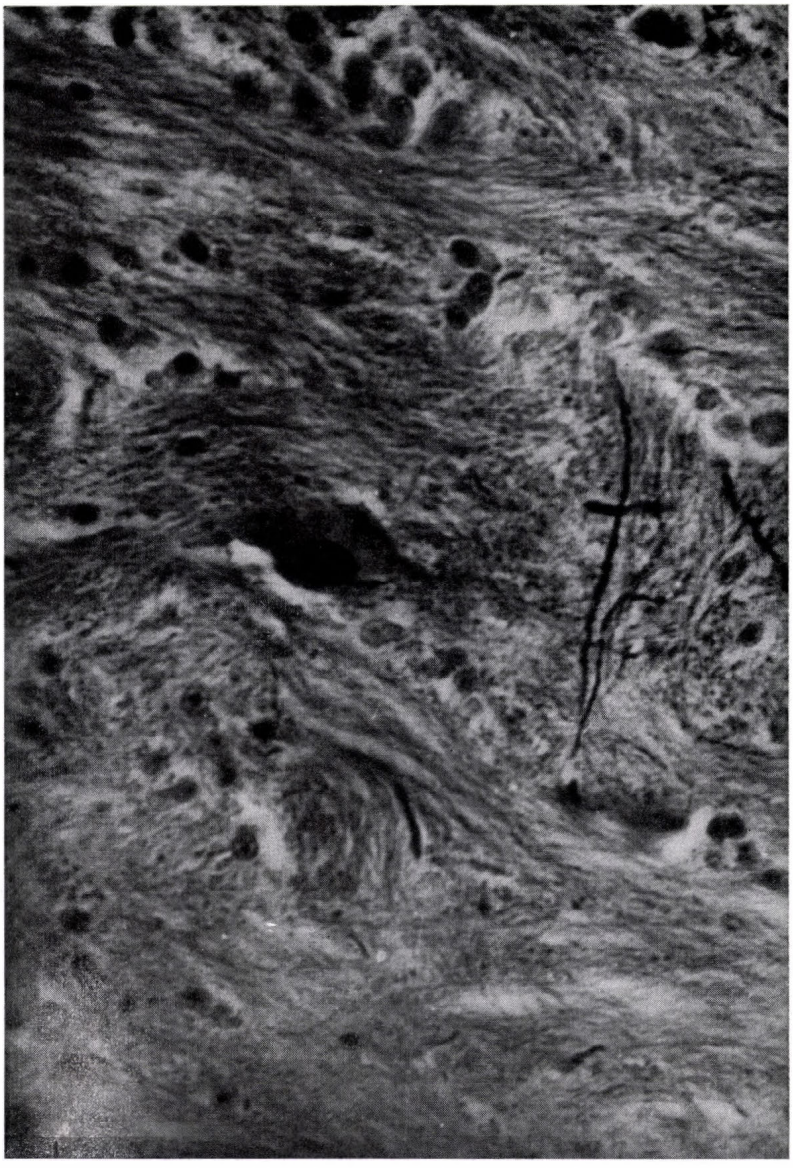

Fig. 12. Neuropile with one neuron (500×)
12. ábra. Neuropil egy idegsejttel (500× nagyítás)

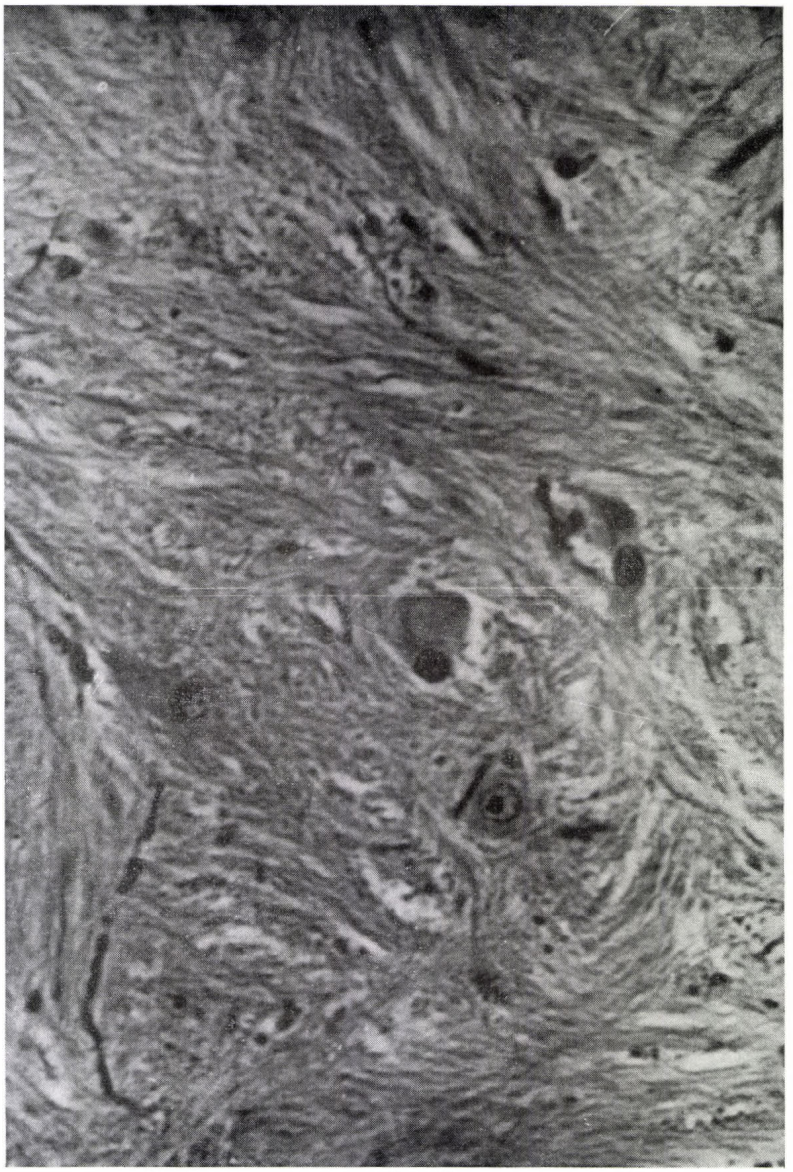

Fig. 13. Neuropile with several neurons (500×)
13. ábra. Neuropil több idegsejttel (500× nagyítás)