

LIGHT AND ELECTRON MICROSCOPICAL INVESTIGATIONS ON THE ADDUCTOR MUSCLE AND NERVOUS ELEMENTS IN THE LARVA OF *ANODONTA CYGNEA* L.

IMRE ZS.-NAGY and ELEMÉR LÁBOS

*Biological Research Institute of the Hungarian Academy of
Sciences, Tihany, Hungary*

Received: 14th February, 1969

The physiological investigation of adductor muscle in adult mussel (PAVLOV, 1895, BARNES, 1955, KOSHTOYANTS and SALÁNKI, 1958, SALÁNKI, 1961, 1963) necessitated to study the larval adductor, too. In these investigations light has been thrown on many new physiological facts concerning the response of the adductor (LÁBOS and SALÁNKI, 1963, LÁBOS et al. 1964a, b, c, LÁBOS, 1964, 1966a, b, 1967, 1969, LÁBOS and TURCSÁNYI, 1966). Interpreting these results the fundamental question has arisen whether the adductor muscle is innervated or it functions automatically. There is no unequivocal answer to this question in the old histological works (SCHIERHOLZ 1888, LILLIE, 1895, HERBERS, 1913). Even recently, the investigations with supravital stainings could hardly carry us farther in the question than the data of old authors. Nevertheless, it has turned out well that certain cells having processes can be stained with methylene blue or crystal violet in scattered or characteristic localizations, the neuronal character of which can not be stated again unequivocally (TÖRÖK and LÁBOS, unpublished). Therefore convincing proofs can only be given by electron microscopic investigations. There are however no such data in the literature.

The aim of our present work was to study the ultrastructure of the glochidia with special interest to the submicroscopic organization of neuromuscular junction. Therefore, our attention was focused first on the adductor muscle and the nervous elements, but several other important characteristics were also touched upon.

Material and method

For experimental purpose the glochidia of *Anodonta cygnea* L. were used between of November and March for in this period glochidia are commonly found in large numbers in the outer gills. They were fixed immediately, together with their mucous groundsubstance. For histological purposes paraffin sections fixed in Susa, stained with routine histological methods (haematoxylin-eosin, azan) and with chrome-haematoxylin-phloxin according to BARGMANN (1949) were used. After a 24 hour fixation even the shells could be cut to a 8-10 micron thickness. For electron microscopy the glochidia were fixed

in 2 per cent OsO_4 solution buffered with *s*-collidine (BENNETT and LUFT, 1959) for 30 min at 0°C and subsequently 10 min at room temperature. This was followed by an alcoholic dehydration and through propylene oxide the material was embedded into Araldite (Durcupan ACM, Fluka). Twenty-thirty glochidia were placed in each block. They all were in closed condition because their adductor contracted immediately on the influence of fixation.

The ultrathin sectioning of the larvae causes great difficulties. The animals at most 200–400 micron size have hard shells of chitin immediately

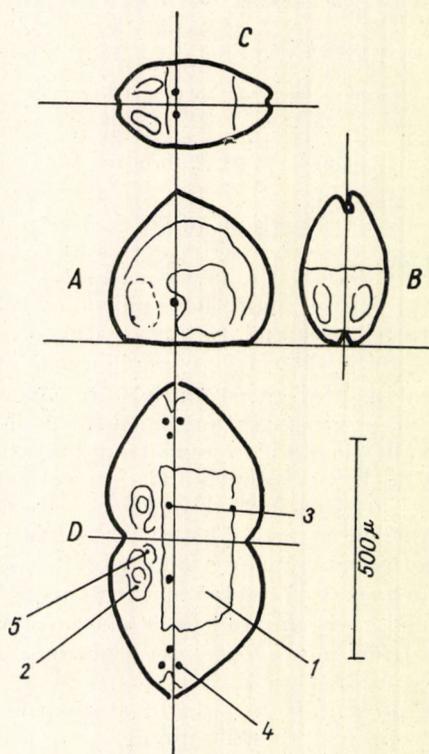


Fig. 1. The proportional sections of glochidia. A — sagittal plane, B — transversal plane, C — horizontal plane, D — opened animal. Meaning of numbers: 1 — adductor muscle, 2 — lateral pit, 3 — solitary sensory cell, 4 — lateral sensory cells, 5 — foot fold.

ruining the glass knives. Thus the following procedure has been adapted: the shells of the suitably oriented larva were trimmed in the block and the surface of cutting was always shaped within the shells of one animal trimming always out the shells. In this way, the sections were made in the mediansagittal, transversal and horizontal planes (*Fig. 1*) on LKB Ultratome III.

Sections were contrasted with uranyl acetate and lead citrate (REYNOLDS, 1963). Micrographs were taken with TESLA BS 413A electron microscope. To identify the elements found in ultrathin sections easily also half-thick (1–2 micron) sections were cut and investigated in phase contrast.

Results

A) *Light microscopic morphology of the glochidium*

Our histological results coincide essentially with that of the old authors (LILLIE, 1895, HERBERS, 1913). The morphological features most important in respect of the interpretation of our electron microscopic results will be related below. The terminology has been wholly taken over from HERBERS (1913).

The larva consists of two halves symmetric to the mediansagittal plane. Its organs are commonly paired except the adductor muscle (*Fig. 2*) adhering on the inner surface of the shells, filling up the middle third of the dorsal half of the body. The adductor consists of smooth muscle cells. Ontogenetically it is not identical with the adductors of the adult animals, namely, it disappears completely during metamorphosis and definitive adductors arise from new anlagen (HERBERS, 1913).

The adductor and the inner parts of the shells, unoccupied by the adductor, are covered by the larval mantle (*Fig. 2*). On the oral and ventral side, the mantle consists of relatively large vacuolated and granulated epithelial cells. It is wholly of ectodermal origin. On its surface a thin stripe of different staining and refraction can be observed. The mantle is not held fast to the shell, there is an interstice between them wherein the myocytes are situated. They keep moving the parts of the mantle and the hooks.

Peculiar organs are situated in the areas behind the adductor (*Fig. 2*). Here, the lateral pit and the foot fold are found, both of extodermal origin and being continuous with the larval mantle. As compared to them the entodermal sac is in dorsal while the mesoderm is in lateral position. This latter represents the common anlage of the heart, the pericardium and the genital organs. In these areas the cells are small, adhering closely and their nuclei stain strongly. The anlagen of all organs of the adult mussel originate from this region. HERBERS (1913) refers to the fact that in overwintered animals the anlagen of cerebral and visceral ganglia are to be found in the form of epithelial thickenings localized in the upper wall of the lateral pit.

Special formations of the larval mantle are represented by the so called sensory cells (*Fig. 3*). There are four pairs of them. One pair is near the adductor, on its ventral side (solitary sensory cells), the other three near the apex of the shell beneath the hooks (lateral sensory cells). These cells overtower the surrounding mantle cells and bear long cilia on their surface. The basal bodies of their cilia are situated side by side and a fibrous bundle extends to the direction of the nucleus from them. This is particularly developed in the solitary sensory cells.

In the vicinity of the lateral pit, cilia can only indistinctly be recognized in histological sections but observing living animals under a microscope it is easy to see that there is an intensive cyliary activity causing an eddy of water here.

Larval nervous centers or diffuse nervous network have been mentioned neither in old literature nor found in our histological material. Chromaematoxylin-phloxin (BARGMANN, 1949) stains all the epithelial cells deep blue, therefore no specific elements can be differentiated by this method.

B) *Electron microscopic results*

1. The adductor muscle

The cells of the adductor muscle are of various shape in transversal section. Round, flattened and what is more stelliform as well as irregularly lobulated cross section figures can be found. The greatest diameter of the muscle cells is about 10 micron. The sarcoplasm is very poor in structure, composed by some mitochondria pressed close against the periphery of the cell, a number of larger or smaller vesicles and free ribosomes. Sometimes the sarcoplasm forms radial septa towards the nucleus thus parcelling up the filamentous substance. The cross sections of the nuclei are also irregular in shape. A denser chromatin substance adheres to the inner surface of the nuclear membrane. Relatively big nucleoli and a wide perinuclear space are seen with regular nuclear pores (*Fig. 4*). The single cells are separated by seemingly empty, extensive intercellular spaces.

The contractile store consists of a thicker (about 200 Å) filament and a thinner (about 40 Å) one. The interfilamentar distance of the thick filaments varies between 800 and 1400 Å. Where the distances are uniform even a regular hexagonal pattern is observable (*Fig. 5*). This is a frequent but not a common phenomenon. The thin filaments are arranged, however irregularly round the thick ones. In cross sections elements of high density and of about 800–1000 Å thickness occur among the filaments sporadically, which correspond to the dense bodies of about 5000 Å length observed in longitudinal sections (*Fig. 6*). Neither is their arrangement regular.

Among muscle cells axon-like structures of about 0,5–1 micron thickness occur frequently. Sometimes they almost completely enclose the muscle cells in cross section pictures (*Fig. 7*), another occasion only their enlarged knob-like endings attach closely to the sarcolemma or invaginate deep into the muscle cell. They always contain commonly empty-core vesicles of about 400–1200 Å diameter (*Fig. 7*), rarely, however, dense-core vesicles occur, too. Mitochondria are also present in the enlarged endings. The axolemma is closely connected with the sarcolemma. The two membranes are on certain places nearer to each other than elsewhere and also a fine stripe appears in the intermembranic space. On the axonal side of these contacts clusters of vesicles (*Fig. 7*) can always be found and, what is more, the coalescence of vesicles into the axolemma can also be observed in some places. These contacts of axons with the muscle cells obviously correspond to the neuromuscular synapses.

The synapses are of various arrangement. There are cases when an axon contacts only one muscle cell, at other times it may even contact two or three muscle cells. We found also a situation when the axon surrounding the muscle cell showed several such junctions with the same muscle cell. It is again a further variation when two or three different axons form synapses on the surface of the same muscle cell.

2. The larval neurones

Flattened cells can be seen in a single layer under the epithelial cells of the larval mantle in close connection with them. These cells bear no resemblance to the mantle cells. In their cytoplasm and processes clumps of vesicles of the

same kind can be seen as in the nerve endings of the adductor muscle. These processes full of vesicles are connected with other ones by synapse-like structures (*Fig. 8*). The connected membranes thicken and there is a cluster of vesicles on one side. Seldom dense-core vesicles occur, too, the great majority of vesicles, however, appears to be empty.

We could observe a nerve fibre entering the adductor muscle. In the immediate neighbourhood of the adductor there was a thick axon-like process containing mitochondria, a great number of microtubuli, membrane profiles of irregular shape and ribosome-like granules. On its surface 8–10 enlarged knob-like endings were situated. These latter were morphologically identical with the processes of the above related cells. The thick axon enters the adductor at right angles to the direction of muscle cells and its branching gives endings forming the neuromuscular junctions.

Axon-like structures can be found in the whole area of the mantle even among the epithelial cells. Some of them can be recognized as processes of the above related flattened cells, others contain in large numbers dense-core vesicles, again others contain no vesicular components, their inside is structureless and seems to be almost empty. All three kind of processes take part in forming synaptic contacts.

3. The sensory cells

The sensory cells are situated among the epithelial cells of the larval mantle, however, the former greatly differ from the latter. On their surface cilia are found among the microvilli. The cilia contain 9 peripheric and 2 central ciliary tubules (*Fig. 9*). There is a special structural element near the basis of cilia but still out of cellular surface. The peripheric ciliary tubules fuse in a single tube of high density (*Fig. 9*) having also a closed basal plate. In its plane the cross section picture corresponds only to a dark spot. Under this plate appearing as a transversal dark stripe in longitudinal sections of the cilia, the peripheric ciliary tubules fused into a tube continue again. The outer membrane of cilia closely adheres to the tube of tubules above the dark stripe while under the level of this stripe it draws off again from the tube and join the cellular membrane only farther (*Fig. 10*). The ciliary tubules penetrating into the inside of the cell, end in the basal body connected with peculiar ciliary rootlets. The rootlets open wide in the shape of a fan and enter deeply the cytoplasm. They are of much higher density than other components of the cytoplasm and there is a dark-bright periodicity in them (*Fig. 10*). The dark stripe seems to be homogeneous and is of about 200 Å in width. The brighter one is about twice as wide and dark elementary filaments of about 20 Å thickness appear in it, oriented parallel to the longitudinal axis. A number of microtubuli, mitochondria of tubular character as well as multivesicular bodies can be found among the rootlets. All these components are embedded into a peculiar mainly smooth endoplasmic reticulum of a vesicular type. The rootlets get more and more thinner in deeper regions of the cytoplasm and completely disappear at the depth of about 5 microns (*Fig. 10*).

The nucleus is localized on the cell basis. In its vicinity the cytoplasm contains Golgi apparatuses of luxuriant structure, profiles of granulated endoplasmic reticulum, a lot of free ribosomes, mitochondria and microtubuli. It is remarkable the presence of many mostly empty, sometimes dense-core

vesicles of about 400–1200 Å diameter, they may also constitute large groups (*Fig. 11*). Such groups of vesicles can be seen attached to the cell membrane in places where axon-like processes surrounding densely the basal part of the cell adjoin to the outer surface of the cell membrane. The cell body of about 25 micron height reaches deeper than the epithelial cells and extends somewhat under them laterally.

4. The cells of the lateral pit and the foot fold

The structure of the epithelial cells of these areas differs in a particular way comparing to that of the larval mantle cells. The cells become narrow and extend to a considerable length into the depth. On their surface they have microvilli. Their nuclei are relatively large, elongate and rich in chromatin. The cytoplasm contains a lot of free ribosomes and mitochondria. There are some weakly developed Golgi apparatuses at places (*Fig. 12*). In several cells the granular endoplasmic reticulum is also to be found in the form of parallel lamellae or Nebenkern-like structures. It was observed several times that the outer lamella of the nuclear membrane was continuous with the lamellae of endoplasmic reticulum (*Fig. 13*). In certain cells structures resembling to the precyosomes of the adult mussels (Zs.-NAGY, 1969) were also found (*Fig. 14.*). Among the epithelial cells there are also cells having no contact with the surface and showing processes as well as a very differentiated cytoplasm. Many dense-core vesicles of about 1000–1500 Å size, well developed Golgi apparatuses, endoplasmic reticulum of vesicular type and mitochondria of tubular character — are the components of these cells (*Fig. 15*). The dense-core vesicles occur in the processes, too. Sometimes, these cells touch the muscle cells of the adductor directly, but we have never succeeded in observing their processes penetrating the adductor.

The processes form a group in several places among the epithelial cells, like a neuropile (*Fig. 16*). Some processes have no vesicular components, others contain dense-core vesicles, again others are filled up with larger vesicles of about 2000–3000 Å in diameter, resembling morphologically to the elementary neurosecretory granules. The empty vesicles found in the nerve fibres of the adductor do not occur here. In several cases we could see the processes of the epithelial cells join the neuropile directly. There are no synapse-like structures between the fibres. There occurs that one or more processes are closely connected to the surface of certain cells, these contactas, however, show no synaptic specialization.

5. Other submicroscopic features

The abundance of microvilli on the surface of all epithelial cells is remarkable. These are of about 1 micron in length and 0,1 micron thick (*Fig. 17*) and covered by a unit-membrane continuous with the cellular membrane. They generally branch near the cell surface forming a branched candlestick-like structure (*Fig. 18*). There is a dense stripe of uncertain contour within, which seems to be empty cored in cross-section, being of tubular structure. Between the microvilli there exists a network consisting of very fine filaments and forming small vesicles on the outer surface of villus membrane (*Fig. 17*).

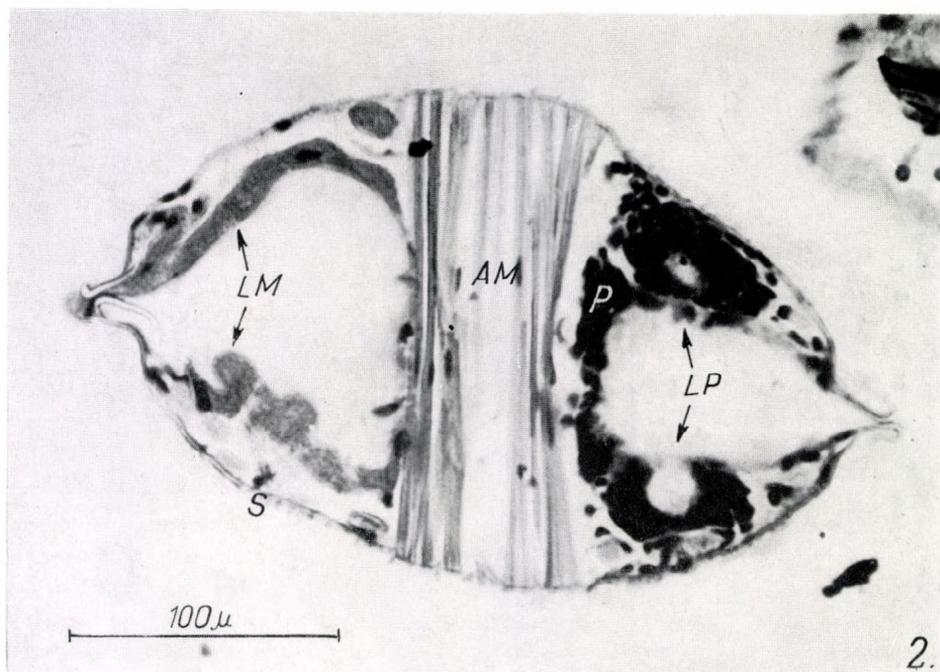


Fig. 2. The histological section of glochidium made in horizontal plane through the lateral pit. AM — adductor muscle, LP — lateral pit, P — foot fold, LM — larval mantle, S — shell, haematoxylin-eosin staining, $\times 370$.

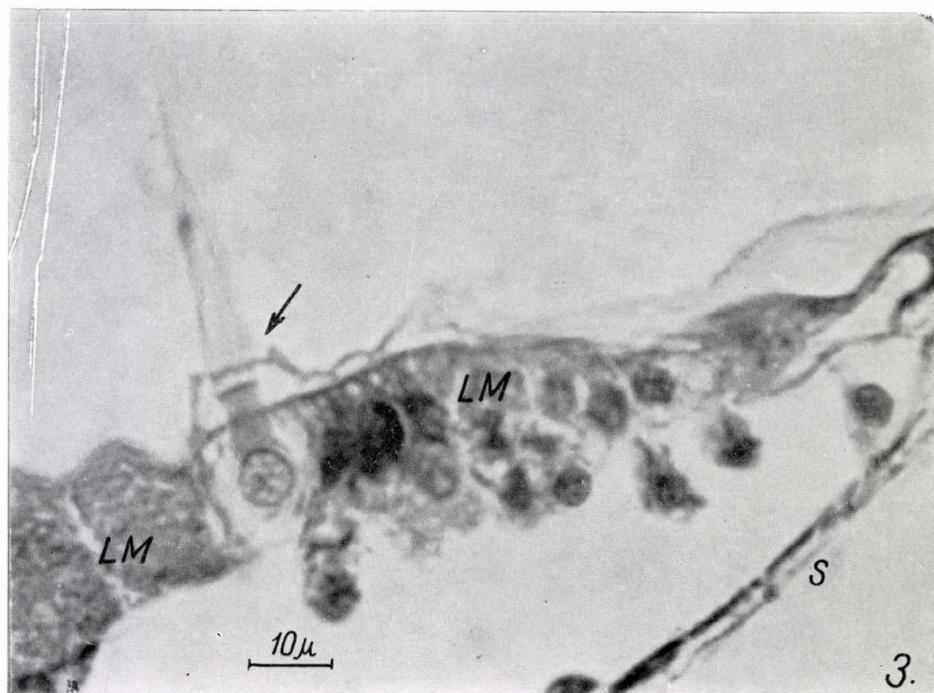


Fig. 3. The histological picture of the solitary sensory cell (arrow). LM — larval mantle, S — shell, haematoxylin-eosin staining, $\times 1000$.

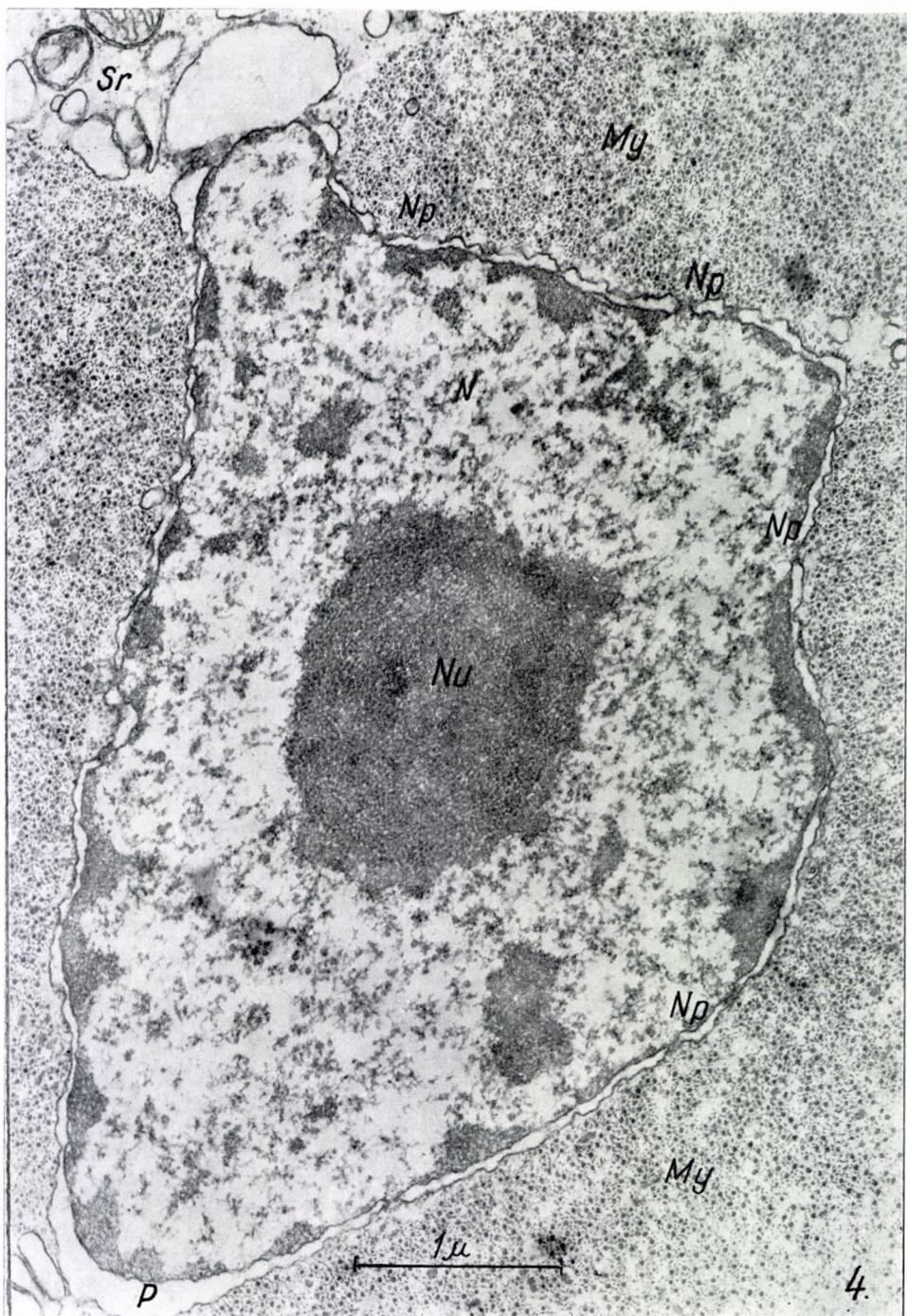


Fig. 4. Nucleus (N) of the adductor muscle cell. Nu — nucleolus, Np — nuclear pores, P — perinuclear space, My — myofilaments, Sr — sarcoplasmic reticulum. $\times 30\ 000$.

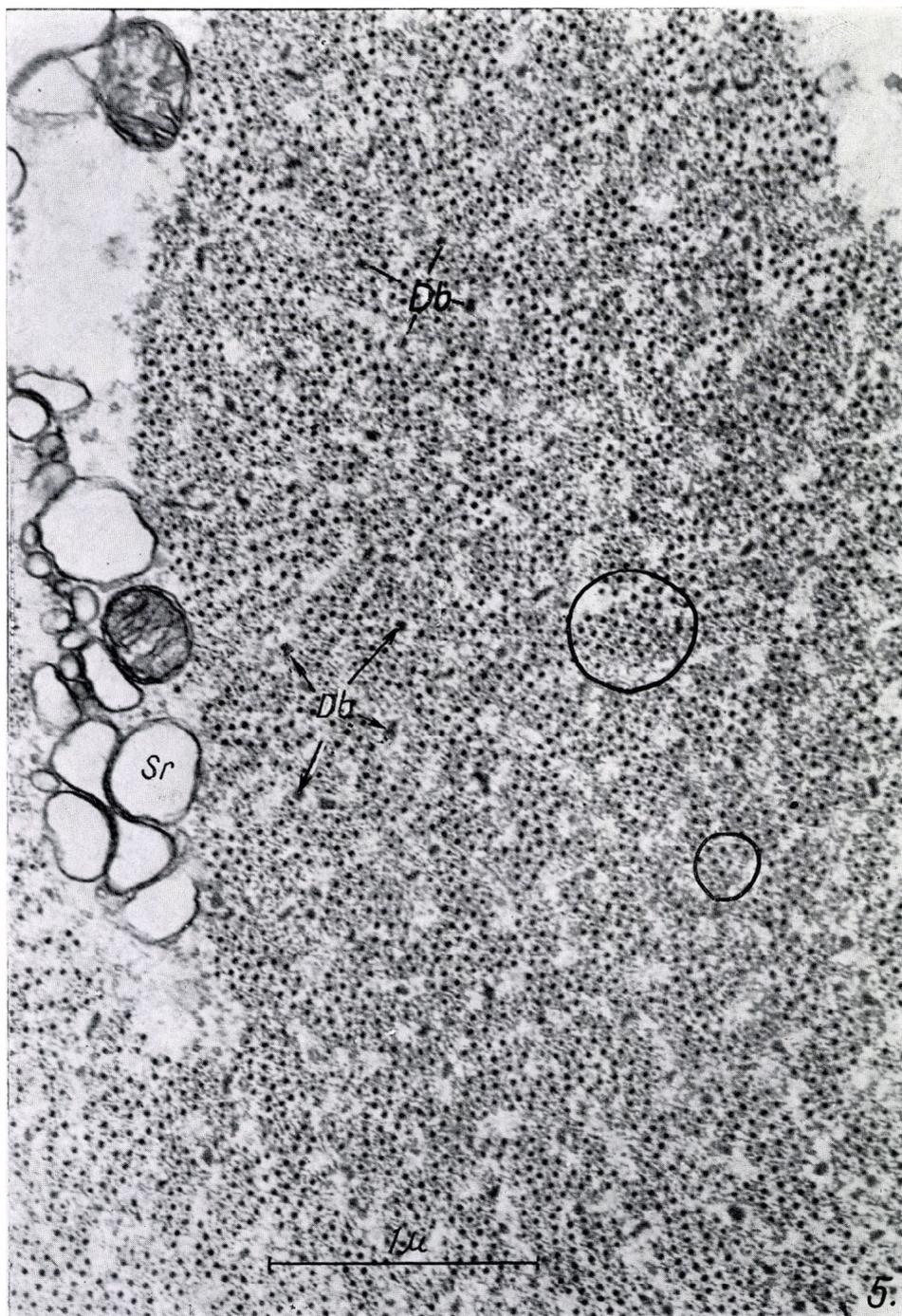


Fig. 5. Cross section of myofilaments in the adductor muscle. The regular pattern is expressed in some places (rings) elsewhere it is absent. Db — dense bodies, Sr — sarcoplasmic reticulum, $\times 40\ 000$.

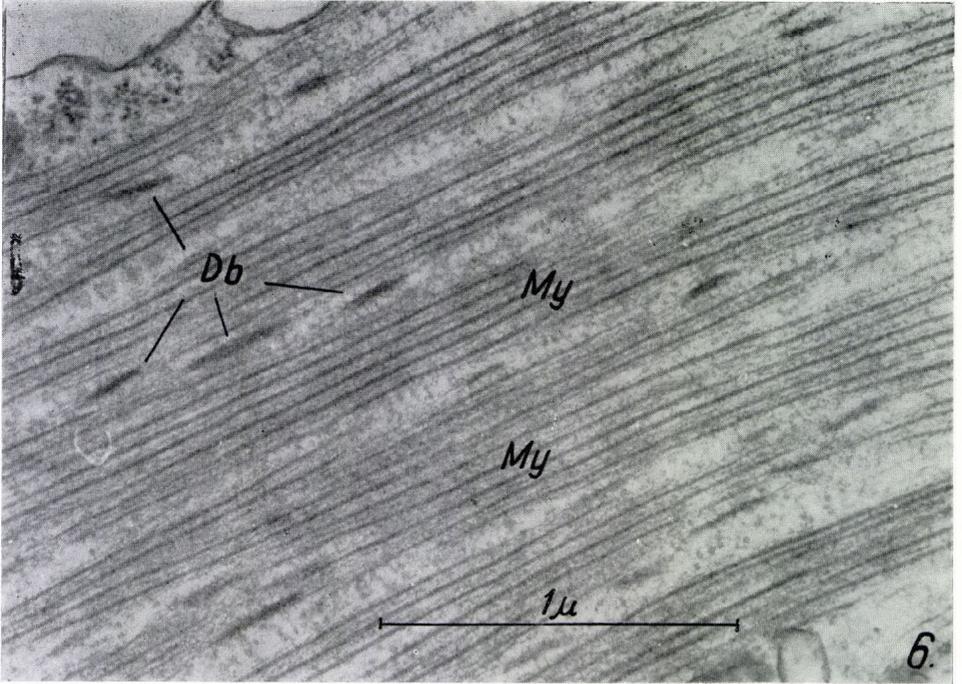


Fig. 6. Longitudinal section of myofilaments in the adductor muscle. Db — dense bodies, $\times 49\ 000$.

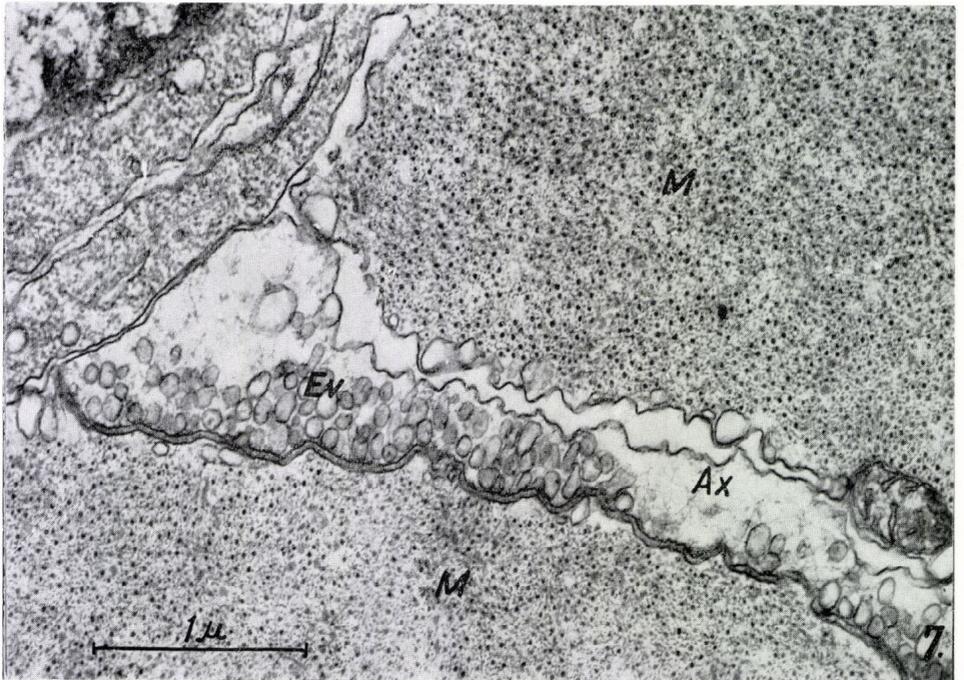
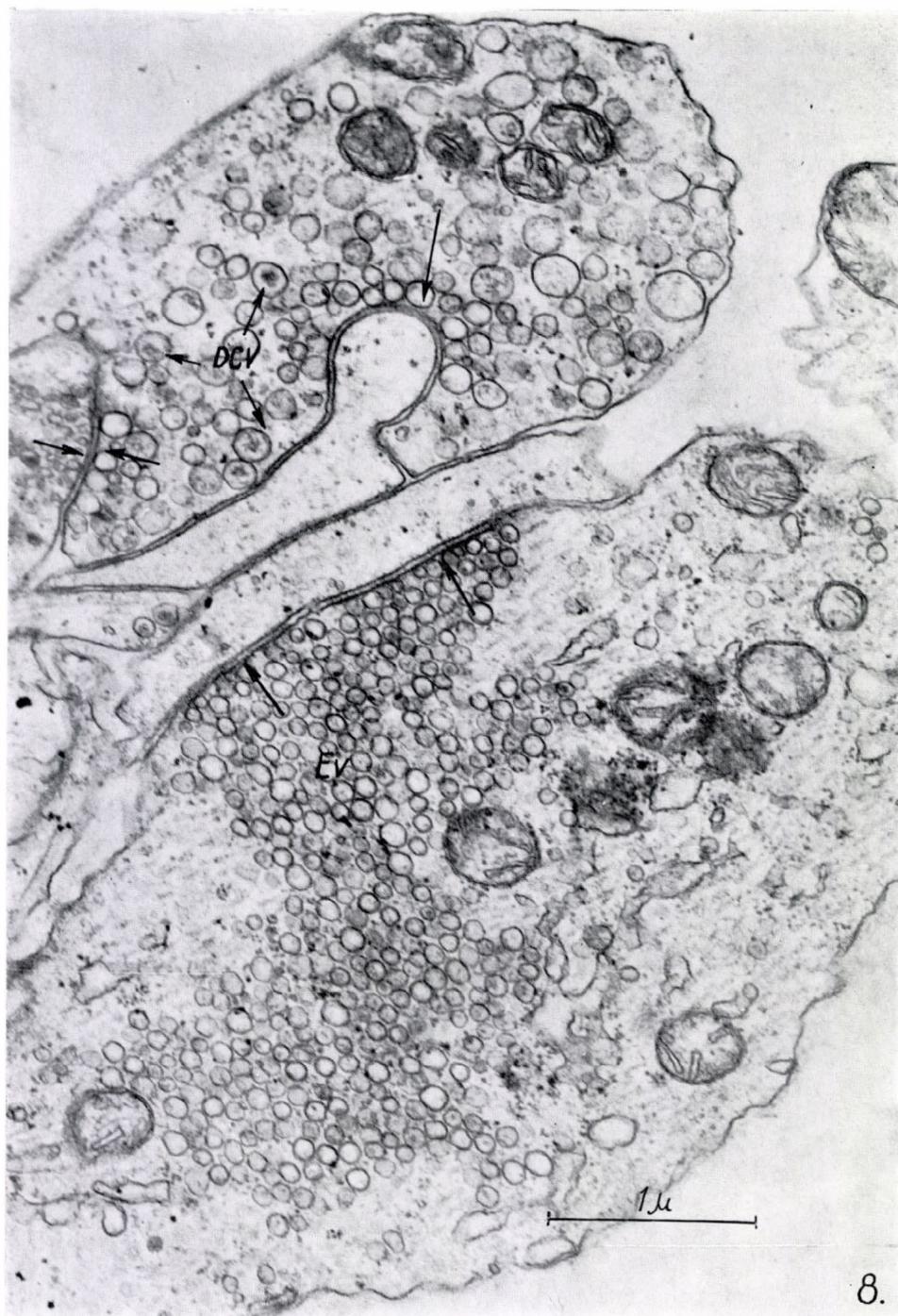


Fig. 7. Picture of a neuromuscular synapse in the adductor muscle. Ax — axon, M — muscle cell, Ev — empty vesicles, $\times 30\ 000$.



8.

Fig. 8. The synaptic contacts of the larval neurones (arrows). The lower part of the picture shows a soma-like structure, the others are processes. Ev — empty-core vesicles, DCV — dense core vesicles, $\times 30\,000$.

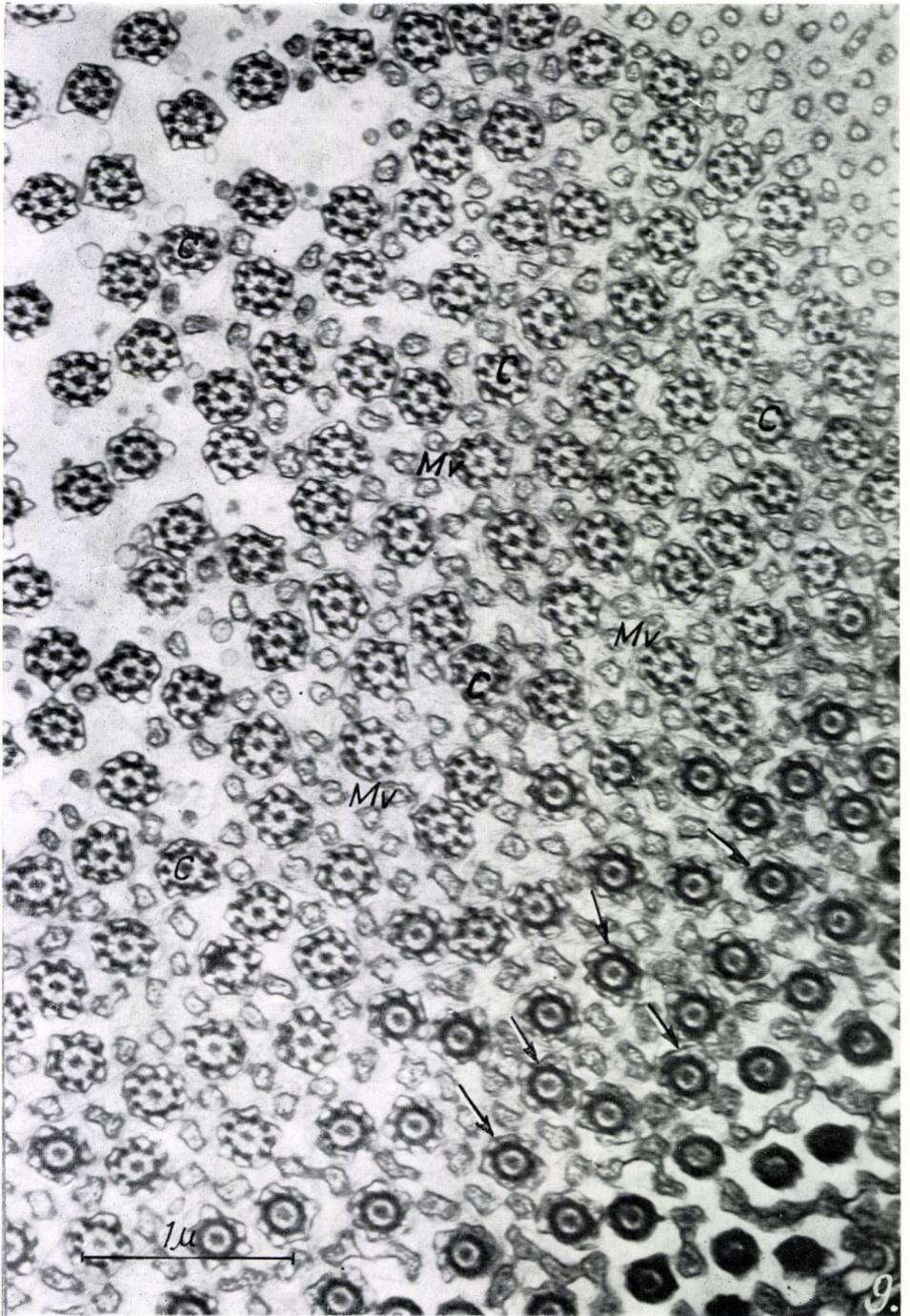


Fig. 9. Transversal, a slightly oblique section of cilia of the sensory cell near the cell surface. Arrows point to cilia in which the ciliary tubules are fused into a tube. Mv — microrvilli, $\times 30\ 000$.

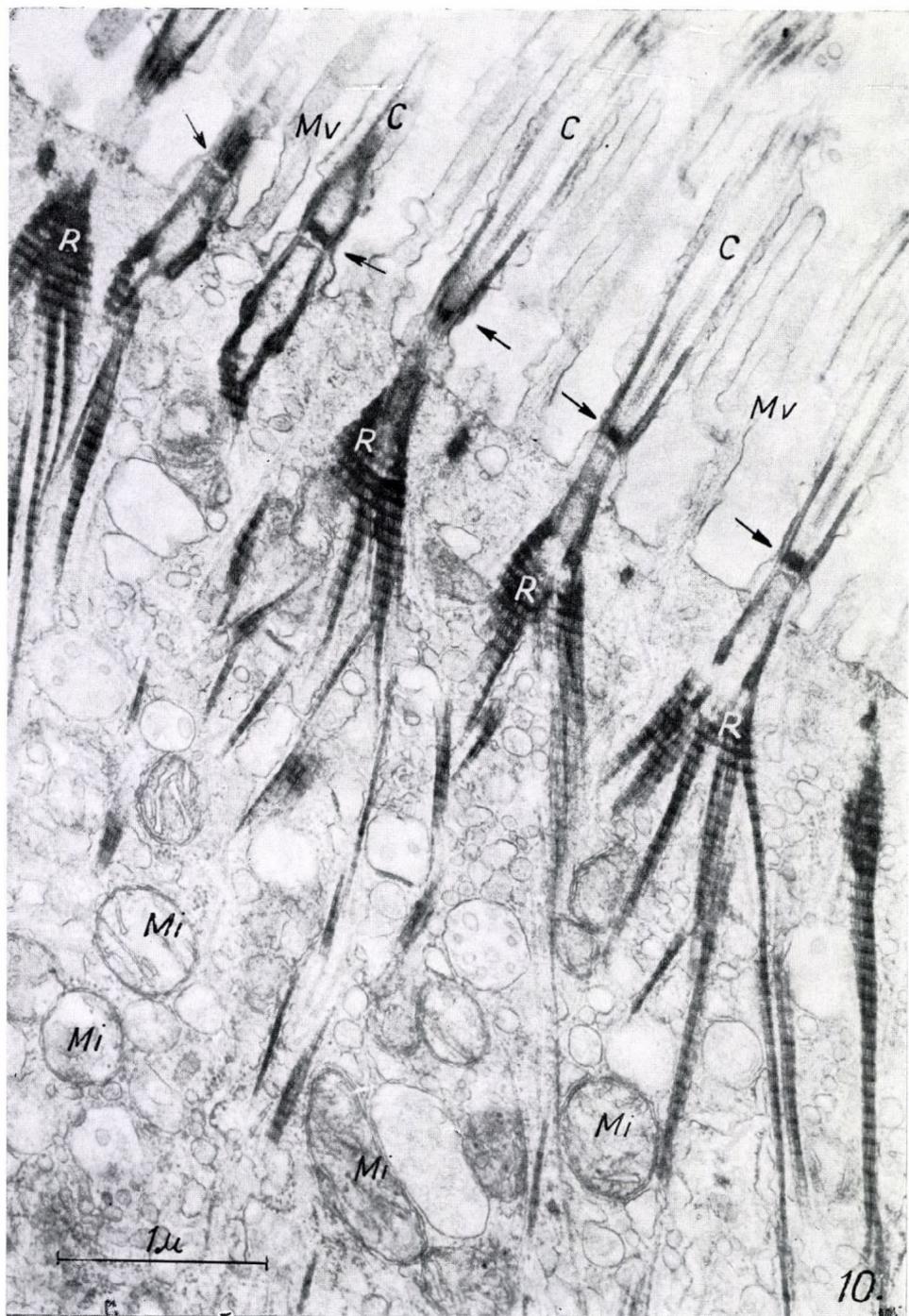


Fig. 10. The superficial part of the solitary sensory cell with the longitudinal section of cilia. Mv — microvilli, R — ciliary rootlets, Mi — mitochondria, Arrows point to the special structures of cilia. $\times 30\ 000$.

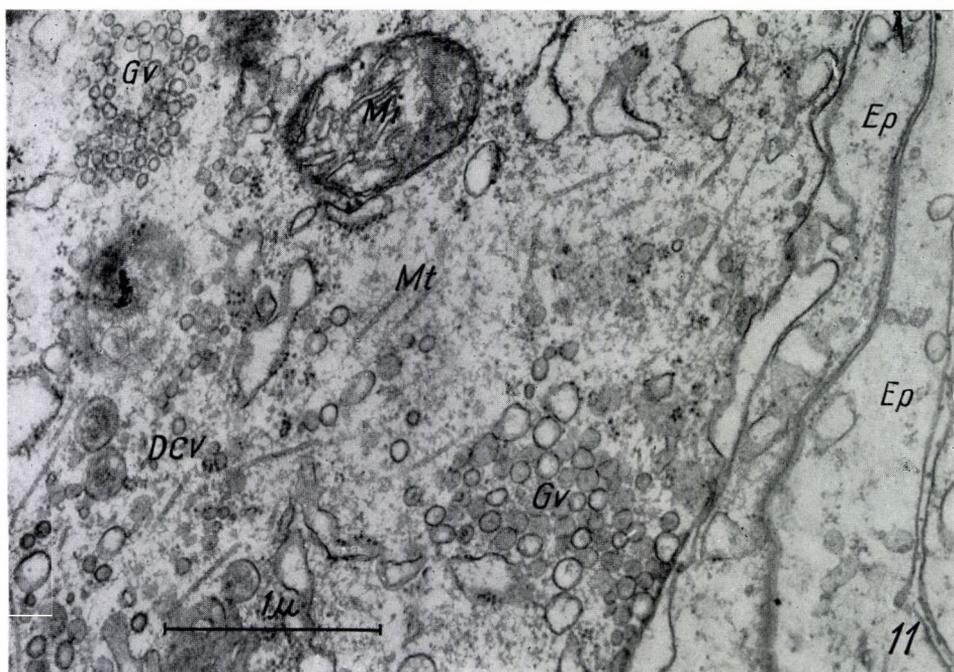


Fig. 11. Detail of basal part of solitary sensory cell. Gv — groups of vesicles, DCV — dense-core vesicles, Mt — microtubuli, Mi — mitochondria, EP — epithelial cell processes, $\times 30\ 000$.

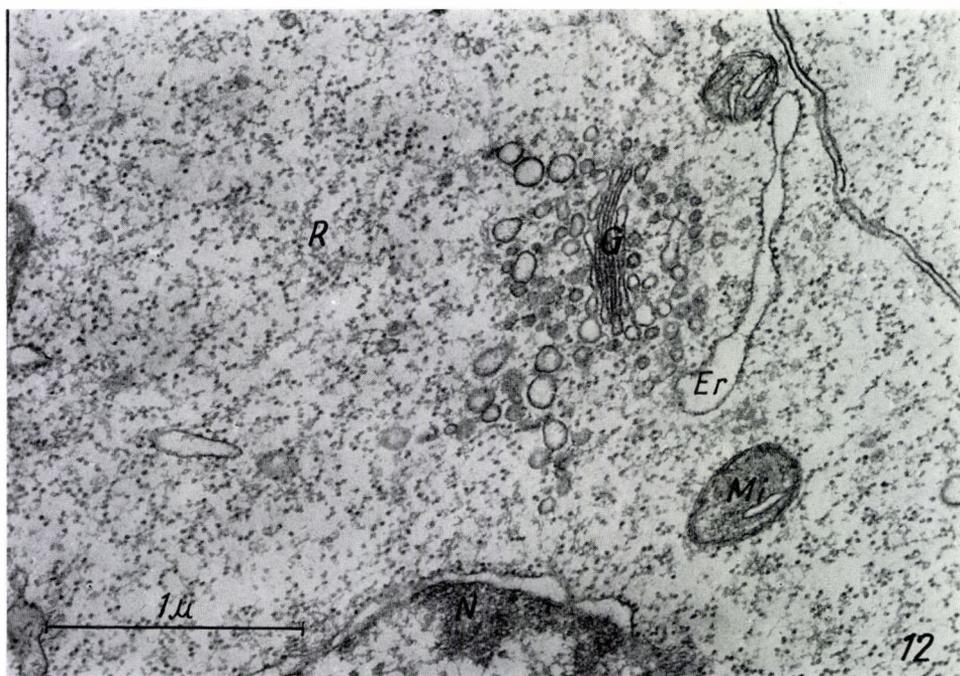


Fig. 12. Detail of the epithelial cells covering the lateral pit. N — nucleus, G — Golgi apparatus, Er — endoplasmic reticulum, Mi — mitochondrium, R — ribosomes, $\times 35\ 000$.

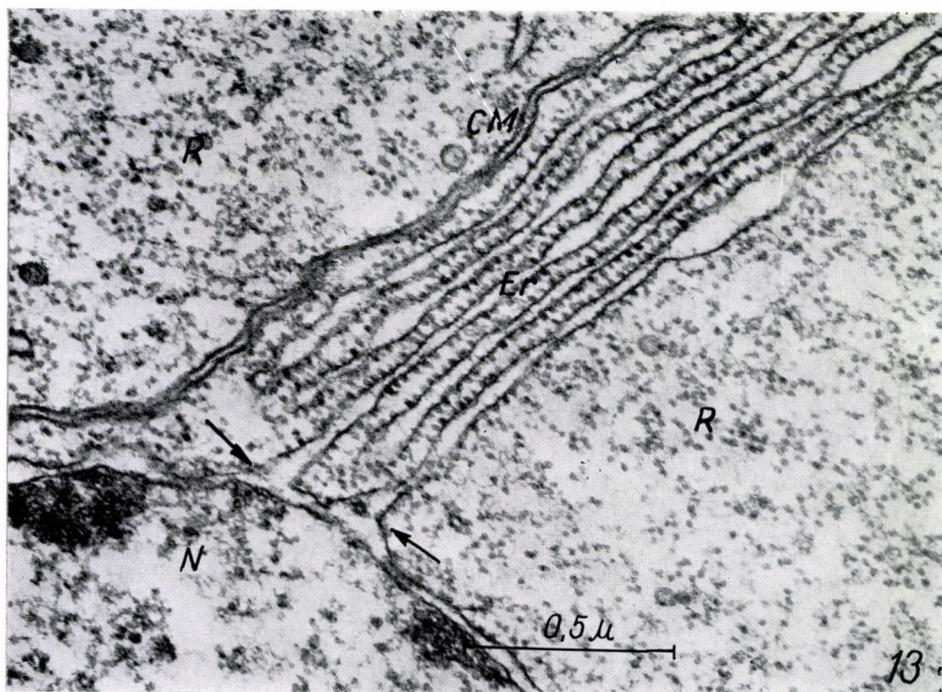


Fig. 13. Parallel oriented lamellae of granulated endoplasmic reticulum (Er) in an epithelial cell of the lateral pit. Arrows point to places where the connection with the nuclear membrane is clearly visible. N — nucleus, CM — cellular membranes, R — ribosomes, $\times 59\ 000$.

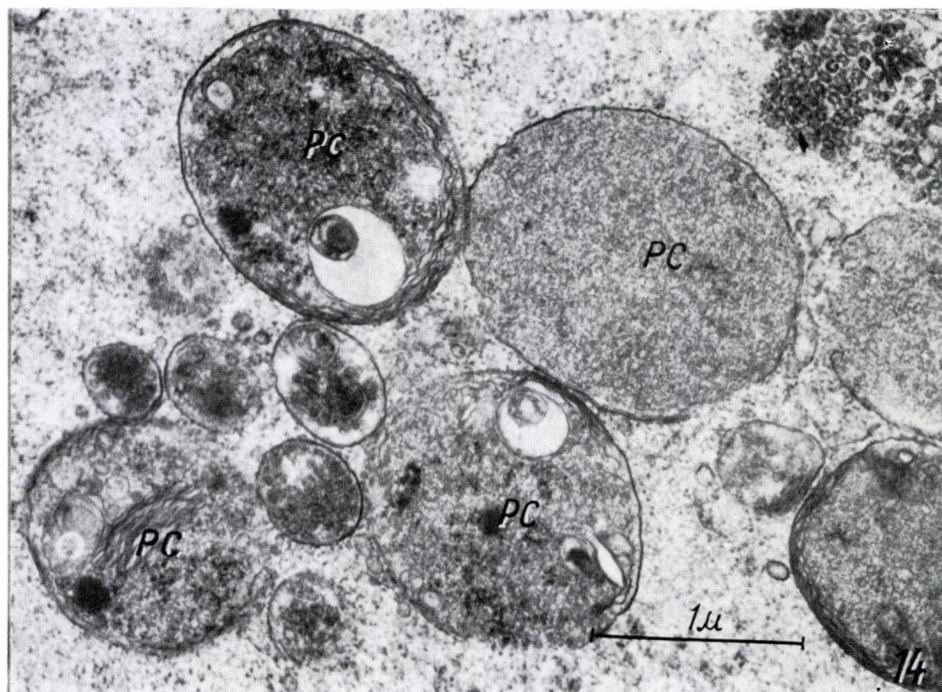


Fig. 14. Precytosomes (PC) in an epithelial cell of the lateral pit. $\times 30\ 000$.

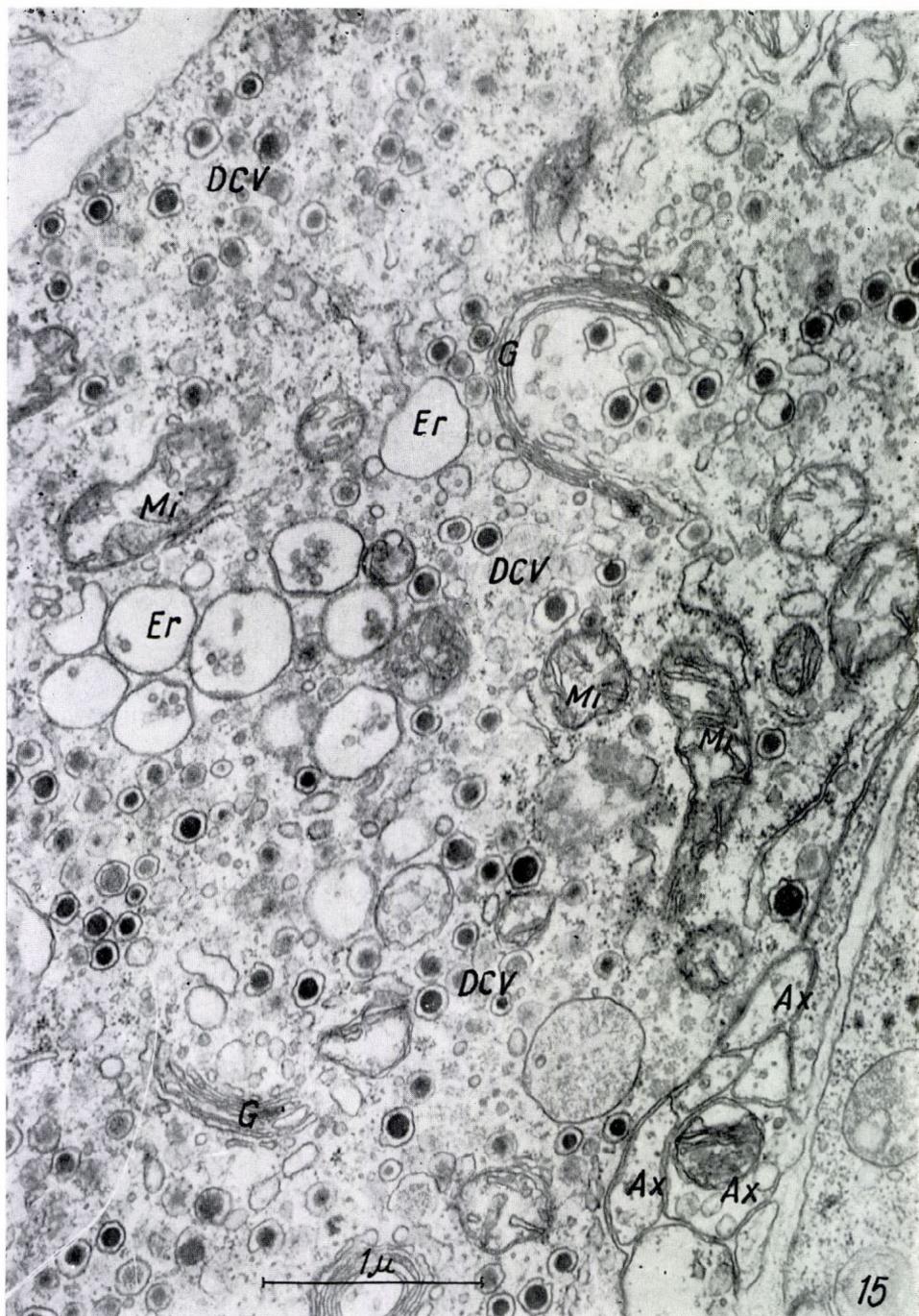


Fig. 15. Detail of a differentiated neuron in the foot fold. G — Golgi apparatus, DCV — dense-core vesicles, Er — endoplasmic reticulum, Mi — mitochondria, Ax — axons coming from other neurons. $\times 30\ 000$.

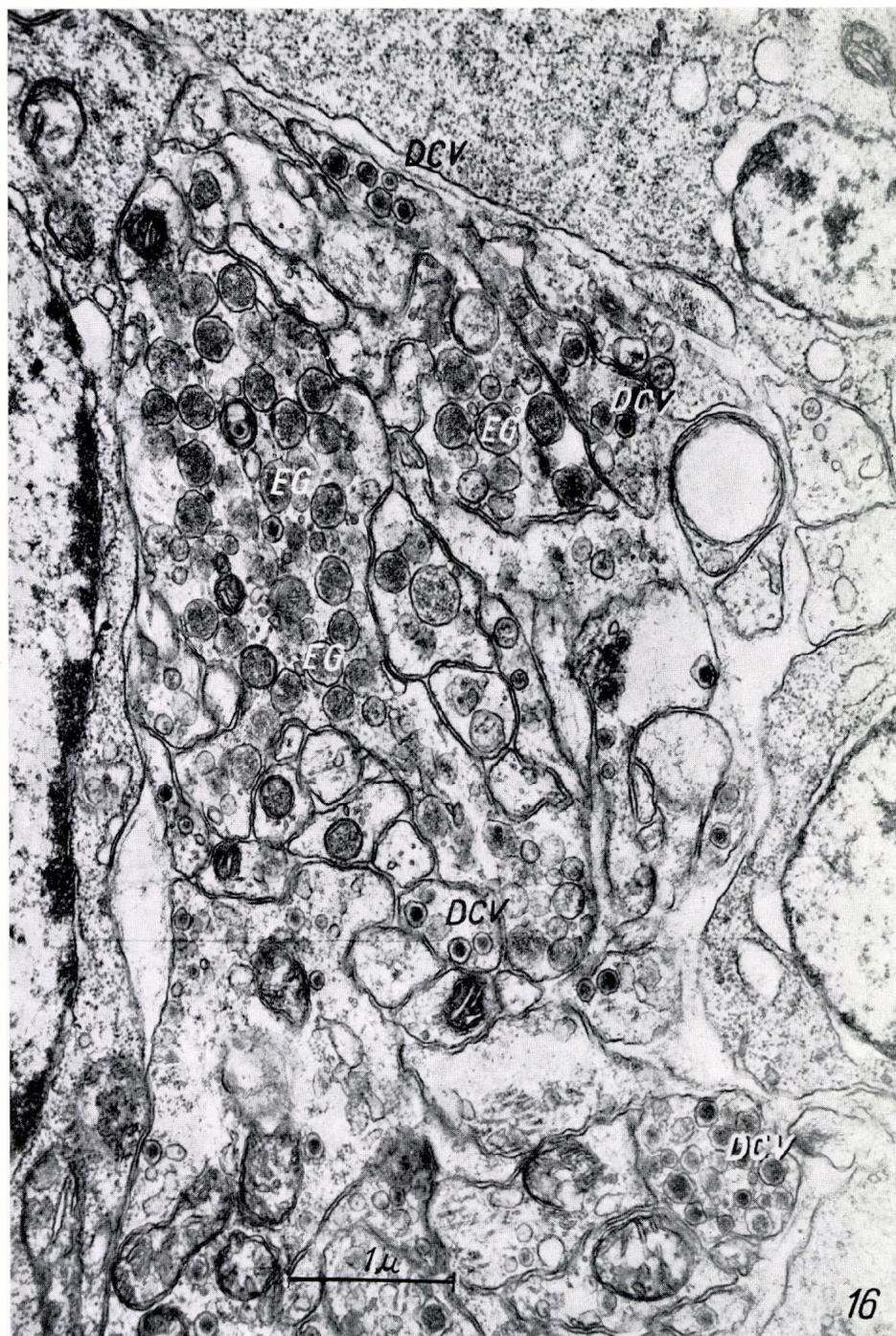


Fig. 16. Neuropile-like clusters of fibres from the foot fold. DCV — dense-core vesicles, EG — elementary neurosecretory granules, $\times 25\ 000$.

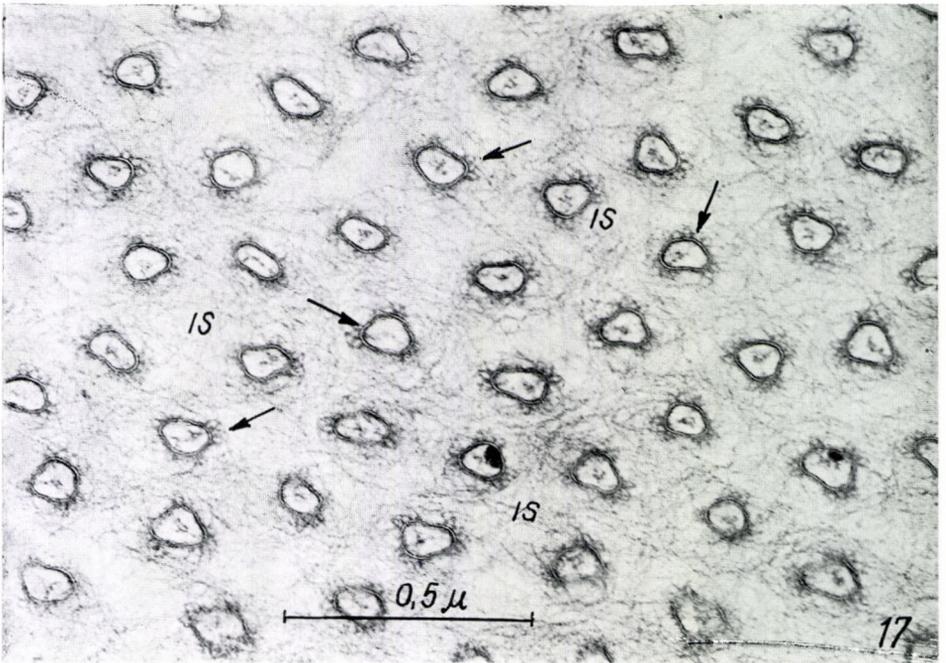


Fig. 17. Cross section of microvilli of epithelial cells from the mantle. IS — intervillous substance forming vesicles near the membrane of microvilli in some places (arrows). $\times 68\ 500$.

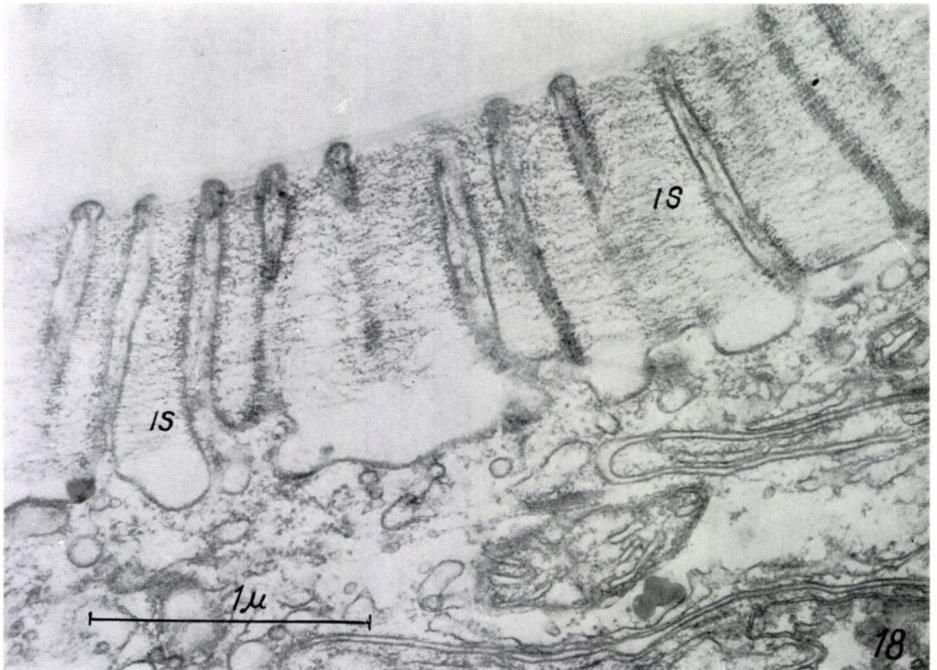


Fig. 18. Longitudinal section of microvilli. IS — intervillous substance. $\times 39\ 000$

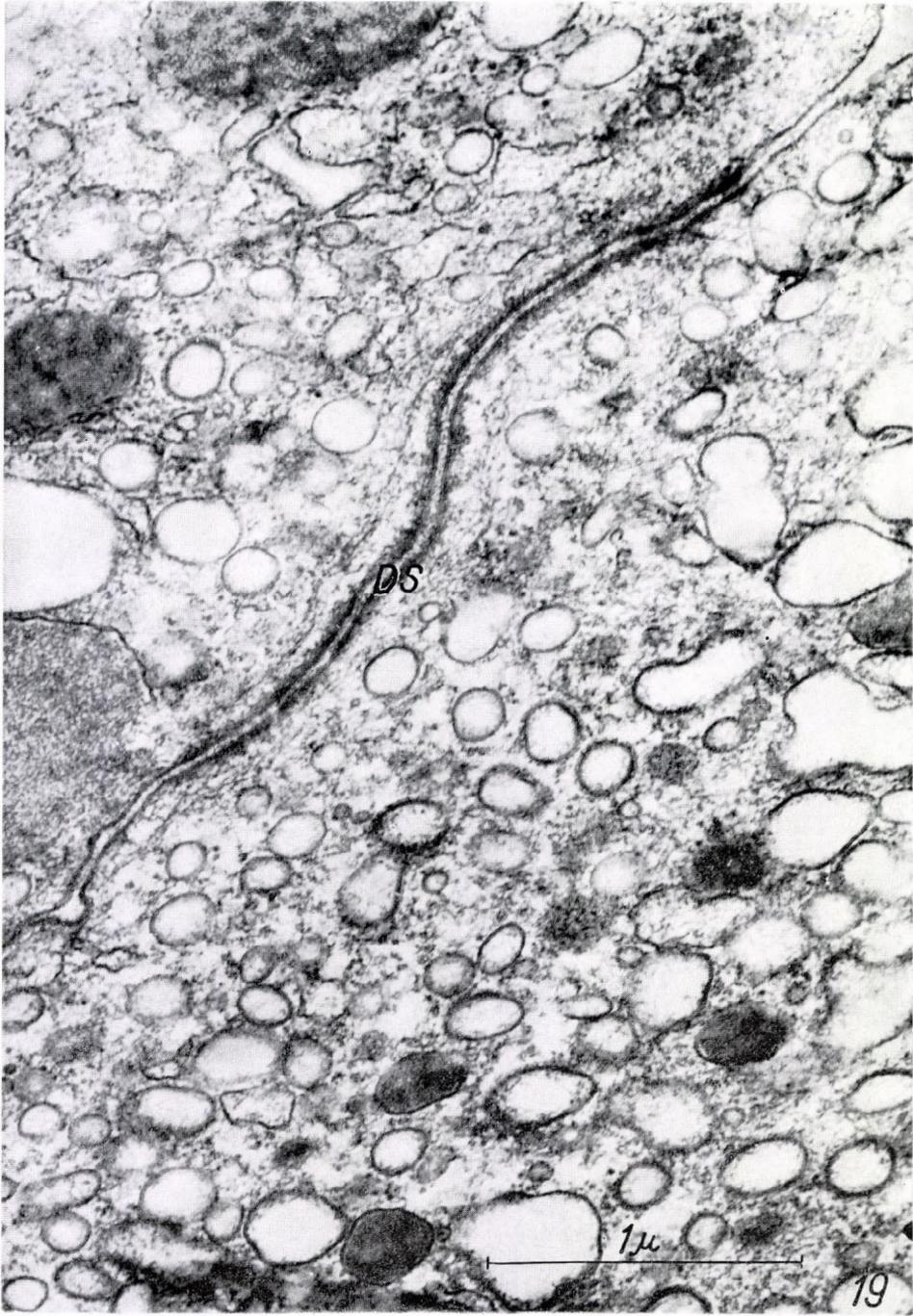


Fig. 19. Long desmosome (DS) connecting epithelial cells of the mantle as well as the typical details of epithelial cells. $\times 45\ 000$.

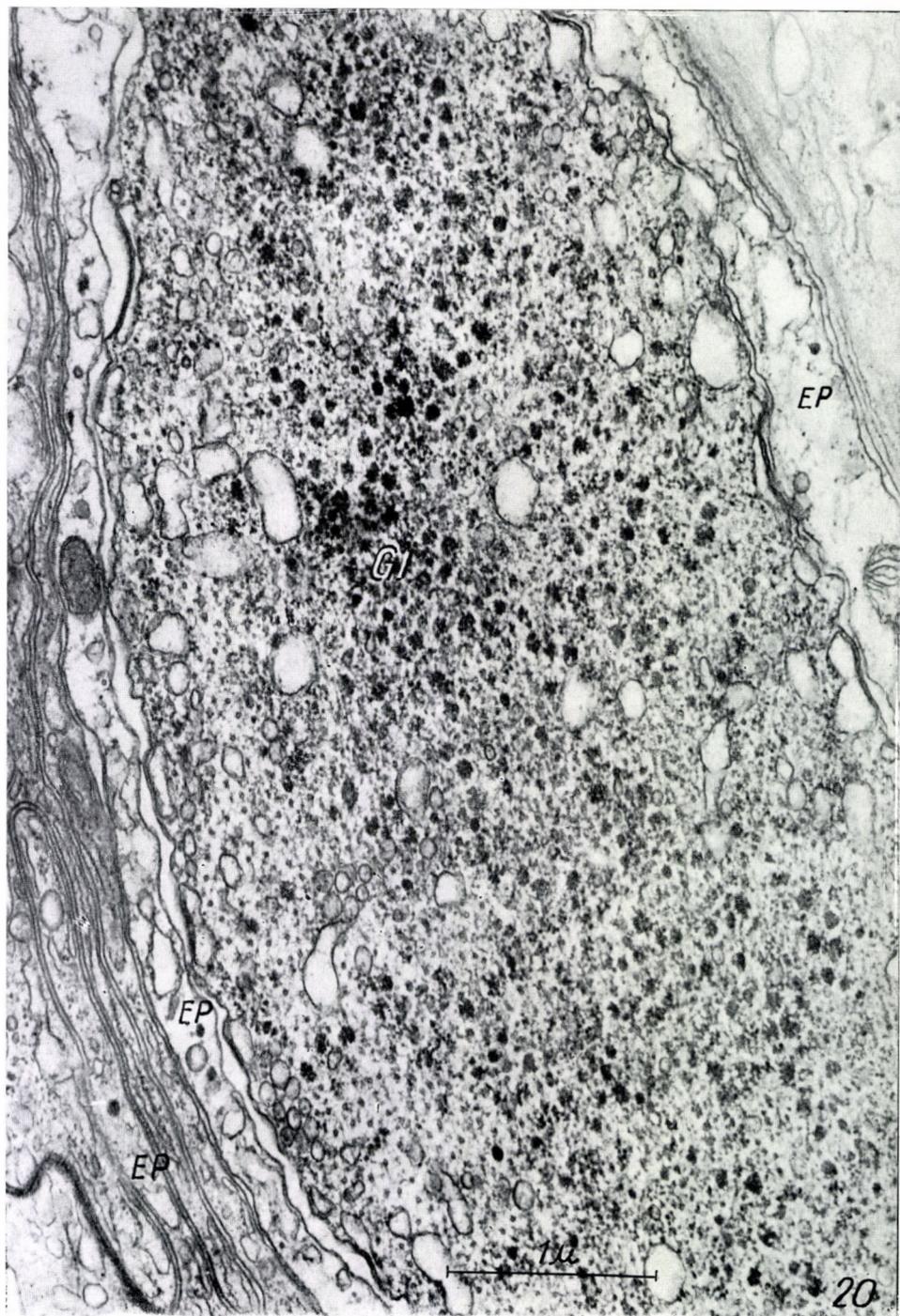


Fig. 20. Epithelial cell having no contact with the surface, containing glycogen granules, surrounded by processes (EP). $\times 30\,000$.

The epithelial cells of the mantle contain a great number of vacuoles and granules of different sizes and density. Golgi apparatuses of a very rich in structure also frequently occur in them. Near the surface the cells are connected by desmosome-like structures while in the deeper regions small intercellular bridges can be seen between the membranes (*Fig. 19*). Certain epithelial cells last their contact with the surface, became branched and their processes can be traced far in the sections. Sometimes in these cells a number of dense granules is accumulated, resembling to the glycogen granules (*Fig. 20*).

Discussion

The histological methods used did not help us to know more about the nervous system of the larva than the old authors. Even the chrome-haematoxylin-phloxin staining (BARGMANN, 1949) was not suitable to differentiate between the neural and other elements. Therefore, in the identification of the structures observed we could rely only on the electron microscopic data and the interpretation was based partly on the nervous system of the adult mussels and partly on the general neurobiological knowledge.

On the basis of its morphology the adductor muscle can be classified as a molluscan muscle of the tropomyosin type (KOMNIZ et al. 1957). There is, however, a significant difference in the diameter of myofilaments. While in the adductor muscle of the adult mussel the diameter of thick filaments touches even the 800 Å (own unpublished observation) in the case of glochidia it is not more than 200 Å. The regular arrangement of the filaments is, however, more striking than in the adult animals. At the same time the phasic contraction and relaxation of the larval adductor is significantly faster as compared to that of the adult animals (LÁBOS, 1964b).

The presence of axon-like structures in the adductor unequivocally indicates that the adductor has a motor innervation. The reality of the observed neuromuscular junctions is beyond doubt. It is noteworthy that the synapses are always of the same structure, i. e. the larval adductor has only one kind of innervation at least morphologically.

The presence of motor innervation has been presumed on the basis of response of adductor, under electric stimulation, for there exists a polarized excitability of direction differing from that of the adductor (LÁBOS, 1964a, 1967). The thick axons are probably the equivalent of that excitable structure running perpendicular to the adductor muscle.

During the previous pharmacological investigations of the adductor response there was a failure of distinction between the myogenic and neurogenic character of the response (LÁBOS and SALÁNKI, 1963, LÁBOS et al. 1964, LÁBOS, 1966). Stimulating effects were to be produced with tryptamine, scopolamine, and cocaine as well as with β -adrenergic antagonists (LÁBOS et al. 1964, LÁBOS, 1966, 1969). Acetylcholine, d-tubocurarine and nicotine were ineffective while atropin in large doses (1 mM) had a stimulating effect, (LÁBOS et al. 1964, LÁBOS, 1969). On the basis of all these data a non-cholinergic, tryptaminergic regulation was presumable. The effect of tryptamine, scopolamine and cocaine was markedly potentiated by the catecholamines (adrenaline, noradrenaline, dopamine) but alone they are ineffective. The morphology of

synaptic vesicles, found in larval adductor, renders the cholinergic mediation improbable for they are significantly greater than the well known cholinergic synaptic vesicles. Furthermore, since the nerve endings innervating the adductor contain mostly empty vesicles the failure of direct adrenergic effect becomes clear. Of course on the basis of our investigations we can not decide whether the effect of endogenous or exogenous tryptamine takes place on the site of neuromuscular transmission, on neural elements or directly on the sarcolemma.

The data relating, though indirectly, to the presence of adrenergic regulation (LÁBOS et al. 1964, LÁBOS, 1966, 1969) agree with the mass occurrence of dense-core vesicles in the axons found outside of the adductor muscle, for the adrenergic character of dense-core vesicles is evidenced in adult animals (Zs.-NAGY, 1967).

After all, the neurohumoral regulation of the adductor muscle may be imagined as a diffuse network connected with processes, giving the efferent innervation. Tryptamine exerts its effect on a yet unknown point of this system. The cells and processes containing dense-core vesicles probably control this motor system directly or indirectly or perhaps in a humoral way. Seeing that the cells containing dense-core vesicles are localized in the ganglion anlagen while processes of similar nature generally also occur in the larval mantle, it should be assumed the differentiated cells of the ganglion anlagen take part in the indirect regulation of the larval adductor muscle. According to our results the ganglion anlagen mentioned only as epithelial thickenings by HERBERS (1913), contain also differentiated elements which are functionally not independent from the larval organs. This may be the reason why HERBERS found significantly developed ganglion anlagen at the very beginning of the metamorphose, taking over the regulation of functions after desintegration of the larval nervous system.

The sensory cells were known by old authors (LILLIE, 1895), too. Their fine structure is described in our present paper. It is noteworthy that the microvilli characteristic for the common epithelial cells and the cilia appear together on their surface. This refers to the relationship with the epithelial cells of resorptive function as well as to the incomplete differentiation of functions. The tactile excitability of the cilia has been evidenced (AREY, 1921, LUKACSOVICS and LÁBOS, 1965). We are in the dark concerning the role of the peculiar structures of cilia observed. Striated ciliary rootlets have also been found by FAWCETT and PORTER (1954) in the epithelia of gill and intestine of the adult mussels, however, their function is not clear either.

Because of the presence of microvilli the sensory cell may particularly be suitable to intake chemical stimuli in the same way as other resorptive epithelial cells. The quick conduction of the excitation, however, is probably better guaranteed by the special structure and environment of the sensory cell than in the resorptive cells. The processes observed in neighbourhood of the sensory cell most likely represent an afferentation. Thus, the sensory cell must be regarded as a secondary receptor cell.

Our investigations reveal some morphological phenomena interesting also from other points of view. Thus, we succeeded in observing surprisingly clear the direct contact between the outer layer of the nuclear membrane and the membranes of granular endoplasmic reticulum in the undifferentiated cells of the ganglion anlagen. It seems likely that these membranes do not come into being *de novo*, but they develop from the nuclear membrane. We are of

the opinion that our results concerning the structure of microvilli as well as the constitution of the larval mantle are of interest from the point of view of better knowledge of larval functions adapted to parasitic life.

Summary

The larval adductor muscle of *Anodonta cygnea* can be classified as a molluscan muscle of the tropomyosin type. The diameter of its thick myofilaments is about 200 Å, while that of the thin ones is 40 Å. The thick myofilaments show a hexagonal arrangement of about 800–1400 Å lateral length. Some dense bodies also occur. A number of neuromuscular junctions can be observed in the adductor. The nerve endings contain a lot of generally empty-core vesicles of about 400–1200 Å. The main direction of axons is perpendicular to the longitudinal axis of the muscle cells. The axons correspond to the processes of cells situated dispersedly under the larval mantle. The processes of these cells are connected to each-other by synapse-like structures containing the same kind of vesicles as those of the neuromuscular junctions.

In the ganglion anlagen, localized in the wall of lateral pits and foot fold, differentiated, branching cells containing dense-core vesicles can also be found. The processes of these cells form primitive neuropile-like clusters of fibres. Processes containing dense-core vesicles can also be found dispersedly outside of the ganglion anlagen but never occur in the adductor muscle.

The sensory cells bear specific cilia on their surface, which are connected with striated ciliray rootlets. The sensory cell itself has no processes, however, there is a lot of other processes surrounding it.

The possibility of functional interpretation of the structures observed on the basis of physiological data is discussed and several other ultrastructural characteristics of the glochidia are also pointed out.

REFERENCES

- AWEY L. B. (1921): An experimental Study on Glochidia and the factors underlying encystment. — *J. Exp. Zool. Philadelphia* **33**, 463–492.
- BARGMANN W. (1949): Über die neurosekretorische Verknüpfung von Hypothalamus und Neurohypophysis. — *Z. Zellforsch.* **34**, 610–634.
- BARNES G. E. (1955): The behaviour of *Anodonta cygnea* L. and its neurophysiological basis. — *J. Exp. Biol.* **32**, 158–174.
- BENNETT H. S., J. H. LUFT (1959): S-collidine as a basis for buffering fixatives. — *J. Biophys. Biochem. Cytol.* **6**, 113–114.
- FAWCETT D. W., K. R. PORTER (1954): A study of the fine structure of ciliated epithelia. — *J. of Morphology* **94**, 221–281.
- HERBERS K. (1913): Entwicklungsgeschichte von *Anodonta cellensis* SCHRÖT. — *Z. f. wiss. Zool.* **103**, 1–174.
- KOMNIZ D. R., F. SAAD, K. LAKI (1957): Vertebrate und invertebrate tropomyosins. — *Nature* **179**, 206–207.
- KOSHTOYANTS H. S., J. SALÁNKI (1958): On the physiological principles underlying the periodical activity of *Anodonta*. — *Acta Biol. Acad. Sci. Hung.* **8**, 361–366.
- LÁBOS E., J. SALÁNKI (1963): The effect of alkali metal ions and alkaline earth metal ions on the rhythmic activity of fresh-water mussel *Anodonta cygnea* L. — *Annal. Biol. Tihany* **30**, 45–57.
- LÁBOS E. (1964a): Studies on the electric excitability of the adductor muscle of glochidia. — *Annal. Biol. Tihany* **31**, 27–37.

- LÁBOS E. (1964b): A new method for recording the rhythmic activity of adductor of larvae of fresh-water mussel (glochidia). — *Annal. Biol. Tihany* **31**, 23–26.
- LÁBOS E., J. SALÁNKI, G. R. KLITYINA (1964): The effect of cholinotropic drugs on the rhythmic activity of glochidia of fresh-water mussel (*Anodonta cygnea* L.). — *Acta Biol. Acad. Sci. Hung.* **15**, 119–128.
- LÁBOS E., J. SALÁNKI, K. S.-RÓZSA (1964): Effect of serotonin and other bioactive agents on the rhythmic activity in the glochidia of fresh-water mussel (*Anodonta cygnea* L.). — *Comp. Biochem. Physiol.* **11**, 161–172.
- LÁBOS E. (1966): Contributions to the mechanism of tryptamine effect on the adductor activity of fresh-water mussel larvae. — *Annal. Biol. Tihany* **33**, 13–35.
- LÁBOS E., B. TURCSÁNYI (1966): On primary and secondary processes of photoinduced muscle responses in *Anodonta*-larvae. — *Annal. Biol. Tihany* **34**, 45–60.
- LÁBOS E. (1967): On the mechanism of anisotropic excitability for the adductor response of glochidia. — *Neurobiology of Invertebrates Proceedings of a Symposium at Tihany. Akadémiai Kiadó, Budapest.* pp: 293–301.
- LÁBOS E. (1970): N,N-dialkyl triptaminok, 5-metoxi-triptamin, β -adrenerg-antagonisták, kokain, szkopolamin és egyéb farmakonok aktiváló hatása *Anodonta*-glochidiumok záróizomtevékenységére. — *Annal. Biol. Tihany* In press.
- LILLIE F. A. (1895): The embryology of the Unionidae. — *J. Morph.* **10**, 1–90.
- LUKACSOVICS F., E. LÁBOS (1965): Chemo-ecological relationship some fish-species of Lake Balaton and the glochidia of *Anodonta cygnea* L. — *Annal. Biol. Tihany* **32**, 37–54.
- PAVLOV I. P. (1895): Wie die Muschel ihre Schale öffnet. — *Pflügers Archiv.* **37**, 6–31.
- RAVEN CHR. P. (1958): Morphogenesis. An Analysis of Molluscan Development. — *Pergamon Press.*
- REYNOLDS E. S. (1964): The use of lead citrate in electron microscopy. — *J. Cell. Biol.* **17**, 208–212.
- S.-RÓZSA K., E. LÁBOS (1967): Biologically active compounds in the glochidia of *Anodonta cygnea* L. I. Identification of tryptamine and some amino acids by paper chromatography. — *Annal. Biol. Tihany* **34**, 51–57.
- SALÁNKI J. (1961): Role of afferentation in the regulation of the rhythm in the periodic activity of fresh-water mussels. — *Acta Biol. Acad. Sci. Hung.* **12**, 161–167.
- SALÁNKI J. (1963): The effect of serotonin and catecholamines on the nervous control of periodic activity in fresh-water mussel (*Anodonta cygnea* L.). — *Comp. Biochem. Physiol.* **8**, 163–171.
- SCHIERHOLZ (1888): Ueber Entwicklung der Najaden. — *Arch. f. Naturg.* **51**, Cit. by LILLIE (1895).
- TÖRÖK L., LÁBOS E. (unpublished): Glochidiumok valószínű ingerlékeny struktúráinak vitális festése.
- ZS.-NAGY I. (1967): Histochemical and electron microscopic studies on the relation between dopamine and dense core vesicles in the neurons of *Anodonta cygnea* L. — *Neurobiology of Invertebrates Proceedings of a Symposium at Tihany. Akadémiai Kiadó, Budapest.* pp: 69–84.
- ZS.-NAGY I. (1969): The morphogenesis of cytosomes in the neurons of *Anodonta cygnea* L. (Mollusca, Pelecypoda). — *Acta Biol. Hung.* (in press).

SZÖVETANI ÉS ELEKTRONMIKROSKÓPOS VIZSGÁLATOK
AZ *ANODONTA CYGNEA* L. LÁRVÁJÁNAK ZÁRÓIZMÁN
ÉS IDEGELEMEIN

Zs.-Nagy Imre és Lábos Elemér

Összefoglalás

Az *Anodonta cygnea* L. lárvális záróizma a tropomyosin típusú puhatestű izmok közé sorolható. Vastag myofilamentumainak átmérője 200 Å, a vékonyaké 40 Å. A vastag filamentumok többnyire 800–1400 Å oldalhosszúságú, hatszögű elrendeződést mutatnak. Densz-bolyok is megtalálhatók. A záróizomban számos neuromuscularis junctió figyelhető meg. Az idegvégzőlésekben 400–1200 Å átmérőjű, általában üres központi vezikulák vannak. A záróizmot beidéző axonok fő lefutási iránya merőleges az izomszövetek hossz tengelyére. Az axonok a köpeny alatt szétszóróan elhelyezkedő sejtek

nyúlványainak felelnek meg. E sejtek nyúlványai egymással is szinapszis-szerű kapcsolatban állnak, amelyekben ugyanolyan vezikulák láthatók, mint a neuromuscularis junctióban.

Az oldalgödör falában és a lábdundorban elhelyezkedő gangliontelepekben már differenciálódott, dense-core vezikulákat is tartalmazó nyúlványos sejtek is fellelhetők. Ezeknek nyúlványai primitív, neuropil-szerű rosttömörülést is képeznek. Dense-core vezikulákat tartalmazó nyúlványok szétszórta a gangliontelepeken kívül is megtalálhatók, de a záróizomban nem fordulnak elő.

Az érzékelősejtek speciális csillókat hordanak a felszínükön, amelyekhez harántcsikolt gyökérostok tartoznak. Maga az érzékelő sejt nem nyúlványos, de körülötte számos más nyúlvány található.

Szerzők diszkutálják a talált struktúrák funkcionális értelmezésének lehetőségeit fiziológiai adatok alapján, továbbá rámutatnak a glochidium ultrastruktúrájának néhány további jellegzetességére is.

ГИСТОЛОГИЧЕСКИЕ И ЭЛЕКТРОННОМИКРОСКОПИЧЕСКИЕ ИССЛЕДОВАНИЯ ЗАПИРАТЕЛЬНЫХ МЫШЦ И НЕРВНЫХ ЭЛЕМЕНТОВ ГЛОХИДИЕВ БЕЗЗУБКИ

И. Ж.-Надь и Э. Лабаш

Личиночную запирательную мышцу беззубки можно отнести к тропомиозиновому типу мышц моллюсков. Диаметр толстых миофиламентов 200 Å а тонких 40 Å. Толстые филаменты показывают шестиугольную упорядоченность, которая характеризуется 800—1400 Å боковой длиной. Обнаруживаются и dense bodies. В запирательной мышце наблюдаются многочисленные нервно-мышечные контакты. В нервных окончаниях имеются везикулы диаметром 400—1200 Å, средняя часть которых обычно пуста. Главное направление нервных волокон, иннервирующих запирательную мышцу, перпендикулярно продольной оси мышечных клеток. Волокна соответствуют отросткам клеток, расположенных разбросано под мантией. Эти отростки создают друг с другом контакты, напоминающие синапсы, и содержат такие же везикулы, как и нервно-мышечная связь.

В стене боковой ямки и ганглиозных массах, находящихся в зачатке ноги, обнаруживаются дифференцированные клетки с отростками, содержащие и dense-core везикулы. Отростки этих клеток образуют примитивное нейропил-подобное накопление волокон. Отростки, содержащие dense-гранулы, расположены рассеяно и вне ганглиозных масс, но в самой запирательной мышце их обнаруживать не удалось.

Поверхность чувствующих клеток снабжена специальными ресничками, связанными поперечно-полосатыми корешками. Чувствующие клетки не обладают отростками, но в их окружности было найдено большое количество отростков иного происхождения.

Авторы обсуждают возможное функциональное значение обнаруженных структур на основе физиологических данных и дают некоторую дальнейшую характеристику ультраструктуры глохидиев.