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THE FINE STRUCTURE OF NEUROMUSCULAR AND INTERMUSCULAR CONNECTIONS IN THE ADDUCTORS OF ANODONTA CYGNEA L. (MOLLUSCA, PELECYPODA)

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The innervation of adductors in bivalves has only been studied at light microscopic level by silver impregnations, methylene blue staining or cholinesterase histochemistry (BOWDEN and LOWY, 1955; BOWDEN, 1958; ÁBRAHÁM and MINKER, 1957, 1959). The published data are contradictory first of all because of the irreproducibility of the impregnation and staining methods. On the other hand, that time the cholinesterase activity was generally believed to indicate only nervous elements. Later, however, it became clear that in the adductors of mussels it is not the case (ZS.-NAGY, 1964a). Therefore, the histological investigations could only evidence that the adductors contain nerve fibres. According to BOWDEN (1958) neuromuscular end plates are present; others could not confirm his findings (ÁBRAHÁM and MINKER, 1957, 1959); BOWDEN (1958) described also nerve cells in the adductors whereas the latter authors denied this finding categorically.

Starting from the above mentioned contradictions we wanted to investigate electron microscopically the structure of axons and neuromuscular junctions as well as the presence or absence of nerve cells. Since, however, during the investigations light has been thrown to some intermuscular connections which can be significant in the muscle function, we treat them also here.

Material and method

The investigations were carried out on the 12—20 cm long specimens of *Anodonta cygnea* L. The adductors were fixed as follows:

a) 2% OsO_4 buffered with s-collidine at pH 7.2 (BENNETT and LUFT, 1959) for 2 hours at 0 °C.

b) 2.5% glutaraldehyde in tap water at pH 7.0 for 16—20 hours at room temperature. The glutaraldehyde was purified by ion-exchange according to VADÁSZ (1966). After the glutaraldehyde fixation we washed the material in tap water for 2 hours than it was postfixed in 2% OsO_4 for 1—2 hours.

After fixation the material was dehydrated by ethanol and embedded in Araldite (Durcupan ACM, Fluka) on the usual way. The sections were cut on an LKB Ultrotome III and contrasted with uranylacetate and lead citrate (REYNOLDS, 1963). The micrographs were taken with a TESLA BS 413A electron microscope.

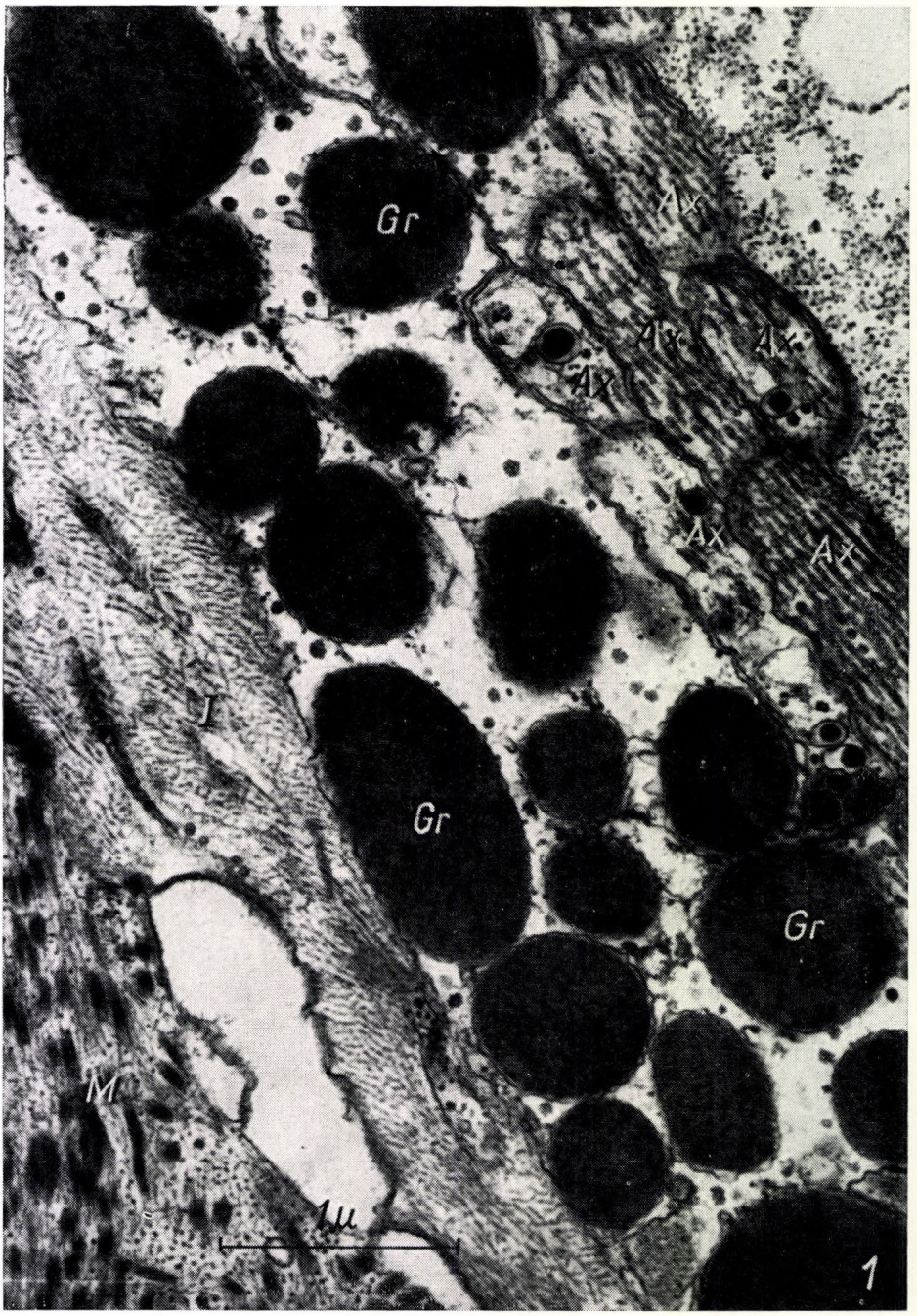

Fig. 1. Nerve fibres (Ax) in the white part of the posterior adductor. Gr — large granules in the cytoplasm of the special cell adjoining the axons. I — interstitial connective tissue; M — detail of a muscle cell. ×30,000

Results

1. The structure of innervating axons

The nerve branches consist of several axons having neither myelin nor Schwann sheaths. There frequently occur cells of special structure containing in their cytoplasm large ovale-shaped granules of high electron density *(Fig. 1)*. Neither these cells form a closed sheath around the axons, only adjoin them on certain places. The structure of axoplasm varies. In some places a lot of parallel arranged microtubuli are to be seen *(Fig. 2)*, however, numerous dense-cored vesicles (DCV) or empty vesicles also occur among them. The vesicles are also present in other parts of axons devoid of microtubuli. The thickness of axons is different; from several tenths of micron it can reach 1—2 micron. The axons of larger diameter have usually a very poor axoplasm.

2. The structure of neuromuscular junction

In some places between the muscle cells solitary axonal enlargements filled in with vesicles can be observed. They usually adjoin muscle cells and correspond to the nerve endings *(Fig. 3)*. On the place of contact both the axolemma and the sarcolemma are intact and of normal thickness. The intermembranic cleft is about 200 Å in width. In the nerve ending clusters of vesicles are frequently seen near the point of contact. The vesicles are always mixed, empty and DCV forms occur together, their diameter is about 400—1200 Å. Sometimes we could observe some morphological characteristics suggesting the fusion of vesicles with the presynaptic membrane *(Fig. 3)*. There are endings forming contacts with more than one muscle cells. The postsynaptic part never showed any specialization.

3. The presence of nerve cells

In either adductors both in white and yellow parts we observed cells *(Fig. 4)* the cytoplasm of which bears a close resemblance to that of the central neurones of mussels. These cells occur individually or in small groups usually together with axons. Sometimes the axons show a neuropile-like gathering near to these cells *(Fig. 4)*. The cells are rather small, we failed to observe any larger than 20 μ. The low number of cytosomes in these cells represents a difference as compared to the central neurones. The neurones of the adductors contain also dense-cored vesicles *(Fig. 5)*. The fibrocytes of the muscular interstitium can be strictly distinguished from the nerve cells on the basis of their characteristic rough endoplasmic reticulum being absent in the nerve cells.

4. The structure of intermuscular connections

Usually between two muscle cells there is a narrow intercellular space filled in by atypic collagen fibres. This space in some places has dilatations containing also fibrocytes. The sarcolemma here and there shows some specific structures resembling desmosomes *(Fig. 6)*. The cell membrane is trilaminated, on its internal side there is a dense material and the membrane itself

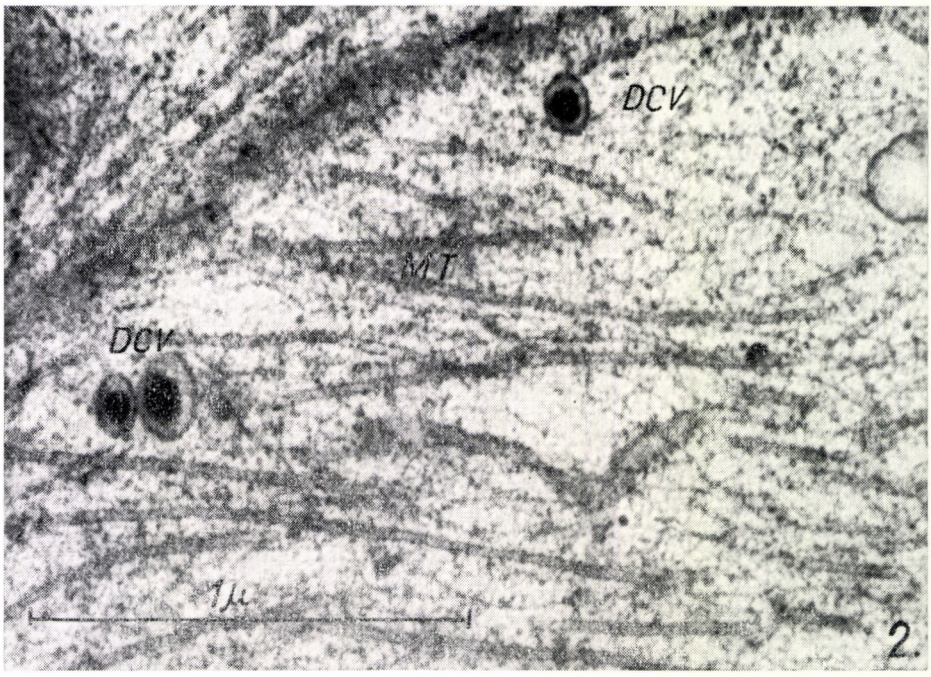

Fig. 2. Structure of the axoplasm in the white part of the posterior adductor. MT — microtubuli, DCV — dense-cored vesicles. ×58,500

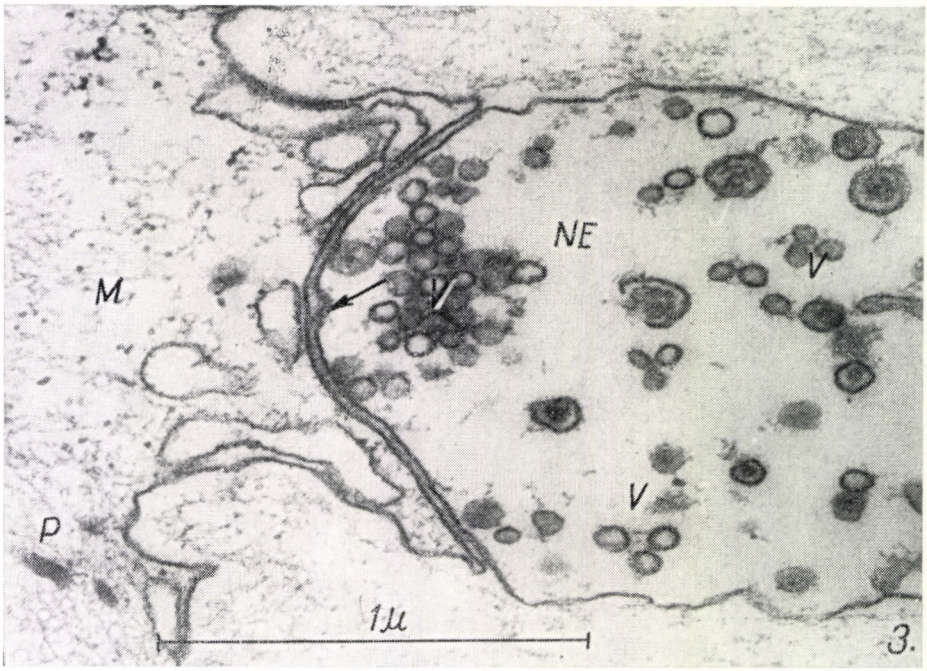

Fig. 3. Neuromuscular junction in the anterior adductor. NE — nerve ending; V — synaptic vesicles; M — muscle cell; P — paramyosin filament. The arrow indicates a possible place uf fusion of vesicles into the presynaptic membrane. ×58,500

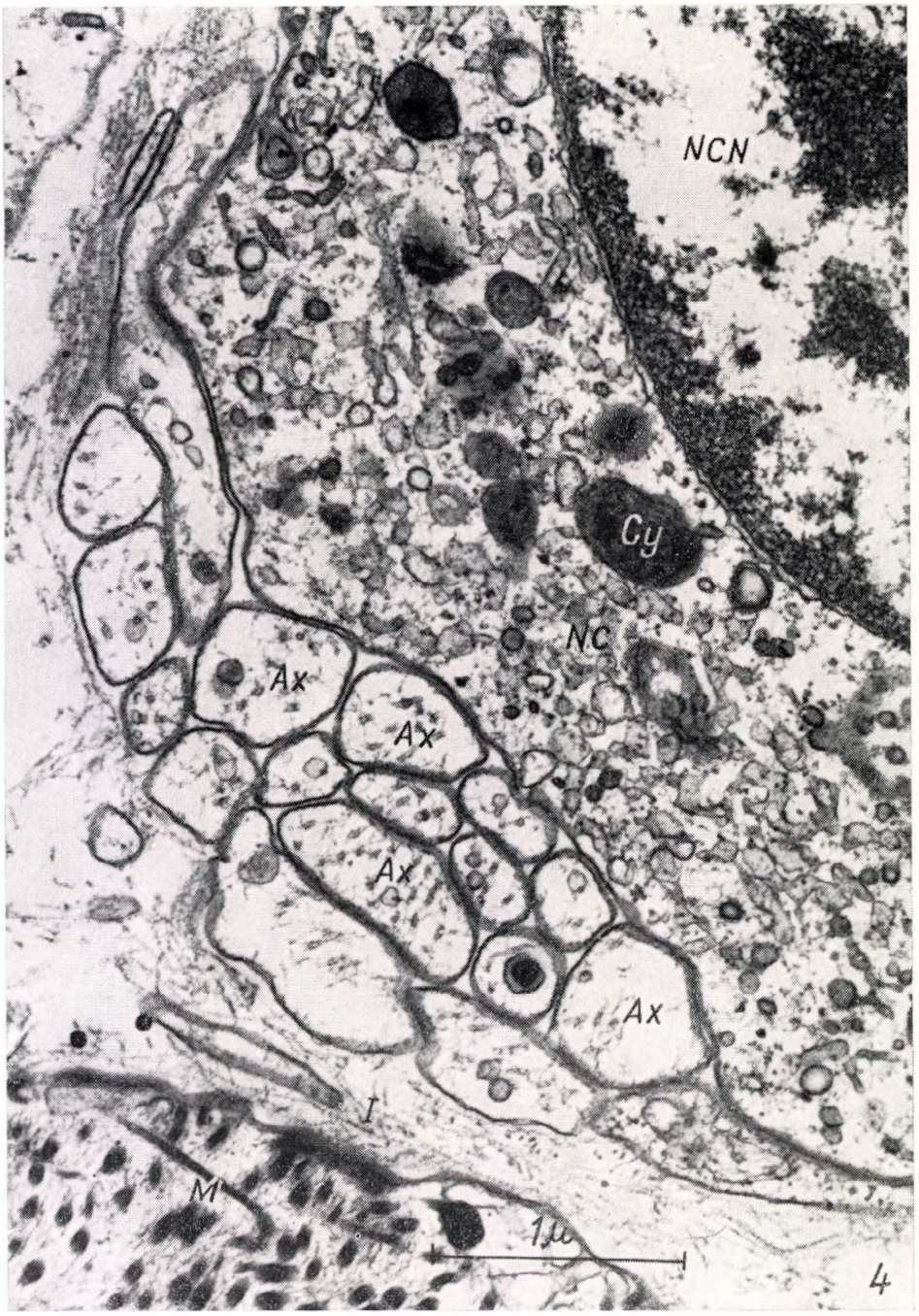

Fig. 4. Detail of a nerve cell in the posterior adductor (NC). NCN — nerve cell nucleus; Cy — small cytosome; AX — axons contacting with the nerve cell; M — muscle cell; I — interstitium. ×35,000

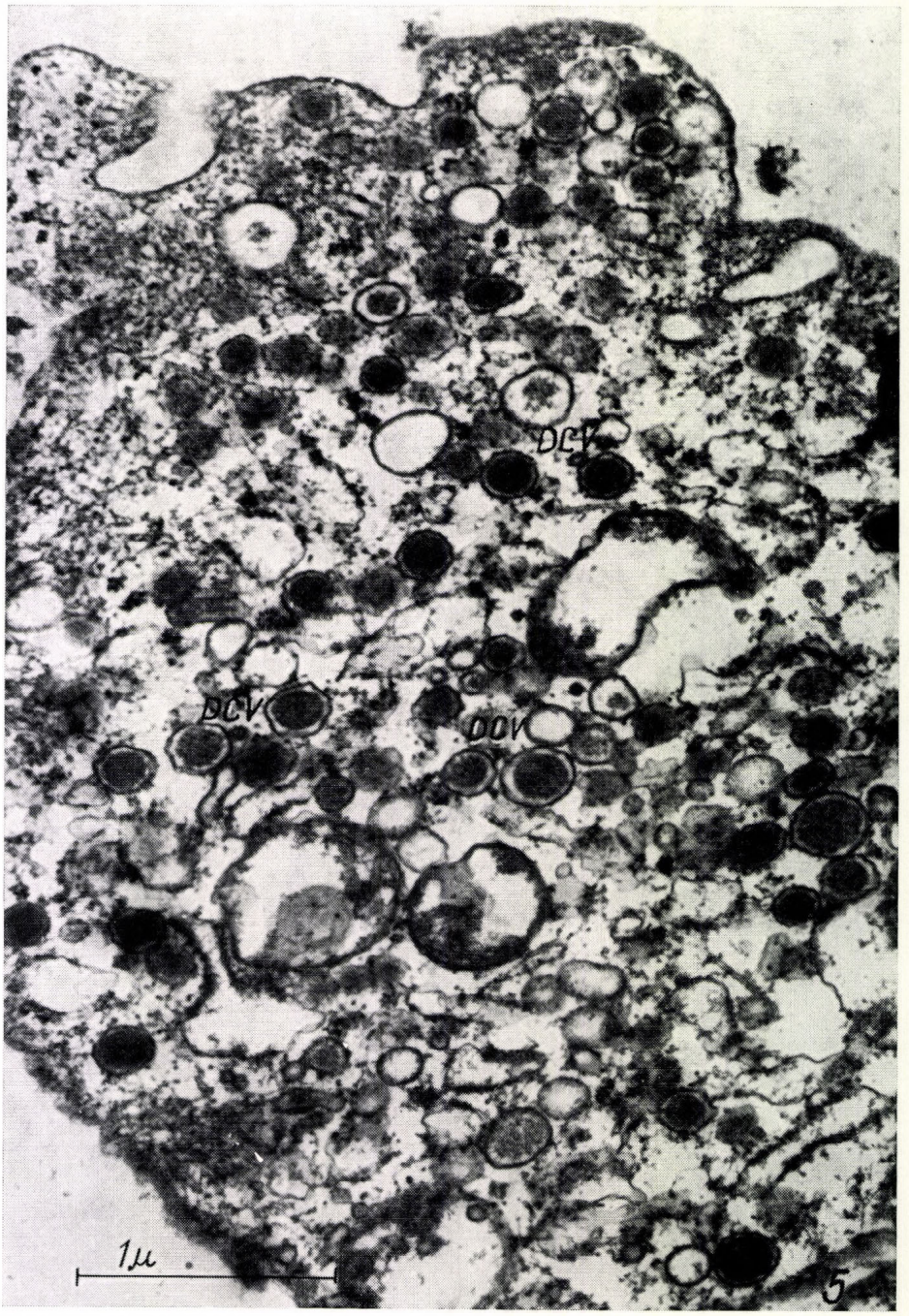

Fig. 5. Dense-cored vesicles (DCV) in the neurones of the posterior adductor. Their diameter is about 2000 Å. ×35,000

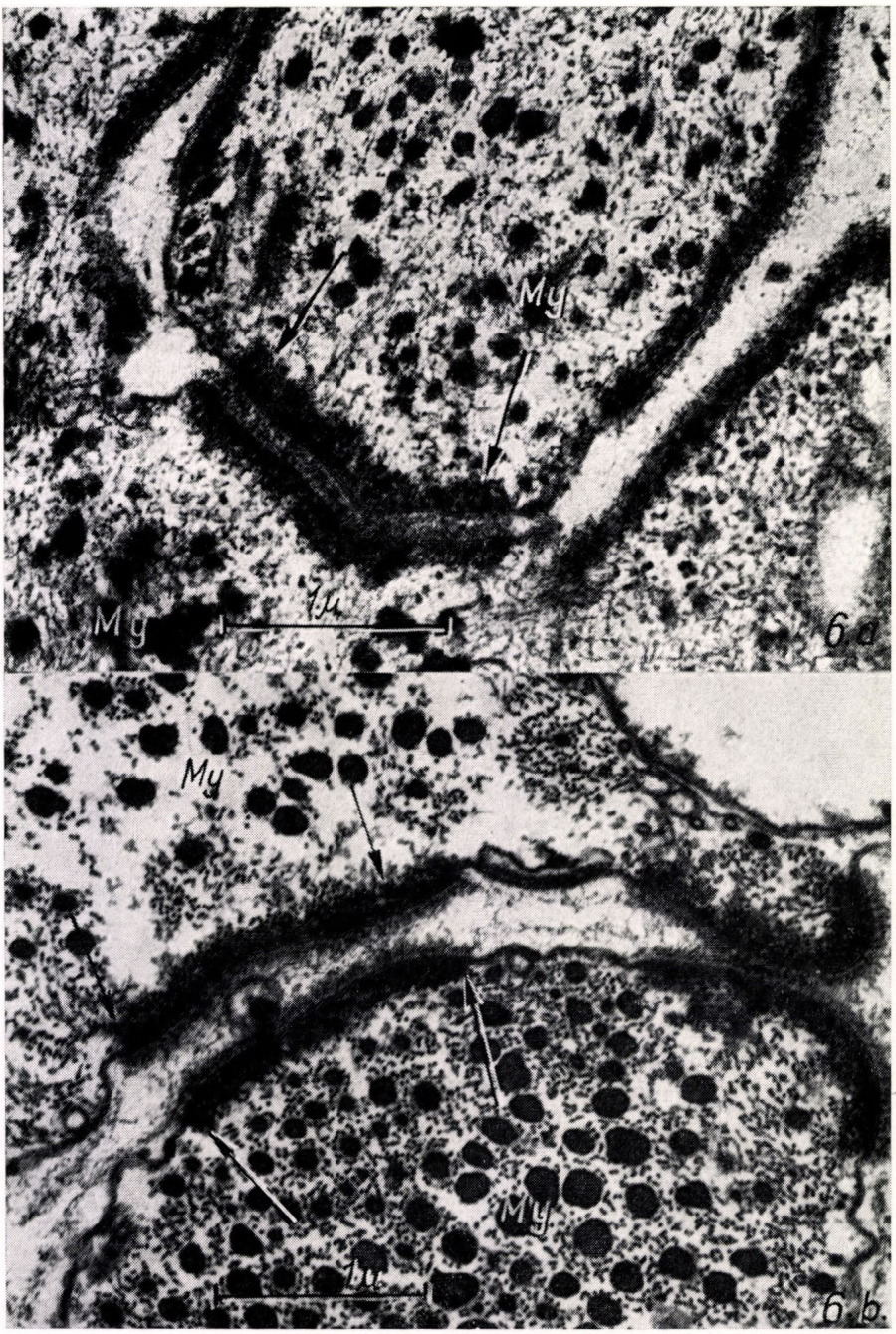

Fig 6. a) Desmosome between two muscle cells (arrows) in the white part of the posterior adductor. My — myofilaments, ×30,000. b) Half-desmosomes from the same muscle (arrows). On the right side of the picture partially connected half-desmosomes are to be seen. ×30,000

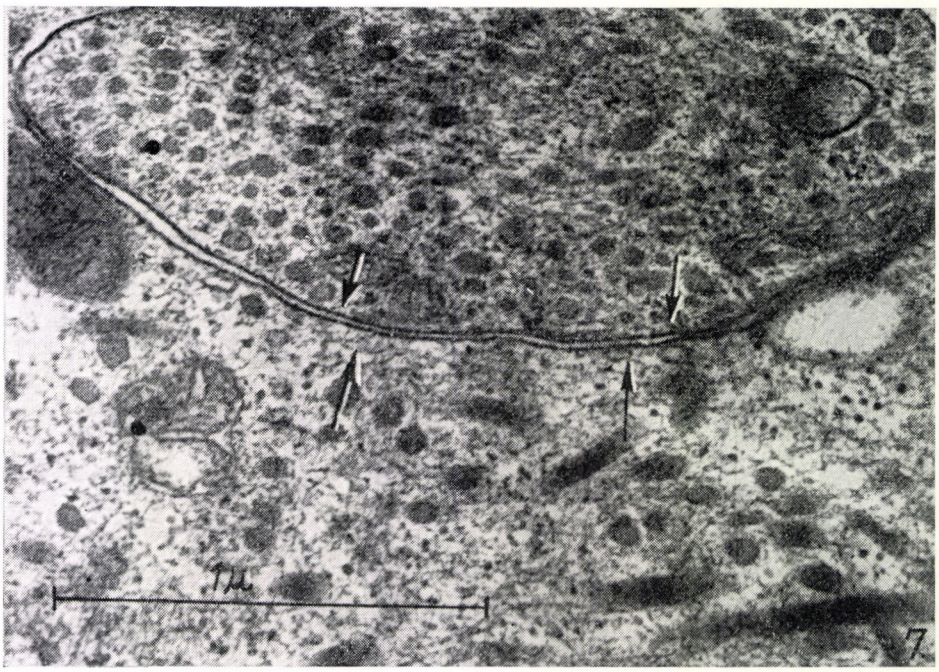

Fig. 7. Membrane appositions between two muscle cells (arrows). ×58,500

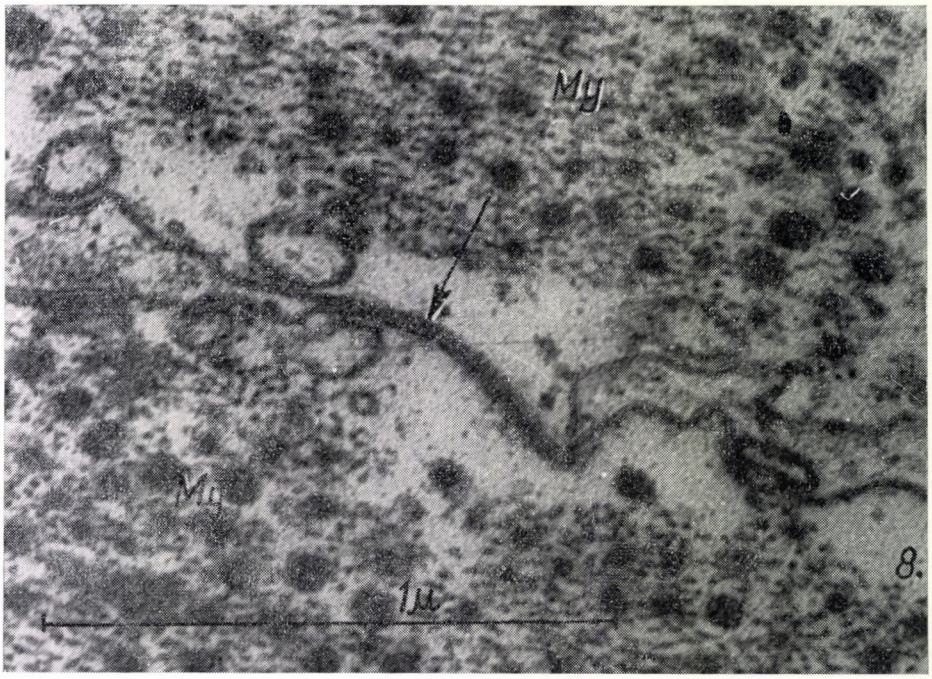

Fig. 8. Nexus between two muscle cells (arrow). My — myofilaments. ×78,000

is also thicker than at other places of the sarcolemma. At these desmosomes the muscle cells come nearer to each other than elsewhere. There occur also halfdesmosomes where the sarcolemma of the opposite muscle cell shows no specialization. At such places the intercellular space is also of normal width. The half-desmosomes can especially frequently be seen on the surface of muscle cells showing a small diameter in cross sections.

Apart from the desmosomes there are, however, intersarcolemmic contacts between the normal muscle membranes, too. The intercellular cleft is only 150—200 Å width on such places *(Fig. 7)*. We could only rarely observe nexuses or "gap junctions" between the muscle cells *(Fig. 8)*.

Discussion

There is no doubt that the adductors have no motor end plate. The innervating axons are similar to those found in the ganglia (Zs.-Nagy, 1964b, 1968a). The structure of neuromuscular contacts is generally identical with that of the neuromuscular (Schlote, 1962, 1963; Graziadei, 1966; Kerkut and cow. 1966; Rogers, 1968; Benjamin and Peat, 1968; Zs.-Nagy and Lábos, 1969) and neuro-interstitial (Nicaise, 1967; Nicaise and cow. 1968) contacts observed in other Molluscs. In the ganglia of *Anodonta cygnea* the DCV and empty vesicles represent the different physiological stages of the same elements (Zs.-Nagy, 1968b). Therefore, we are of the opinion that only on the basis of the morphological difference they cannot be assumed to contain different transmitter substances either in the adductors. The DCVs contain dopamine in the ganglia (Zs.-Nagy, 1968b), thus, also the innervation of the adductors is most probably monoaminergic. We failed to observe morphologically neuromuscular junctions of cholinergic character. This contrasts with the presence of cholinesterase activity in the adductors (Bowden, 1958; Zs.-Nagy, 1964a; Salánki and cow. 1967). It is extremely difficult to interpret the significance of this enzyme taking into consideration that its general sarcolemmar localization (Zs.-Nagy, 1964a) by no means agrees with the distribution of nervous elements.

On the basis of their physiological behaviour the adductors must be assumed to have a double innervation, namely the stimulation of the motor nerves causes either a tonic contraction or relaxation depending on the parameters of the stimulation. The former effect can experimentally be brought about by catecholamines whereas the latter by serotonin (Salánki and Lábos, 1969). As we mentioned above the nerve endings containing DCVs must function with catecholamines, i.e. they represent the stimulating factor. Nothing is known, however about the localization of serotonin. Since the stimulation of the cerebro-visceral connectives causes an increase of the serotonin level in the posterior adductor (Salánki and Hiripi, 1970) meanwhile the adductor relaxes, one cannot call in question the physiological significance of serotonin. This is why the innervating axon-endings are to be considered functionally of two different kinds even despite of the morphological identity. One cannot deny, however, that the catecholamines and the serotonin are localized together in the same nerve endings, only the character of the nerve impulse determines their liberation.

Our investigations show that the adductors contain also nerve cells which are not negligible in the interpretation of the muscle function. Their presence in the adductors elucidates the physiological fact, that even after a total denervation a rhythmic activity of the adductors remains, which is not a myogen rhythm (PAVLOV, 1885; SALÁNKI and ZS.-NAGY, 1970). It is curious that they were not seen in silver impregnated sections (ÁBRAHÁM and MINKER, 1957, 1959). We do not know wether the cholinesterase-positive structures described by BOWDEN (1958) as nerve cells are identical or not with the nerve cells found by us in the electron microscope. Anyway the occurrence of these nerve cells is not so frequent that it could explain — even if they contain cholinesterase — the high activity of this enzyme in the adductors. It should be noted that recently FOH and BOGUSCH (1969) described light microscopically nerve cells of the same size in the penis retractor muscle of *Helix pomatia*.

The presence of desmosomes and half-desmosomes show that the muscle cells are connected mechanically to each other and to the interstitial connective tissue; i.e. they do not extend from shell to shell. HANSON and LOWY (1961) failed to find desmosomes in the yellow adductor of an oyster. It is possible, however, that in the glycerinized muscle used mainly by them the desmosomes are not well preserved. Desmosomes have been found also in smooth muscle of vertebrates (FAWCETT and SELBY, 1958; SHOENBERG, 1958). The mechanical connecting function of desmosomes is also indicated by their frequent occurrence at the taper ends of the spindle-shaped muscle cell. It should be noted, however, that EBARA (1964) holds as possible that the desmosomes take part in the electric coupling in the heart of the oyster.

The other intersarcolemmic connection corresponds to a usual membrane apposition and as such it probably does not take part in the electric coupling. The very infrequent places where the membranes form a nexus (DEWEY and BARR, 1962) or "gap junction" (REVEL and KARNOVSKY, 1967) the electric coupling can take place since these structures represent a well-known low electric resistance. Since the neuromuscular connection itself is not more specialized than the usual membrane apposition and the chemical impulse-transmission takes place in it, it seems to be possible that also the intermuscular membrane appositions play some role in a chemical transmission process maintaining the synchronized function of the muscle cells in the adductors. One may assume that the cholinesterase activity localized diffusely in the sarcolemma has also some functional significance in such a process.

Summary

The anterior and posterior adductors of *Anodonta cygnea* have been studied by the usual electron microscopic techniques. It was found that the axons between the muscle cells are unmyelinated and no Schwann sheath is present. They are morphologically similar to the axons of the central nervous system. They contain empty or dense-cored vesicles of about 400—1200 Å diameter. The enlarged endings of axons are especially rich in vesicles. The endings are in connection with the sarcolemma on some places forming unspecialized membrane appositions representing the neuromuscular synapses. Near these contacts there often occur clusters of vesicles in the endings.

We failed to find any specialized neuromuscular end plate in the adductors. Nerve cells of small size are also present in the adductors.

There are membrane appositions between the muscle cells and rarely nexuses or "gap junctions" can also be seen. These intersarcolemmic contacts can have some functional significance in the maintenance of synchronity of muscle contraction by chemical or electrical transmission of impulses. Desmosomes and half-desmosomes occur in great number on the surface of the muscle cells.

REFERENCES

ÁBRAHÁM, A., E., MINKER (1957): Innervation of the lamellibranch muscle. — *Nature* **180,** 925—926.

ÁBRAHÁM, A., E., MINKER (1959): Experimentell-morphologische Untersuchungen über die Innervation der Schließmuskeln von Süsswaßermuscheln. — *Z. Zellforsch.* **49,** 638—654.

BENJAMIN, P. R., A., PEAT (1968): Myoneural junctions in the connective tissue sheath of a molluscan ganglion. — *Nature* **219,** 1371—1372.

BENNETT, H. S., J. H., LUFT (1959): S-collidine as a basis for buffering fixatives. — *J. Biophys. Biochem. Cytol.* **6,** 113—114.

BOWDEN, J., J., LOWY (1955): The lamellibranch muscle. Innervation. — *Nature* **176,** 346.

BOWDEN, J. (1958): The structure and innervation of lamellibranch muscle. — *Intern. Rev. Cytol.* **7,** 295—335.

DEWEY, M. M., L., BARR (1962): Intercellular Connection between Smooth Muscle Cells: the Nexus. — *Science* **137,** 670—672.

EBARA, A. (1964): Effect of restricted conduction pathway on the transmembrane potential of oyster myocardium, *Crassostrea gigas.* — *Sci. Rep. Tokyo Kyoiku Daigaku, B.* **12,** 1—8.

FAWCETT, D. W., C. C., SELBY (1958): Observations on the fine structure of the turtle atrium. — *J. biophys. biochem. Cytol.* **4,** 63—72.

FOH, E., G., BOGUSCH (1969): Die Nervennetze im Penisretract orvon *Helix pomatia.* — *Z. Zellforsch.* **93,** 439—446.

GRAZIADEI, P. (1966): The ultrastructure of the motor nerve endings in the muscles of Cephalopods. — *J. Ultrastruct. Res.* **15,** 1—13.

HANSON, J., J., LOWY (1961): The structure of the muscle fibres in the translucent part of the adductor of the oyster *Crassostrea angulata.* — *Proc. Roy. Soc. Ser. B.* **154,** 173—196.

KERKUT, G. A., M., WOODHOUSE, G. R., NEWMAN (1966): Nerve-muscle junction in the snail *Helix aspersa.* — *Comp. Biochem. Physiol.* **19,** 309—311.

NICAISE, G. (1967): Neuro-interstitial junction in a Gastropod, *Glossodoris.* — *Nature* **216,** 1222—1223.

NICAISE, G., M., PAVANS de CECCATTY, C., BALEYDIER (1968): Ultrastructures des connexions entre cellules nerveuses, musculaires et gliolinterstitielles chez *Glossodoris. Z. Zellforsch.* **88,** 470—486.

PAVLOV, I. P. (1885): Wie die Muschel ihre Schale öffnet. — *Pflüger's Archiv* **37,** 6—13.

REVEL, J. P., M. J., KARNOVSKY (1967): Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. — *J. Cell. Biol.* **33,** C7.

REYNOLDS, E. S. (1963): The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. — *J. Cell Biol.* **17,** 208—212.

ROGERS, D. C. (1968): Fine structure of smooth muscle and neuromuscular junctions in the optic tentacles of *Helix aspersa* and *Limax flavus.* — *Z. Zellforsch.* **89,** 80—94.

SALÁNKI, J., I., VARANKA, L., HIRIPI (1967): Comparative study on the cholinesterase activity of different tissues of freshwater mussel *(Anodonta cygnea* L.). — *Annal. Biol. Tihany* **34,** 99—116.

SALÁNKI, J., E., LÁBOS (1969): On the role of cholinergic, adrenergic tryptaminergic mechanisms in the regulation of a "catch" muscle *(Anodonta cygnea* L.). — *Annal. Biol. Tihany* **36,** 77—93.

Salánki, J., L., Hiripi (1970): Increase of serotonin in the adductors of *Anodonta cygnea* L. (Pelecypoda) relaxed by nerve stimulation and in relation to the periodic activity. — *Comp. Biochem. Physiol.* 32,629—636.
Salánki, J., I., Zs.-Nagy (1970): Physiological and morphological evidences on the intramuscular regulation of rhytmic activity in the adductors of *Anodonta cygnea* L. — *Acta biol. Acad. Sci. Hung.* 21,329—330.
Schlote, F. W. (1962): Die Muskulatur von *Helix pomatia* und ihre Innervation. — *Proc. First. Malac. Congr.* 113—151.
Schlote, F. W. (1963): Neurosekretartige grana in den peripheren Nerven und in den Nerv-Muskel-Verbindungen von *Helix pomatia*. — *Z. Zellforsch.* **60,** 325—347.
Shoenberg, C. F. (1958): An electron microscopic study of smooth muscle in pregnant uterus of the rabbit. — *J. biophys. biochem. Cytol.* **4,** 609—614.
Vadász Gy. (1966): Glutáraldehid savmentesítése elektronmikroszkópos felhasználásra. — *Kísérletes Orvostudomány* **18,** 334—336.
Zs.-Nagy, I. (1964a): A histochemical study of cholinesterase on the adductor muscle of the fresh water mussel (*Anodonta cygna* L.). — *Annal. Biol. Tihany* **31,** 153—157.
Zs.-Nagy, I. (1964b): Electronmicroscopic observations on the cerebral ganglion of the fresh water mussel (*Anodonta cygnea* L.). — *Annal. Biol. Tihany* **31,** 147—152.
Zs.-Nagy, I. (1968a): Fine structural analysis of the neurons of *Anodonta cygnea* L. (Pelecypoda). — *Annal. Biol. Tihany* **35,** 35—59.
Zs.-Nagy, I. (1968b): Histochemical and electron microscopic studies on the relation between dopamine and dense core vesicles in the neurones of *Anodonta cygnea* L. In: *Neurobiology of Invertebrates*, Proceedings of a Symposium at Tihany, Ed. by J. Salánki, *Akadémiai Kiadó, Budapest*, pp. 69—84.
Zs.-Nagy, I., E., Lábos (1969): Light and electron microscopical investigations on the adductor muscle and nervous elements in the larva of *Anodonta cygnea* L. — *Annal. Biol. Tihany* **36,** 123—133.

A NEURO-MUSZKULÁRIS ÉS INTERMUSZKULÁRIS KAPCSOLATOK FINOM SZERKEZETE AZ ANODONTA CYGNEA L. (MOLLUSCA, PELECYPODA) ZÁRÓIZMAIBAN

Zs.-Nagy Imre és *Salánki János*

Összefoglalás

Vizsgáltatott az *Anodonta cygnea* elülső és hátsó záróizmának beidegzése szokásos elektronmikroszkópos technika segítségével. Megállapítást nyert, hogy az izomsejtek között található axonok velőtlenek és Schwann-hüvellyel sem rendelkeznek, morfológiailag hasonlítanak a központi idegrendszer axonjaira. Bennük 400—1200 Å átmérőjű üres vagy dense-core vezikulák láthatók. Az axonok tágult végződései különsen sok vezikulát tartalmaznak. E végződések helyenként hozzáfekszenek az izomsejtekhez, azok membránjaival appozíciós kontaktusokat képeznek, amelyek specializációt nem mutatnak. Ezek felelnek meg a neuromuszkuláris szinapszisoknak. E helyeken gyakori a vezikuláris állomány tömörülése az idegvégződésekben. Neuromuszkuláris véglemezt nem találtunk a záróizomban. Sikerült megfigyelni az izomsejtek között kisméretű idegsejtek jelenlétét is.

Membránappozíciók az izomsejtek között is találhatók, sőt ritkán ugyan, de nexusok, illetve „gap junction"-ok is előfordulnak. Ezen interszarkolemmáris érintkezési formáknak jelentőségük lehet a záróizomműködés szinkronizásának biztosításában ingerületi folyamatok kémiai vagy elektromos átadása révén. Desmosomák és féldesmosomák nagy számban láthatók az izomsejteken.

ТОНКАЯ СТРУКТУРА НЕРВНО-МЫШЕЧНЫХ И МЕЖМЫШЕЧНЫХ СВЯЗЕЙ В АДДУКТОРАХ ANODONTA CYGNEA

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Были исследованы передние и задние аддукторы Anodonta cygnea методом обычной электронноймикроскопии. Установлено, что аксоны среди мышечных клеток безмиэлиновые и не обладают даже Шванновской оболочкой, морфологически они сходны с аксонами центральной нервной системы. Внутри них видны пустые или dense-core везикулы диаметром 400—1200 Å. Особенно много везикул содержится в расширенных концах аксонов. Эти окончания в некоторых участках прилегают к мышечным клеткам, их мембраны образуют аппозиционные контакты, которые не показывают специализации. Эти контакты соответствуют нервно-мышечным синапсам. В этих местах часто наблюдается скопление везикул в нервных окончаниях. В аддукторе не нашли концевую пластинку. Удалось наблюдать наличие мелких нейронов среди мышечных клеток. Изредка наблюдались мембранные аппозиции между мышечными волокнами, nexus или «gap junction» тоже могут встречатся. Эти интерсерколеммарные формы связей, может быть, играют значительную роль в синхронизации аддукторов, помощью химической или электрической передачи возбуждения. Десмосомы и полу-десмосомы в большом количестве видны в мышечных клетках.