ELECTRON MICROSCOPE EXAMINATIONS ON THE BRAIN OF WATER BEETLE (DYTISCUS MARGINALIS)

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(Received December 13, 1966)

In the course of the past times, apart from our comparative neurohistological investigations of a different kind, we dealt much with the central nervous system of the higher invertebrate, first of all with the brain. During the relevant examinations we took generally two main points of view into consideration. One of them was to investigate, as far as possible, even in the smallest details, the exact structure of the brain part that is in the service of production and conduction of *stimuli*. The other point of view of ours was to recognize the structure and function of neurons that, inside the cerebral substance, had entered the service of production and conduction of secretion in the course of the phylogeny. For such examinations the brain of *Coleoptera* of bigger body proved generally to be suitable; first of all the water beetle has been found appropriate and proper in this respect. The cause of that can be outlined as follows:

1) The water beetle lives in the fish-ponds in the environs of Szeged, in large number, it can easily be gathered and survive for a comparatively long time if properly cared for.

2) The brain of the animal is well-developed, can be removed, stained, and impregnated easily; and in it the central switching systems, which have developed because of the great number and maturity of the organs of sense and of the fast and complicated ways of motility, can be studied excellently.

3) The cells that serve to produce neurosecretions are large, can be limited sharply from the neurons being in service of the production and receiving *stimuli*, and the process of the production of secretion in them and the way of secretion in their processes can be followed exactly.

The results of our examinations, obtained in regard to the above mentioned aspects, are partly already published, partly going to be published. We are giving now account about the examinations carried out on the brain of water beetle by electron microscope, as follows.

Material, methods

The brain removed from animals narcotized by chloroform was fixed with 1 p. c. osmium acid in veronal acetate buffer. The brain was rinsed, dehydrated and embedded in metakrilat, as usual. The sections and micrographs were made partly in the Electron Microscopic Laboratory of the Zoological Institute in Leipzig, partly in the Central Laboratory of the University Medical School in Szeged, and partly in the Electron Microscopic Laboratory of the Attila József University in Szeged. The elements obtained by help of the micrographs will be treated of in the following order: 1) nerve cells, 2) neurosecretory cells, 3) neuropil, 4) interneuronal synapses.

Nerve cells

The nerve cells take place under the unilamellar subcapsular *glia* which surrounds the brain, forming the cortical part of it. Under light microscope they are unipolar and form smaller or bigger groups. Their size changes in the

Fig. 1. *Dytiscus marginalis:* cross section of the brain. a — *trachea,* b — myelin body, c — cell membrane, d — GOLGI complex, e — endoplasmatic *reticulum,* f — *vesiculum,* g — *mitochondrium.* x 14 000

single groups and cannot be considered to be identical even inside the groups. The typical pear-shape can be observed under electron microscope, as well, especially using lower magnification at examination. The cell is surrounded by an obvious cell-membrane. The latter one is joined by a secondary membrane consisting of a glial process. Both of the membranes seem to be unitary and continuous, although it is possible, too, that there are some breaks in the internal one or in both of them. In a few cases also a third membrane can be seen between the two ones with smaller or bigger thickenings.

In the cytoplasm there are granules and vesicles of different site and size, either scatterred or grouped in masses here and there.

The *cisternae* of the endoplasmatic *reticulum* do not show any regularity. They are essentially *tubuli* of longer or shorter course appearing generally piece by piece in the micrograph (Fig. 1).

The constituents of GOLGI's complex are thin *tubuli* joined by smaller or bigger longitudinal vesicle groups. Such kinds of formations can generally be defined but with difficulty and, as they can be observed, as a rule, only in details, it is difficult to recognize them.

In some cells the myelinbodies can be observed in a rather high number. They are obviously large, some of them are oblong and homogeneous, others are spherical and show an arched incision. In the cytoplasm the number of *mitochondria* is comparatively not high. They are in general large, oblong, and belong to the *crista* type. There are smaller, globular, and irregular ones. The nucleus is surrounded by a double nuclear membrane. The membranes are sometimes sharp outlined, sometimes, however, they are fused. It is a frequent case that in a unitary cell-membrane the pores of a comparatively uniform diameter are lying side by side. The latter ones are here and there double ones, in other cases, however, not a single gap may be seen in a rather large section of the membrane. The *chromatin* of the *nucleus* consists of smaller or bigger granules. Sometimes under the nuclear membrane, and in some cases even deeper, dark *chromatin* masses can be observed, differring in their structure from the light, granular substance (Fig. 2).

Neurosecretory cells

In the neurosecretory cells contained in the intercerebral part of *protocerebrum* the compact, globular *resp.* ovoid secretory granules can be observed well. Apart from them also the larger vesicles limited by a sharp membrane are frequent, containing small globular or ovoid homogeneous corpuscles. These include globular bodies, limited by well-discernible walls. The number of the latter ones is proportional to the size of vesicle. It is highly probable that these small corpuscles are precursors of the neurosecretory granules, are gradually discharged from the vesicles and become definitive secretory granules, *resp.* compact drops. Such kinds of formations can be seen *en masse* in the cytoplasm scatterred or longitudinally arranged.

The nucleus is surrounded by a double membrane. The membranes seem to be of sharp contour. The two membranes are separated from each other by an obvious empty space. In the membranes, first of all in that lying towards the cytoplasm, the pores can be seen through which the nucleoplasm and the cyto-

plasm approach each other. We could not observe any case in which the cytoplasmatic *cisternae* join the pores (Fig. 3).

Neuropil

The neuropil is a dense network of fibres of different thickness, belonging to different course systems. Among the fibres there are obviously thick fibres of light contour, reaching the field of brain through the pharyngeal connective

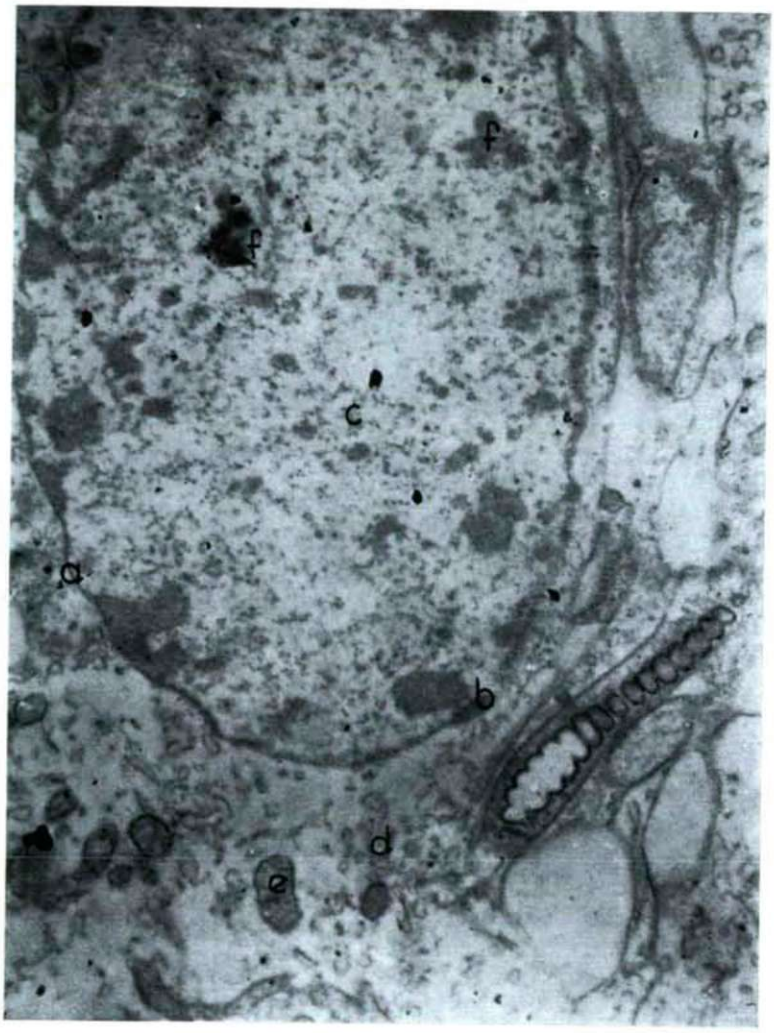

Fig. 2. *Dytiscus marginalis:* cross section of the brain. a — nuclear membrane, b — *pori*, c — *chromatin* granules, d — endoplasmatic *reticulum*, e — *mitochondrium*, f — *chromatin* packets. x 20 000

as processes of the granglion cells lying in the field of *thorax*. There they ramify gradually and, as observed during light microscope examinations, join the end-fibre systems of cortical origin with a rich end-knobsynapsis system. Among

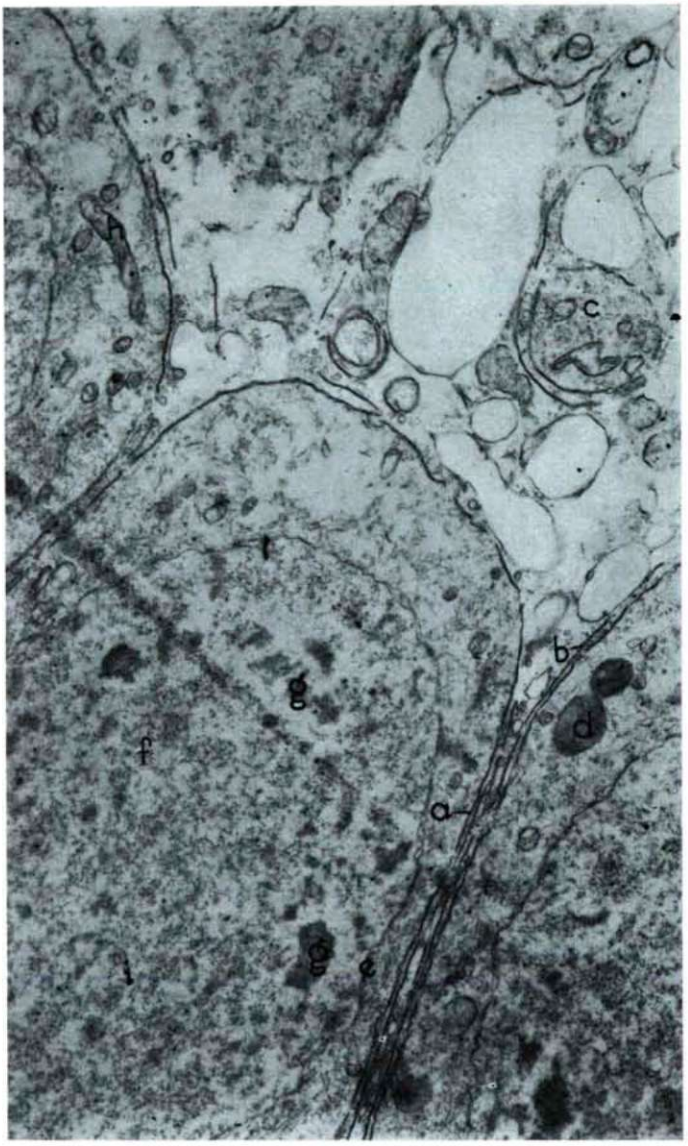

Fig. 3. *Dytiscus marginalis:* cross section of the brain. a — cell membrane, b — *glia* membrane, c — neurosecrete granules, d — vesicle with presecrete granules, e — nuclear membrane, f — *chromatin* granules, g — *chromatin* packets, h — *mitochondrium*, i — neurosecrete granules. Magnified x 18 000

the fibres there are frequently to be found some oval, likely oblong, compact *nuclei* which are the *nuclei* of glial cells, occuring here in a high number.

The nerve fibres are surrounded by glial membrane of changing thickness. In the fibres small vesicles, sporadically *mitochondria* of major size can be observed. On the cross section the cross sections of neurofilaments can be seen, among them not rarely also smaller or bigger rings (Fig. 4).

The *mitochondria* occur in the neuropil in much greater number than in the nerve cells. As to their shape, many of them have oblong, elliptic forms in which the ribs get thoroughly across the body (Fig. 5). Sometimes they appear

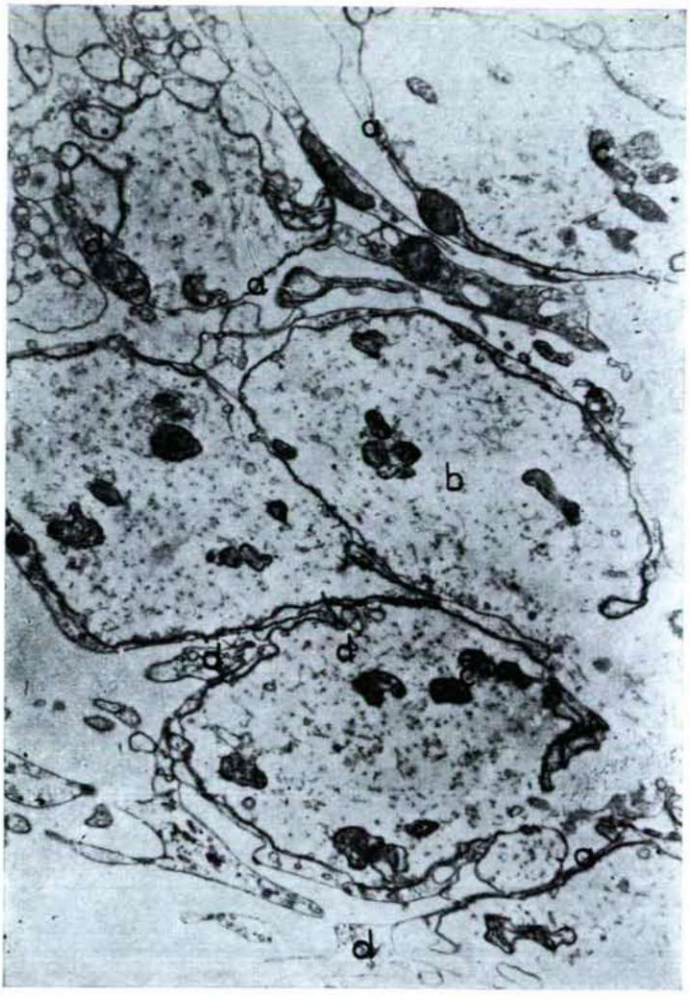

Fig. 4. *Dytiscus marginalis:* brain, cross sected nerve fibres in neuropil. a — *glia* membrane, b — *neurofilamentum*, c — *mitochondrium*, d — *vesiculum*. Magnified x 14 000

in huge masses and differ highly from one another even in the same group concerning both their shape and size (Fig. 6).

Sometimes the mitochondrial groups are confined but to one side details of the fibre, and their whole accumulation refers to a wandering. This is referred to also by the typical arrangement into series, the pecularity and variety of shapes. Among the highly varied mitochondrial forms some refer also to a division (Fig. 7). The dumb-bell shaped and *crista* forms are not rare, and some have thoroughly special shapes, represented in Fig. 8, in the extreme right lower detail of the picture (Fig. 8). Again emphasizing that in the neuropil there are a great lot of *mitochondria* appearing in the most different forms of groupings, we have to write about an obvious mitochondrial form, not mentioned as yet, as we know, in the relevant literature. That is the ring-shaped *mitochondrium* two forms of which can be seen in Fig. 9. Both are on the left side of the figure, one of them above in the corner the other at the very edge in the middle (Fig. 9). In the nerve fibres there occur microvesicular bodies. These are essentially vesicles of smaller size containing a lot of smaller, globular vesicles. Vesicles occur not only in the microvesicular bodies but also in fibres, first of all in thick ones, in a free state. They are frequent in the synaptic area of the axial threads, and even in places where no synaptic formations can be observed. Sometimes they form major groups in the centre of the fibre.

Interneuronal synapses

There is a continuity neither between the nerve cells nor between the nerve fibres. The morphologic basis of stimulation is in every case the contact. As, because of the glial membrane covering the cell membrane, there is no axosomatic synapsis, the most part of the contacts fall to the field of neuropil, belong-

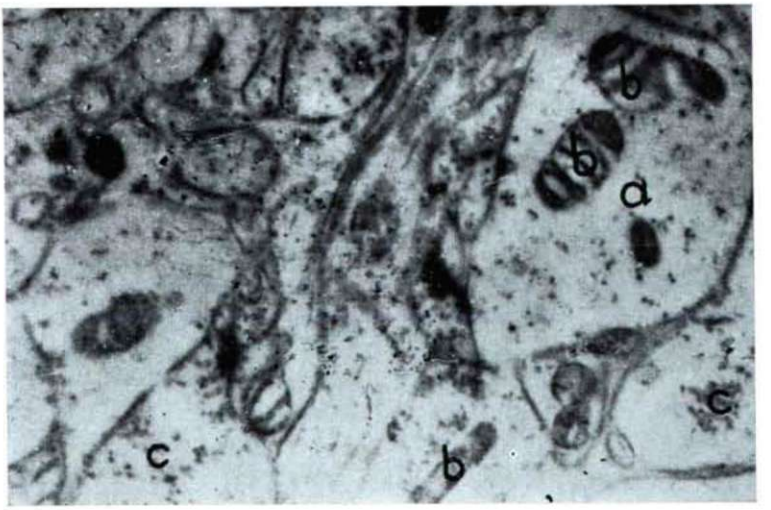

Fig. 5. *Dytiscus marginalis:* cross section of the brain, *mitochondria* in the nerve fibre, a — nerve fibre, b — *mitochondria*, c — *vesiculum*. x 25 000

ing to the axoaxonic, resp. axodendritic contacts. The question which of them is, in fact, the axoaxonic and which the axodendritic contact could not be decided even under the electron microscope. The cause is that we use extremely fine sections for the electron microscope examinations in which the ramifications cannot be closely followed. Thus it is impossible to ascertain which of the fibres is a dendrite and which a neurite. Even under a light microscope it is rather difficult to solve that question because the ramification of a neurite, leaving the cell, can be traced but in the rarest cases. The question is still more difficult if we realize that, according to CAJAL's theory the long jugular part

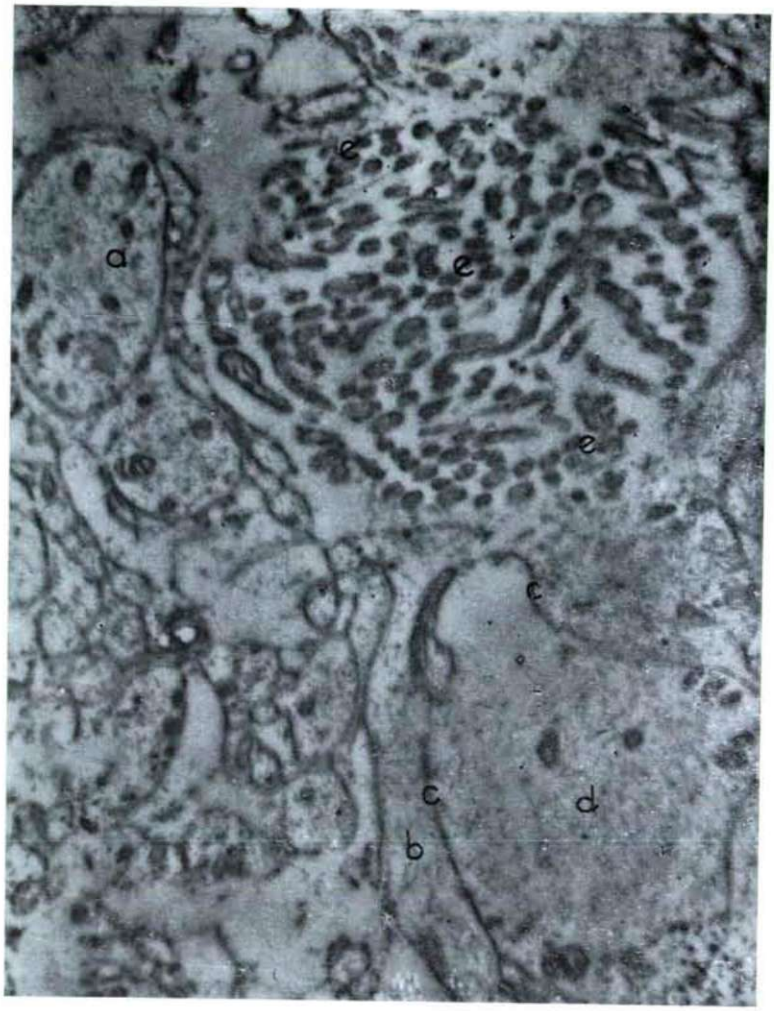

Fig. 6. *Dytiscus marginalis:* cross section of the brain; nerve fibres in the neuropil. a — cross sected nerve fibre, b — nerve fibre longitudinal sected, c — *glia* membrane, d — *neurofilamentum*, e — *mitochondrium*. Magnified x 15 000

of the pear-shaped cells in the central nervous system of insects must be considered as an indifferent part of the cell without any polarization. In CAJAL's opinion the ramification begins by the end of the jugular part. Both the axon and the dendrite depart from there both having a rich ramification. In this sense we may distinguish three, *resp.* four parts in the neuron of insects, *viz.*, the proper *soma*, the indifferent non-polarized jugular part, and the neurite and dendrite departing from the distal part of the jugular part. As neither the neurite nor the dendrite has a typical character, mainly in the field of synapses, they can not be distinguished from each other. Therefore we may speak about axo-axonic and axodentritic synapses only in general. We cannot decide, however, what is, in fact, one of them and what is the other. TRUJILLO-CENOZ (1962) distinguishes three forms of the interneuronal synapses in the neuropil of Arthropods. They are as follows: 1) long contacts, 2) cross contacts, and 3) button-like contacts. TRUJILLO-CENOZ calls longitudinal contact the form of nerve fibre contacts in the case of which „ . . . two or more nerve fibres gathered to-

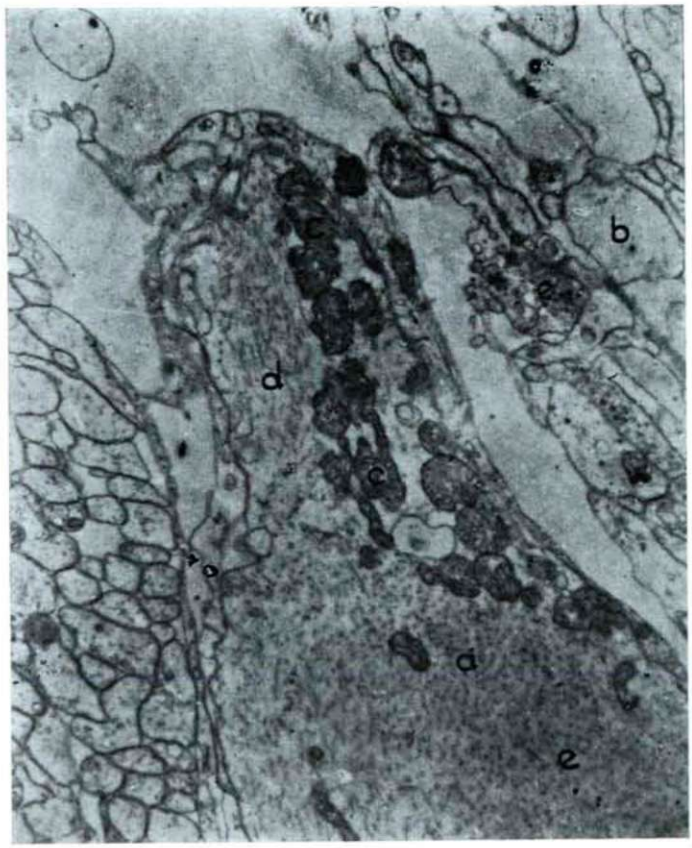

Fig. 7. *Dytiscus marginalis:* cross section of the brain, nerve fibres in the neuropil. a — thick nerve fibre in longitudinal section, b — thin nerve fibre in cross section c — *mitochondrium*, d — *neurofilamentum*, e — *vesiculum*. Magnified x 14 000

gether without the interposition of glial membranes . . ." and the membrane separating the two fibres is about 350—400 Å. It is characteristic of this form of fibre contact, as well, „ . . . that the neuroplasm at this level shows no increase in the number of vesicles or mitochondria," These forms of contact can frequently enough be observed also in the neuropil of the brain of water beetle (Fig. 10). It is, in general, characteristic of them that there are but few synaptic vesicles and the *mitochondria* are practically fully missing in the contact fibres.

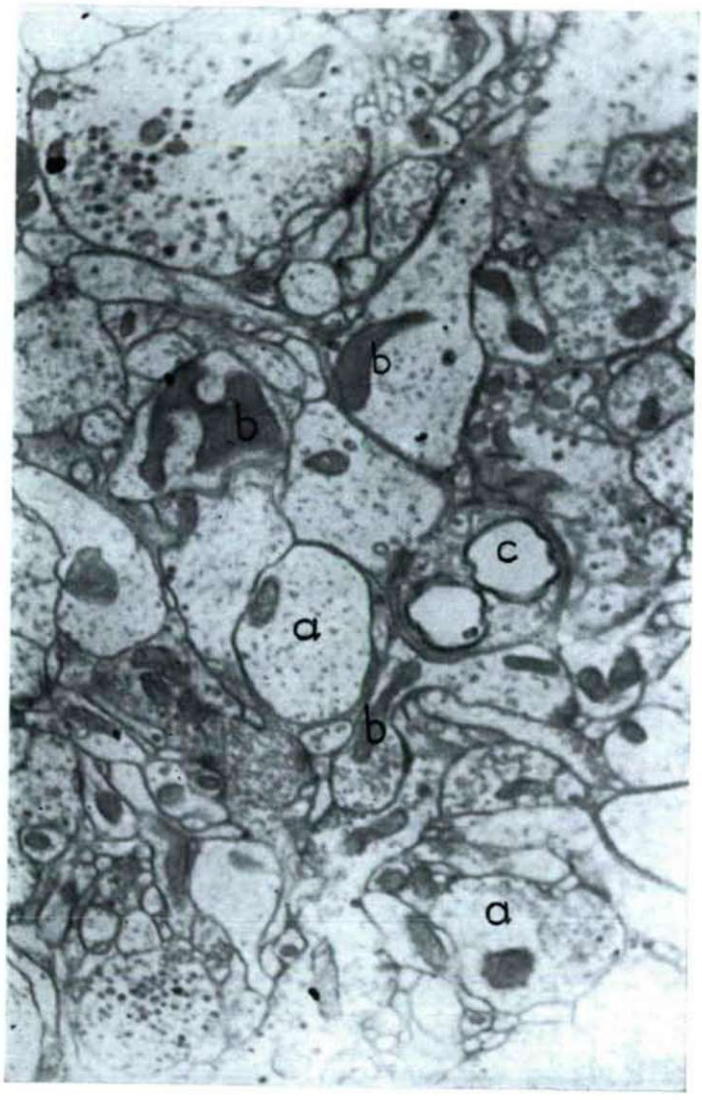

Fig. 8. *Dytiscus marginalis:* brain; cross sected nerve fibres in the neuropil. a — cross sected nerve fibre, b — *mitochondria,* c — *trachea.* x 20 000

As cross-contact is named by TRUJILLO-CENOZ the contact form where a nerve fibre passes immediately over the surface of the other, and so the place between the contact surfaces is small enough for enabling the naked axial threads to pass the nerve impulse to one another. Such kinds of formations can be found in a high number in the neuropil of the brain of water beetle. That form of contact is performed in the brain of the water beetle, as a rule, by two thick fibres so that one of them passes over the other. Also the case is not rare where the branches of a thick fibre produced by bifurcation form a cross synapsis with another thick fibre. In the fibres forming the cross contacts the vesicles and *mitochondria* are missing. Only the pale endoplasmatic reticles and the thin neurofilaments are formations appearing more or less in the fibres with such a kind of contacts (Fig. 11).

A third form of the interneuronal contacts is the contact with end-soles. At this form of contact the widened distal piece of a nerve fibre joins to the end of another fibre. At this form of contact there are lots of vesicles and *mitochondria* in the presynaptic fibre area. In our opinion, this from of synapses reflects the most the morphologic and physiologic character of the synapsis, and is frequent as well (Fig. 12).

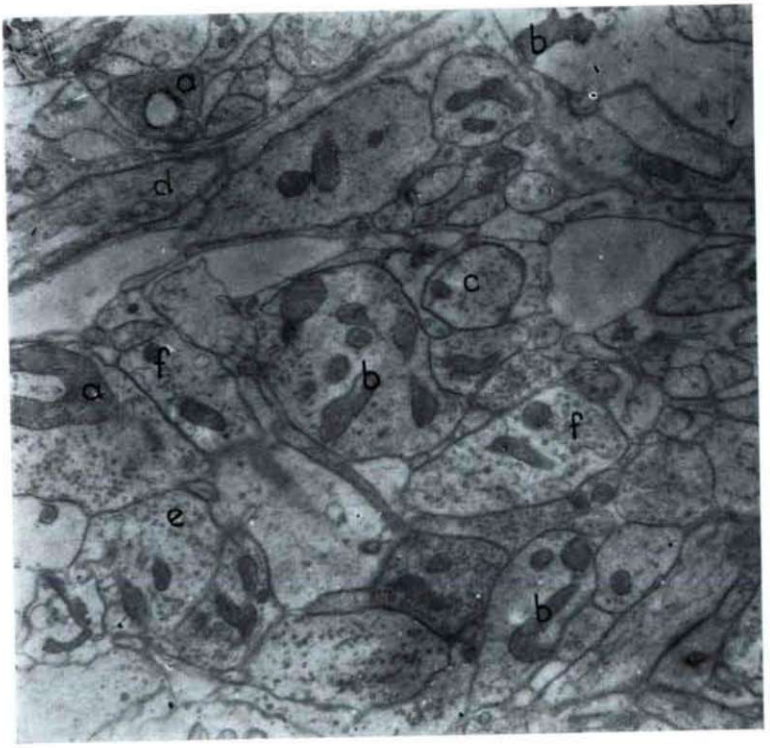

Fig. 9. *Dytiscus marginalis:* brain, cross sected nerve fibres in the neuropil. a — peculiar *mitochondria,* b — *mitochondria,* c — cross sected nerve fibre, d — longitudinal sected nerve fibre, e — *neurofilamentum,* f — *vesiculum.* Magnified x 19 000

Discussion

The researchers, examining the structure of the synaptic contacts in the nervous system of the higher *Metazoa* with electron microscope, consider the synaptic vesicles to be the most characteristic constituents of synapses (E. De Robertis, 1959). According to their examinations these are the constituents that produce the transmit substance for transferring the nervous *stimuli*. This conclusion may be valid for the nervous system of higher animal organisms; it lacks, however, ground in the case of the brain of water beetles. According to our examinations, Trujillo-Cenoz may be right considering to be „ . . . pro-

Fig. 10. *Dytiscus marginalis:* cross section of the brain, nerve fibres in the neuropil. Longitudinal contact. a — cross sected nerve fibre, b — along sected nerve fibre, c — *glia* membrane, d — *mitochondrium*, e — *vesiculum*, f — longitudinal contact. x 7000

bable that a functional interneuron connection may result not only from contacts between fibres containing vesicles but also between fibres in which vesicles are absent (ESTABLE 1961)." In the brain of water beetles there are comparatively many contact forms lacking vesicles. This statement is verified by almost all above published figures, especially convincingly by Fig. 10. In this figure there are comparatively many longitudinal contacts where the thickness of the membrane separating the contact surfaces doesn't exceed 500 Å. In this meaning they are, therefore, synapses. Vesicles can, however, not be observed in either of the fibres or they can be seen in only one of them without being able to ascertain even in that case whether they are presynaptic or postsynaptic vesicles. The same can be seen in the case of cross synapses, as well, where

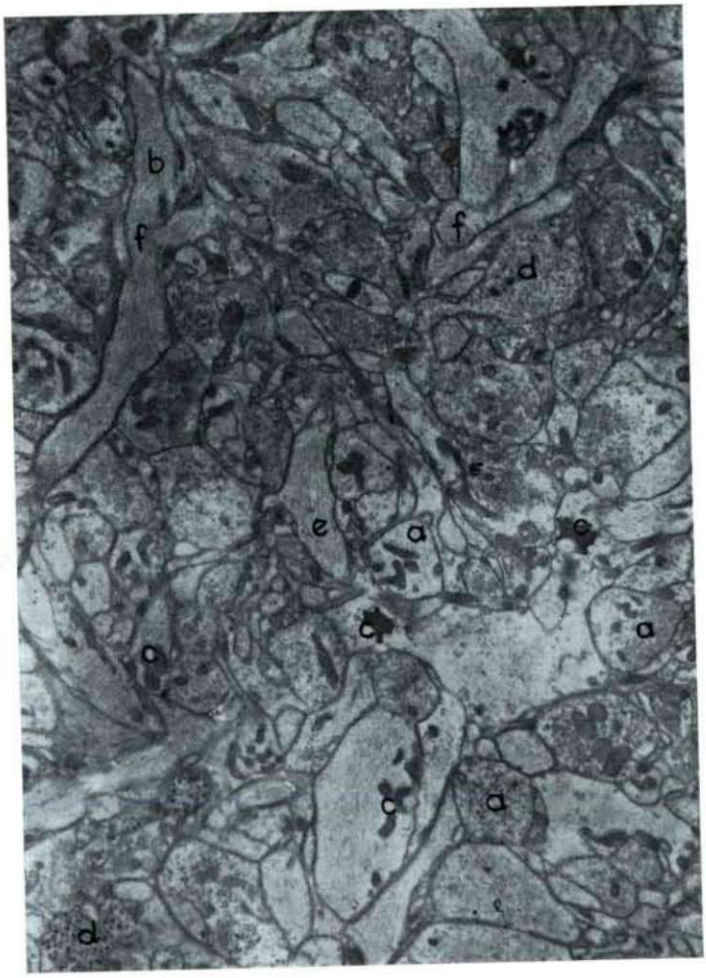

Fig. 11. *Dystiscus marginalis:* cross section of the brain; nerve fibres in the neuropil. a — cross sected nerve fibre, b — nerve fibre in longitudinal section, c — *mitochondria*, d — *vesiculum*, f — cross contact. Magnified x 7000

there are generally no vesicles in any of the contact fibres (Fig. 11). A typical synapsis form is the end-sphere, *resp.* end-knob contact, discussed above in the third place. This form appears here and there in classical shapes (Fig. 12). In such a case it may well be seen, indeed, that the end of the presynaptic fibre-end becomes knoblike thicker, being rounded off at the end, and the terminal region is full of vesicles and *mitochondria*. It is interesting that in the central part of the fibre a comparatively well limited globular space seems to be vesicle-free; the *mitochondria*, variegating both in size and in structure, take place there. In the picture we can see the two synaptic membranes, the intersynaptic

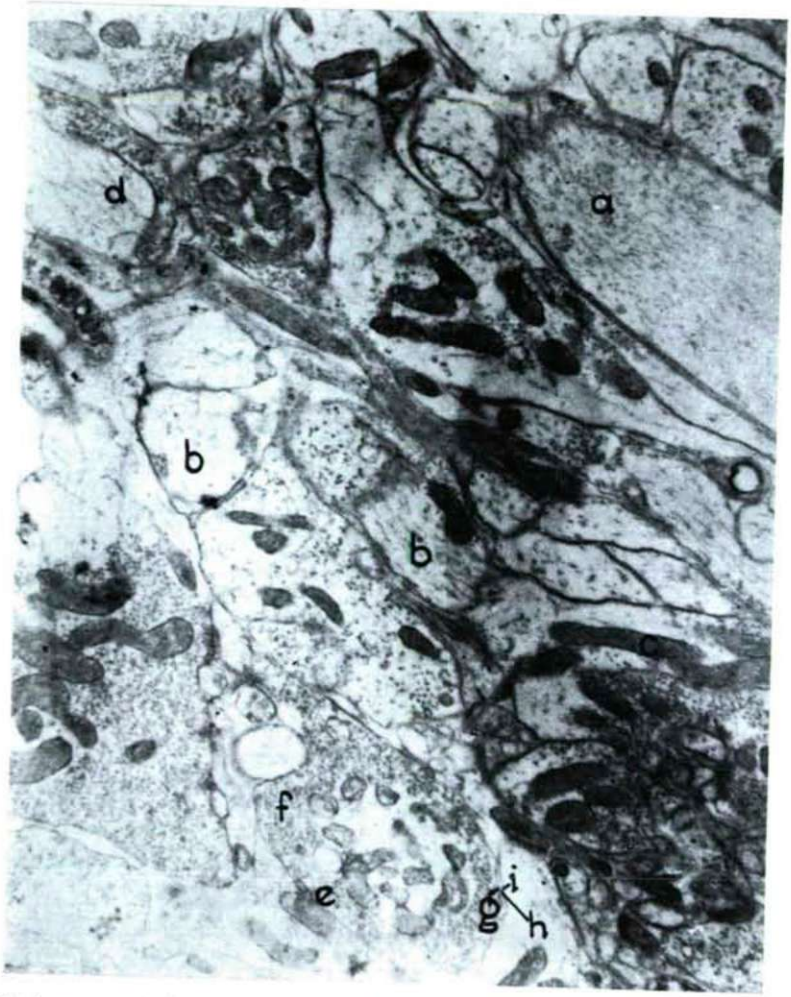

Fig. 12. *Dytiscus marginalis:* cross section of the brain, nerve fibres in the neuropil. Endknob contact. a — nerve fibre in longitudinal section, b — cross sected nerve fibre, c — *mitochondria*, d — *neurofilamentum*, e — end-knob contact, f — presynaptic *vesicula*, g — presynaptic membrane, h — synaptic space, i — postsynaptic membrane. x 14 000

space, and the postsynaptic fibre containing *mitochondria*, anyhow without vesicles. These observations and conclusions confirm the supposition that in the central nervous system of Arthropods the vesicles are not constant and so essential components of the synapses. There are synapses containing vesicles and there are some ones containing none. As, however, the nervous system is uniform both morphologically and physiologically for the whole animal kingdom, we are not reluctant to assert that from the contact forms introduced in literature we cannot consider as real synapses but the knoblike contacts, without taking the presence or absence of synaptic vesicles to be characteristic. That form corresponds to the pictures obtained by a light microscope after impregnation and it can ensure, by its structure, the course of the one-way conduction and transfer of *stimuli*. This supposition is supported by the development of the nervous system and confirmed by the rich ramifications of the nerve fibres and by the sharply obvious and peculiar formations which form the end parts of the nerve fibres.

Summary

The results of the examinations carried out on the brain of water beetle by electron microscope can be summarized as follows:

(1) The nerve cells arranged in groups at the edge of cerebral substance are pear-shaped. The cell-body is surrounded by two membranes, one of them is the cell-membrane, the other is formed by the processes of the glial cells.

(2) In the cytoplasm of nerve cells we can observe, in shape of convoluted *tubuli*, the endoplasmatic *reticulum* and GOLGI's complex consisting of *tubuli* and vesicles. The vesicles, myelinbodies and *mitochondria* can be seen, as well.

(3) The *nucleus* of the nerve cell is surrounded by a double membrane. The membranes grow together here and there forming a unitary homogeneous structure, in other places they are separated and are full of pores. The pores, whose diameter is by and large of the same size, are arranged in two lines in some places. The *chromatin* consists mostly of small, irregular clods, forming, however, here and there, mainly under the membrane, compact konts showing very different shapes.

(4) In the neurosecretory cells the double membrane is sharply conspicuous; the presecretory granules which are confined by thin membranes, can be observed in the cytoplasm free or closed into a greater vesicle with other ones.

(5) The nerve fibres that form the neuropil are bordered by a double glial membrane. In their protoplasm they contain a lot of pale, thin neurofilaments and *mitochondria*. The latter ones are large their shape is extremely varied. The forms reminding of division are frequent, and the hollow, rhomboid forms of thick walls are characteristic and particular.

(6) In the neuropil both the longitudinal contact forms and the cross-shaped ones, as well as the end-knoblike forms can be found. The observations and theoretical conclusions, however, support the assumption that from those only the latter ones may be considered as real synapses.

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