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The nervous system of ctenophores III. Ultrastructure of synapses

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Summary

The ultrastructure of synapses from the nervous systems of seven species of ctenophores has been studied. All synaptic contacts so far observed show a peculiar organization characteristic of this phylum: the pre-synaptic neurite contains in sequence from the plasma membrane: a row of vesicles lining the synaptic membrane, a sac of endoplasmic reticulum which is connected with the rough reticulum of the neuron, and one or several mitochondria. The arrangement of these closely associated organelles is constant, forming a complex referred to as the *pre-synaptic triad.*

The pre-synaptic triad is found both in interneural synapses – which may be polarized, symmetrical or reciprocal – and in various neuro-receptor and neuro-effector junctions.

The possible functional implications of these features are discussed.

Introduction

The Ctenophora are pelagic animals characterized by eight meridional rows of locomotory cilia arranged in 'combs'. They have interested biologists because of their phylogenetic position, intermediate between diploblastic and triploblastic phyla. Like Cnidaria most of their nervous system consists of a true nerve net spread under the ectodermal epithelium, but their highly developed muscular system embedded in the mesoglea is reminiscent of that occurring in flatworms. Neurophysiologists have been particularly concerned with the nervous control of the cilia and muscles of Ctenophora.

Ultrastructural evidence for synaptic contacts in ctenophores was first given by Horridge and Mackay (1964) for the cydippid *Pleurobrachia pileus*, and later by Horridge (1965) for the lobate *Leucothea multicornis*. Our own investigations have confirmed these findings and have extended them to seven species belonging to three distinct sub-orders, namely: *Beroe ovata* and *Beroe forskalii* (sub-order Beroidea), *Pleurobrachia rhodopis*, *Hormiphora plumosa*, *Lampetia pancerina* and *Callianira bialata* (sub-order Cydippidea) and *Bolina hydatina* (sub-order Lobata).

The characteristic organization displayed by the supposed pre-synaptic element at these contacts has been described briefly (Hernandez-Nicaise, 1968a and b, 1973). This organization is constant, appearing to be unaffected by either physical factors (fixation) or biological factors (species, age, localization of the tissue, nature of the post-synaptic element). In all our work, it has been assumed that these ultrastructural features represent chemical synapses and offer an unequivocal criterion for recognizing nerve cells or nerve fibres in any ctenophore species.

This paper presents a detailed ultrastructural study of synaptic components based on observation of *several hundred synapses* with the electron microscope. In order to provide a basis for discussing functional and evolutionary correlations, we also give a classification of the observed synapses.

Table 1. Classification of fixation procedures utilized on ctenophores in this study.

Steps in fixation	Fixation procedures						
	A		B		C		D
	A1	A2	B1	B2	C1	C2	
Glutaraldehyde	none		3% in cacodylate buffer 1100 mosmol (calculated)	1400 mosmol (calculated)	3% in cacodylate buffer 1100 mosmol (calculated)		3% in cacodylate buffer 0.2% $CaCl_2$ added to buffer 1100 mosmol (measured)
Wash in buffer	none		cacodylate 550 mosmol (calculated)	1100 mosmol (calculated)	cacodylate 1100 mosmol (calculated)		cacodylate + $CaCl_2$ 1050 mosmol (measured)
Post-fixation with osmium tetroxide	1% in veronal-acetate 1100 mosmol (calculated)		1% in cacodylate 450 mosmol (calculated)	1100 mosmol (calculated)	1% in cacodylate 1100 mosmol (calculated)		1% in cacodylate + $CaCl_2$ 970 mosmol (measured)
'En bloc' staining	none	P.T.A. in absolute ethanol (1 h)	none	none		uranyl acetate in maleate buffer*	none
Embedding medium	Araldite		Epon		Epon		Epon
Corresponding figures	12	2	6, 13	10	8	1, 4, 5, 9	3, 7, 11

* According to Karnovsky (1967).

Methods

All specimens studied were collected, fixed and embedded for electron microscopy at the Station Zoologique, Villefranche-sur-Mer (06, France), Université de Paris.

In order to facilitate comparison between the figures of this paper we have summarized, in Table 1, the steps of various fixation procedures tried during the course of our study. Details of the most satisfactory procedure (fixation D) for electron microscopy were described in a previous paper (Hernandez-Nicaise, 1973).

Thin sections were usually stained with uranyl acetate and lead citrate. For grids from series C2, uranyl acetate was naturally omitted.

Observations

MORPHOLOGY OF SYNAPTIC COMPONENTS

The synaptic junction is characterized by the presence of a pre-synaptic complex of organelles. In addition, the synaptic membranes are modified as in higher phyla: they appear parallel and thickened (especially the post-synaptic membrane: see, in particular, Figs. 1 and 7) and are separated by a synaptic cleft. The width of the cleft varies from 120 to 170 Å. The cleft possesses a filamentous, moderately dense material in which cross-bridges are occasionally observed (Figs. 1 and 2). Following glutaraldehyde fixation, if the plane of section is normal to both synaptic membranes, the intra-cleft material appears to be organized in the form of a thin dense lamina, equidistant from both plasma membranes (Fig. 3). The total length of synaptic contact varies from 0.5 to 7 μm.

It is noteworthy that the thickening of the post-synaptic membrane is clearly enhanced by phosphotungstic acid (P.T.A.) 'en bloc' staining after simple osmium fixation (compare Figs. 2 and 12). This thickening appears mostly as a dense coat on the inner side of the plasma membrane. The filamentous bridges of the synaptic cleft are also revealed by this staining method.

The pre-synaptic triad

As previously noted (Hernandez-Nicaise, 1973), passing inwards from the plasma membrane the pre-synaptic neurite exhibits: a row of vesicles lining the synaptic membrane, a sac of agranular reticulum and a mitochondrion. These organelles build a complex, the *pre-synaptic triad*, whose arrangement is constant (Figs. 1–13).

The synaptic vesicles are in intimate contact with the reticulum so that they often depress the cisterna wall. The vesicular and reticular membranes may even appear partially fused, forming a pentalaminar structure. Furthermore, in some preparations the internal cavities of both organelles are in continuity, with the vesicles presenting a characteristic drop-like profile (Fig. 5).

The other surface of the endoplasmic sac tightly enfolds or is applied to a mitochondrion. The endoplasmic membrane and the outer mitochondrial membranes are concentric, separated by a very narrow interval of constant width (50–80 Å) filled with a fuzzy, electron-dense material. Only that side or sector of the mitochondrion nearest the synapse is enveloped in such a way. The cisterna of the reticulum extends further around the mitochondrion

than the immediate synaptic zone and away into the cytoplasm. In the cytoplasm it bears unequivocal ribosomes (Figs. 4 and 12) and one may consider the synaptic portion of the reticulum to be part of the rough endoplasmic reticulum, locally agranular. In tangential sections of some synapses, we have even observed continuity between the bag of synaptic reticulum and the nuclear membrane (Fig. 6).

Most of the triads involve a single mitochondrion. But triads including several mitochondria are not uncommon, especially at neuro-muscular junctions where synapses containing a row of five mitochondria have been encountered (Fig. 13).

In all observed synapses the mitochondrion of the pre-synaptic triad presents very few cristae. This feature appears specific to these pre-synaptic mitochondria since mitochondria of the neighbouring cells may possess a large number of cristae. The matrix is generally clear and contains a few dense aggregates.

The synaptic vesicles

Every nerve fibre and nerve cell of a ctenophore usually appears to be more or less crowded with vesicles. A morphometric study of these vesicles in the nerve-net of *Beroe ovata* has led us to distinguish *cytoplasmic vesicles* from *synaptic vesicles* (unpublished results).

Figs. 1–7. The basic features of synapses in the Ctenophora.

Fig. 1. Terminal synapse between a neurite and an epithelial cell in external epithelium of *Pleurobrachia rhodopis*. The terminal is entirely filled by the presynaptic triad: *mi* – mitochondrion; *e.r.* – endoplasmic reticulum; *s.v.* – synaptic vesicle. The high magnification allows one to distinguish filamentous components of the fuzzy coat (*co*) lining the post-synaptic membrane, and of the intracleft material, as well as the pre-synaptic projections (*p*). Fixation C2. × 100 000.

Fig. 2. Two neurite-to-epithelial cell synapses under the aboral organ of *Pleurobrachia rhodopis*. Fixation A2: the P.T.A. staining enhances mainly the post-synaptic thickening and dense materials between the synaptic vesicles and the neuronal membrane. Same abbreviations as Fig. 1. × 50 000.

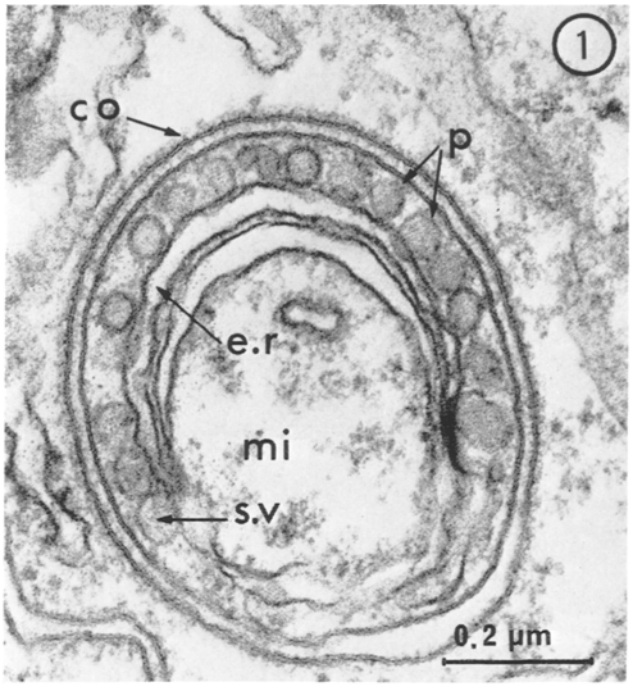
Fig. 3. Neuro-muscular synapse in the mesoglea (*M*) of *Beroe ovata*. Note the electron dense line (▶) in the synaptic cleft. Fixation D. × 50 000.

Fig. 4. Synapses between a neurite and an epithelial cell (*ep*) in the external epithelium of *Pleurobrachia rhodopis*. Note the differentiated contact between reticulum and mitochondrial membranes, and ribosomes attached on the reticulum membrane at the periphery of the cisterna (*arrows*). *M* – mesoglea. Fixation C2. × 50 000.

Fig. 5. 'En passant' neurite-to-neurite synapse in the external nerve-net of *Pleurobrachia rhodopis*. Note the apparent budding of synaptic vesicles from the membrane of the reticulum (small arrows), the striking differences between the cytoplasmic vesicles (*c.v.*) and synaptic vesicles, and the presence of microtubules (*t*) and ribosomes (*r*) in the cytoplasm. Fixation C2. × 50 000.

Fig. 6. Tangential section of the periphery of a nerve cell body of *Beroe ovata*. Note the continuity between the nuclear envelope (*n.e.*) and the cisterna of the reticulum in a pre-synaptic triad. *N* – nucleus. Fixation B1. × 25 000.

Fig. 7. Neurite-to-mesenchymal cell (*me*) synapse, under the labial epithelium of *Beroe ovata*. Most of the synaptic vesicles open into the synaptic cleft (small arrows). Note the intervesicular evaginations of the reticulum and the presence of flattened profiles suggesting the depleted remnants of vesicles. Fixation D. × 50 000.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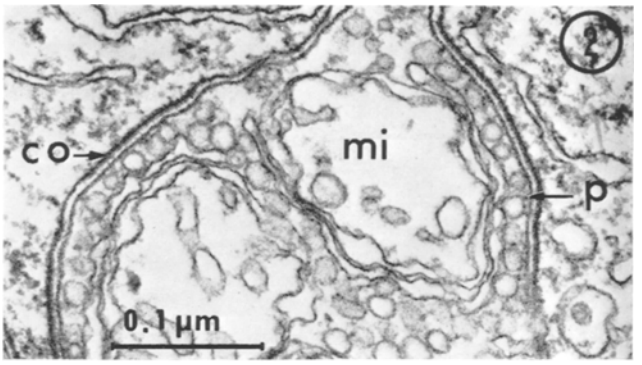
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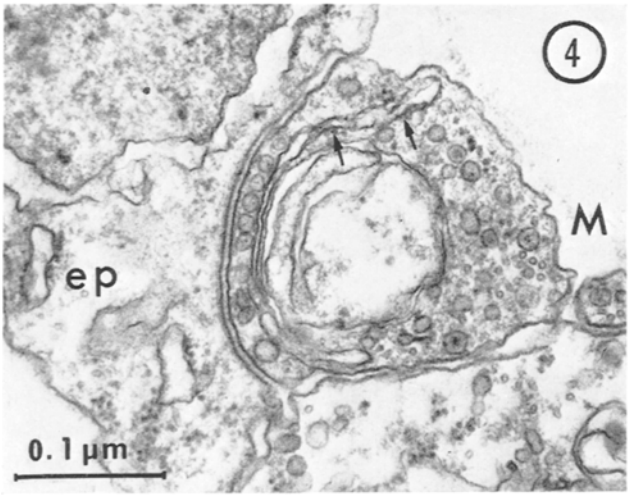
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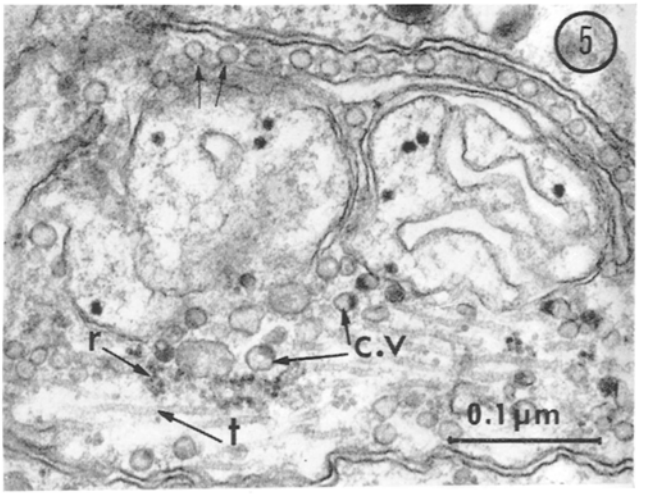
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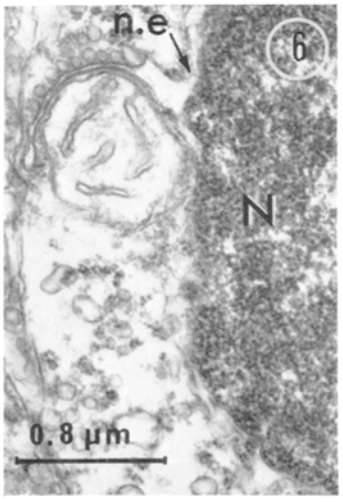
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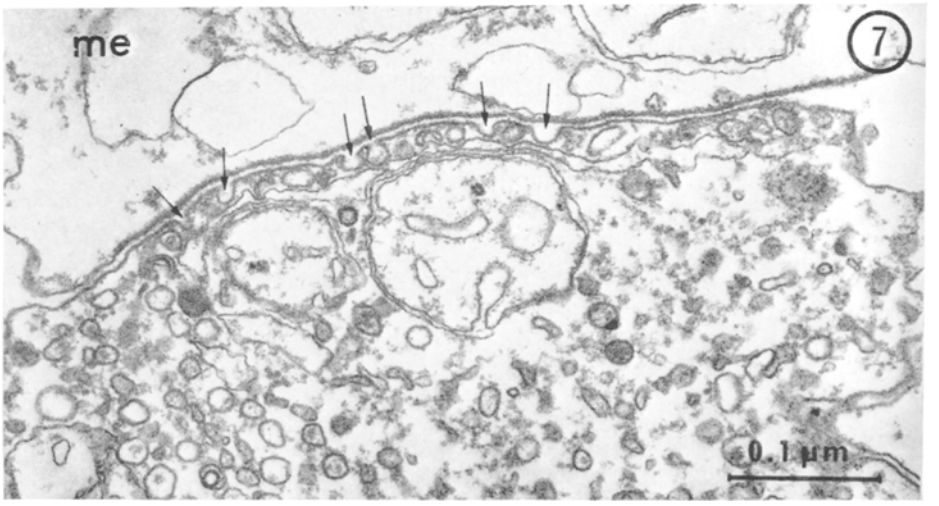
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Consequently the term 'synaptic vesicle' has been exclusively restricted to those vesicles constituting the pre-synaptic triads described above.

The shape of synaptic vesicles is spheroid, or sometimes slightly ovoid. This spheroid shape is resistant to flattening irrespective of variations in fixing procedure.

Most of the vesicles occur at some distance from the plasma membrane (about 80 Å). This space is filled by an electron-opaque filamentous substance revealed in osmium-fixed tissues by P.T.A. staining and apparent in all glutaraldehyde-fixed tissues. The fibrillar material is arranged in small columns which show some similarities to the well known pre-synaptic 'dense projections' (Gray, 1961) of higher phyla.

In some cases, however, the vesicular and the cellular membranes look tightly apposed or even fused and the vesicle may protrude into the synaptic cleft. Moreover, in a small proportion of synapses from tissues fixed with glutaraldehyde (without addition of Ca^{2+} ions) 'omega' profiles were observed, each suggesting the opening of a vesicle into the synaptic cleft. The proportion of synapses exhibiting 'opening' synaptic vesicles was dramatically increased in animals fixed in presence of Ca^{2+} ions, and in each synapse the number of 'opened' vesicles also increased (Fig. 7).

As described for cytoplasmic vesicles (Hernandez-Nicaise, 1973), all synaptic vesicles appear agranular after primary osmium fixation (Figs. 2 and 12). However, the use of glutaraldehyde prior to post-fixation with osmium allows both agranular *and* granular vesicles to be seen.

Our unpublished study of *Beroe ovata* neurites after standard glutaraldehyde fixation indicates the presence of at least four classes of neurites. Our classification is based on the characteristics of cytoplasmic vesicles. In addition, the synaptic vesicles in each postulated neurite population have been measured. Our preliminary results indicate that each 'neurite type' corresponds to a 'synaptic vesicle type'. We can distinguish:

(a) type I neurites: very large granular synaptic vesicles, 1200 Å in diameter, enclosing a very small dense eccentric granule (Fig. 11);

(b) type II neurites: large synaptic vesicles approximately 800 Å in diameter, that may appear empty or show a small dense eccentric granule;

(c) type III neurites: small clear round vesicles, 600 Å in diameter;

(d) type IV neurites: small round dense-cored vesicles, 550 Å in diameter.

A detailed study of this type has not yet been carried out on *Pleurobrachia rhodopis* although the preparations from this species so far examined show noticeable variations between the vesicles of synapses. Present evidence indicates that there are at least two types of synaptic vesicles: small agranular (Figs. 1 and 5) and large granular ones (Fig. 8).

We are now attempting to correlate the morphological characteristics of synaptic vesicles in ctenophores with the possible distribution of postulated transmitters such as ACh and monoamines.

CLASSIFICATION OF SYNAPSES

Preparations for light microscopy suggest that the neurons are isopolar. Our ultrastructural observations strengthen this view since *any part of the neuronal membrane including the*

neuronal perikaryon membrane is able to synapse with another nervous element or with an effector (Figs. 6, 11 and 12). The only distinctive features of the nerve cell body are thus its nucleus and the poorly developed Golgi apparatus.

As classical terminology (e.g. axo-dendritic synapse) does not fit with such peculiar features, we suggest the following terms for interneural synapses: soma-to-soma synapse, soma-to-neurite synapse, neurite-to-soma synapse, neurite-to-neurite synapse.

Both 'en-passant' synapses (Figs. 3 and 5) and nerve terminal synapses (Fig. 1) have been observed along neurites and upon neuronal perikarya and effector cells. There are no ultrastructural differences between these two kinds of synaptic contact. 'En-passant' contacts are often located at vesicle-filled swellings of the neurites which could represent the varicosities observed in silver preparations and after vital staining.

As we reported previously, interneural synapses appear morphologically either polarized (asymmetrical) (Fig. 8), or non-polarized (symmetrical) (Fig. 10), or bi-polarized (reciprocal) (Figs. 9 and 12) (Hernandez-Nicaise, 1968a). A symmetrical synapse can be considered as an interneural or neurosensory contact between two pre-synaptic endings facing each other. This contact is completely symmetrical as each neurite possesses a pre-synaptic triad. Reciprocal synapses give the appearance of two adjacent synaptic contacts with opposite polarities. The ultrastructure of both synapses taking part in this reciprocal arrangement appears to be the same. Each reciprocally-synapsing neurite exhibits the pre-synaptic triad characteristic of ctenophores.

Symmetrical and reciprocal synapses are widespread. They occur, however, less often than asymmetrical ones except under the aboral sense organ and in the nervous core of the tentacles. We have found them in all species so far studied, in young larvae as in mature animals, in every location of the sub-epidermal nerve fibres.

Neuronal perikarya and fibres have been observed to synapse upon other nervous perikarya and fibres, and various receptors and effectors. To date, six histologically well-defined non-neuronal post-synaptic elements have been observed: sensory cells, ciliated cells of the locomotory tracts (balancer supporting cells, groove ciliated cells and comb ciliated cells), epithelial glandular cells, chromatophores, muscle cells (fibres included in the epidermal sheet *and* mesogleal fibres) and 'mesenchymal cells'.

As emphasized above, the pre-synaptic elements of all these synaptic contacts exhibit identical organizations. No specialized organization of the post-synaptic region typical of any given effector has been observed. There seem to be no differences between synapses from epithelium-ensheathed nerve fibres and synapses from the naked fibres running in the mesoglea.

Discussion and conclusions

It appears from our study that while being a primitive nervous system, anatomically, the nerve-net of ctenophores possesses highly organized interneural and neuroeffector contacts.

These contacts had already been noticed and considered to be synapses by Horridge (1964, 1965), although he did not mention the constant presence of the endoplasmic reticulum between the synaptic vesicles and the mitochondrion.

As we pointed out previously, the situation is not fundamentally different from what we know of Cnidaria (see Jha and Mackie, 1967; Westfall, 1970 and 1973; Westfall *et al.*, 1971; Buisson, 1970). The singularity of Ctenophora lies in the great abundance of synaptic contacts and particularly in the special arrangement that we have named the pre-synaptic triad.

It has to be kept in mind that the term 'synapse' now commonly assumes a functional connotation for ultrastructural features. In many instances, physiological evidence for the reality of a supposed synapse is not yet available.

Throughout the animal kingdom, the pre-synaptic element of any presumed chemical synapse is characterized by the presence of vesicles associated with a locally differentiated neuronal plasma membrane (see reviews in Tauc, 1967; Bloom, 1970). Moreover, there seems to be a general agreement about the importance of discrete but fairly universal differentiations in the synaptic arrangement such as: the dense pre-synaptic projections, the middle dense line of the synaptic cleft and a post-synaptic thickening (see for instance the early reviews of Palay, 1958; Couteaux, 1961 and Whittaker and Gray, 1962, and Pfenninger's recent cytochemical investigations, 1971a and b). It is noteworthy that similar features are present in the junctions that we consider to be synapses in the nerve-nets of ctenophores.

In the case of Ctenophora this basic ultrastructural scheme is complicated by the synaptic vesicles forming a triad together with the endoplasmic reticulum and one or several mitochondria. We shall now try to interpret the pre-synaptic triad of Ctenophora in terms of a functional association of its three basic components taking into consideration current hypotheses about synaptic physiology. Four points will be discussed: the association between the mitochondrion and the endoplasmic reticulum, the connections between the reticulum and the synaptic vesicles, the opening of these vesicles into the synaptic cleft and the differences and relations between cytoplasmic and synaptic vesicles.

Figs. 8–13. Same abbreviations as Figs. 1–7.

Fig. 8. Polarized neurite-to-soma synapse in the nervous core of a tentacle of *Pleurobrachia rhodopis*. Note the larger diameter of two cytoplasmic vesicles (*c.v.*) at the periphery of the synaptic area. Fixation C1. × 50 000.

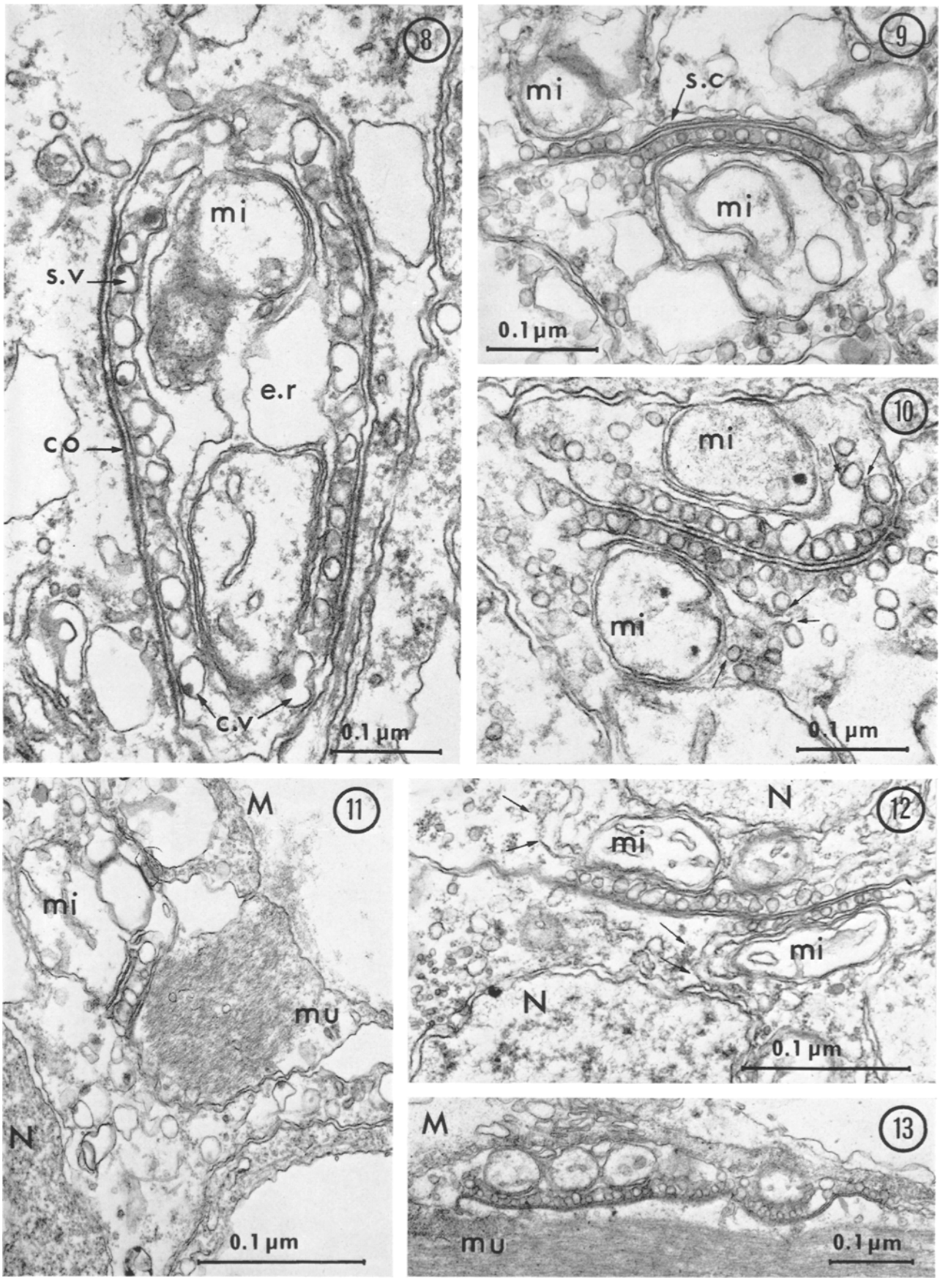
Fig. 9. Reciprocal neurite-to-neurite synapse in the external nerve-net of *Beroe ovata*. Note the subsurface cisterna (*s.c.*) in the upper neurite. Fixation C2. × 50 000.

Fig. 10. Symmetrical neurite-to-neurite synapse in the pharyngeal nerve-net of *Beroe ovata*. Note in both synapses the connections between cytoplasmic vesicles and the reticulum cisternae (*arrows*). Fixation B2. × 50 000.

Fig. 11. Soma-to-muscle synapse in the pharyngeal external muscular sheet of *Beroe ovata*. Note the difference in size between cytoplasmic and synaptic vesicles. Fixation D. × 30 000.

Fig. 12. Soma-to-soma reciprocal synapse in the epithelium of a *Bolina hydatina* larva. Note the abundance of ribosomes on the membrane of the reticulum cisterna that does not face the mitochondrion (arrows). Fixation A1. × 30 000.

Fig. 13. A synapse containing five mitochondria: a neuro-muscular junction in the mesoglea of *Beroe ovata*. The juxtaposition of five pre-synaptic triads builds a small motor end-plate. Fixation B1. × 15 000.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The association between mitochondrion and endoplasmic reticulum

A functional relation between these two organelles is suggested by the narrowness of the space separating their membranes (50–80 Å) and the electron-density of this cleft. Current data allow us to consider two possibilities.

Firstly, the junctional area between the mitochondrion and reticulum might be a site of ionic exchanges and in particular of Ca^{2+} ions. The role of the reticulum in the uptake and release of Ca^{2+} during the course of muscular contraction is now generally accepted. Some authors, moreover, referring to Lehninger's review (1970), consider that sarcoplasmic mitochondria 'may be the major Ca^{2+} segregating organelle in some types of muscle'. Lehninger himself suggests that 'similarly, mitochondria may play a role in sequestration and release of Ca^{2+} at nerve endings'.

Secondly, the junctional area might also be a preferred pathway for transfer of newly synthesized enzymes and other proteins. Mitochondrial enzymes might possibly participate in the final synthesis of transmitters either directly or indirectly by providing energy for any preliminary synthesis carried into the reticulum cisterna of the pre-synaptic triad. Intimate relationship between mitochondria and the endoplasmic reticulum is established in cells of various tissues and animals (see review in Szöllösi and Hunter, 1973). It has to be noted that these complexes occur particularly in oocytes, i.e. cells engaged in a high rate of synthesis.

Connections between the endoplasmic reticulum and synaptic vesicles

The close contact between these two organelles and the occasional continuity between their internal cavities lead us to consider the possible existence of pathways serving for the passage of metabolites here too, perhaps from membrane to membrane, or from cavity to cavity. Direct transfer from endoplasmic reticulum to synaptic vesicle in such a manner could then represent the final packaging of the transmitter.

The electron microscopical evidence of continuity between the two organelles suggests, furthermore, that vesicles may bud from the reticulum of the pre-synaptic triad. The reticular origin of synaptic vesicles has been repeatedly investigated and discussed since De Robertis and Bennett (1955) put forward their hypothesis. It is now considered probable by many authors (see Taxi, 1969; Koelle, 1970). Budding of dense cored vesicles during ontogenesis has been described by Machado (1971). From whatever organelle the synaptic vesicles may originate – endoplasmic reticulum, plasma membrane (Bunt, 1969; Akert *et al.*, 1969; Gray, 1971), or other vesicles (Nagasawa *et al.*, 1970; Fillenz, 1971) – it is generally accepted that they undergo differentiation in the nerve terminal.

In the case of Ctenophora, the possible differentiation of synaptic vesicles from cytoplasmic ones cannot be excluded on the basis of existing morphological evidence. It is, nevertheless, a rather attractive hypothesis, that they may arise by budding from the cisterna of the reticulum in the pre-synaptic triad.

The opening of synaptic vesicles into the synaptic cleft

In our preparations the frequent occurrence of vesicles opening into synaptic clefts can be interpreted as resulting from exocytosis of the contents of synaptic vesicles. It is noteworthy

that we observed a dramatically increased proportion of synapses exhibiting such 'omega profiles' after we started adding $CaCl_2$ to our fixing and washing solutions. Ample evidence now exists that the release of a wide range of secretions stored in vesicles, including ACh and biogenic amines, depends upon the presence of calcium ions in the extracellular fluid (see review in Smith, 1971b).

The idea that the contents of synaptic vesicles are released into the synaptic cleft by exocytosis has recently received wide support (see reviews in Hubbard, 1971; Smith, 1971a and b, and the ultrastructural findings of Courteaux and Pécot-Dechavassine, 1970a and b, 1972). On the other hand, Bunt (1969), Holtzman *et al.* (1971) and Ceccarelli *et al.* (1972) indicate that synaptic vesicles may withdraw substances from the extracellular fluid into pinocytic vesicles. In the absence of appropriate experimental evidence this possibility cannot be excluded in the case of Ctenophora synapses. In our opinion synaptic vesicles probably undergo exo- and endocytosis in succession, as already pointed out by Couteaux and Pécot-Dechavassine (1970) with regard to vertebrate motor end-plates.

Differences and relations between the cytoplasmic synaptic vesicles

Our study of the species *Beroe ovata* has led us to distinguish cytoplasmic from synaptic vesicles. We infer from our measurements that each type of cytoplasmic vesicle is associated with a particular type of synaptic vesicle. Besides their different locations, these two sorts of vesicle differ mainly in size (the cytoplasmic ones showing a larger diameter in sections) and also, in some cases, in their granular content. Cytoplasmic vesicles are scattered through the whole neuronal perikaryon, but they are particularly abundant in the vicinity of the Golgi apparatus. The latter, therefore, may perhaps be involved in their genesis. We have previously noted the abundance of such vesicles in neurites along with numerous microtubules (Hernandez-Nicaise, 1973), an association consistent with the hypothesis of transport of these vesicles from the perikaryon. Transport by means of microtubules has been assumed in a number of investigations (see review in Dahlström, 1971). Finally, our investigations on the distribution of biogenic amines in the nervous system of ctenophores (unpublished study) seem to indicate the presence of such amines in some types of granular cytoplasmic vesicles.

The situation is strongly reminiscent of the organization of peripheral adrenergic fibres in vertebrates (see review in Dahlström, 1971). In those fibres, two types of vesicles have been described: large granular vesicles originating in the neuronal soma and small granular synaptic vesicles which are present only at the nerve terminals (see Fillenz, 1970, 1971). The presence of noradrenaline has been demonstrated in both kinds of vesicles (see Tranzer and Thoenen, 1968; Bisby and Fillenz, 1970; Fillenz, 1971).

Although it may be misleading to compare the ultrastructural features of the nervous system of Ctenophora to peripheral nerve fibres of vertebrates, such comparison reveals similarities which are helpful in the interpretation of our observations. If the large cytoplasmic vesicles of Ctenophora carry to the synapse substances that are synthesized in the perikaryon and are necessary for synaptic transmission (as do the large granular vesicles of vertebrates), there are two possibilities: (a) synaptic vesicles originate from cytoplasmic vesicles and, taking into account evidence from our electron micrographs cytoplasmic

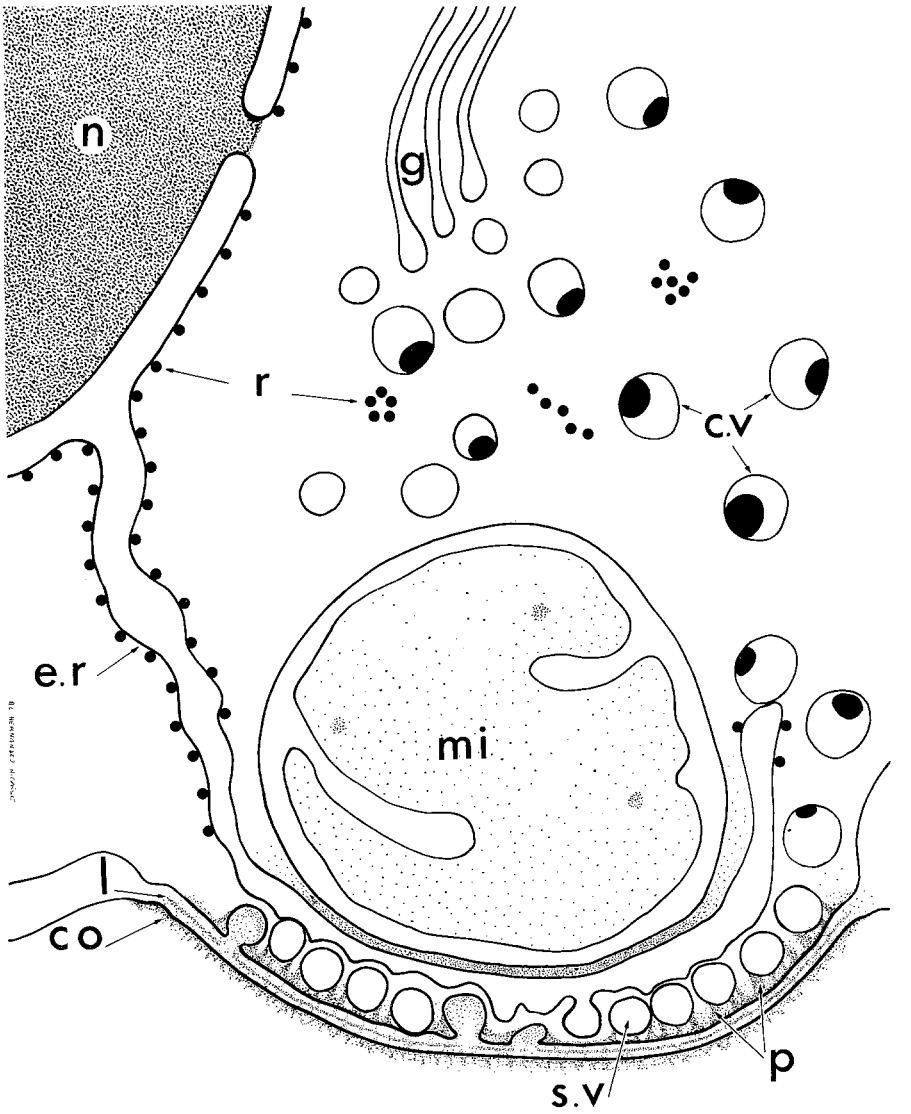

Fig. 14. Diagram of the ctenophore synapse; *c.v.* – cytoplasmic vesicles; *co* – dense coat on the post-synaptic membrane; *e.r.* – endoplasmic reticulum; *g* – Golgi apparatus; *l* – intra-cleft dense line; *mi* – mitochondrion; *n* – nucleus; *p* – pre-synaptic dense projection; *r* – ribosome; *s.v.* – synaptic vesicle.

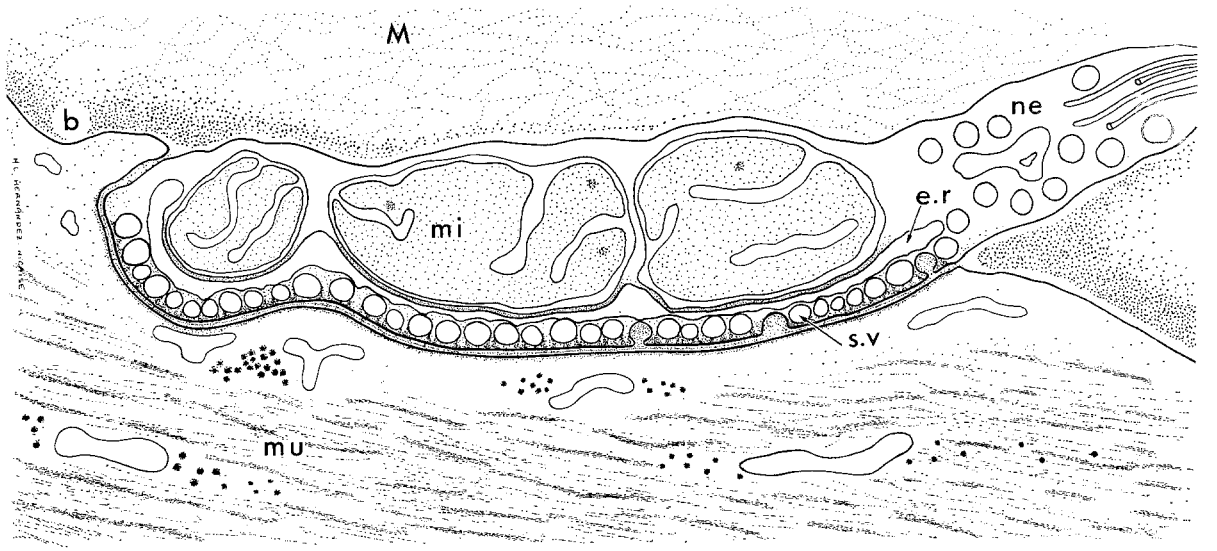

Fig. 15. Diagram of a neuro-muscular junction of a ctenophore, involving a mesogleal muscle cell. *b* – basement membrane; *e.r.* – endoplasmic reticulum; *M* – mesoglea; *mi* – mitochondrion; *mu* – muscle cell; *ne* – nerve fibre; *s.v.* – synaptic vesicle. Note that the nerve fibre is naked and has no specific basement membrane.

vesicles undergo their transformation at the synaptic area itself; or (b) two distinct vesicle populations do exist. The profiles observed in our preparations (indicating possible continuity of the vesicular membrane with reticular and plasmic membranes) would account for an *in situ* turn-over of the synaptic vesicles.

In conclusion, the pre-synaptic triad of the synapses of Ctenophora appears essentially to be a unique site where the synthesis of metabolites necessary for synaptic transmission is likely to take place. The triad could then provide, *in situ*, a rapid, continuously adjustable supply of these metabolites. Furthermore, this unique feature may reflect the isopolar morphology of the neuron at a physiological level, since most of the synthesis traditionally attributed to the cell body is carried out by synaptic triads that may occur anywhere on the neuron surface. Fig. 14 gives a diagrammatic representation of a synaptic junction in a ctenophore as inferred from our observations. Fig. 15 represents the particular case of the neuro-muscular junction.

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