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Ultrastructural studies of neuromuscular junctions in visceral and skeletal muscles of the chaetognath *Sagitta setosa*

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Summary. The ultrastructural characteristics of the neuromuscular junctions were studied in oesophageal (visceral) muscle and in four skeletal muscles of the head and trunk in *Sagitta*.

Three types of neuromuscular junctions were encountered. The first is made up of nerve terminals which synapse with the surface of the muscle fiber, in a deep or in a slight depression. The second is characterized by muscle fiber protrusions that cross the connective tissue and form synapses with nerve endings; in this type, numerous post-junctional membrane folds are noted. In the third type, the synaptic cleft is very large (>0.2 µm) and contains bundles of connective fibers.

Nerve endings are partially ensheathed in glial cells; they contain mostly clear synaptic vesicles, though some dense-cored vesicles are noted. In many muscle fibers post-junctional membrane thickenings are also observed. All observed neuromuscular junctions resemble chemical synapses.

Chaetognaths thus show a great variety of neuromuscular junction ultrastructure as do for instance Arthropods.

Key words: Skeletal muscle – Visceral muscle – Neuromuscular junctions – Motor end-plates – Chaetognaths

In adult *Sagitta,* the body is constructed around three cavities (Hertwig 1880; Burfield 1927; Hyman 1959); the wall is mainly composed of a superficial stratified epidermis containing the nervous system. Nervous elements comprise ganglia, nerve trunks and a large intraepidermal network (Hertwig 1880; Grassi 1883). In the trunk, a thin layer mostly composed of collagenous fibers (basement membrane) separates the epidermis from the muscular

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tissue and the epithelial lateral fields (Burfield 1927; Duvert and Salat 1979); it also divides the body musculature into two halves along the sagittal plane, these partitions being the so-called mesenteries (an inadequate term, since they are not homologous with true mesenteries). In the head, the connective tissue is made of sheets of basement membrane and its derivates, which branch out around many muscles (Burfield 1927). As in the trunk, these muscles are inserted into the connective tissue. In both the head and, especially, the trunk, muscles are separated from nerves by these skeletal structures; the problem of innervation of the muscles remains.

In the trunk the problem is complicated by the presence of a large ventral ganglion. Some authors e.g. Bullock, after De Beauchamp (1960), state that "the ventral ganglion probably innervates the main locomotor muscles" (Bullock and Horridge 1965). This opinion is not shared by others, such as Burfield (1927). We have thus investigated the ventral ganglion and the lateral nerve trunk zones to see if motor end-plates are located and more abundant in their vicinity.

This is the first report of neuromuscular junctions in a Chaetognath. We have used the terminology of Burfield (1927).

Materials and methods

Specimens of *Sagitta setosa* were collected in the plankton from the Bassin d'Arcachon. Because of the great difficulty in achieving a good and homogeneous preservation of the epidermis and of the nervous system, various fixatives were tried. The best results were obtained with animals fixed in an aldehyde mixture (Karnovsky 1965) or in 2.5% glutaraldehyde in a sodium cacodylate buffer 0.1 M, pH 7.8, containing sucrose to give a total osmolarity $\geq$1,200 mOsm. The animals were postfixed in 1% osmium tetroxide in sodium cacodylate buffer, pH 7.5, then impregnated with an uranyl acetate solution (Silva et al. 1971) and embedded. Thin sections were examined with a Philips EM 300 electron microscope.

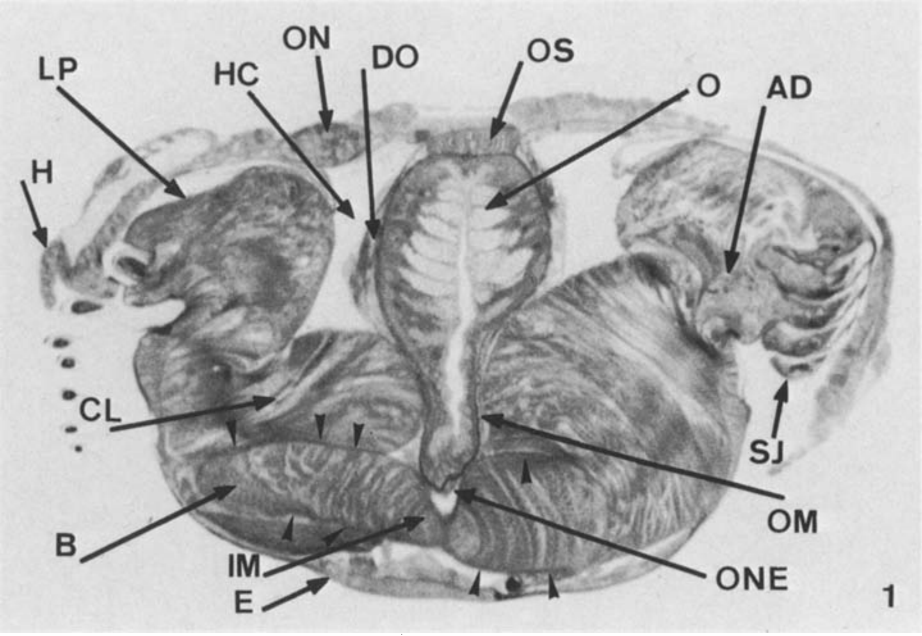
Results

I. Oesophageal muscle

We studied oesophageal muscle (Fig. 1), because it is made up of two layers, one longitudinal, the other circular. This musculature is more developed than that of the intestine, so that motor nerve endings were expected in greater number.

The oesophageal muscle is probably innervated by oesophageal nerves and ganglia. The nerves penetrate deeply into the muscle tissue and the contact area between nerve endings and muscles is very large (Figs. 2, 3). Nerve motor endings spread on many adjoining muscle fibers. Together with their glial cells they penetrate the cortex of the muscle fibers or wedge in between the cells (Figs. 2, 6).

Nerve endings are clearly seen. Schwann cells extend between axons; they partially ensheath the nerve endings in the synaptic zone (Figs. 4, 6) and are sometimes poorly developed, the nerve endings then sharing a large



AD Adductor uncinorum muscle; *AF* A fiber; *AX* axon; *B* bicornis muscle; *BF* B. fiber; *BM* basement membrane; *CL* complexus lateralis muscle; *DO* dilatator oris muscle; *E* epidermis; *H* hood; *HC* head cavity; *IM* intra-muscular organ; *LP* lateral plate; *O* oesophagus; *OM* circular and longitudinal muscles of oesophagus; *ON* optic nerve; *ONE* oesophageal nerve; *OS* obliquus superficialis muscle; *SC* Schwann cell; SJ seizing jaws

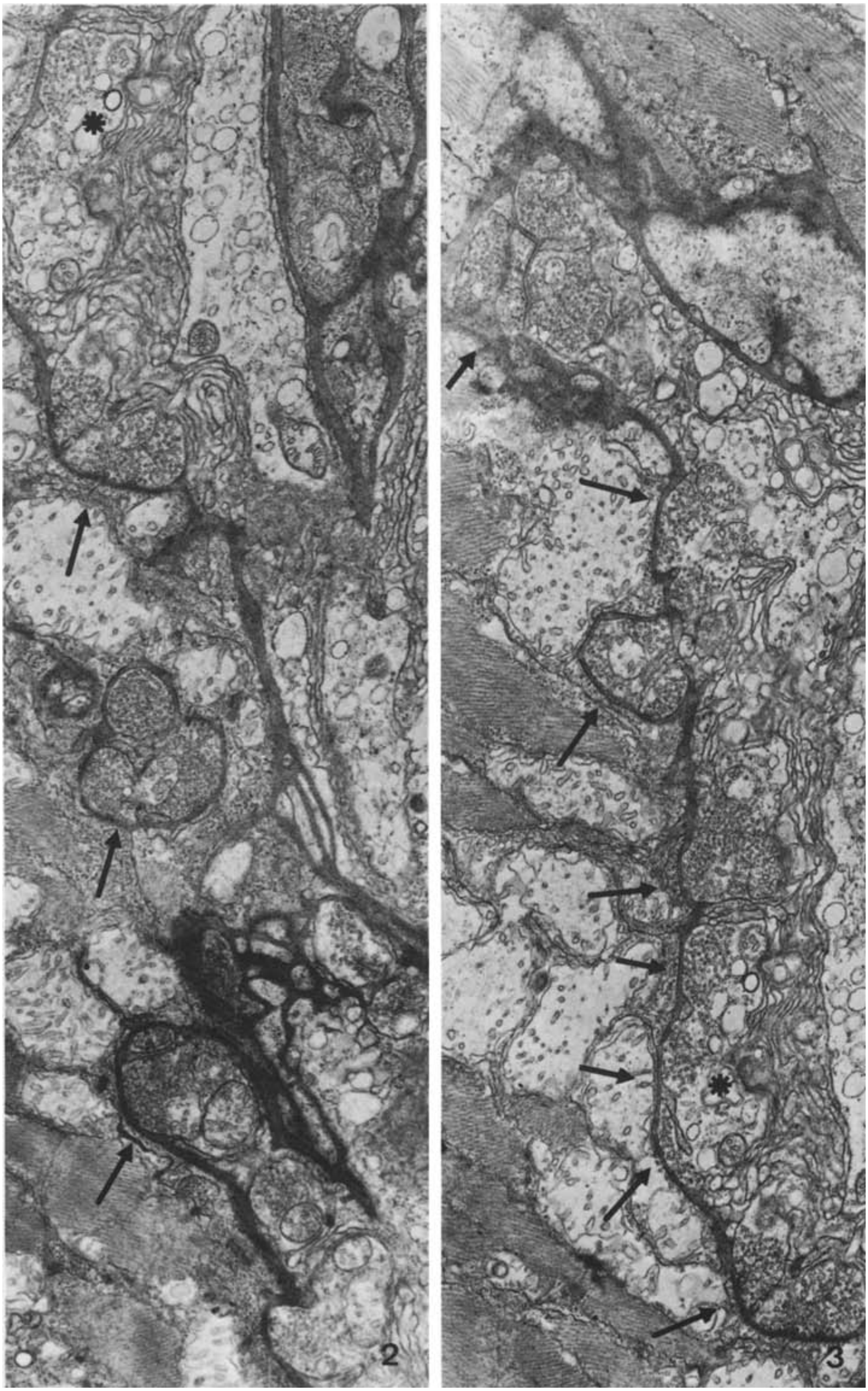
Fig. 1. Transverse section of the head, behind the mouth. Head muscles studied in this paper are shown (except m. transversus dorsalis). Semithin section. × 300

contact area with the muscle fibers (Figs. 2, 3). Nerve endings may ramify (Figs. 2, 6, asterisks), the digitations being separated by Schwann cell processes. Synaptic vesicles and mitochondria are abundant; the vesicles are about 60 nm in diameter, mostly clear, and relatively homogenous. Facing the muscle fiber, a membrane thickening is sometimes observed (Fig. 5). The synaptic cleft is about 50 nm; a dense material, continuous with the basal lamina, surrounds the nerves and the muscle; facing membrane thickenings, this material has a stratified appearance (Fig. 5). In the muscle fibers, one can see a great accumulation of mitochondria (Figs. 2, 3). Facing the nerve ending, the plasma membrane shows a local thickening (about 13 nm) mainly due to an enlargement of the cytoplasmic leaflet. Membrane folds are present against the synaptic zone, in the muscle fiber (Figs. 4–6).

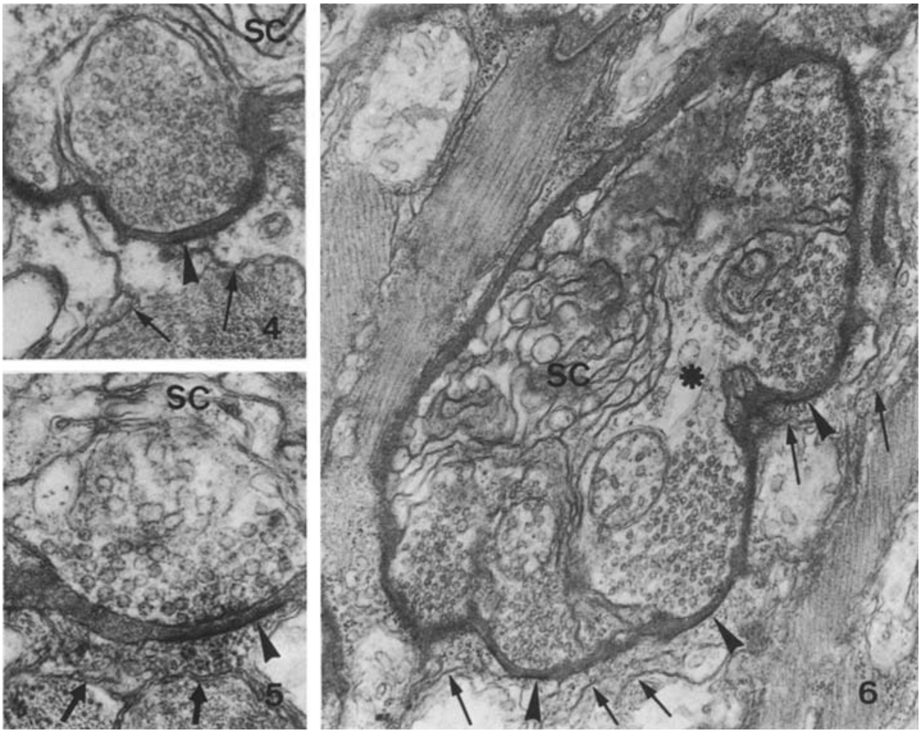
II. Muscles of the head

A. Bicornis. This is an unpaired muscle, below the mouth (Fig. 1). It is totally surrounded by a basement membrane (Fig. 1, arrowheads).

Using the electron microscope, one can see a medio-ventral nerve (Figs. 7, 12) in the vicinity of Bicornis. Near this nerve we localized a motor



Figs. 2, 3. Transverse section in the oesophageal muscle layers showing numerous motor endplates (*arrows*) The nervous tissue is at the right and the muscle fibers at the left. *Asterisks* show ramifications of the nerve endings, often separated by Schwann cells. ×9900; ×9300



Figs. 4–6. Details showing the motor end-plates in oesophageal muscles. *Arrows* show post-junctional foldings; *arrowheads* show post-junctional membrane thickenings, facing extracellular differentiations. Fig. 4: ×23700; Fig. 5: ×30000; Fig. 6: ×17700

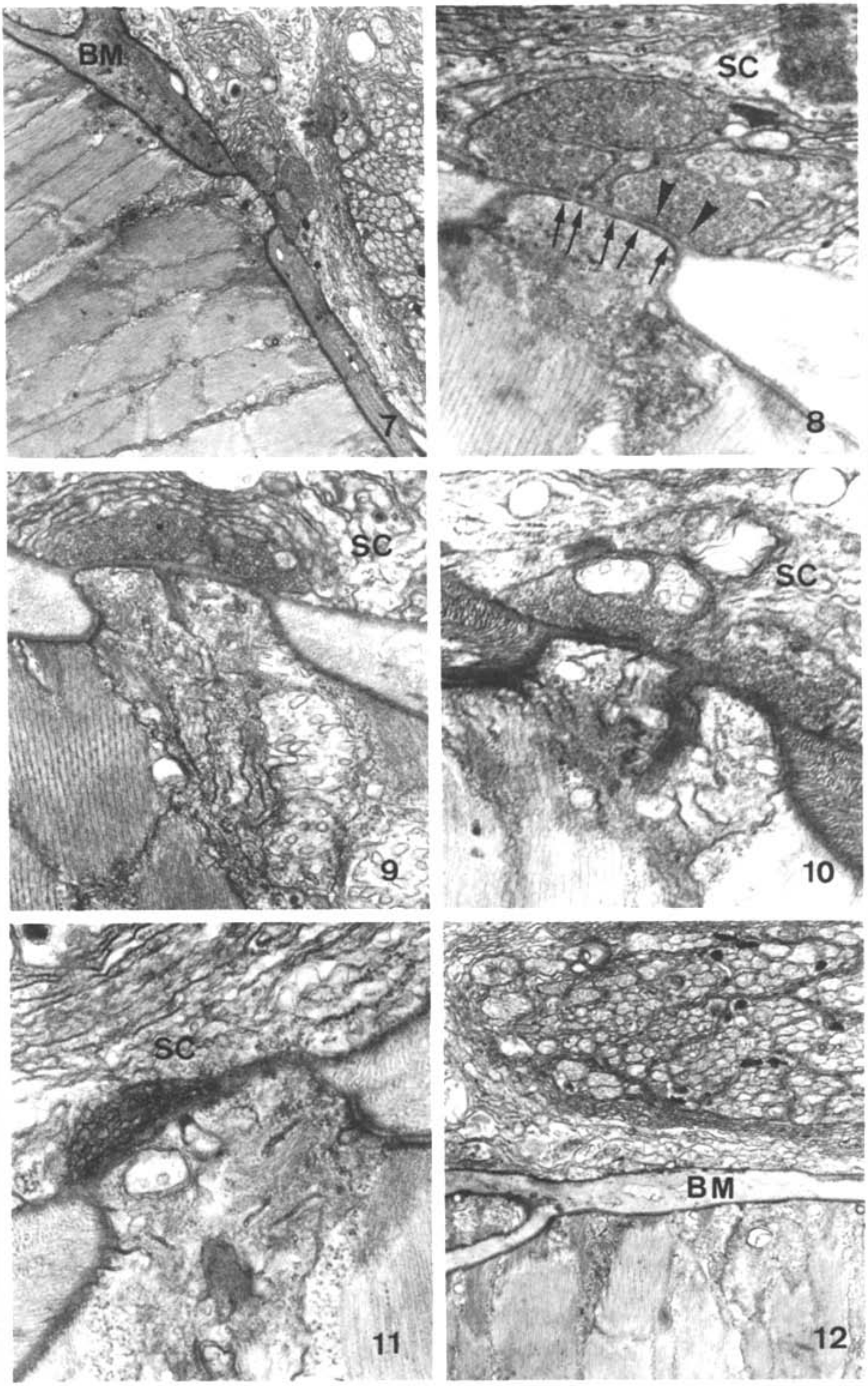
endplate, which we studied in serial sections (Figs. 7–12). This motor nerve ending is very narrow and seems to make contact with only one fiber.

At this level the nerve ending ramifies (Figs. 7, 8) and is closely ensheathed in glial cells, except where it contacts the muscle fiber. The muscle cell protrudes through the basement membrane, the nerve always remaining on the surface of the connective tissue.

In the nerve one can see numerous clear vesicles, and some mitochondria. The vesicles are homogeneous with a diameter ranging from 48 nm to 70 nm. Discrete membrane thickenings are sometimes noted (Fig. 8).

The synaptic cleft is about 50 nm in width. It contains a dense material, continuous with basal laminas (Figs 8, 9). Facing the nerve endings the muscle fiber shows some differentiation. Mitochondria are noted (Fig. 9), but the most striking feature is the development of plasma membrane folds and an abundant cytoskeleton. Two kinds of folds are noted, one >0.1 μm wide (Figs. 9, 10) and the other made of numerous narrow clefts, about 30 nm wide, ramified in the sarcoplasm (Fig. 9). Local plasma membrane thickenings are observed in both the nerve endings and muscle fibers (Figs. 8, 10).

This neuromuscular junction is located in the myotendinous zone.



Figs. 7–12. Serial sections of a motor end-plate in Bicornis. It is situated at the myotendinous zone near a superficial nerve (**Figs. 7, 12**). The muscle crosses the basement membrane and synapses with a ramified ending. Post-junctional folds are seen (**Figs. 9–11**); membrane thickenings in the prejunctional and post-junctional membranes (**Fig. 8**) are noted. **Figure 12** is a section immediately after the end of the synapse (compare with Fig. 7). Fig. 7: ×4300; Fig. 8: ×18800; Fig. 9: ×16000; Fig. 10: ×20300; Fig. 11: ×21200; Fig. 12: ×5600

B. Complexus lateralis. This muscle is well developed on both sides of the digestive tube; it surrounds Bicornis, both ventrally and laterally (Fig. 1).

We found motor end-plates at two different levels. First, in conjunction with a nerve located near the vestibular ganglion (Figs. 13, 14); second, in conjunction with the medioventral nerve already noted in the vicinity of Bicornis. In both cases the muscle fibers cross the basement membrane and penetrate more or less deeply in the nervous tissue. Schwann cells partially ensheathe the nerve endings, except in the zone facing the muscle fiber (Figs 14, 15). The nerve endings contain mitochondria together with many clear vesicles. These vesicles seem homogeneous, with a diameter of about 40 nm.

The synaptic cleft is reduced, it contains an electron dense substance which is continuous with the basal laminas surrounding the nerve and the muscle.

In the muscle fibers facing the nerve ending some mitochondria and a very large subsynaptic apparatus are to be found. The latter is composed of plasma membrane foldings which ramify in the sarcoplasm, together with an abundant cytoskeleton (Fig. 15). These clefts are about 20 nm wide; larger clefts are also noted (Fig. 13).

Motor end-plates are to be found along many fibers (Fig. 13) crossing the basal lamina.

C. Transversus dorsalis. This powerful muscle is antagonistic with Expansus superior and may help in the seizing jaw movements. We were able to observe motor end-plates related to a nerve located between Transversus dorsalis and Obliquus superficialis.

The nerve endings are superficially located and ensheathed in Schwann cells, except in the zone facing the muscle plasma membrane (Figs. 16, 17), and penetrates the muscle tissue. In the nerve, mitochondria can be observed, together with heterogeneous vesicles. The latter have a diameter of 60–70 nm.

The synaptic cleft is narrow, about 30 nm, and contains a dense substance continuous with basal lamina.

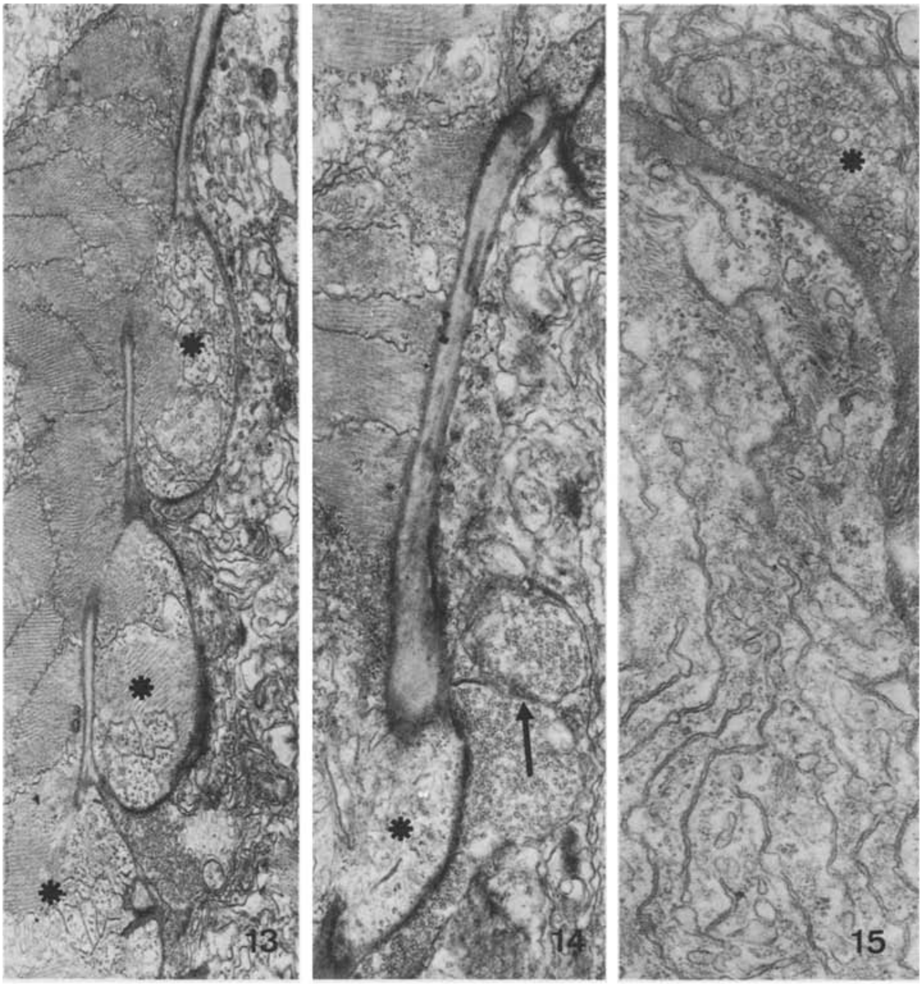
In the muscle, mitochondria may be abundant (Fig. 16) and membrane thickenings are noted (Fig. 17, arrowheads).

Identical motor end-plates were also found in other, not clearly identified, head muscles (as perhaps Dilatator vestibuli internus with a nerve from the vestibular ganglion).

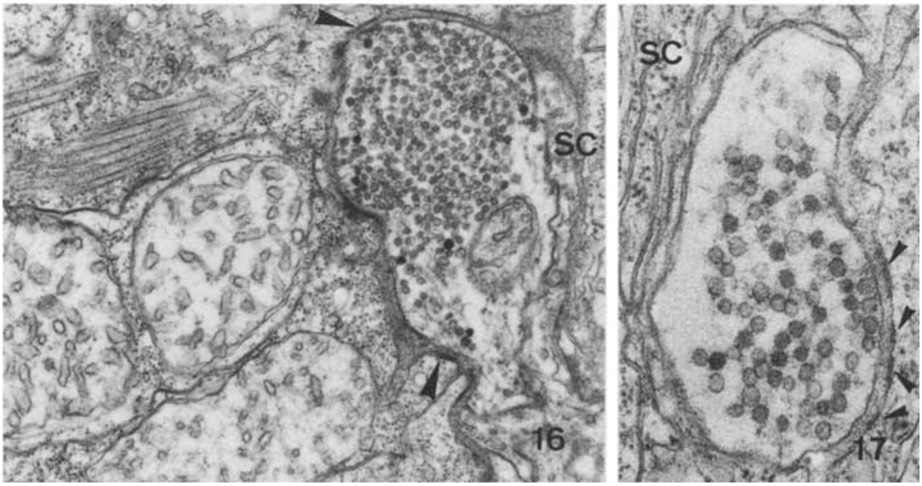
III. Primary musculature of the trunk

This constitutes the main part of the musculature of the trunk and the tail. It is always separated from the epidermis and the nervous system by the thick basement membrane, about 0.2 µm to 0.5 µm, and even more in the lateral fin zones (Figs. 18–20).

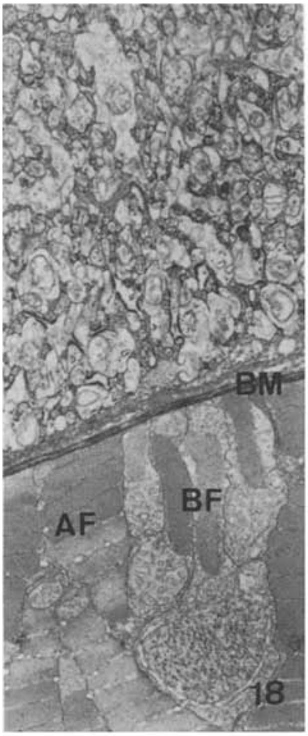
No nerve or muscle fibers cross the basement membrane at the ventral ganglion level (Fig. 18), either in the neuropile or in the cellular part. At

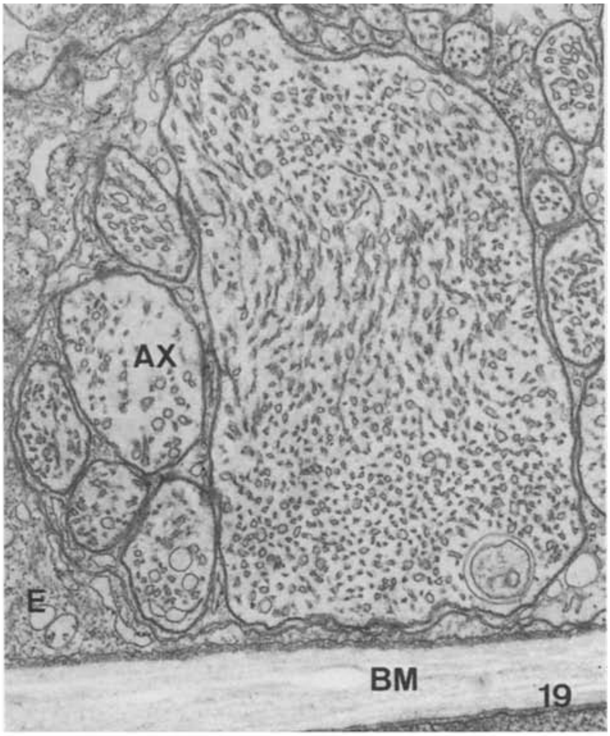


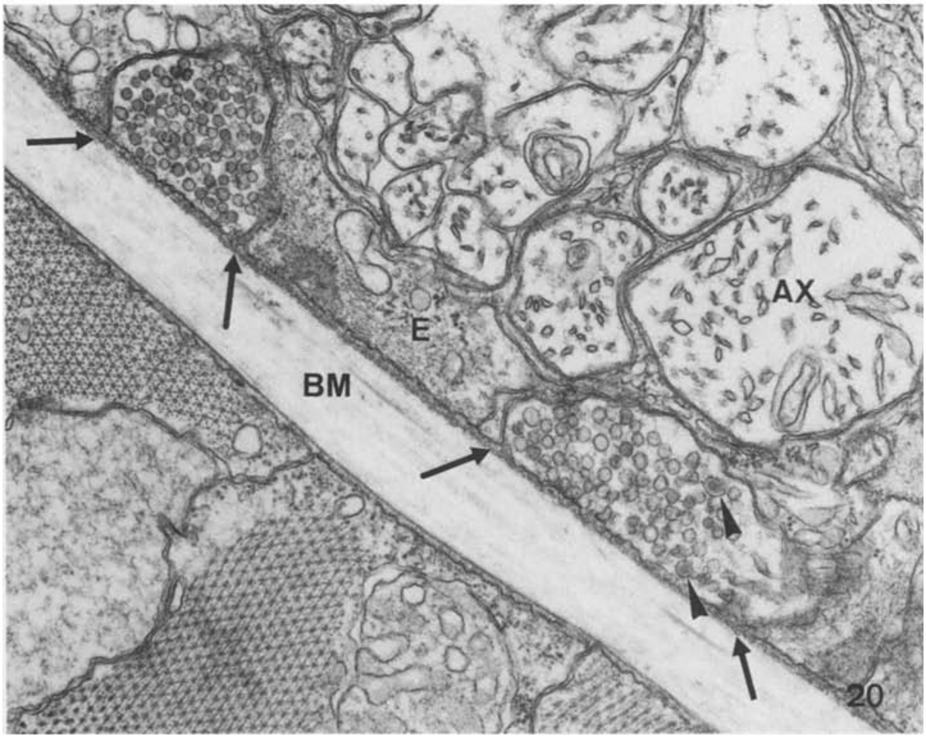
Figs. 13–15. Sections in the neuromuscular junction in Complexus lateralis. Muscle fibers cross the basement membrane, spread largely against the nerve (*asterisks*). Post-junctional folds are well developed. Note the close apposition between two nerve sections (**Fig. 14,** *arrow*), out of the synaptic zone. Fig. 13: ×9100; Fig. 14: ×15100; Fig. 15: ×38400



Figs. 16, 17. Neuromuscular junction at Transversus dorsalis level. Synaptic vesicles, seem heterogeneous; note the thickenings of the plasma membrane in the muscle fibers (*arrowheads*). ×17000; ×37400







Figs. 18, 19. Transverse sections in the trunk, in the ventral ganglion (at the neuropile level) and at a nerve trunk level. No motor end-plate can be seen between nerves and muscle fibers. ×5400; ×16300

Fig. 20. Two nerve endings (between *arrows*) in the intra-epidermal nerve plexus. Synaptic vesicles are very abundant; one can see clear vesicles and some dense-cored vesicles (*arrowheads*). ×28800

the level of the main nerve trunks the basement membrane is also continuous (Fig. 19).

Nerve ending-like structures are regularly found, disseminated between the epidermis and the basement membrane (Fig. 20). No epidermal or glial cell is to be found between these nerve endings and the basement membrane facing the primary musculature. Synaptic vesicles are abundant and about 60–70 nm in diameter. Clear vesicles are very numerous; some dense cored vesicles are regularly found (Fig. 20, arrowheads).

Both the sarcoplasm and the plasma membrane of the muscle fiber facing the nerve endings lack morphological differentiation. These motor end-plate-like structures are found at the myotendinous level.

Discussion

Motor end-plates in *Sagitta* show morphological differentiations and polarization similar to those in many animals (Osborne 1975; Pappas 1975; Huddart 1975): glial cells partly wrapping nerve endings; an accumulation of synaptic vesicles; a synaptic cleft with a basal lamina-like material and membrane thickenings, particularly on the muscle membrane (except in primary musculature). In the muscle fiber one can see an accumulation of mitochondria, and sometimes of other organelles such as plasma membrane invaginations and the cytoskeleton (except in primary musculature); but no "aposynaptic granules" (Bullock and Horridge 1965; Osborne 1975) were found. So, in oesophageal and head muscles, the fine structure of the synapses is like that of classical chemical synapses (Pappas 1975).

Three kinds of neuromuscular junctions are found in *Sagitta:*

1) In the oesophageal muscle and Transversus dorsalis nerves give protrusions in muscle tissue and come in contact with the muscle fibers; the synaptic cleft is narrow, as in many other animal muscles (Osborne 1975).

2) In Complexus lateralis and in Bicornis, the fibers give rise to more or less developed protrusions, crossing the basement membrane and making synaptic contact with nerves. This kind of innervation is also known in other animals unrelated to each other and to Chaetognath, as for instance: Kamptozoa (Emschermann 1982), Annelids (Mill and Knapp 1970), Echinoderms (Coob and Laverack 1967), Nematods (De Bell 1965), Arthropoda (Lang 1972) and Chordata (Flood 1966, 1970).

3) In the primary musculature of the trunk, nerve endings and muscle fibers are separated by a large space crossed by connective fibers of the basement membrane (see below).

Since no studies have been made of the nature of the synaptic transmitter(s) in chaetognaths or of the physiological characteristics of the muscles, we do not know the significance of such diversity; perhaps it is correlated with some physiological peculiarities as in other invertebrate muscles (Crabtree and Sherman 1981).

In the second kind of neuromuscular junction, where the muscle fibers give rise to protrusions making contact with nerves, junctional folds are present: they seem more developed in Complexus lateralis than in Bicornis and much more than in oesophageal muscle (compare Fig. 15 with 10 and with 4 to 6). Postjunctional folds are known to exist in some vertebrate muscles (Couteaux 1973), and a subsynaptic reticulum has been reported in some invertebrate muscles (Osborne 1975); these structures increase the surface between the two tissues. In Invertebrates the subsynaptic apparatus is not analogous to that found in Vertebrates; its structure seems different and, as in *Sagitta,* it can be found in both visceral and skeletal muscles (Osborne 1975). Some of the synapses described in this paper resemble other neuromuscular junctions already reported in invertebrate visceral, cardiac and skeletal muscles and in some vertebrate muscles (Osborne 1975; Couteaux 1973). Since no physiological data are available in chaetognath muscles or nerves, we cannot speculate about morphological characteristics found in different motor end plates. At the ultrastructural level great differences exist between the primary musculature of the trunk (Duvert 1969) and oesophageal and head muscles. Differences are obvious in the contractile apparatus. In the head muscles, myofibrils are not so regular, sarcomeres are longer and have a higher F2:F1 ratio than in primary musculature; Z lines sometimes show strong differences and primary musculature myofibrils look like flight muscle myofibrils in insects (Auber and Couteaux 1963). It is generally agreed that there is some kind of correlation between ultrastructural characteristics of the contractile apparatus and the speed of contraction (Elder 1975); one may think that primary musculature is composed of fast muscle fibers, and head musculature, like oesophageal muscles, of slow muscle fibers. This hypothesis seems to be confirmed by coarse in vivo observations. Ultrastructural variation of the motor end-plate is to be noted in these comparatively slow muscles, but not in the rapid trunk musculature.

Primary musculature of the trunk is the only kind of muscle where very numerous nerve endings do not lie against plasma membrane of the muscle fiber, the two tissues being separated by the connective tissue; moreover no obvious differentiations are seen at this level. The same characteristics are noted in nerve endings from muscular tissue in other animals, such as Pogonophora (Gupta and Little 1970, 1975) and Annelids (Smith et al. 1973). In these animals the intraepidermal nervous system is separated from the muscle by connective tissue as in *Sagitta.* As in the vertebrate sympathetic nervous system (Burnstock 1970) close apposition between nervous elements and their effector does not seem indispensable for contraction in the primary musculature of the trunk.

Unfortunately the way in which *Sagitta* swims is not known. *Sagitta* has an hydroskeleton (Duvert and Salat 1979); one can hypothesize that muscle fibers, which are linked via numerous gap junctions (Duvert and Salat 1980), act as a syncytium. Perhaps each quadrant of the primary musculature (Burfield 1927) may act in an antagonistic way, bending the body during swimming. In this hypothesis, close apposition between nerve and muscle is not indispensable for initiation of contraction; the motor end-plate may increase the concentration of neurotransmitters in the vicinity of the fiber bundles. Our observations are in agreement with this assumption. The innervation of the secondary musculature (Duvert and Salat 1979) remains to be established.

We know nothing about the innervation pattern of Chaetognath muscles (Burfield 1927). Our results suggest that it may be polyneuronal in oesophageal muscles and in the primary musculature. Conversely in Bicornis, only some fibers (perhaps only one) are innervated; the excitation is probably propagated via gap junctions as in the primary musculature (Duvert et al. 1980), or in other electrically interconnected muscle of Nematodes (De Bell 1965) or in vertebrate cardiac and smooth muscles (Huddart 1975).

Chaetognaths show a great diversity of motor end-plate structure, according to the muscle studied. This diversity is especially noticeable in slow muscle types in the head and intestine; it may be related to physiological characteristics, as yet unknown, and does not seem to be useful for phylogenetic studies (Grimstone 1959). All these peculiarities point to many specialisations in this intriguing phylum.

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