

The Ultrastructure of the Motor Nerve Endings in the Muscles of Cephalopods

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The motor innervation of muscles of the sucker of *Octopus* and the lip of *Sepia* has been described. Definite nerve endings containing synaptic vesicles and mitochondria and with circumscribed synaptic specializations are frequently observed in both the species studied. The presence of nerve fibers running in grooves into the muscle cells and bounded by an infolding of the surface membrane of the muscle is also reported. An overall thickness of the synaptic membrane complex of 250 Å, with an intervening gap of approximately 100 Å, is also a standard feature in these neuromuscular contacts. These findings are briefly discussed, and a comparison is made with the structure of the motor end plates in other animals.

No extensive observations of the ultrastructure of the nerve terminals in molluscan muscles have yet been reported (cf. references 22 and 30 for a review of the subject). Investigators who have studied the motor nervous system of molluscan muscles by using the light microscope are divided into two main groups. Some of them (29, 42, 43, 47, 48) have reported a fine plexus of nerve fibers without definite endings. These authors have tried to correlate this plexiform structure with the *terminalreticulum* of Stoehr (49) found in vertebrate smooth muscles. Other authors (1, 2, 6-8, 10, 15, 19-22, 32, 34, 51, 52) described the presence of free nerve endings in molluscan muscles. It must be pointed out, however, that these different light microscopic observations are very difficult to compare as they are often very poorly documented and without photographic evidence.

An electron microscopic investigation would help in the understanding of these structures, and research has been undertaken in an attempt to elucidate the morphology of the neuromuscular contacts in some of these animals.

METHODS

Adult, untreated *Octopus vulgaris* and *Sepia officinalis* were used. Suitable small pieces of suckers from the octopus and of lips from the cuttlefish were cut with sharp scissors

from live, unanesthetized animals and immediately transferred to 1% osmium tetroxide in Ringer's solution, buffered at pH 7.4 with Veronal acetate, and maintained at about 4°C. Fixation was continued for 4 hours with continuous agitation. The pieces were dehydrated in graded ethanols, stained in 1% phosphotungstic acid in absolute ethanol for 3 hours (17), and then embedded in Araldite (16). Blocks were sectioned on a Porter-Blum ultramicrotome with glass knives, and sections exhibiting gold or silver interference colors were picked up on carbon-coated grids. Micrographs were taken at the magnification of 2000–30,000 on a Siemens Elmiskop 1b electron microscope. Histological sections for comparative observations were obtained from material fixed in 10% neutral formol in sea water and then stained with Bielschowsky-Gros and Cajal-Young (55) methods.

RESULTS

The present study is not concerned with the structure of the muscles of these animals, and this will be reported in full later. However, light- as well as electron-microscopic observations show that they are helical smooth muscles (27) in both the species studied.

The nerve supply to the lip of the cuttlefish is provided by motor neurons lying in the superior buccal ganglion, which send their axons through the labial nerves to the lip muscles (23, 28). The sucker of the octopus is of particular interest as it receives its motor innervation from two separate ganglia (24, 25).

Both in the sucker and in the lip, bundles of nerve fibers branch and form a complicated plexus among the muscle fibers. Smaller bundles of nerve fibers arise from the plexus and contact muscle fascicles along which finer bundles branch (Fig. 1a). These then separate and each fiber ends on the contractile units (Fig. 1a and b). The electron micrographs show that the fine nerve bundles are composed of some ten nerve fibers of different diameter ($0.1\text{--}1 \mu$) enclosed in a single Schwann cell (Fig. 7). In cross section a mesaxon can be seen. Its infolding is sometimes very complicated and closely resembles the *tunicated nerves* described by Edwards *et al.* (14) in the muscles of the wasp leg.

Thin fibers, containing synaptic vesicles and mitochondria, run in close contact with the muscle fibers (Fig. 2) or in a groove of a single muscle fiber (Fig. 4). Frequently, more than one nerve fiber (up to five in some cases) run through tunnels in the muscle fiber, suspended by a mesaxon-like structure formed by the infolded surface membrane of the muscle fiber (Figs. 3 and 4). Where a close membrane to membrane apposition between the muscle fiber and the axon is seen, increased density of the opposed nerve-muscle membranes can be observed (Figs. 3, 4, and 8). Where these specializations occur the axons contain synaptic vesicles (300–800 Å in diameter) and mitochondria. The former, if not very numerous, clump near the thickening of the nerve membrane (Figs. 3 and 8). These regions have the charac-

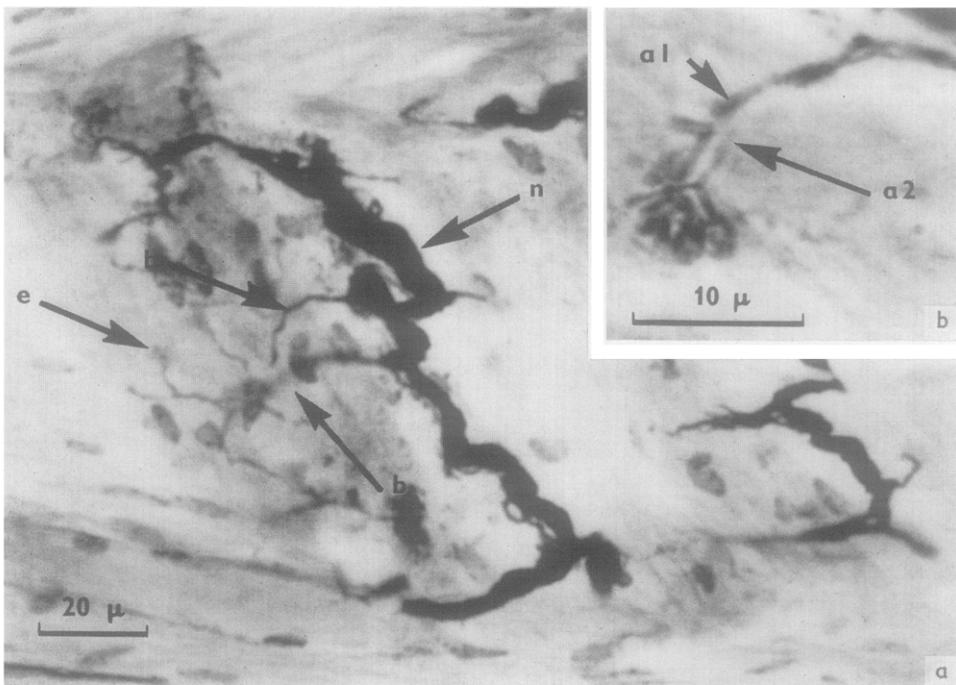


FIG. 1 a. Longitudinal section of a muscle bundle (*Octopus*) with a nerve fascicle (*n*) running alongside. Finer fascicles (*b*) branch and then separate, each fiber ending (*e*) on the contractile units. Cajal-Young staining. $\times 700$.

FIG. 1 b. Enlarged detail of a presumed nerve ending (*Octopus*). Two nerve fibers (*a1* and *a2*) end branching in an end plate apparatus. Notice the different diameter of the two nerve fibers. Bielschowsky-Gros staining. $\times 2250$.

teristics of synaptic apparatus. Nerve fiber profiles with synaptic specializations can frequently be observed penetrating the surface of the muscle cell (Figs. 4 and 6). The overall thickness of the synaptic membrane complex is 250 Å with a synaptic gap 100 Å wide (Figs. 3, 5 and 8). At certain regions where the opposite nerve muscle membranes show increased density, the gap sometimes appears to be less (Fig. 5), but this may be due to the plane of section. Some of these specialized regions (Figs. 3 and 8) show dense, septum-like structures running across the gap perpendicularly to the nerve-muscle membranes, rather in the fashion of septate desmosomes (53). Further studies are in progress designed to elucidate these structures. The pre-synaptic membrane often shows dense projections (Fig. 5) which may correspond to those described by Gray (18) in the synapses of the central nervous system in mammals. Serial sections (Fig. 5a and b) show that in some endings these structures are limited to circumscribed areas and have probably a ribbon-like course inside the

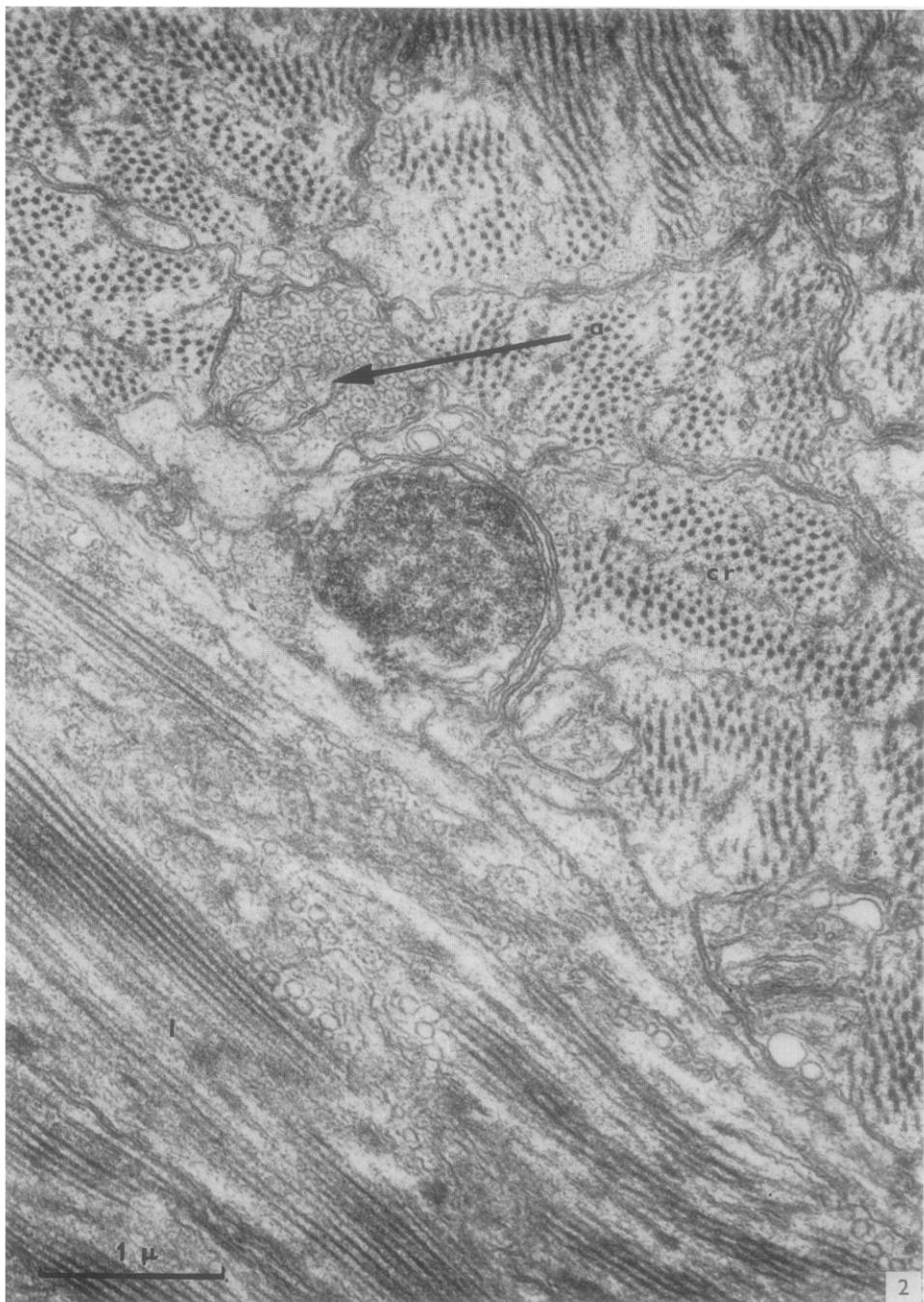


FIG. 2. Cross (*cr*) and longitudinal (*l*) section through the muscles of the sucker of *Octopus*. A small axon (*a*) full of synaptic vesicles run free from Schwann ensheathing cells and is completely enveloped by plasma membranes of the adjacent muscle cells. $\times 25,000$.

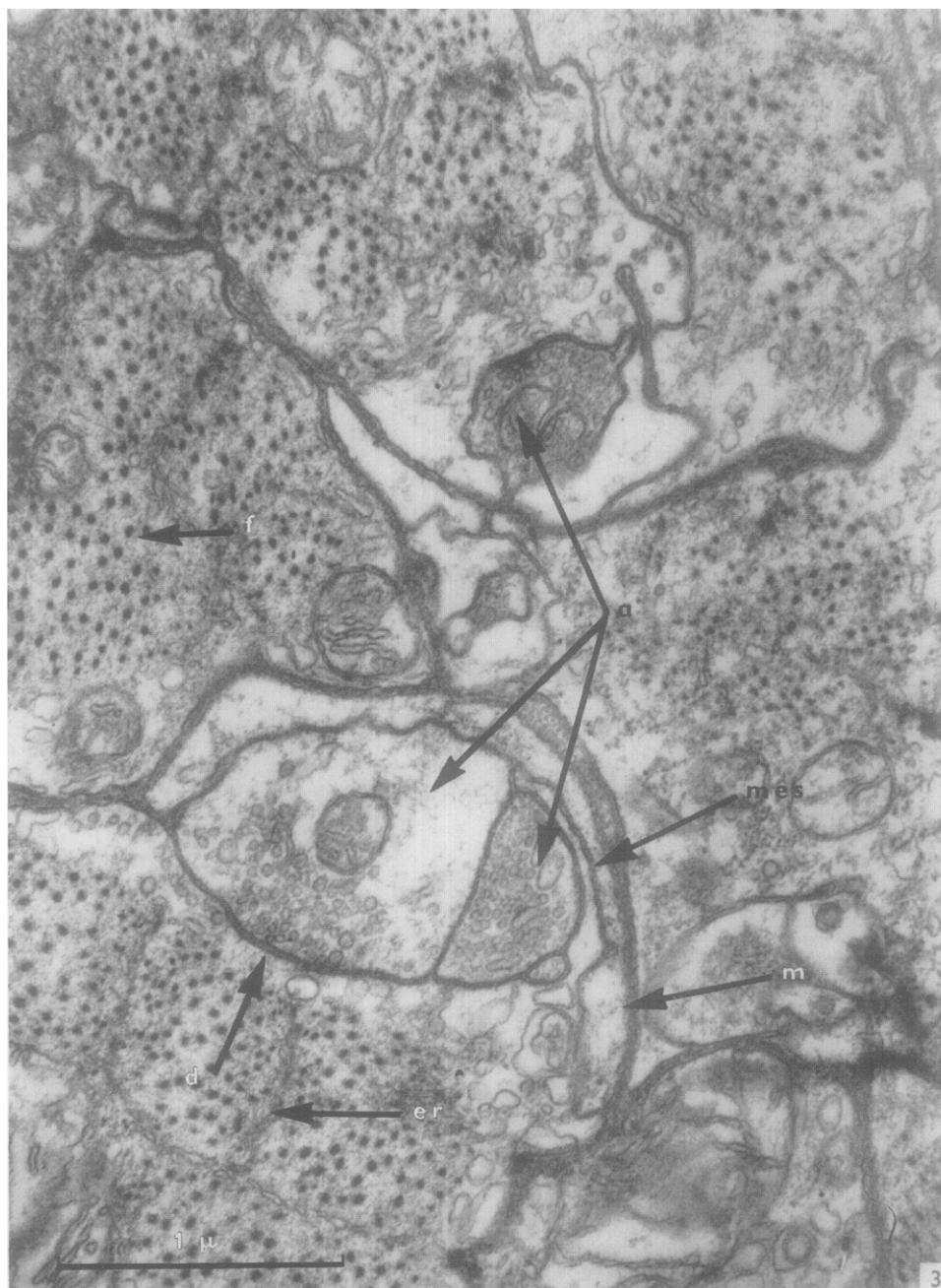


FIG. 3. Cross section of lip muscle fibers (*Sepia*) showing several axons (a) running in two muscle fibers and suspended by a mesaxon-like structure (mes) of the infolded surface membrane (m) of the muscle cell. Notice in (d) a zone of increased density of the opposite nerve-muscle surface membranes with septum-like structures running across the gap (see also Fig. 8). f, myofilaments; er, sarcoplasmic reticulum. $\times 38,000$.

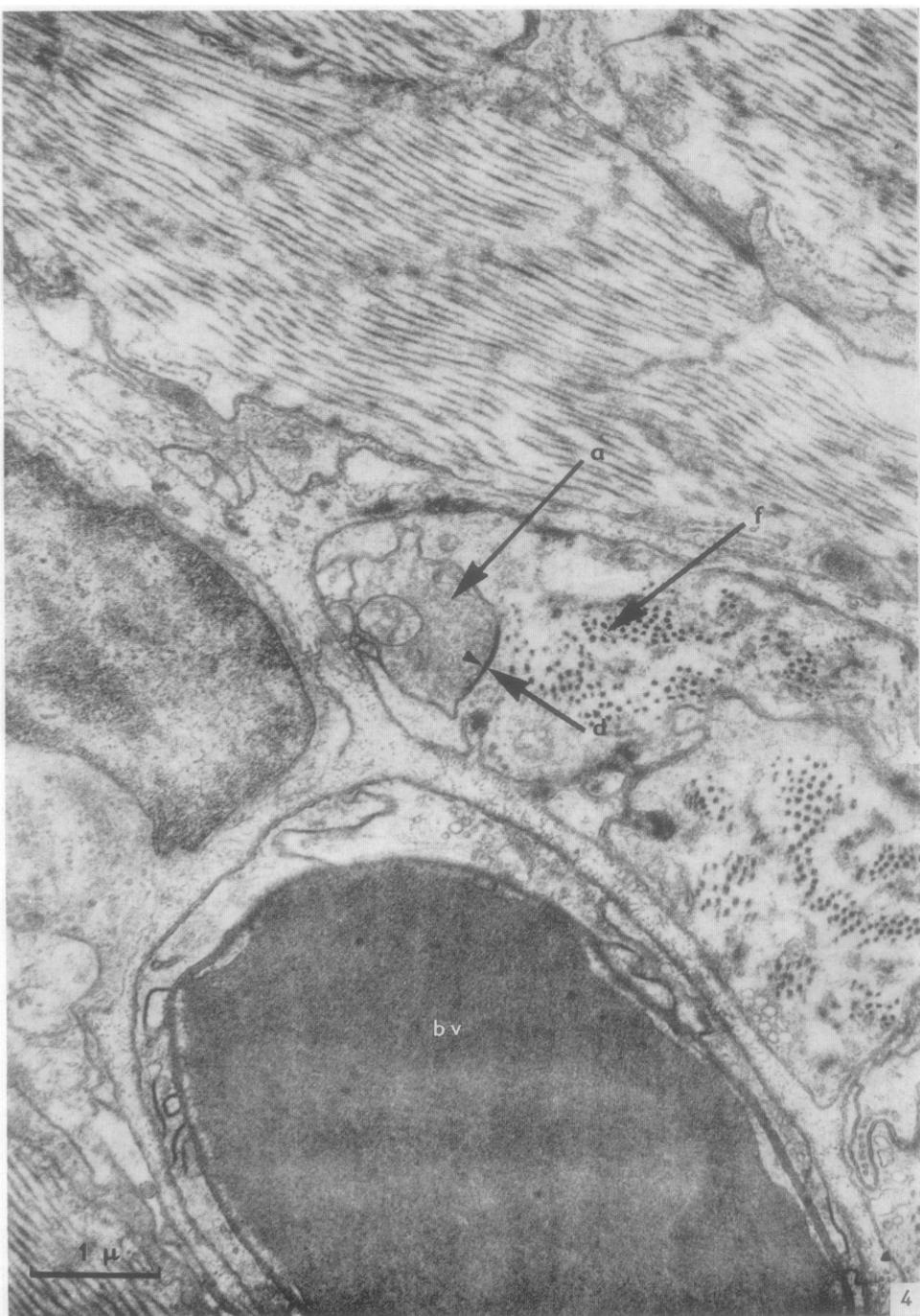


FIG. 4. Cross section of a muscle fiber (*f*) showing one axon (*a*) running in a groove (*Octopus*). A zone of increased density of the opposite membranes is observed in (*d*). *bv*, blood vessel. $\times 18,000$.

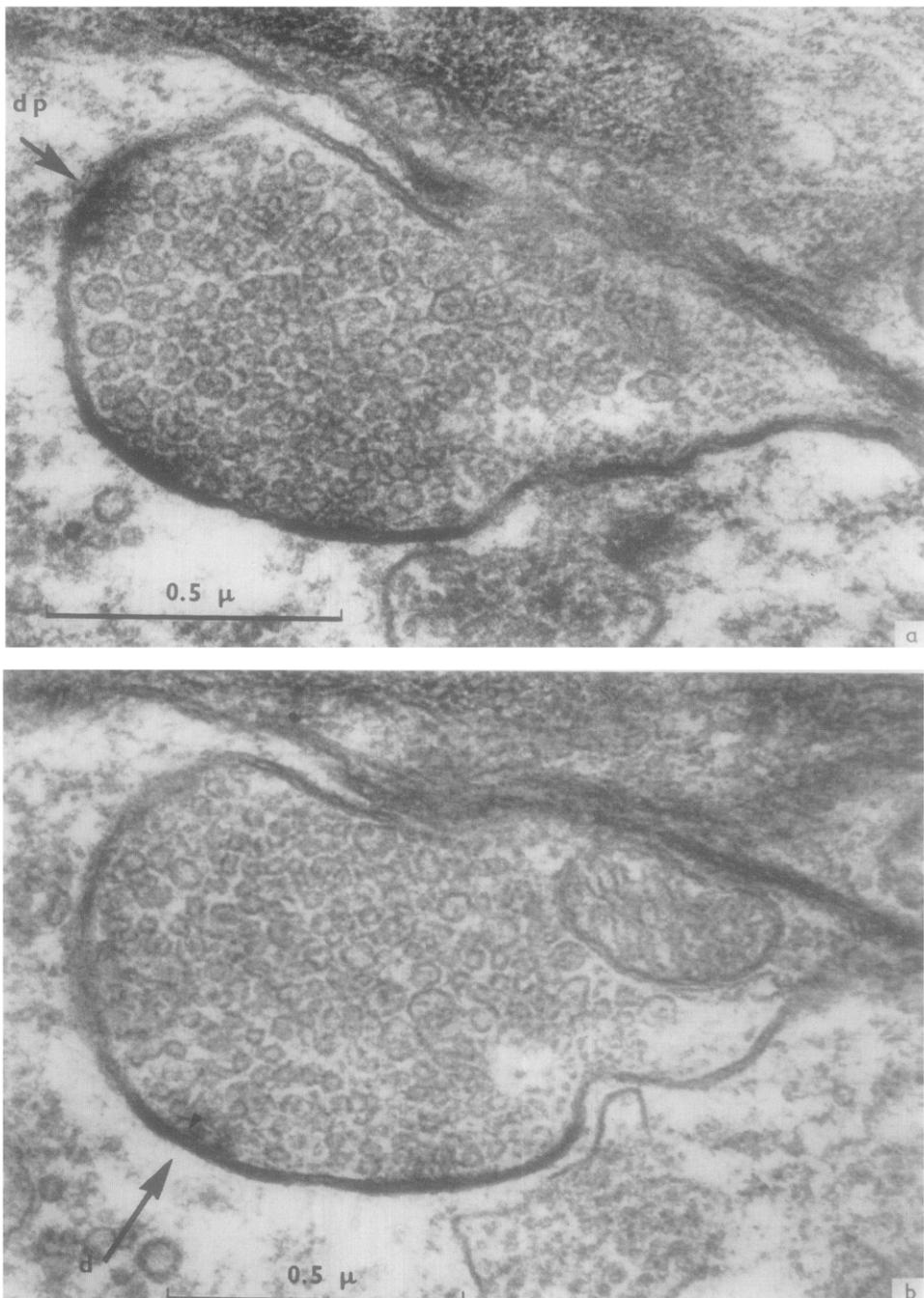


FIG. 5. Serial section through a terminal ending on a muscle fiber (*Octopus*). Increased density of the opposite membranes is observed in (d) and dense projections of the presynaptic membranes in (dp) (see 5 a and 5 b). $\times 80,000$.

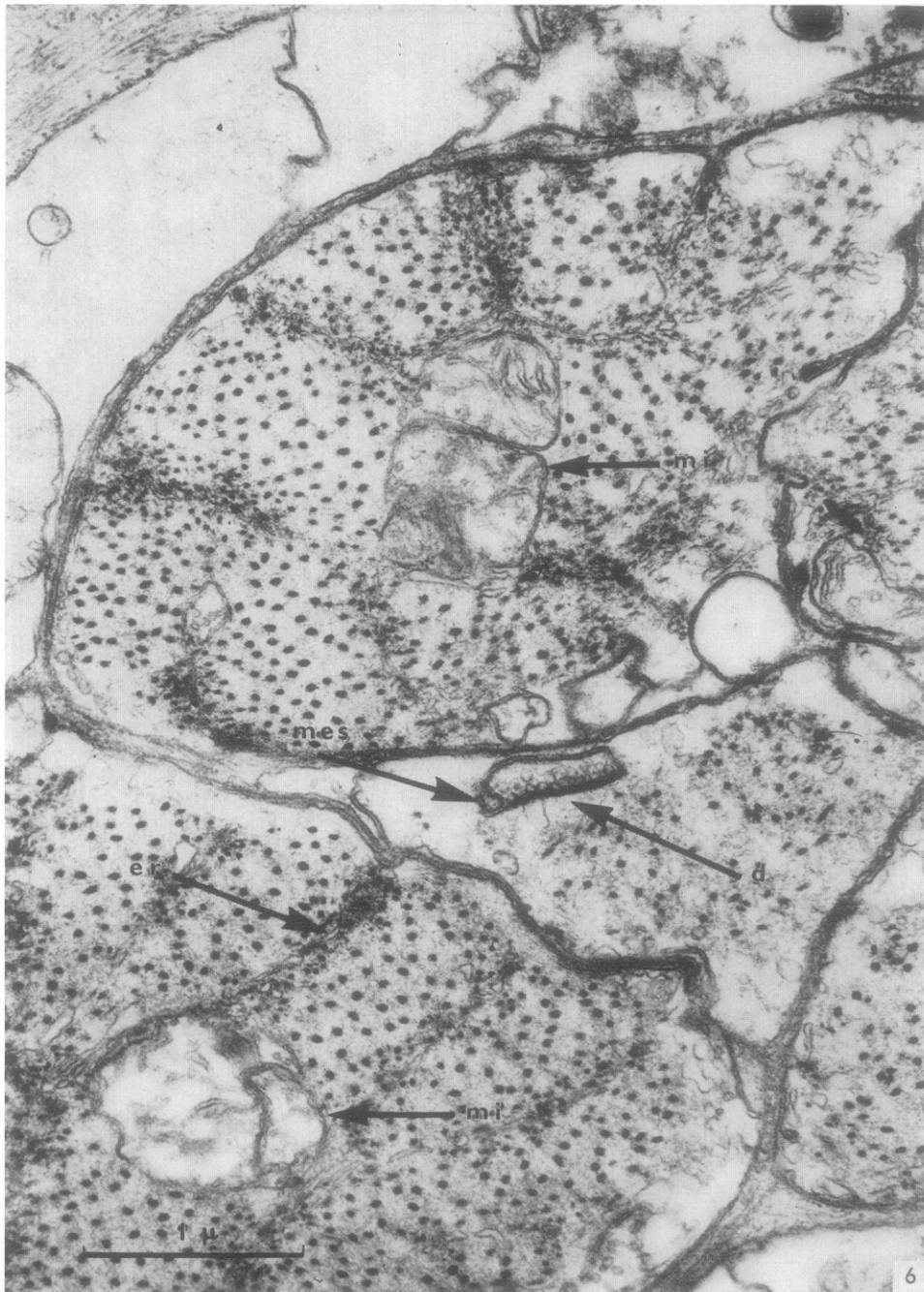


FIG. 6. Cross section of two muscle cells showing mitochondria at their center (*mi*) and the endoplasmic reticulum (*er*) (*Sepia*). In (*d*) tangential section of an invaginated nerve ending containing synaptic vesicles and showing increased density of the apposite membranes. *mes*, mesaxon. $\times 30,000$.



FIG. 7. Cross section of a nerve bundle from the lip of *Sepia*. The arrow (a) shows two axons of different diameter, the upper one containing two mitochondria and one synaptic vesicle with a dense core. $\times 41,000$.

terminal knob. The sizes and shapes of the terminal apparatuses vary greatly from one to another. They are very frequent, but any attempt to classify them into different categories seems premature. There is no definite zone of the muscle fiber where synapses occur. The contacts are observed in the middle, near the nucleus as well as on a pole of the muscle cell. One nerve fiber simultaneously contacting two muscle fibers can also be observed (Fig. 8). The sarcoplasm near the synapse may or may not contain myofilaments. No mitochondria have so far been observed in the synaptic region of the muscle cytoplasm. There is a close relationship between the postsynaptic membrane and the smooth membrane profiles of the sarcoplasmic reticulum (Fig. 3), which shows in cross sections of the muscle fibers a quite regular pattern (Fig. 6).

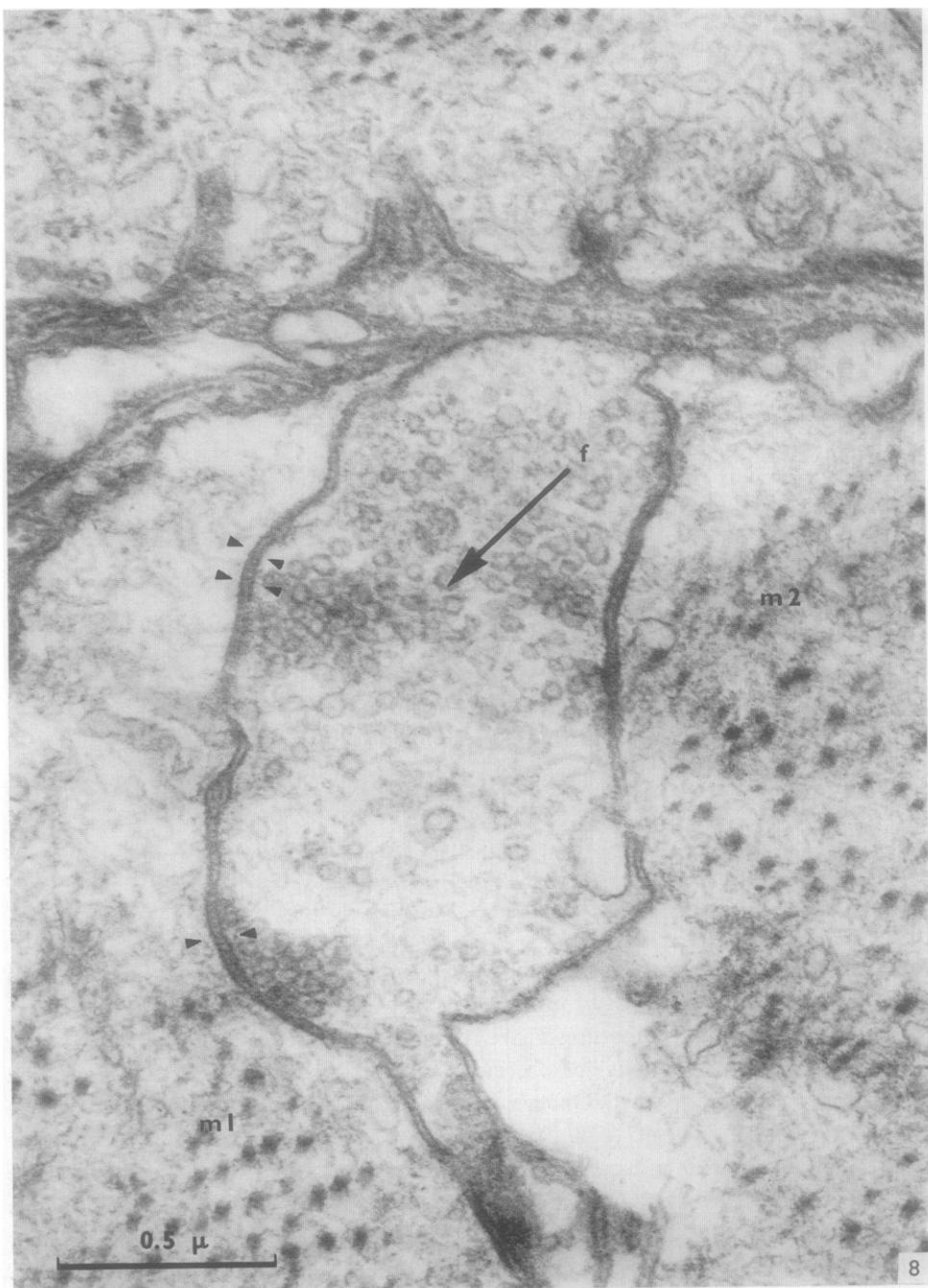


FIG. 8. Cross section of a nerve fiber (*f*) filled with synaptic vesicles, contacting two discrete muscle fibers (*m*1 and *m*2) (*Sepia*). In the gap between the zones where increased density of the surface membranes occurs (small arrows) discrete, septum-like, dense bars are seen. $\times 60,000$.

DISCUSSION

The present observations suggest that the motor innervation of the muscles of the sucker of the octopus and of the lip of *Sepia* takes the form of definite nerve endings. This possibility was previously suggested on the basis of light microscopic observations in the muscles of the fin and the stomach of *Sepia* (21, 22). The presence of nerve fibers running in pockets inside the contractile elements, surrounded by the limiting membrane of the muscle cell, was previously described in vertebrate smooth muscles (9, 33, 39, 54) as well as in some invertebrate muscles (35) and is a very common feature indeed in these cephalopods. The postulate of some authors (5, 46, 49) of an intracellular ending of the nerve fibers in smooth muscle fibers of various animals is now quite understandable.

Electron microscopy clearly demonstrates the presence of apposed membranes of the two elements (nerve and muscle) as well as definite areas where synaptic transmission is thought to occur. The neuromuscular junctions so far examined in these somatic muscles of *Cephalopods* show the basic features of synaptic apparatus, with increased membrane density, synaptic vesicles, and mitochondria in the presynaptic process. But it must be pointed out that they differ in many details from the neuromuscular junctions of vertebrates. Structures such as the subneural apparatus or junctional folds of vertebrate striated muscles are not seen, nor teloglia surrounding the terminal apparatus. It seems likely that in cephalopods the axons run a long way free from any Schwann cell sheath, and directly enveloped or in intimate contact with the plasma membranes of adjacent muscle cells. It is not known at present whether these regions of unspecialized contact are functionally important. It seems likely that the synaptic endings of cephalopods resemble those described in some mammal smooth muscle (9, 33, 39, 50) or striated muscles of fish (38). The muscles of the stretch receptors of crayfish (35) and the muscles of insects (13, 45) also show discrete nerve endings with structures having the basic features previously described in cephalopods. But it seems important to emphasize that the overall thickness of the synaptic membrane complex in the present material is 250 Å, with a gap between the pre- and postsynaptic membranes of 100 Å. These measurements of the synaptic cleft are smaller than those so far reported for the neuromuscular junctions (in the order of 150–200 Å or more) (cf. 3, 4, 9, 13, 33, 38, 45) and may be compared with some electrically transmitting synapses (11, 26, 40, 41) of which synaptic clefts range from 150 Å down [see Eccles (12) for a review]. Moreover, the presence of dense, septum-like bars lying across the pre- and postsynaptic membrane as observed in some of these endings suggests the necessity of more structural (and physiological as well) study before considering their functional role.

The presence of a developed sarcoplasmic reticulum near the synaptic apparatus as

well as in proximity to the nerve fibers running in a groove of the muscle fiber needs more careful study in view of the hypothesis suggesting the importance of this apparatus in the conduction of the stimulus into the contractile element (31, 36, 37, 44). In *Octopus* the sucker is innervated by fibers that arise from the ganglion of the sucker and from the ganglion of the arm (24, 25). This double source of fibers may explain the observation of more than one fiber running in a groove or canal inside the muscle cell. These latter axons show synaptic contacts with the muscle fiber, and this suggests that more than one fiber may innervate each muscle cell. That the fibers as shown in Fig. 3 could arise from the branching of a single axon must be carefully considered, although silver techniques show (Fig. 1b and c) that these branches normally diverge at various angles from the main fiber, do not run close together, and do not vary greatly in diameter.

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