The Fine Structure of a Multiterminal Innervation of an Insect Muscle

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PLATES 97 TO 101

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ABSTRACT

The detailed structure of nerve branches, neuromuscular junctions, and muscle fibers of a multiterminal innervation of cockroach abdominal muscle has been studied with the electron microscope. The muscle fiber is of the banded myofibril type; with paired mitochondria and abundant endoplasmic reticulum. The peripheral nerve branches are multiaxonal with large central axon and several small peripheral tunicated axons. Tracheoblasts closely accompany the nerve branches. The multiple neuromuscular junctions show typical axonal vesicles, muscle aposynaptic granules, and close plasma membrane apposition with no interposition of basement membrane material.

INTRODUCTION

Previous electron microscopic studies of neuromuscular junctions from mammals (Palade, 1954, 1957; Caesar, Edwards, and Ruska, 1957; Reger, 1954, 1955), from reptiles (Robertson, 1956), amphibia (Reger, 1957) and fish (Couteaux and Laurent, 1957) among the vertebrates, and from several different types of insect muscles (Edwards, Ruska and de Harven, 1958 *a*, 1958 *b*) have shown uniaxonal, unijunctional relations between synapsing peripheral nerve ending and single muscle fiber. In the earthworm body wall muscle and in frog heart, multiaxonal, unijunctional relations may occur (unpublished observations).

Physiologic evidence has been accumulating for some years, particularly through the use of microelectrodes, that multiterminal innervation occurs in vertebrates (Kuffler and Gerard, 1947), crustaceans (Wiersma, 1952), and insects (Roeder, 1953; Hoyle, 1957 *a*, 1957 *b*). Indeed the evidence suggests that a given muscle may be quintuply innervated (van Harreveld, 1939).

The present study gives electron microscopic evidence for a multiterminal innervation of a cockroach abdominal muscle. The several peripheral nerves are multiaxonal, and the single muscle fiber exhibits up to four, widely separated neuromuscular junctions.

Material and Methods

The abdomen of the cockroach (*Blatta germanica*) was slit open and immediately filled with 2 per cent osmium tetroxide buffered to pH 7.4 with veronal-acetate. After 10 minutes the longitudinal ventral abdominal muscle, with attached nerves and tracheoles, was dissected out and cut into small bits. Those bits believed to contain the nerve endings were then further fixed for 1 hour, washed with buffer, dehydrated in graded ethanols, and embedded in a mixture of 90 parts butyl to 10 parts methyl methacrylate containing one and one-half per cent lucidol initiator for 48 hours at 46°C. Sections were cut with a diamond knife in a Porter-Blum microtome, mounted on carbon film, and observed in the Siemens Elmiskop I at initial magnifications of 1,000 to 20,000.

RESULTS

A number of muscle fibers showed innervation by one to three peripheral nerve branches (Fig. 1) and up to four widely spaced neuromuscular junctions per fiber. The distance between individual nerve branches averaged 15 to 20 μ, and the neuromuscular junctions varied in spacing from 4 to 30 μ. The nerve branches were usually accompanied by tracheoblasts.

Peripheral Nerve.—The peripheral nerve branches were seen in a group abutting the muscle fiber (Figs. 1 to 3) or in the interstitium close to the muscle fiber (*cf.* N_1 in Fig. 1). Each nerve branch comprises generally a large central axon (Figs. 1 and 3) and several smaller peripheral axons, ensheathed by a common lemnoblast.

The lemnoblast possesses a filamentous basement membrane of variable thickness (Figs. 2, 3, 5, and 6). The fine filaments, averaging 50 A in diameter, form a dense feltwork, but in general run parallel to the long axis of the nerve. The basement membrane is thinnest on the interstitial side of the abutting nerve and widens considerably on the muscle side where it fuses with the basement membrane (sarcolemma) of the muscle fiber (Figs. 2 and 5). The plasma membrane of the lemnoblast may invaginate at several points (Fig. 3) to form the mesaxons. The lemnoblast possesses a large, generally bean-shaped nucleus (Fig. 3) having a well developed nuclear envelope and peripherally aggregated chromatin. The cytoplasm of the lemnoblast (Figs. 2 to 5) contains small mitochondria, reticular apparatus of Golgi, and fine granules and filaments ranging from 60 to 150 A in diameter. In the larger nerve branches a homogeneous substance, resembling the chitinous bars around the giant axons in the ventral nerve cord, has been observed in the mesaxon, generally close to the larger axons (Figs. 2 and 3).

The number of axons in the abutting nerve branches varies from 4 to 8, depending upon size. The central axon may be up to 8 μ in diameter. The peripheral axons vary from 0.5 to 1.5 μ in average diameter. All axons of the nerve branches possess a plasma membrane, and contain mitochondria and filaments, rarely vesicles. The axons are surrounded respectively by loose windings of mesaxon, the number of mesaxonal turns varying from one to five or more (*cf.* Fig. 3). Thus, the nerves are "tunicated" (Edwards, Ruska, and de Harven, 1958 *a*) rather than "myelinated." The peripheral nerve branches join the muscle fiber by fusion of the respective basement membranes of lemnoblast and muscle (Figs. 1 to 3, 6, and 8).

Tracheoblasts.—An adequate oxygen supply to both nerve and muscle, and particularly to junctional regions, is provided by numerous tracheoblasts containing tracheoles and small tracheae (Figs. 1, 4, and 5) of various sizes. The larger, peripheral tracheoblasts closely accompany the nerves, the basement membranes of lemnoblast and tracheoblast often being fused so as to form a continuum of nerve and oxygen supply (Fig. 1). In rare instances trachea-containing portions of a tracheoblast may be interposed between nerve and muscle (Fig. 4).

The tracheoblast basement membrane compares in thickness and composition with that of the lemnoblast. The plasma membrane invaginates at irregular intervals to form the mesotracheole that encloses the tracheoles (Fig. 5). The cytoplasm of the tracheoblast contains mitochondria, granules, and membrane-bound vesicles of the endoplasmic reticulum (Figs. 4 and 5). The nucleus is generally large and eccentric (Fig. 1). The tracheae and tracheoles may be differentiated on the basis of their size and chitinous components (for details see Edwards, Ruska, and de Harven, 1958 *c*).

Neuromuscular Junctions.—The number of junctions per muscle fiber varied from one to four in the planes of the sections observed. The muscle fiber shown in Fig. 1 possessed four junctions. Three of these were situated in muscle grooves beneath the nerve-tracheole net, and one near the junction of the muscle cell with its neighbor. The details of the four neuromuscular junctions are shown in Figs. 6 to 8.

The synapsing axons contain numerous vesicles, averaging 50 mμ in diameter, and several cristae-filled mitochondria in the sectioning plane. On the interstitial side the axon is covered by cytoplasm and membranes of the lemnoblast. On the muscle side of the synapsing axon there occurs only the plasma membrane. At the most intimate synaptic regions the plasma membrane of the axon is directly apposed to the plasma membrane of the muscle fiber, with an interspace of only 15 mμ intervening. No basement membrane material is seen between axon and muscle. The muscle cytoplasm in the synaptic region contains aposynaptic granules, vesicles and tubules of the endoplasmic reticulum, and but few mitochondria. The aposynaptic region thus differs from that of the wasp leg muscle, or the cicada flight and tymbal muscles, in the distribution of mitochondria. As earlier observed in other junctions, there appears to be a clumping of vesicles in the axon and an aggregation of granules in the muscle cytoplasm (double arrows in Fig. 8) at the region of most intimate synapsis. Interestingly enough, the synapse does not always occur opposite the Z region of the sarcomere of the peripheral fibril, but may be at mid-sarcomere level (Figs. 6 and 8).

Muscle.—The abdominal intersegmental muscle fiber is of the banded type; *i.e.*, the fibrils are flattened cylinders. The individual myofibril possesses a thick, irregular Z band, distinct A and I regions, a variable H band, but no M line. The mitochondria occur generally in pairs (Figs. 1

and 8) between the fibrils, one on either side of the Z line. The myofibrils are widely separated by granules and also by tubules and vesicles of the endoplasmic reticulum. The endoplasmic reticulum shows aggregates of tubules at Z line level, triads at the level of the junction of A and I regions, and of particular interest, it shows a definite connection with the plasma membrane (Figs. 2, 7, and 8). At such connections the plasma membrane is infolded, forming a deep funnel or groove (Figs. 7 and 8). The membrane may be discontinuous along the walls of the funnel (Fig. 7), or may be continuous but showing vesiculation (Fig. 8). In either case, the continuity with the reticulum is established by means of a series of vesicles, which become continuous with the reticulum at the A-I junction and with those vesicles and tubules surrounding the myofibril at Z level (Fig. 8). Such a relationship between reticulum and plasma membrane has been shown earlier in more detail by Porter and Palade (1957) in amphibian muscle. The significance of the relation is not yet understood.

SUMMARY AND CONCLUSIONS

The interpretation of the results shown above must, of necessity, be limited to the observations themselves. It is tempting to suggest that the large, central axon could be the "fast" fiber and the smaller peripheral axons, either "slow" or "inhibitory" or other "fast" fibers of a multiterminal arrangement of nerve branches. One could further speculate, and it would only be speculation, that the several neuromuscular junctions could be the synapses of the several different types of nerve fibers identified by electrophysiologic means in other arthropod muscles. However, at present it is not possible to link the well documented physiological evidence and this one set of observations of possible multiterminal innervation of single muscle fibers. It can only be stated that this is the first example of a multiaxonal, multijunctional relation between nerve and muscle observed in this laboratory. It is hoped that this information, along with future microelectrode, further electron microscopic and histochemical studies may help to clarify the difficult question of whether the various muscle responses are due to variation in type of axon, synapse, or muscle fiber, or to some combination of the three.

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Explanation of Plates

Plate 97

Fig. 1. Composite of several micrographs showing multiterminal innervation of a single abdominal intersegmental muscle fiber of a cockroach. Two small branches of a nerve (N_1) lie in the interstitium. Two larger nerve branches (N_2 and N_3) adjoin the muscle fiber by fusion of the basement membranes of their sheath cells with the basement membrane of the muscle cell. The nerve branches are accompanied by tracheoblasts (T_1, T_2, and T_3). Note that nerve three comprises one large central axon and several, smaller peripheral axons. A similar arrangement of axons was observed in nerve two in parallel sections. × 2,000.

Fig. 2. Detail of abutment of nerve and muscle. The basement membranes of lemnoblast (*BML*) and muscle cell (*BMM*) fuse to form a common membrane between nerve and muscle. The micrograph shows four of the small, peripheral axons (*A*) of the nerve, each surrounded by one or two windings only of mesaxon. The lemnoblast cytoplasm (*L*) contains small mitochondria, few components of the endoplasmic reticulum, and scattered granules. The muscle fiber (*M*) shows a connection between plasma membrane and endoplasmic reticulum (*ER*). × 30,000.

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PLATE 97
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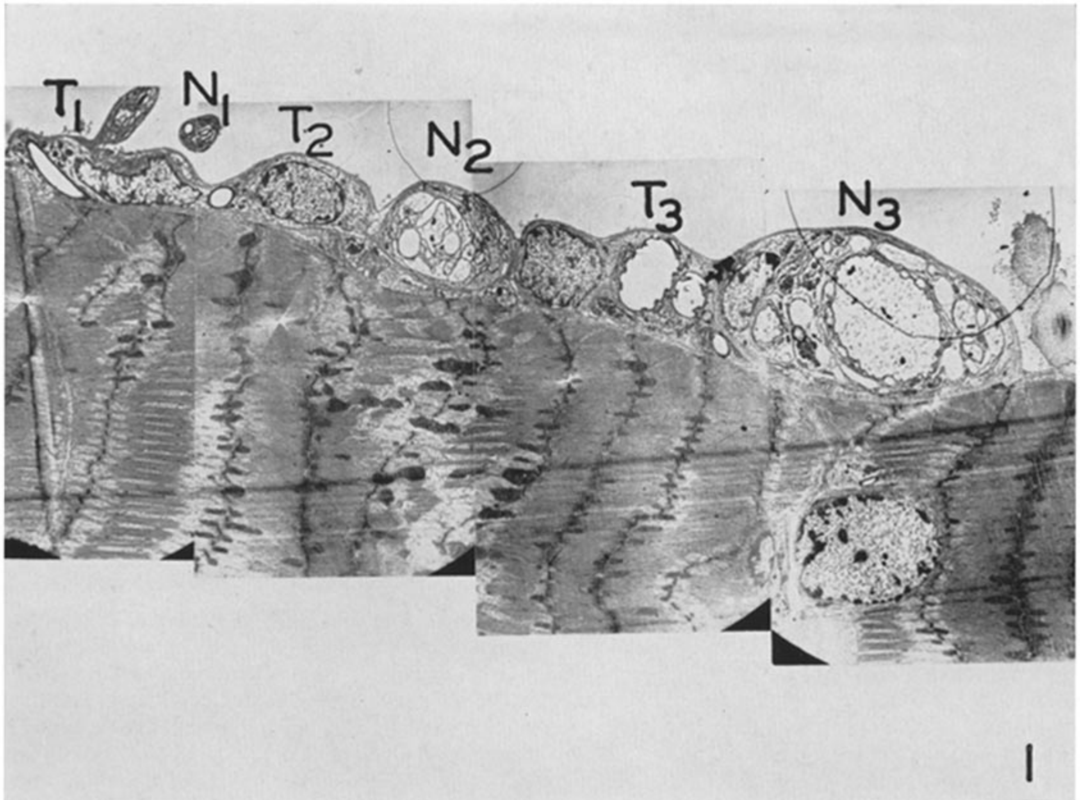
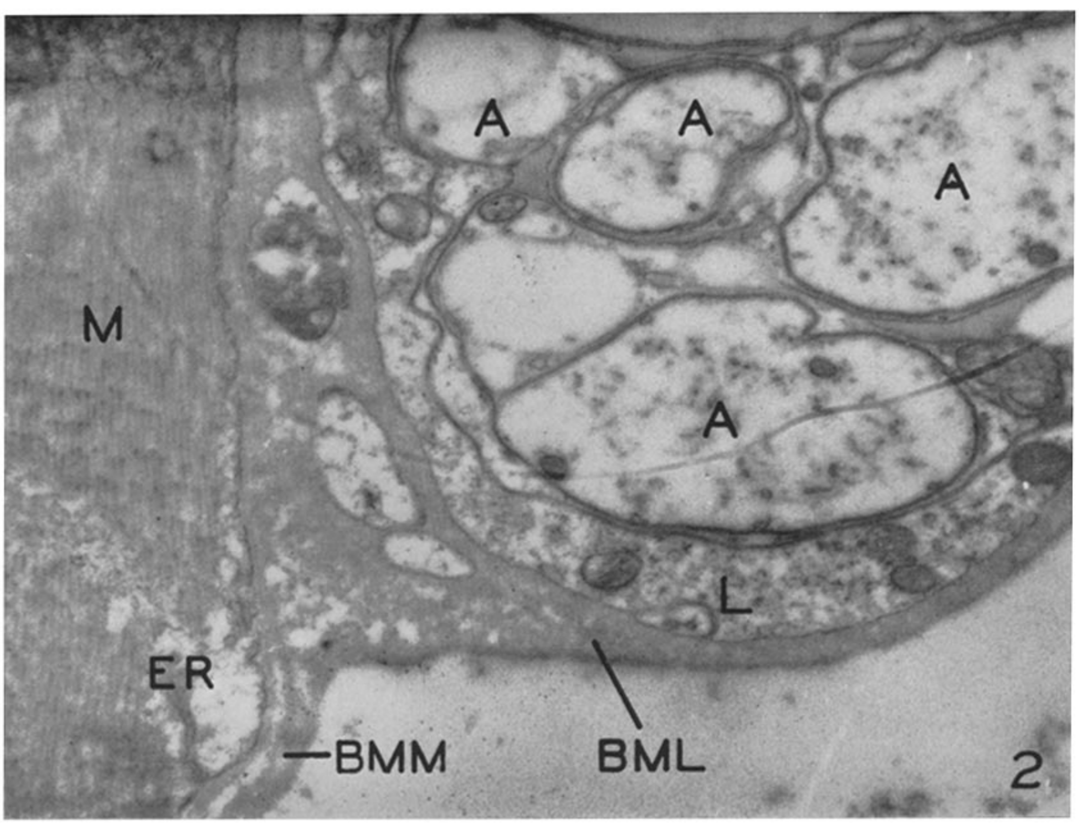




(Edwards: Fine structure of multiterminal innervation)

PLATE 98

FIG. 3. Portion of muscle fiber (*M*) and adjoining large, peripheral nerve branch. The basement membrane of the muscle fiber fuses with that of lemnoblast at lower right. The nerve lemnoblast possesses a filamentous basement membrane. The plasma membrane invaginates (arrows) at irregular intervals to form the mesaxons (*MA*) which course in meandering fashion through the cytoplasm and make one or more turns around the individual axons. The lemnoblast possesses a large, bean-shaped nucleus (*NU*) eccentrically placed in the cell. The cytoplasm (*L*) contains granules, vesicles, and filaments. A small portion of the reticular apparatus of Golgi (*GA*) is visible. In addition there appears a homogeneous component of medium electron density (to left of Golgi apparatus) between the mesaxonal membranes usually close to the axons. Pictured are three axons: 2 small, peripheral (A_1 and A_2), and part of the large central axon (A_3). × 30,000.

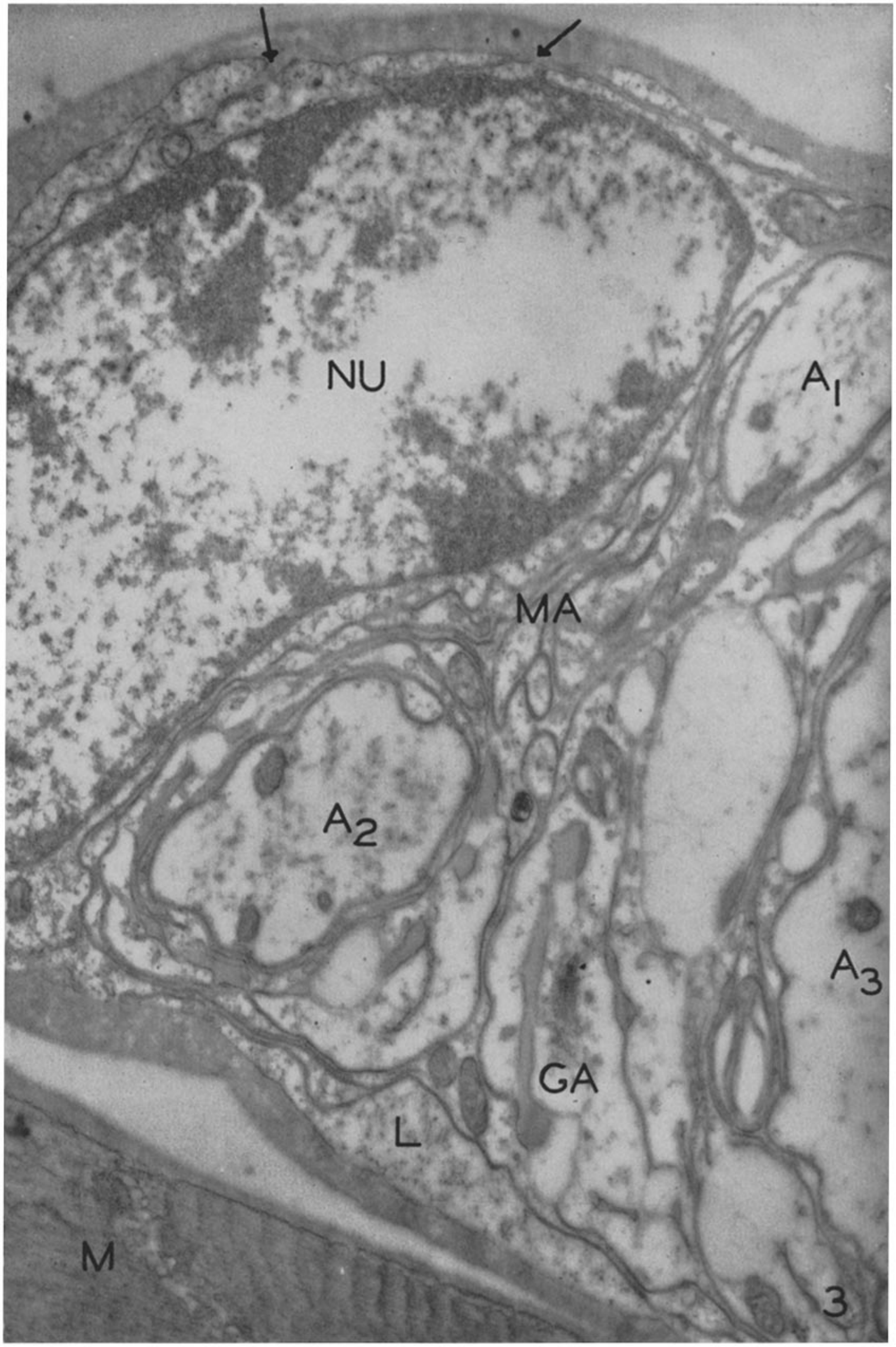
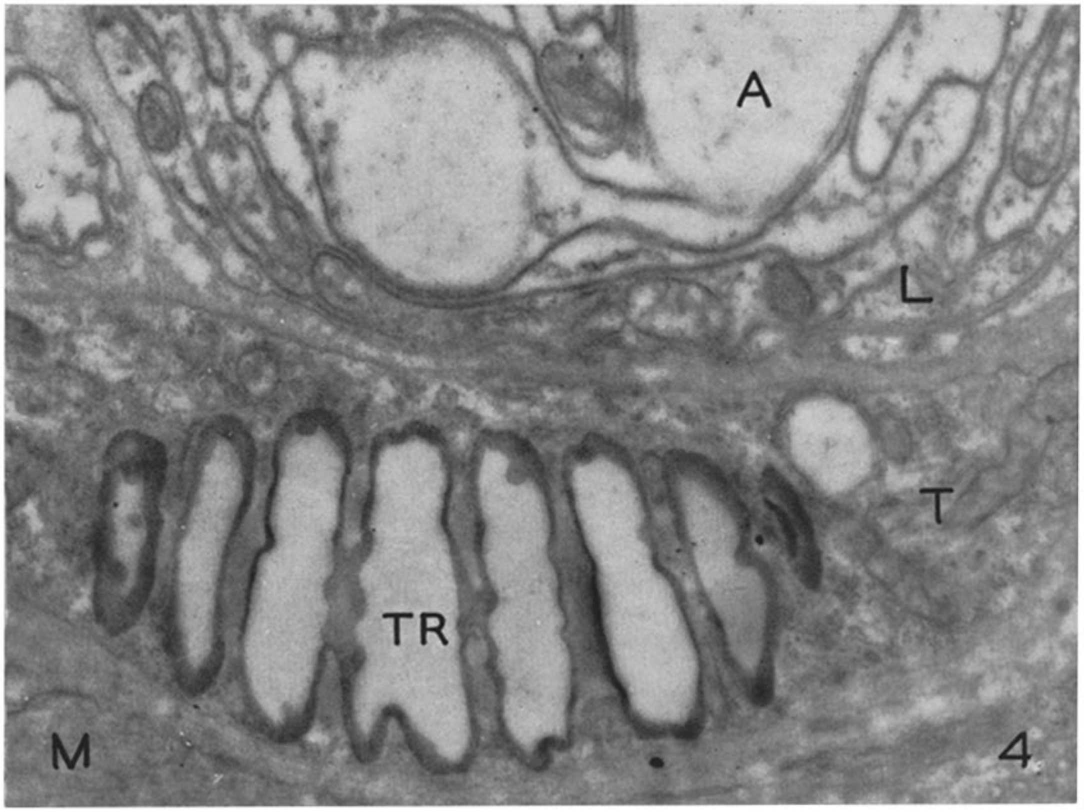
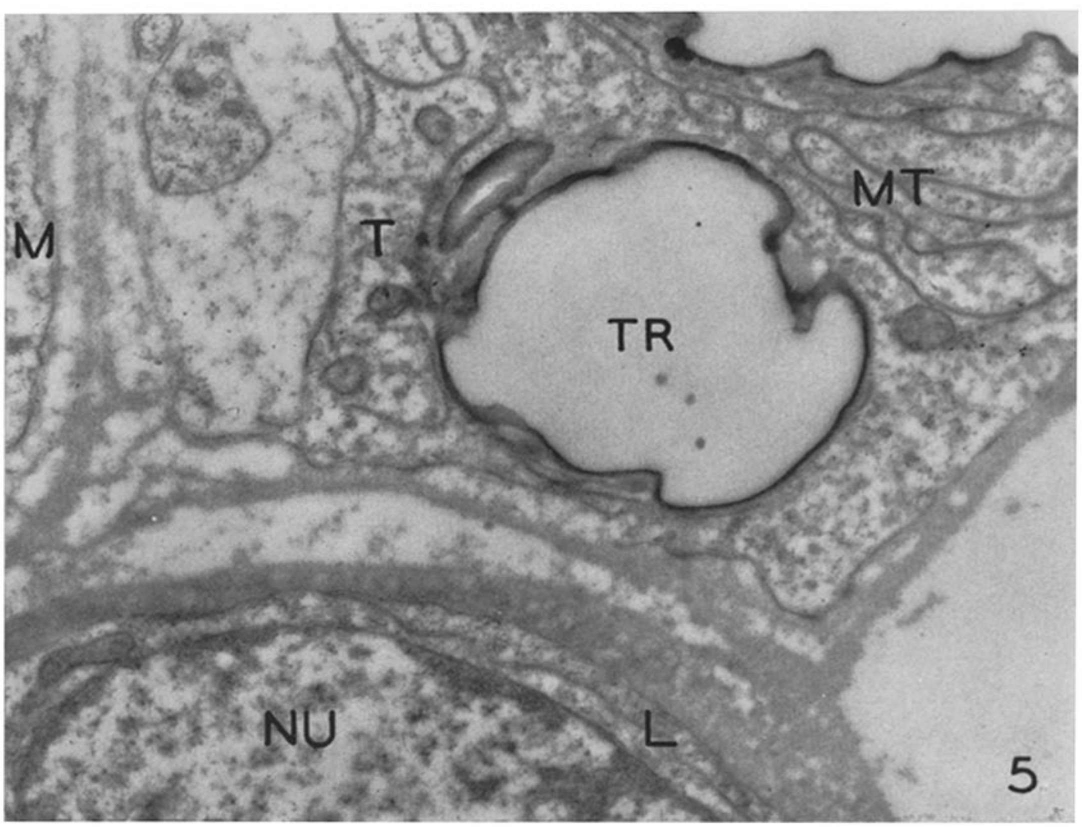

NU
A1
MA
A2
A3
GA
L
M
3

PLATE 99

FIG. 4. Portions of muscle fiber (*M*), tracheoblast (*T*) interposed between muscle and nerve, and peripheral nerve showing part of its lemnoblast (*L*) and a peripheral axon (*A*). The filamentous basement membranes of nerve lemnoblast and tracheoblast are fused (center) as are those of tracheoblast and muscle fiber (lower). The tracheoblast cytoplasm contains mitochondria, granules, and vesicles. The taenidia of the small trachea (*TR*) possess papillate processes. × 30,000.

FIG. 5. Detail of junction of muscle fiber (*M*), nerve lemnoblast (*L*), and tracheoblast (*T*). The basement membranes of all three cells form a loose, filamentous membrane, at their point of fusion, of such nature that one cannot easily determine its cellular origin. Part of a mesaxon and of a nucleus (*NU*) are visible in the lemnoblast. The tracheoblast shows two tracheoles (*TR*) surrounded by mesotracheoles (*MT*), which form in a manner similar to the mesaxon of the lemnoblast. × 30,000.





(Edwards: Fine structure of multiterminal innervation)

PLATE 100

FIG. 6. Detail of two of the neuromuscular junctions seen in the muscle fiber pictured in Fig. 1. The relation of synapsing axon to muscle fiber is easily distinguished from that of peripheral nerve to muscle. The multiaxonal, peripheral nerve (*PN*) at right is widely separated from the muscle fiber (*M*) by the fused basement membranes (*BM*). The synapsing axons (A_1 and A_2), however, are separated from the muscle fiber by only the respective plasma membranes and the interspace. The axons of the peripheral nerve contain mitochondria and neurofilaments. The synapsing axons contain mitochondria (A_1) and synaptic vesicles (well shown in A_2). The muscle cytoplasm in the synaptic region (arrows) contains elements of the endoplasmic reticulum, and aposynaptic granules. × 32,000.

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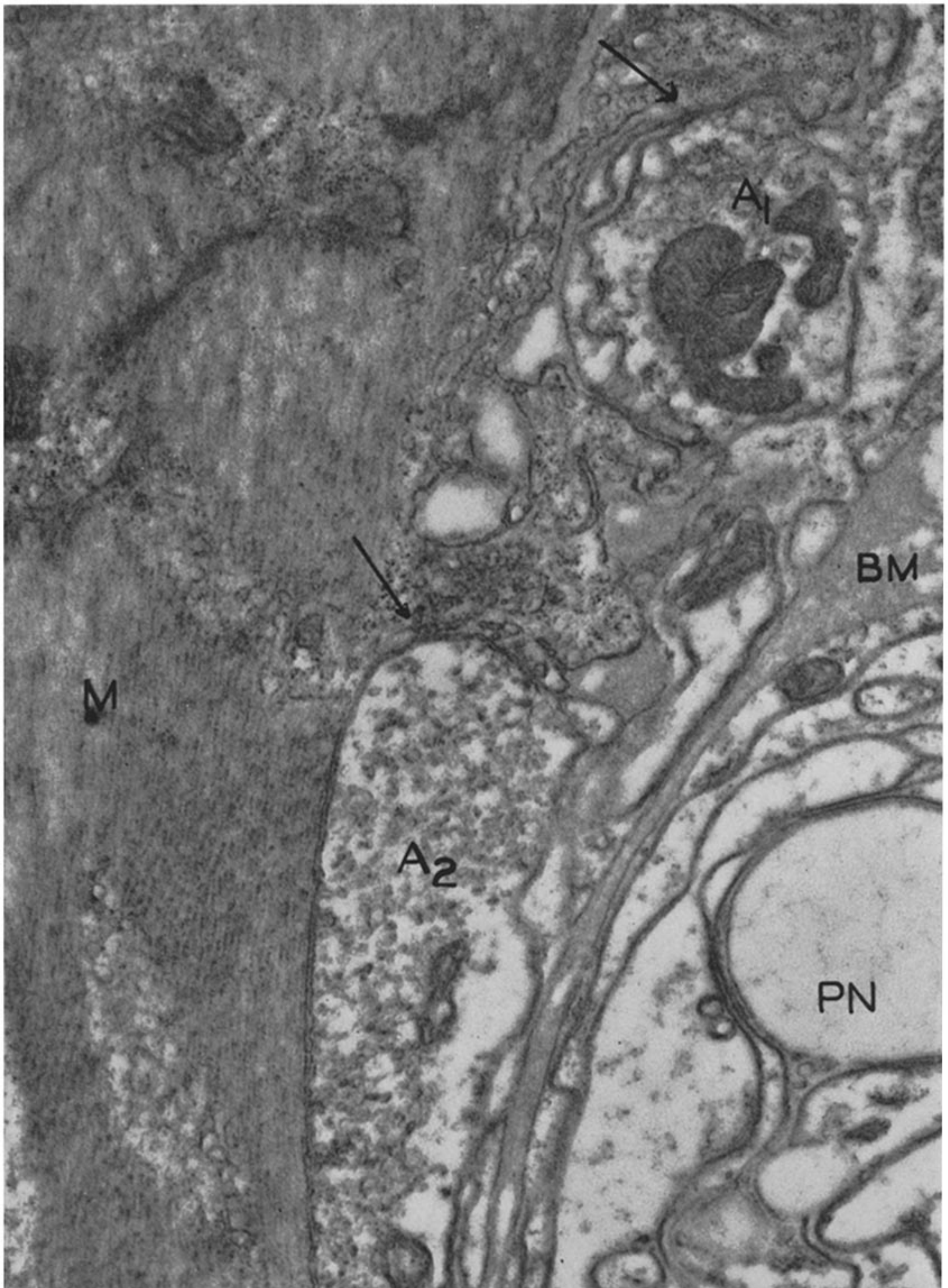
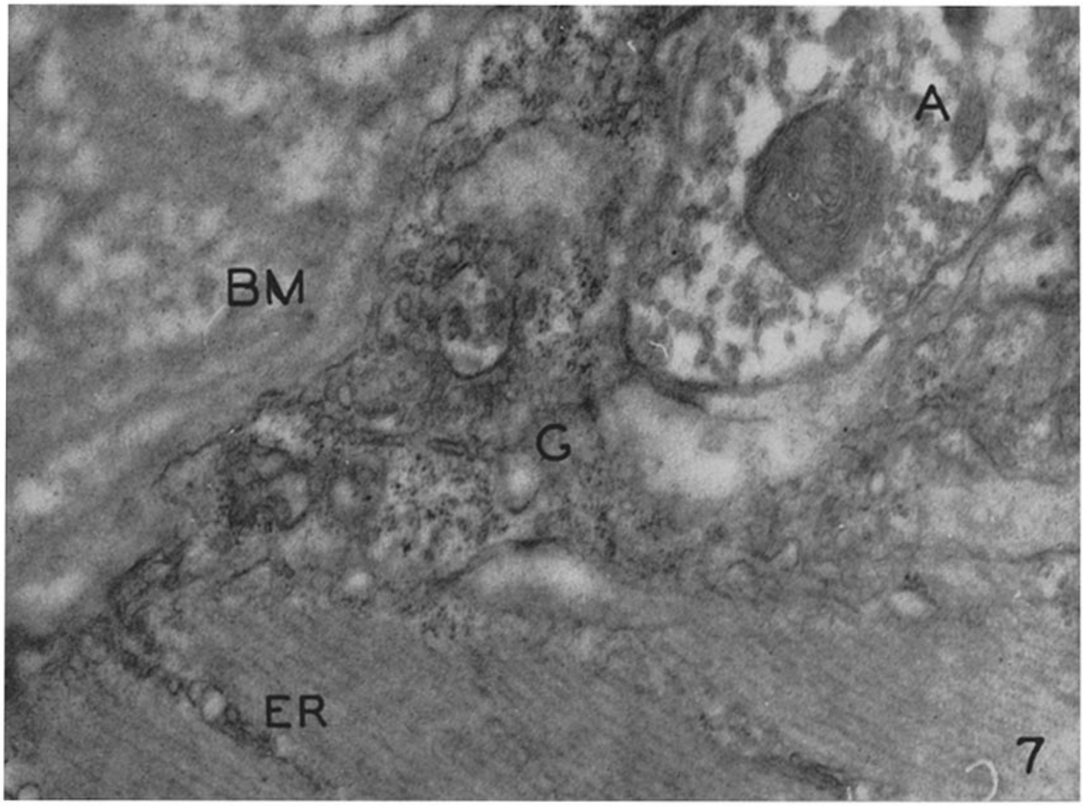


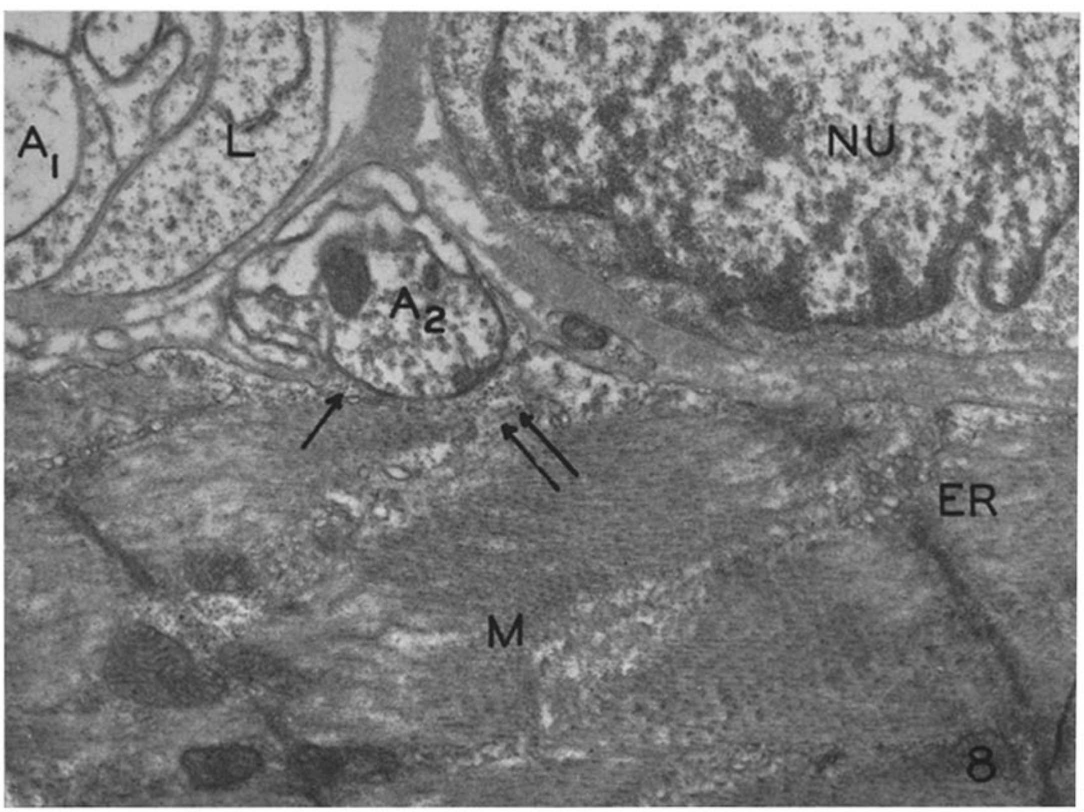
(Edwards: Fine structure of multiterminal innervation)

Plate 101

Fig. 7. Detail of neuromuscular junction from muscle fiber shown in Fig. 1, at a region about 20 μ distant from the nearest peripheral nerve branch. The synapsing axon (*A*) contains several mitochondria and numerous synaptic vesicles. The synaptic region of the muscle fiber contains tubules and vesicles of the endoplasmic reticulum, as well as dense aggregates of aposynaptic granules (*G*). In the muscle fiber (lower portion of micrograph) may be seen myofilaments, endoplasmic reticulum (*ER*) continuous with the plasma membrane, and at lower right a reticular triad. At upper left is the fused basement membrane material (*BM*) from muscle fiber and lemnoblast. × 40,000.

Fig. 8. Detail of neuromuscular junction in muscle fiber (*M*) directly beneath abutment of peripheral nerve (upper left) and accompanying tracheoblast, whose nucleus (*NU*) may be seen at upper right. Part of a small axon (A_1), mesaxon, and cytoplasm (*L*) may be seen in the peripheral nerve. Note continuity of lemnoblast basement membrane and intramesaxonal homogeneous material. The lemnoblast is separated from muscle fiber by their fused basement membranes. The portion of the muscle fiber shown contains several myofibrils, having A and I regions, Z line, and a light H region. The mitochondria line up in pairs at I region level (left). The endoplasmic reticulum surrounds each myofibril, and its tubules and vesicles are particularly abundant (*ER*) at *Z* and mid-sarcomere levels. At right it can be seen that the reticular vesicles are continuous with, or formed from, the plasma membrane. The synapsing axon (A_2) is surrounded peripherally by lemnoblast cytoplasm. On the muscle side the plasma membranes of axon and muscle fiber and their interspace separate axon and muscle. Note the membranes of the endoplasmic reticulum near the synapse (single arrow), and the clumps of axonal synaptic vesicles and of muscle aposynaptic granules (double arrows) at the region of close synapsis. × 20,000.


A
BM
G
ER
7


A1
L
NU
A2
ER
M
8