

The Fine Structure of Muscle Spindles in the Lumbrical Muscles of the Rat

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ABSTRACT

Lumbrical muscles of young rats were fixed with OsO₄ and embedded in methacrylate for electron microscopy. The spindle capsule was found to be continuous with and similar in structure to the sheath of Henle surrounding the nerves supplying the spindle. The capsule consists of several closely applied concentric cytoplasmic sheets. Each sheet is about 1,000 Å thick and has no fenestrations. Many caveolae and vesicles in the cytoplasm suggest active transport through the sheets. The periaxial-space fluid contains much solid material. It is suggested that the capsule and periaxial space regulate internal chemical environment.

The interfibrillar structures are less evident in the polar regions of intrafusal fibres than in extrafusal fibres. Simple motor end-plates occur on the polar regions of intrafusal fibres. In the myotube region of the intrafusal fibre a peripheral zone of myofibrils surrounds a cytoplasmic core containing nuclei, mitochondria, Golgi bodies, reticulum, and a few lipid-like granules. Naked sensory endings lie on the myotube "in parallel" with the underlying myofilaments. Naked processes of the primary sensory ending deeply indent the muscle plasma membrane and the underlying wisps of myofilament in the nuclear-bag region.

The plasma membranes of sensory nerve ending and intrafusal muscle fibre are about 200 Å apart.

INTRODUCTION

In recent years the physiological examination of the sensory organs in skeletal muscle has been more productive than the morphological. The sensory role of the muscle spindle was not deduced until 1894, but since then has been proved by a substantial background of observations. Yet since that date, until very recently, anatomical studies have added only minor details of morphology, or have confirmed or denied, through more thorough

investigation, earlier claims that had inadequate grounds.

Sherrington in 1894-95, in the early stages of his work that forms the basis of our present knowledge of reflexes, studied the morphology of skeletal muscle and peripheral nerves, paying particular attention to the muscle spindles and Golgi tendon organs. He confirmed and enlarged upon the descriptions of Kühne (1863 *a* and *b*), Kölliker (1862-63), and others. What was more important, he demonstrated that the endings in the equatorial region of the spindle do not degenerate following section of the ventral roots. He stated that "the majority, perhaps all, of the large root-ganglion fibers terminate in the muscle spindles." Ruffini (1898-99) described in great detail muscle spindles from unspecified muscles of the cat. His description of "annulo-spiral" and "flower-spray" nerve endings caused argument in later years.

A brief description of the gross features of the mammalian spindle is included here to make the later observations more understandable. It is necessarily a condensed account and, therefore, a number of details are

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lost in generalities. The description is based on observations of sections and whole-mounts stained with gold, silver, or methylene blue, and studied with the light microscope by Sherrington (1894-95), Ruffini (1898-99), Hinsey (1927-28), Hines and Tower (1928), Barker (1948), and others.

Essentially, the mammalian muscle spindle consists of several fine, modified, striated muscle fibers with their associated nerve endings, all enclosed in a delicate fusiform capsule. The fibers so enclosed are called intrafusal fibers. Such spindles are scattered through most skeletal muscles. They are supported by endomysium and lie amongst the ordinary muscle fibers, which may be called extrafusal fibers. The number, size, distribution, and some details of structure, depend on the particular muscle and the species. For example, the number of intrafusal fibers within a spindle is usually about six, but may be from one to twenty; the length may be one or several millimeters; the capsule is usually delicate, but may be thick. The nerve endings on the intrafusal fibers vary considerably in their gross morphology and in their number, but there is a general basic pattern common to mammals. Ruffini had no doubt that the spindles and tendon organs were part of a highly complex sensory apparatus.

The capsule consists of about six concentric sheets of very delicate connective tissue thought to contain circularly disposed fibrils. It is continuous with the intra- and extrafusal endomysium and also with the perineurium of the small nerve trunks passing to the spindle. The perineurium, or sheath of Henle, also consists of a number of concentric tubular coats of delicate connective tissue. The sheath is well developed around the spindle nerve trunks.

The central segment of the spindle is the equatorial region, and the two ends are the poles. The capsule frequently closely invests the long polar parts of the intrafusal fibers, but in the generally shorter equatorial region, the capsule parts from the intrafusal endomysium and encloses the so called periaxial space, which is a large fusiform cavity up to $200\ \mu$ in diameter round the central axis of intrafusal fibers and endomysium. Sherrington (1894-95) claimed that this cavity is connected with the lymphatic system. One or more large myelinated sensory nerves pass through the capsular space to enter the equatorial endomysium. The motor supply may enter the capsule in company with the afferents, or enter separately through the polar capsule.

Small arterioles and capillaries are associated with the capsule.

The intrafusal muscle fibers attach to connective tissue at either end of the spindle. The attachment may be to tendon, fascia, or endomysium. Intrafusal muscle fibers are usually between 1 and 5 mm. long and can be

divided into zones or regions. In both polar regions the fiber is small and well striated; it has a circular outline in cross-section, and has nuclei scattered along the axis. The two myotube regions are at either end of the central equatorial region, within the confines of the periaxial space. In the myotube the contractile material is reduced to a peripheral shell enclosing a central core of sarcoplasm containing numerous nuclei. The middle segment of the equatorial region may be called the nuclear bag. It is packed with a mass of small spheroidal nuclei, which is enclosed within a thin peripheral shell of sarcoplasm containing myofilaments; the latter are very difficult to see.

Each of the three zones of the fiber is associated with a particular form of nerve ending. The morphology, extent, and number of endings vary with the particular muscle and species. Thus, there may be an ending on one or both myotube regions or there may be none. The ending on the nuclear bag is invariably present in some form. The polar endings have been the subject of considerable difference of opinion regarding morphology and distribution. They are generally held to be motor.

The nuclear bag ending has also been named "primary" or "annulo-spiral" by Ruffini (1898-99) and "A2" by Matthews (1933). It may show considerable order in a spiral or annular form. It may, however, be a random spread of processes with swellings. It is supplied from the largest afferents (group I, 12 to $20\ \mu$ in diameter) in the muscle nerves. Usually one group I afferent fiber passes to each spindle, and, having made several dichotomous divisions, supplies the nuclear bag region of each intrafusal fiber in the spindle.

One or both of the myotubes may have nerve endings. These may be called simply "myotube endings" but were named "secondary" or "flower-spray" by Ruffini (1898-99) and "A1" by Matthews (1933). This type may show some spiral or annular order, but is usually in the form of spreading processes with swellings. It is supplied from the medium-sized group II afferents, 4 to $12\ \mu$ in diameter.

The motor innervation of the intrafusal fibers is at one or both poles, and is supplied almost exclusively by the small (gamma) group of efferent myelinated fibers, which supply nothing else. In the mammal these small efferent fibers have a diameter of less than $8\ \mu$ and a conduction velocity of less than 55 m./sec. Hines and Tower (1928) maintained that the motor supply is confined to one pole, and that the motor ending is of the "terminaison en grappe" form; that is, it resembles a small bunch of partly developed grapes. Barker (1948) reported a bipolar motor innervation by a number of individual small efferents. He found large motor end-plates, and reported that the motor fibers divide to supply end-plates on different muscle fibers.

Recently, brief reports (Boyd, 1959) have indicated a more complex spindle structure, in that the arrangement of the myotube and motor endings depends on the

diameter of the intrafusal fibers, which fall into two distinct size groups. In the particular rabbit spindle that he used for his main reconstruction, Barker found that one pole was innervated by one medium-sized fiber only. The diameter of this fiber near the spindle was 4.8μ in the silver preparation. He quoted other observers who had seen similar one-axon innervation of a pole.

Apart from the last example, the diameters of the sensory fibers given above refer to measurements of the fiber diameters in peripheral trunks and dorsal roots; in and around the spindle the nerve fibers are smaller. Barker did not see any spindle nerve fiber greater than 12μ in diameter in rabbits, but found one of nearly 15μ supplying a cat spindle. Sherrington (1894-95), using silver preparations of cat and monkey, measured two sensory spindle nerve fibers of 8 and 12μ in diameter. Batten (1897), using Sihler's technique, reported afferents with diameters of 8 to 10μ in man and 8μ in dog. Barker found the afferents from rabbit and cat myotube endings to be from 6 to 9μ in diameter.

Barker agreed with Sherrington, who reported that the afferent nerves, approaching their first subdivision at the spindle, increase in total diameter because, although the myelin becomes thinner, the axis cylinder becomes thicker. The first division may be outside or inside the spindle.

To follow the development of the physiological concepts relating to the muscle spindle would require a lengthy review. It is not possible to do justice to the physiological work in this brief account, and a number of workers who have made significant contributions cannot be mentioned. Thus, the following introductory remarks are restricted to observations that are basic and that seem to be relevant to the morphology to be described below.

Adrian and Zotterman in 1926 described the electrical response resulting from stretching a single end organ in a muscle.

Matthews (1929, 1931 *a* and *b*, 1933), working with amphibian and mammal, demonstrated the pattern of response of the Golgi tendon organ, and of the muscle spindle through its slow and fast afferents. His work is unchallenged. These patterns of response are now used in physiological work to determine the origin of a specific signal in a muscle system.

This work confirmed the prediction of Fulton and Pi-Suñer (1927-28) that the Golgi tendon organ and the muscle spindle would behave differently when the muscle is contracting. During the contraction of a muscle the tendon organ, being in series with the load on the attachments of the muscle fibers, is presumably distorted, and it discharges. The rate of discharge is proportional to the tension. The spindle fibers, however, are in parallel with the extrafusal fibers, and when the

latter contract they relieve the load on the spindle, which momentarily ceases to discharge, or reduces its rate of discharge.

Leksell (1945) recorded the action potentials of the group of small (gamma) motor fibers, which have a diameter of less than 8μ and conduct at velocities of less than 55 m./sec. in the mammal. He found that they have a very high threshold in the peripheral nervous system, that they cause no visible muscular contraction, but that they have a profound effect on the nature of the afferent discharge from the muscle. He concluded that they consist entirely of motor fibers for the muscle spindles. This work laid the foundation for the very important concept of the part played by the small-fiber motor system in the control of muscle activity. It provided evidence that the contraction of the intrafusal fibers can stretch the sensory receptors on the central regions of the fibers, thus causing an afferent discharge. Hence the response of the receptor to outside forces can be modified by the central nervous system.

Lloyd (1943) investigated the hind limb reflexes of the cat. He found that large rapidly conducting afferent fibers mediate the two-neuron myotatic reflex. In this reflex the afferents discharge the motoneurons of the same muscle, and subliminally excite the motoneurons of the synergists of that muscle. The two-neuron myotatic reflex includes the extensor reflex (the extensor tendon-jerk) and the flexor tendon-jerk. He also found that the flexion reflex proper is mediated by a more slowly conducting afferent limb of a reflex arc of at least three neurons. The afferents of this arc excite the motoneurons of ipsilateral physiological flexor muscles, and inhibit the motoneurons of ipsilateral physiological extensor or antigravity muscles. The works of Matthews (1933) and of Hunt (1953), taken with this work of Lloyd, suggest that the nuclear bag is the origin of the rapidly conducting afferent of the extensor reflex; that the myotube is the origin of the slower afferent of the flexion reflex; and that the Golgi tendon organ gives rise to a fast afferent impulse, which probably inhibits the extensor reflex.

Eldred, Granit, and Merton (1953), in discussing supraspinal control of the spindles, suggest that the gamma efferents are influenced by higher centers that can cause them to produce intrafusal contraction, which would then initiate the spinal reflexes. Thus the gamma efferent system may be used in the higher control of motor activity.

Kuffler and Hunt (1952) have prepared an account of the early work on the small-nerve motor system of the mammal, and of its probable role.

Thus the spindle has been shown to be a complex sensory organ. Like a strain gauge, it detects an increase in length of the nuclear bag, and, in many cases, of the myotube region as well. A change in length of these regions may be caused

by a change in an external force altering the length of the whole muscle, by an effective contraction of the extrafusal fibres shortening the muscle, or by a change in length of the contractile polar regions of the intrafusal fibres under the influence of the gamma efferent fibres. If the resultant of these length changes causes a stretch of the equatorial region, the spindle afferents discharge. The frequency of the discharge is proportional to the tension on the intrafusal fibre, and may vary over a range from zero in relaxed muscle to hundreds of impulses per second at maximum stretch.

For the spindle to be an effective detector, the nuclear bag must always be under sufficient tension to bring the stretch-receptor endings at least close to the threshold of firing. Thus, in a contracted muscle there is considerable activity in the gamma efferents, and even in relaxed muscle there is some.

The spindle and Golgi tendon organ are but two of the receptors concerned with the feed-back loops involved in regulation of muscular contraction. The sensory endings of skin, connective tissues, joints, and the special senses, play their part in sending information to the central nervous system. The precision with which this complex works is, of course, the basis of survival and mechanical skills. The understanding of it will be increasingly important in the study of pathological states of the motor system.

Some details of spindle morphology have been the cause of argument. Evidence is conflicting or inadequate concerning the following aspects of mammalian muscle spindles:

1. The morphology of the motor endings and the extent of the motor innervation.
2. The morphology of the sensory endings and their relationships to the intrafusal fibre.
3. The nature of the intrafusal muscle fibre in its different zones; whether it belongs to either "red" or "white" muscle; and whether the fibres may show any feature that might lend support to certain physiological evidence (Hunt and Kuffler 1951 *a*) that they are similar to the muscle fibres of the small-nerve motor system of the frog, which are capable of slow non-propagated contractions only.

There are excellent reviews of the anatomical literature by Sherrington (1894-95), Batten (1897), Hinsey (1927-28), Hines (1927), Barker (1948), and Tiegs (1953). Hines also reviewed the literature on "red" and "white" muscle in 1927.

An excellent approach to the physiological

literature up to 1955 can be found in the published volume of the Silliman Lectures delivered by Granit (1955). This contains a most comprehensive bibliography and sound conjecture in a well reasoned presentation.

Robertson (1956 *b*) has made some observations on the spindles of frogs with the electron microscope. There are some gross differences, however, between frog and mammalian spindles.

The present paper reports on an attempt to use the high resolution of the electron microscope on some of the problems relating to the fine structure of the muscle spindle in the paw muscles of the rat. Owing to the complex nature of the muscle spindles encountered, and to the technical difficulties in specimen preparation, many problems remain. Hence, this study is no more than introductory in nature.

Materials and Methods

Two- to 3-month-old albino rats were used.

Sometimes fixation which was accomplished while the animals were under ether anesthesia was by aortic perfusion of 1 per cent ice cold Palade's (1952) OsO_4 fixative at pH 7.5. Better results were obtained by directly injecting into the area an ice cold mixture of 5 per cent OsO_4 diluted to 4 per cent by addition of standard Michaelis buffer (1931) to produce a pH of 7.5. In the case of lumbricals and interossei the fixative was injected into the carpal tunnel of the forepaw. This distended the fascial spaces along the flexor tendons. The toes were immediately severed through the proximal phalanges, and the paw, still attached to the loaded needle and syringe, was severed above the wrist. Paw and syringe were then covered with crushed ice. Every few minutes a few drops were forced from the syringe. This caused fixative to ooze from the stumps of the toes. The aim was to force the fixative to flow along the region of the lumbricals, which pass from flexor tendons in the palm to extensor expansions over the proximal phalanges. In the hind foot the procedure was the same, except that the injection was into the flexor tendon area in the center of the sole. The deeper interossei seemed to be fixed quite as well as the lumbricals.

The dissection after aortic perfusion was commenced immediately the injection was finished, and as the muscles were removed they were dropped into ice cold fixative. The dissection after local injection was delayed until fixation was completed and dehydration had commenced: the paws were carried into the low alcohols at room temperature by changing the contents of the syringe to water and then to 30 per cent ethanol. The muscles were then dissected as rapidly as possible under 30 per cent ethanol.

As it seemed pointless to establish a state of equilibrium throughout the tissue with each change of alcohol,

the muscles were left in each concentration only so long as would probably establish a reasonable gradient of concentration through the surface layers. If this gradient could be maintained in small steps to the center of the tissue until the block was in absolute alcohol, the most rapid dehydration would be achieved with, perhaps, the least damage. The alcohol was increased in strength every 2 or 3 minutes by 10 per cent steps from 30 per cent to absolute. The muscles were left in absolute alcohol for 15 minutes, then transferred to *n*-butyl methacrylate, and embedded after several hours. Polymerization was initiated at 50°C. with pure crystalline 2,4-dichlorobenzoyl peroxide.

Spindles could not be identified in blocks of osmium-fixed mammalian muscle by examination with a dissecting microscope. Orientation of the block for cutting was difficult.

The embedded muscle was fixed with dental modelling wax to a piece of plastic rod held in the microtome chuck. Successive sections, 1 μ thick, were cut transversely to the muscle fibers and examined by phase contrast microscope until a spindle could be recognized as it was first cut through the tip of a polar region. The block was then removed from the wax bed, and the cut face was examined with an incident-light microscope at about $\times 200$ magnification. After adjustment of the angle of the cut face to the axis of the microscope the outlines of the tissue could be seen faintly. The image of the face was compared with the phase contrast image of the section, and the spindle identified in the block. The block was then trimmed, both by hand and in a microtome, until most of the muscle had been removed from three faces around what was judged to be the axis of the spindle. The use of wax allowed the block to be reset when necessary. Finally, the trimmed block was oriented for oblique longitudinal sectioning. This type of section was chosen because it is more informative than a transverse one while still providing for many sections through the spindle. An entire longitudinal section of a spindle is too long for the electron microscope grid. Moreover, it gives little margin for error in adjusting the block. Even with oblique orientation, adjustments were extremely critical when the knife was changed. Preparing a block successfully for sectioning usually occupied 2 days. Unfortunately, those spindles that were cut in the most favorable planes were, for the most part, from muscles that had been fixed by vascular perfusion rather than from the better preserved muscles that had been fixed and dehydrated *in situ*.

Sections reflecting interference colors from pale to deep gold were chosen and examined with a modified RCA EMU2C electron microscope fitted with a 250 μ condenser aperture, a 50 μ objective aperture in the back focal plane of a compensated objective lens, and a special stabilized power supply. Canalco equipment was used for compensation.

Kodak contrast and Kodak experimental fine grain positive emulsions, and D19 developer, were used.

OBSERVATIONS

The Capsule

The capsule is made up of about six remarkably thin, concentric, tubular sheets of the cytoplasm of specialized fibroblasts. These cells are here termed "capsular-sheet cells" (Figs. 1 to 6). In most areas, the cytoplasm of capsular-sheet cells varies from 1000 to 3000 Å in thickness, but small portions of it may be as thin as 300 Å between plasma membranes. Owing to the possibility of there being significant angles of obliquity in thin sections, the dimensions given are only approximate. The concentric sheets are separated by spaces that range from a few hundreds to a few thousands of Angstrom units in width. In these spaces are scattered collagen fibres and the amorphous material that tends to concentrate in a narrow layer with vague external limits against the plasma membranes of cells where they have a free surface in a tissue space. For want of a better name this amorphous layer is usually referred to as the "basement membrane." The dense portion of the basement membrane is separated from the dense portion of the plasma membrane by a less dense layer that is about 200 Å thick. Porter (1954) described this lighter layer in the sarcolemma and called it the "cuticular layer."

In the spindle capsule the basement membrane is well developed on both sides of each sheet, and is especially thick on the inner surface of the innermost capsular-sheet cells lining the periaxial space (Fig. 2).

Conditions found between neighbouring sheets vary. Where the plasma membranes of neighbouring sheets are separated by a distance of 1000 Å or more, the amorphous basement-membrane material shows a ragged separation as though torn apart (Figs. 2, 3, and 5). When the plasma membranes are from 400 to 600 Å apart the interspace is filled with basement-membrane material, which may be of uniform density, or may have a faint dense line in the position of the apposed surfaces of the two fused basement membranes (Fig. 2). In some places the plasma membranes are from 200 to 300 Å apart. Isolated desmosomes or attachment plaques serve to bring the sheets into intimate contact (Fig. 2).

The capsular-sheet cell cytoplasm contains small mitochondria, scattered small particles, caveolae intracellulares, and oval vesicles a fraction of a micron across. Some vesicles are filled with many very fine dense granules. The large number of

caveolae is very striking, and many of them nearly extend from one side of the sheet to the other (Fig. 2).

The capsular-sheet cells of each layer are closely apposed at the edges (Fig. 3) to form a continuous sheet. Except where the nerves enter the capsule, the sheets formed by the cells seem to be complete and without pores or fenestrations. These capsular-sheet cells are associated with collagen fibres and are similar to those in the capsule of the muscle spindles of the frog (Robertson, 1956) and to those in the capsule of the mesenteric Pacinian corpuscles of the cat (Pease and Quilliam, 1957). Furthermore, the capsule cells seem to be similar to those that form the sheath of Henle round small nerve trunks. The Henle sheaths of the nerves entering the spindle are continuous with the capsule.

A flocculent precipitate often fills the periaxial space (Figs. 1, 2, and 15). It resembles the precipitate from plasma in blood vessels. It is found in no other part of the muscle, neither inside nor outside the spindle.

Around the poles, the capsule usually closely invests the intrafusal muscle fibres and nerves, which lie in the delicate sheath of endomysium. Bands of material tentatively identified as elastic tissue, of irregular outline and medium density, lie in the capsule and within the endomysium (often against the muscle fibres) in the polar regions (Figs. 5, 10, and 22). No sections have been obtained through the attachments of either end of the organ to tendon or endomysium.

Small arterioles and capillaries have been seen in the outer layers of the capsule, but none have been found to pass through the capsule (Fig. 1).

The largest myelinated nerves are seen to penetrate the capsule towards the axis in the equatorial region. In this series the profiles of the largest nerves found were no greater than about 5μ in diameter. In sections their outlines vary from almost circular to bizarre shapes (Figs. 1 and 5). These variations may be due, in part, to the relation of the plane of section to normal convolutions, and partly to distortions arising during preparation. Sometimes two fibres of very different sizes lie close together, but when the myelin lamellae of each are counted the numbers may be the same. The range of number of lamellae has been found to be from 15 to 27, with a peak around 18. An occasional axon, with the lower number of lamellae, has been found in the polar capsule just beyond the end of the myotube.

Two or three unmyelinated axons, of 1μ or less in diameter, are usually found in the same sheath with the larger nerves (Fig. 5). Small unmyelinated axons, either partly or wholly enclosed in Schwann-cell cytoplasm are scattered in the capsule and endomysium (Figs. 4, 6, and 12).

Unmyelinated axons, at least 3μ in diameter, devoid of Schwann-cell covering, and with many mitochondria, were found in one spindle, in a small Henle sheath deep in the capsule in what was probably the equatorial region.

It should be emphasized here that, owing to the chance relationships of the plane of section to the axis of the nerve, and to artefacts of preparation, dimensions recorded from thin sections through a winding nerve are not very reliable.

The endomysium is continuous with the innermost layer of the capsule in the polar regions (Figs. 5 and 8). In the equatorial zone, the endomysium separates the intrafusal fibres from the periaxial space. The intrafusal fibres are separated from each other by single sheets of endomysial cell cytoplasm. The sheets are generally without fenestrations, but were frequently damaged in preparation of these specimens (Fig. 8). An example of a discontinuous endomysial sheet is seen in Fig. 15. The endomysial and capsular-sheet cells seem to have a similar structure, although there tend to be fewer vesicles and caveolae in the endomysial cytoplasm (Figs. 2 and 16).

Intrafusal Muscle Fibres

No spindles were identified at the attachments to the connective tissue; therefore, it is not known yet whether the structure of the attachments of the intrafusal fibre differs from that of the ordinary myotendinal junctions of the extrafusal fibre.

Polar Regions:

In their polar regions the intrafusal fibres are nearly filled with myofilaments. The elongated nuclei of the fibres are usually centrally placed and widely spaced, and lie with their long axes parallel to those of the fibres. The textures of the intrafusal and neighbouring extrafusal fibres are strikingly different: with one exception (Figs. 9 and 20) the spindle fibre in the polar region was found to have much less interfibrillar sarcoplasm. The margins between the myofibrils of ordinary mammalian skeletal muscle are well defined by sarcoplasmic material. This definition is not nearly so apparent in the polar regions of most spindle

fibres because the myofibrils are tightly packed, and contain little interfibrillar reticulum (Figs. 8 and 10). In the polar regions of the spindle fibres in Fig. 20, however, division into myofibrillar units is much more definite. The fibrils vary in size even in the most regular array; and branching of myofibrils is common, but is usually not conspicuous because of the poor development of interfibrillar substance.

The arrangement of the sarcoplasmic reticulum in rat sartorius muscle has been described in great detail by Porter and Palade (1957). They recognize, in particular, a triad of elements of the reticulum that partly encircles the myofibrils at the junction of the A and I bands. Similar "triads" are found in the polar region of the intrafusal fibre (Fig. 8). In the intrafusal fibres the triads are frequently conspicuous because the peripheral vesicular components (the *er*₂ of Porter and Palade (1957)) tend to be dilated and to have low internal density (Figs. 7 and 8). All the micrographs in this series, however, suggest that the intrafusal triad does not extend nearly so far around the circumference of its myofibril as does the extrafusal triad. The extent of the intermediary vesicles in the triad labelled *t* in Fig. 9 is greater than any seen in other intrafusal fibres, whereas considerably longer lengths or rows of intermediary vesicles are commonly seen in the extrafusal fibres.

Scattered small mitochondria occur. Sometimes they are found in clumps, many of which may have been close to nuclei out of the plane of section. As in other muscle fibres, the nuclei are associated with very active-looking cytoplasm (Figs. 8 and 9). Around them are many mitochondria of various shapes, stacks of smooth vesicles suggestive of Golgi bodies, an occasional array of ergastoplasm or vesicles studded with external granules, and scattered small vesicles and particles. Sometimes vesicles up to $\frac{1}{3} \mu$ in diameter, associated with Golgi bodies and containing a pale amorphous material, are continuous with slit-like membrane-bounded channels that are lost among a number of small vesicles immediately beneath the sarcolemma (Fig. 9). The perinuclear cytoplasm of the intrafusal fibres is more complex and extensive than that of the extrafusal fibres.

Myotube Region:

The full development of the myotube in which myofilaments are confined to a narrow peripheral zone is seen only for a short distance adjacent to

the nuclear bag. There is a gradual transition from the morphology of the polar region to that of the myotube. There is a progressive increase in the number of the centrally placed nuclei, and in the volume of the cytoplasm surrounding them, with increasing distance along the myotube from its polar end (Figs. 11, 12, and 15). At the same time there is a progressive loss of myofilaments until, near the nuclear-bag end, they are confined to a narrow and continuous peripheral zone (Fig. 12). In a longitudinal section of the nuclear-bag end of the myotube, the peripheral shell of myofilaments looks like a single myofibril at both borders of the fibre. It is not known how the myofilaments terminate in those fibrils that fail to pass through the length of the myotube. It cannot be seen in the micrographs whether myofilaments end at a particular part of the sarcomere, or even whether there is a fusion of one fibril with its neighbour during the reduction process.

Generally, there are more interfibrillar formed elements in the myotube regions than at the poles, but there are evidently exceptions (Fig. 15). These interfibrillar elements are continuous with those of the subsarcolemmal and central-core cytoplasm. Nothing definite can be said concerning the relationships, if any, between the Golgi bodies and granular ergastoplasm, on the one hand, and portions of the reticulum peculiar to the different zones of the sarcomere on the other, since consistent associations have not been recognized. The reticulum around the myotube sarcomeres is well developed (that in Fig. 15 excepted), and the outer vesicles of the triad at the A-I junction are conspicuous and often have very low internal density (Figs. 7, 11, and 15).

The central core of cytoplasm contains a mass of formed elements (Figs. 11 and 15). There are many round, oval, or elongated smooth vesicles, which may be 700 Å or less in diameter, and many elongated nuclei. There are large vesicles of various types: those associated with the zones of the sarcomeres are seen along the inner surface of the contractile tube (Fig. 7). Towards the centre of the core are large vesicles, $\frac{1}{3}$ to $\frac{1}{2} \mu$ in diameter: they are round or oval and contain either nothing recognizable or a number of small vesicles of about 600 Å in diameter. They are similar to the vesicular conglomerates of Yamada (1955) and the compound vesicles of Sager and Palade (1957), and are probably parts of Golgi bodies. Several portions of Golgi body are visible in a section of myotube a few microns long. Sometimes these, or

similar large vesicles, contain very fine dense particles, which obscure the interior of the vesicles (Fig. 11).

A few granules (Fig. 15) are found in the core of the myotube and occasionally under the sarcolemma of the myotube or polar regions. In the sections the granules are about 1.5μ in diameter, but they appear to have shrunk away from the surrounding cytoplasm. They are of unknown composition. They probably contain lipid, although they frequently have a lower density than might be expected of pure lipid.

Nuclear-Bag Region:

At the junction between the myotube and the nuclear bag there is a sudden change in the relative volumes of the components. In the nuclear-bag region (Figs. 13 and 14) the peripheral shell of myofilaments is still present, but is thinner than in the myotube. In the myotube the nuclei lie singly in the axis, and they are surrounded by much sarcoplasm. In the nuclear-bag region the number of nuclei is very much increased at the expense of the sarcoplasm, which is confined to narrow and irregular areas between some of the nuclei, around the myofilaments, and beneath the sarcolemma. All the sarcoplasmic elements described above are present; the relative proportions are hard to estimate, but sections of mitochondria seem smaller and fewer compared with those in the myotube sarcoplasm.

The shell of myofilaments, in some if not all places, has Z bands which are very inconspicuous (Fig. 13). The density of this contractile shell is low in all sections of the nuclear bag in this series; but this does not seem to be due to anything lacking in the structure of the filaments, because thick A and thin I band filaments have been identified. However, sections through the shell are patchy in appearance, and outlines of vesicles of the cytoplasm can be seen in irregular concentrations through the images of the filaments (Fig. 14). This indicates that the fine sarcoplasmic reticulum is running amongst very small bundles of filaments, and in a thin section, the effect of this is to displace some of the myofilaments and to produce a confusing picture. In one very small intra-fusal fibre, which had an unusually short nuclear bag, there were wisps of myofilaments between the nuclei (Fig. 13); however, internuclear myofilaments were not seen in any other nuclear bag.

The nuclei seem unremarkable apart from their number. Their membranes and contents show

no specializations beyond those of most mammalian nuclei. They are polyhedral owing to close packing.

Nerve Endings

Nuclear-Bag Region:

In a longitudinal section of the nuclear bag, the nerve ending appears as a series of transversely cut sections deeply indenting the borders of the bag at intervals on both sides (Fig. 13). Thus, the plasma membrane and myofilament shell of the nuclear bag are forced down into valleys between the bulky nuclei. It can be seen in grazing sections that these endings often extend in a parallel array of narrow bands round the circumference of the muscle at an angle nearly perpendicular to the long axis. These bands are the annular or annulo-spiral type of nuclear-bag endings.

In this fixed material the endings indent the muscle fibre so that only a small bulge of nerve substance rises above the general surface level. Cross-sections of the endings (in a longitudinal section of muscle) are somewhat triangular with rounded angles and convex sides. They are bounded by a plasma membrane. Many dense mitochondria make the endings conspicuous. The endings also contain a few vesicles from 200 to 700 A in diameter lying amongst a general background of patchy amorphous material (Fig. 14).

The endings are devoid of Schwann cells. The basement membrane of the muscle is not depressed with the plasma membrane, and is continuous over the free surface of the ending. There is no basement membrane between the plasma membranes of nerve and muscle. The visible dense portions of the plasma membranes of nerve and muscle are separated by a distance of about 200 A at their closest approach. In all sections obtained of this area, however, the preservation of detail left much to be desired: all the neuromuscular junctions are thrown into many small folds, so that, in many places on the perimeters of the endings, the plasma membranes were cut so obliquely that they cannot be identified (Fig. 14). The cytoplasmic vesicles lying against the plasma membranes of nerve and muscle may overlie the obliquely cut membranes in a thick section, and may give the impression that the vesicles are penetrating through breaks in the membranes (Fig. 14). Until pores are proved from micrographs of better resolution, the plasma membranes should be considered to be intact, and the vesicles to be confined to their own cells. A

fusion of nerve and muscle plasma membranes around a well defined pore has not been seen in this series.

Under the nerve ending myofilaments are very close to the plasma membranes, and, as stated above, vesicles seem to lie among small bundles of the filaments.

The shape and dimensions of cross-sections of the nerve endings on the nuclear bag are uncertain. There are sources of error from irregularities in the shape of the nerve and muscle, and from unknown obliquities of planes of section, as well as from any distortions arising during preparation. Making reasonable allowance for these uncertainties, the outlines of sections through the nerve endings suggest considerable variation in cross-sectional area and less variation in cross-sectional contour (Fig. 13). The shapes of the cross-sections range from lenticular to irregularly triangular with rounded corners. Allowing for geometrical uncertainties, the micrographs suggest diameters ranging from $1\frac{1}{2}$ to 3μ , and the greatest and least diameters of any one of the cross-sections of the endings would fall between these limits. The endings less than 1μ in diameter were found at one or other end of the nuclear-bag region (Fig. 13). These small endings lie in slightly raised gutters of muscle substance; they contain a few clumps of vesicles, but usually no mitochondria; otherwise the description of the large ending applies to them.

Myotube Region:

The myotube endings resemble those of the nuclear-bag region in that plasma membranes of muscle and nerve are about 200 A apart and have no interposed basement membranes, myofilaments underlie them closely, and no Schwann cells are associated with them. Here, also, some of the plasma membranes tend to be corrugated, so that continuity of each cannot be followed with certainty, and "pores" should be regarded as artefacts until proved otherwise.

In the figures, the myotube endings close to the nuclear-bag region have an appearance different from those closer to the poles. The former lie in raised gutters of muscle surface (Fig. 15). Sometimes the gutters have low walls, but occasionally the lips meet over the top of a nerve ending. Apart from being more rounded, cross-sections of these endings resemble those on the nuclear bag. They contain many small dense mitochondria lying in a background of scattered amorphous material

and small vesicles. These myotube endings vary in size and show no regular pattern of distribution (Fig. 15). There is no suggestion of a regular spiral arrangement, but grazing sections show that some branches make at least a half circle round the fibre. One example of an ending in contact with two muscle fibres was found (Fig. 15).

The endings characteristic of the polar end of the myotube extend over the vague junctional zone between the polar and myotube regions of the fibre. They are elongated parallel to the axis of the muscle fibre, and often cover a considerable portion of its circumference. In general, they hardly bulge above the surface of the fibre, being sunk into it in shallow depressions (Figs. 16 and 17 to 19). The larger sections through endings contain many dense mitochondria, although smaller ones do not, but the latter may be grazing sections through the edges of larger endings.

In one specimen the endings on the polar end of the myotube contain closely packed masses of minute curled bodies that resemble portions of curved and flattened vesicles or short sections of twisted tubules (Figs. 17 to 19). In Fig. 18 these bodies are the dominant feature of the ending. Mitochondria with obliquely sectioned walls tend to be lost among them because their outlines are similar to those of mitochondrial cristae. The "tubules" are non-granular and about 300 A in diameter and are probably segments of endoplasmic reticulum of a very delicate type. Dense bodies that resemble lipid and are difficult to cut are associated with this "tubular" reticulum. The dense bodies are scattered amongst the mitochondria (Fig. 17), sometimes closely applied to them; and because the ending also contains dense mitochondria that have been damaged or otherwise modified, the identification and classification of the bodies is difficult. Ross *et al.* (1958) reported finding a similar mass of fine tubular endoplasmic reticulum, also associated with lipid droplets, in the foetal zone of the steroid-producing human foetal adrenal; and they note that, according to Palade (1955), the smooth surfaced rather than the rough surfaced reticulum is found to predominate in cells active in lipid metabolism.

The raised and rounded, and the low and elongated forms of myotube ending have not been found on the same fibre. However, as complete lengths of a single myotube were not sectioned successfully it cannot be proved whether or not both forms occur on one myotube.

Polar Region:

The elongated form of myotube ending extends onto the ill defined junctional zone between myotube and pole. Again, small sections of ending devoid of mitochondria are seen, but they may be grazing sections of larger endings.

Motor end-plates have been found in the polar region (Figs. 20 and 22), but in this series no spindle was sectioned successfully from end to end, and therefore, both poles could not be examined on any one spindle. Hence, it could not be determined if motor endings supply both poles. The basic structure of the intrafusal motor endings is the same as that seen on the neighbouring extrafusal fibres, and described in skeletal muscle of the rat by Palade (1954, 1957), the chameleon by Robertson (1956 *a*), and the mouse by Reger (1957, 1958). The intrafusal endings are much simpler than the extrafusal. Six have been identified, all from different spindles.

Infolding of the plasma and basement membranes of the muscle under the ending is conspicuous in the extrafusal end-plates. In the intrafusal end-plates infolding is greatly reduced or non-existent. The few infoldings that are present are rudimentary, and the enclosed basement-membrane layer of the sarcolemma lines a cavity in the slightly dilated end of the fold (Fig. 22). Robertson described similar dilatations in reptilian end-plates, but this feature is not pronounced in the long and complex infolding associated with the neighbouring extrafusal motor end-plates in rat lumbricals (Fig. 23).

The pad of sarcoplasm under the intrafusal motor ending is not developed into a large eminence. Thus, the ending is a micron or less from the myofilaments. All the usual sarcoplasmic structures are present in the small pad. In a section of the latter, Golgi bodies, ergastoplasm, reticulum, large mitochondria, complex vesicles, and vesicles containing very fine dense particles, lie amongst small particles and vesicles surrounding one or two large nuclei (Fig. 20).

The smallest distance between the plasma membranes of nerve and muscle at the junction is about 500 Å. In some places the nerve plasma membrane is smooth and in others it shows small ripples. Some of the deeper of these ripples resemble caveolae (Fig. 20). Massed synaptic vesicles of about 500 Å in diameter cluster close to this membrane. At no time has a vesicle been seen in

the membrane complex of the myoneural junction in this material.

Processes of a Schwann cell form an almost complete cap for each intrafusal motor nerve ending. The basement membrane does not run between the Schwann cell and the nerve ending, but extends from the free surface of one to the other at the perimeter of the end-plate. The separation of the plasma membranes between Schwann cell and nerve ending is about 200 Å.

DISCUSSION

Since there are numerous gaps in the observations, it has not been possible to construct a complete picture of the fine structure of the muscle spindle. The gaps were due largely to loss of lengths of the spindle in the course of alignment of the face of the block to the edge of a new knife or to fresh portions of the knife edge. Because the glass knives were changed frequently during the several days required to section a spindle, no single zone of any one spindle has been examined completely.

The Capsule

The capsule is well developed in these spindles and its structure seems to be similar to that of the sheath of Henle of small nerve trunks. The epineurial coat of large trunks is able to regulate the internal environment to some extent. It was shown by Feng and Gerard (1929-30) that the connective tissue sheath of a frog nerve acts as a diffusion barrier. The observation is supported by other workers using different animals. Causey and Palmer (1953) using P_{32} , showed that the main barrier to diffusion is the epineurium; the perineurium is not a barrier. It seems reasonable to suppose that, as the trunks become smaller and progressively lose their coats, the barrier property is retained by the outer layers of the sheath, no matter how delicate they may be. Krnjević (1955) demonstrated a high concentration of Na and K in the fluid of cat nerves and considered it to be associated with connective tissue protein. He also concluded that a mechanism in the sheath pumped water out of the system; thus, a high internal hydrostatic pressure was prevented.

The structure of the thin continuous sheets of cytoplasm in the capsule suggests that their function is to maintain and activate the two plasma membranes of lipoprotein in each sheet. The presence of numerous caveolae and vesicles suggests active transport in one or both directions (Bennett,

1956). These observations suggest that the capsular-sheet cells have an active role in regulating the internal environment of the spindle. Intra-fusal or extrafusal metabolic products, or nervous innervation of the capsule, may influence the passage of materials across the capsular-sheet cells. If the intracapsular environment could be altered, the response of both intrafusal muscle and nerve might be varied. Unfortunately, it would be very difficult to insert microelectrodes or pipettes through the mammalian spindle capsule in order to analyse the potentials and fluids. Yet the need for analysis has become important in the light of such observations as Howarth's (1957), that even changes in the tonicity of solutions surrounding an ordinary muscle fibre can affect the speed and duration of its contraction.

In the contractile polar regions, the capsule is usually closely applied to the intrafusal endomysium, and the volume of intracapsular fluid is very small. Here, movements of relatively small amounts of substance into or out of the intracapsular fluid would result in relatively large concentration changes in the fluid. Hence, in this region, the regulating effect of the capsular-sheet cells on the composition of the fluid surrounding the intrafusal fibres might be of special importance.

The environmental conditions of the equatorial regions are obviously different from those of the poles. In the equatorial periaxial space, there is a considerable volume of fluid that is distinct from the ordinary extrafusal intercellular fluid and bathes the thin endomysium of the nuclear bag and myotube regions. This volume would tend to maintain a constant environment for the equatorial sensory portions of the fibres in the face of fluxes of substance into and out of the fibre during activity. Sherrington (1894-95) suggested that this capsular-space fluid might be lymph, since he reported finding India ink in the space after injecting the lymphatics of the limb. If the capsular space is drained by the lymphatic system, the capsular fluid would be a more effective buffer or sink guarding against concentration changes engendered by fibres or capsule.

In the sections this capsular-space fluid shows a flocculent precipitate, which indicates an appreciable concentration of solid, and which distinguishes the contents of this space from ordinary extrafusal fluid and from the fluid in the compartments between intrafusal endomysium and fibres (Fig. 2). This precipitate may represent a residue of a protein or polysaccharide complex, which

could have ion-exchange or other binding properties. Such material would increase the effectiveness of the presumed buffering function of the intracapsular fluid with respect to rapid fluctuations in concentration. It would appear, therefore, that the capsule and the capsular space should not be regarded as merely forming a bursa for the equalization of local external forces, or a simple mechanical protection for the fibres.

It is generally stated that lymphatics do not penetrate muscle further than the fascial planes (Yoffey and Courtice, 1956). Sherrington did not state whether the spindles he saw were associated with such planes. If mammalian spindles are all connected with the lymphatic system, and if skeletal muscle lymphatics are generally confined to the fascial planes, it would mean that a special system of lymphatics is provided for the spindles. Clearly, investigation of a possible connection between the capsular space and the lymphatics should be repeated. Furthermore, as suggested by Hines and Tower in 1928, the physiological effect of alteration of the capsule pressure through the medium of the lymphatic vessels should be explored.

The collagen fibres are circumferential in the capsule and longitudinal in the endomysium. This would imply that the capsular fibres limit expansion of the spindle, and that the endomysial fibres limit extension of the intrafusal muscle. The fibres could not be followed for more than a short distance in these oblique sections.

The bands of material tentatively labelled elastic should be considered in relation to function. These bands seem to lie longitudinally, and, therefore, would be in parallel with the intrafusal fibres and tend to unload them in a shortening muscle. They would also tend to hold the spindle in the shortest path between its attachments. On the other hand, the bands may not be elastic material. If they are composed of a visco-elastic or viscous material, they would act as mechanical dampers, and would prevent the rapid return to rest length of the contractile polar regions. This could lead to the continued discharge recorded from spindles by Hunt and Kuffler (1951 *a*, see below) after cessation of stimulation.

Intrafusal Muscle Fibres

It may be worthwhile here to list some of the important differences between mammalian intrafusal and extrafusal muscle fibres. First, the intrafusal fibres are much smaller in diameter than most

extrafusal fibres. Second, they have a more complex innervation. Third, they vary in structure along the length of a single fibre more than do extrafusal fibres. Fourth, each individual is enclosed in a tubular sheath of flattened enomysial cells, surrounded in turn by the cellular capsule: thus, they may be bathed in a closely regulated fluid medium that differs from the ordinary intercellular fluid surrounding the extrafusal fibres. Fifth, Coërs and Durand (1956) reported that the polar and myotube regions of the intrafusal muscle fibre give a diffuse positive histochemical reaction for acetylcholinesterase. The nerve endings, other than those on the nuclear bag, also gave this reaction. In contrast, Koelle and Friedenwald (1949), Gerebtzoff (1953), Couteaux (1955), and others, found that in the extrafusal fibre cholinesterase activity is confined to the motor end-plate. Sixth, with respect to physiological behaviour, Kuffler, Hunt, and Quilliam (1951) demonstrated afferent responses that suggest that spindle fibres can be driven to follow stimulation of the motor nerves at frequencies considerably higher than those to which the extrafusal fibres will respond with discrete twitches. Moreover, Hunt and Kuffler (1951 *a*) postulated that, following prolonged contraction, intrafusal fibres relaxed more slowly than extrafusal fibres. This was their interpretation of the observation that the afferent discharge from the spindle continued for several seconds following cessation of prolonged stimulation of the efferents to the intrafusal fibres. In the same paper they suggested that the contraction is not propagated along the intrafusal fibre.

The picture is complicated by brief reports of more than one type of intrafusal fibre in some spindles (Boyd, 1959). There are references in the literature to similarities that seem to exist between the intrafusal fibres and one or other type of extrafusal fibre. Granit (1955) mentions this question as being of some physiological interest because some "red" muscles are generally said to contract more slowly than "white." The relationships between the structure, as seen with the naked eye and the light microscope, and the function of skeletal muscle fibres are not simple (Denny-Brown, 1929). The picture becomes most confusing when the muscles of different species are compared. Bullard (1912-13), Hines (1927), and Denny-Brown (1929), preferred to speak of "granular" and "agranular" fibres rather than of "red" and "white." The smaller extrafusal fibres

in a mixed muscle tend to be of "granular" or "red" type, whereas the larger fibres are prevalently "agranular" or "white."

Sherrington (1894-95) spoke of the intrafusal fibres as belonging to the "red" group. Hines (1927) noted that the "granular" extrafusal fibres stained with methylene blue more quickly and more intensely than did the "agranular." The intrafusal fibres, when stained in a like manner, were found to occupy an intermediate position between "granular" and "agranular" fibres with respect to speed and intensity of staining. However, Denny-Brown (1929), who used alkaline Sudan III for contrast, presented the intrafusal fibre as an example of a highly differentiated "clear" or "agranular" fibre.

The electron microscope study reported here has failed to settle this question of classification of intrafusal fibres. Myoglobin, which is the characteristic colouring matter of many "red" muscles, has not been recognized in electron micrographs. The myoglobin content of intrafusal fibres is not known. The large vesicles containing fine dense particles, seen in the intrafusal fibres in these micrographs, resemble the "siderosomes" described by Richter (1957) in liver cells. If the particles prove to be ferritin they may have significance in relation to myoglobin concentration. "Red" muscles have been described as containing more "dark" fibres than do "white" muscles. These "dark" fibres contain many granules such as fat droplets and mitochondria (Krause, 1864; Knoll, 1889, 1891; Bullard, 1912-13; Denny-Brown, 1929). Such differences have not been investigated with the electron microscope. In the myotubes studied here, there are many organelles and some lipid-like granules. This suggests a very active metabolism, with lipid as an important participant. Many sections through the polar regions, however, show very few mitochondria and no lipid granules. The distribution and abundance of the sarcoplasmic reticulum may eventually be correlated with the colour or granularity of muscle. But the problem of the sarcoplasmic reticulum was neglected after the classical work of Veratti in 1902, until interest in this structure was renewed by Bennett and Porter in 1953. Although Porter and Palade (1957) have advanced considerably our knowledge of the reticulum, no details of fine structure peculiar to "red," "white," or "granular" muscle fibres have been recognized. The function of the reticulum is not known. Porter and Palade (1957) suggested

that it may have a role in impulse transmission. The degree of development of the reticulum in relation to its function is relevant to the problems of the nature of the intrafusal fibre; for example, Hunt and Kuffler (1951 *a*) suggested that the contraction wave is not propagated. In this series of micrographs, the intrafusal sarcoplasmic reticulum of the poles is less conspicuous than that of the myotubes and neighbouring extrafusal fibres.

The presence of many myofibrils in the polar end of the myotube suggests that this region is capable of an effective contraction. The possible effect of contraction on the overlying nerve endings is discussed later.

A stretch receptor is invariably present upon the nuclear bag, and the virtual absence of contractile material from this zone is in keeping with the function. The reason for the mass of nuclei in the bag is unknown. There is no evidence of division of these nuclei in the adult spindle. No activity of the nuclei is recognizable in the micrographs. It might be supposed that the nuclei form a mobile elastic mass that tends to distend the bag between the loops of the nerve ending. Thus, when the plasma membrane of the fibre is stretched, it may tend to straighten and to lift the endings out of the valleys they occupy. The nuclear bag, however, is present in the frog intrafusal fibre, in which the branches of the sensory ending are disposed parallel to the long axis of the fibre. In such an arrangement, there would be little mechanical advantage gained from the nuclei. A strictly mechanical role for the nuclei seems unlikely.

Nerves and Nerve Endings

Nerves in the Capsule:

In the present investigation, nerve endings in the capsule have not been recognized. Sections were seen of unmyelinated axons, partly or wholly within Schwann-cell cytoplasm, ranging from 1 to 3 μ in diameter. Although these axons may have been related to functional nerve endings in the capsule, the differences in fine structure between a section of an unmyelinated axon in transit and a section of a sensory nerve ending have not been defined. One or two unmyelinated axons of about 1 μ in diameter, each enclosed in a little Schwann-cell cytoplasm lie within the Henle sheaths of the larger myelinated nerves (Fig. 5) and are probably in transit to deeper parts of the spindle. Small unmyelinated axons lie isolated in the capsule or

endomysium; their destination and function are unknown.

There has been no published work on the diameters of the sensory nerve fibres of the spindles in rat lumbricals. In this study, many sections of nerve fibres of about 3 μ in diameter were seen in the capsule in the equatorial region of spindles. In one specimen one of these fibres was estimated to be about 5 μ in diameter. These measurements are very much less than the published diameters of the large sensory nerve fibres as they leave the spindles of other laboratory animals. Diameters measured by Sherrington (1894-95), Batten (1897), and Barker (1948) were, in general, between 8 and 12 μ . Furthermore, the speed of conduction of a nerve fibre is a function of its diameter (Erlanger and Gasser, 1937), and physiological measurements of conduction rates in nerve trunks by Hunt and Kuffler (1951 *b*) confirmed that the large afferents from spindles belong to the largest fibres (group A1 (Lloyd, 1943)), which range from 12 to 20 μ in diameter in the cat. Peripheral tapering of the fibres accounts for the different ranges in diameters at the spindles and in the trunks. Shrinkage during preparation of specimens for direct measurement must also be considered. A possible explanation for the failure to find larger nerves in these sections is that, by chance, none of those examined was through a favourable plane. Further investigation is necessary.

Some endings found on the polar regions have been identified as motor end-plates because they resemble those found on the extrafusal fibres. These extrafusal endings are similar to those described by Palade (1954, 1957), Robertson (1956 *a*), and by Reger (1957, 1958). There is no doubt that the nuclear-bag ending is sensory. The nuclear-bag endings and the motor end-plates differ in the following respects. First, there is no basement membrane between the plasma membranes of nerve and muscle at the sensory ending. Second, no Schwann cell covers the sensory ending. Third, the motor ending has by far the greater number of synaptic vesicles. Fourth, the underlying myofilaments lie much closer to the sensory than to the motor ending. In order to classify any nerve ending on an intrafusal fibre I am tentatively using the above criteria. Thus, all bodies with nerve-like cytoplasm, which lie in contact with a muscle fibre, are assumed to be nerve endings. If the plasma membrane of the ending is separated from that of the muscle by about 200 A, the end-

ing is named sensory. If the plasma membranes of nerve and muscle are separated by basement-membrane material to a distance of 500 to 600 Å the ending is named motor.

There have been conflicting claims concerning the extent of the intrafusal motor innervation in the mammal. Hines and Tower (1928), using methylene blue and whole-mounts of a number of different muscles from different animals, concluded that the innervation is discrete and unipolar. Barker (1948) described a bipolar motor supply derived from a number of small axons that sometimes branched; furthermore, some axons supplied more than one intrafusal fibre, and some intrafusal fibres received more than one end-plate at one pole. He also described a rabbit spindle in which a single medium-sized motor fibre innervated all muscle fibres of one pole. Barker's work is quoted by the physiologists, who have many records interpreted as demonstrating extensive branching of the gamma efferents. All the spindles whose afferents were isolated by Hunt and Kuffler (1951 *a*) were innervated by from 3 to 5 gamma efferents.

Coërs and Durand (1956) obtained a strong positive acetylcholinesterase reaction from the nerve endings on the myotube and polar zones of the fibres, and suggested that all the endings on those two zones are motor. The myotube endings have generally been considered to be sensory. Matthews (1933) suggested that this A1 ending, which is supplied by medium-sized nerve fibres, is responsible for the slow component that he was able to demonstrate in the afferent volley from a stretched muscle.

There is some disagreement in the literature concerning the shape of the motor ending on the intrafusal fibre. In the experience of Hines and Tower (1928) the ending was of the "terminaison en grappe" form, while Barker (1948) and Coërs and Durand (1956) reported typical motor end-plates. Ruffini spoke of "plate endings."

In the present study of thin sections, neither the distribution nor the gross form of the motor endings could be determined. In each series of sections, designed to cover the length of a spindle, there were gaps and imperfections. In no case were both poles of a spindle cut successfully. It is, therefore, not known whether these spindles had a bipolar motor innervation. In spite of the gaps, however, long lengths of a polar region were sometimes followed; yet in no case was more than one end-plate found in a spindle although there

are several intrafusal fibres in each spindle. In section, all these end-plates were small and simple. It is probable, therefore, that the end-plates seen did not cover a large area, and that end-plates were not numerous in these spindles. The simple profiles that were seen could equally well have belonged either to the small bulbous enlargements of the "terminaison en grappe" type of ending or to the processes of simple end-plates.

Satisfactory micrographs were not obtained from both ends of a myotube. The figures of the myotube region suggest that its endings are raised on the end towards the nuclear bag, and long and low on the end towards the pole. It will be seen, however, that the state of contraction and the quality of fixation are not the same in all the examples of myotube shown in the figures, and these and other accidents during preparation may be responsible for the differences in the outlines of the endings.

The arrangement of the sensory endings on the intrafusal fibre would suggest that the myotube ending and the nuclear bag ending stand in the same relationships to the intrafusal fibre as the muscle spindle and the Golgi tendon organ stand to the whole muscle. The muscle spindle is in parallel with the extrafusal fibres, the myotube ending is in parallel with the underlying myofibrils. The nuclear bag and the tendon organ are in series with the contractile elements. Structurally, the arrangement is suitable for the independent measurement of both the state of contraction and the state of tension of the intrafusal fibre, and if motor activity in the spindle does indeed initiate reflex activity, one would expect that information concerning both the state of contraction and the degree of tension would be of equal importance. If the myotube ending responds directly to stretch, and if its threshold length is the rest length of the myotube without extension, the ending would cease to fire whenever the contraction of the myotube overcame the external forces tending to stretch that portion of the fibre. The contraction of the poles and myotubes would be at the expense of the nuclear bag, and would cause the ending on the latter to increase its rate of discharge. Matthews (1933) observed this difference in the behaviour of the two sensory endings and attributed it to the contractility of the myotube. Hunt (1954), however, found no significant difference in the receptor characteristics of the large and medium spindle afferent fibres, other than a slightly higher threshold of

the receptors of the medium-sized fibres to steady stretch of the muscle. It must be remembered that the response of the myotube ending may be complicated by the fact that, whereas the polar end of the myotube contains much contractile material, the nuclear-bag end contains little.

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BIBLIOGRAPHY

- Adrian, E. D., and Zotterman, Y., The impulses produced by sensory nerve endings. Part II. The response of a single end-organ, *J. Physiol.*, 1926, **61**, 151-71.
- Barker, D., The innervation of the muscle spindles, *Quart. J. Micr. Sc.*, 1948, series 3, **89**, 143-86.
- Batten, F. E., The muscle spindle under pathological conditions, *Brain*, 1897, **20**, 138-79.
- Bennett, H. S., The concepts of membrane flow and membrane vesiculation as mechanisms for active transport and ion pumping, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 99-103.
- Bennett, H. S., and Porter, K. R., Electron microscope study of sectioned breast muscle of the domestic fowl, *Am. J. Anat.*, 1953, **93**, 61-89.
- Boyd, I. A., Simple and compound mammalian muscle spindles, *J. Physiol.*, 1959, **145**, 55-56P.
- Bullard, H. H., On the interstitial granules and fat droplets of striated muscle, *Am. J. Anat.*, 1912-13, **14**, 1-46.
- Causey, G., and Palmer, E., The epineural sheath of a nerve as a barrier to the diffusion of phosphate ions, *J. Anat.*, 1953, **87**, 30-36.
- Coërs, C., and Durand, J., Données morphologiques nouvelles sur l'innervation des fuseaux neuromusculaires, *Arch. Biol. (Liège)*, 1956, **67**, 685-715.
- Couteaux, R., Localization of cholinesterases at neuromuscular junctions, *Internat. Rev. Cytol.*, 1955, **4**, 335-375.
- Denny-Brown, D. E., The histological features of striped muscle in relation to its functional activity, *Proc. Roy. Soc. London, Series B*, 1929, **104**, 371-411.
- Eldred, E., Granit, R., and Merton, P. A., Supraspinal control of the muscle spindles and its significance, *J. Physiol.*, 1953, **122**, 498-523.
- Erlanger, J., and Gasser, H. S., *Electrical Signs of Nervous Activity*, Philadelphia, University of Pennsylvania Press, 1937.
- Feng, T. P., and Gerard, R. W., Mechanism of nerve asphyxiation: with a note on the nerve sheath as a diffusion barrier, *Proc. Soc. Exp. Biol.*, 1929-30, **27**, 1073-76.
- Fulton, J. F., and Pi-Suñer, J., A note concerning the probable function of various afferent end-organs in skeletal muscle, *Am. J. Physiol.*, 1927-28, **83**, 554-62.
- Gerebtzoff, M. A., Recherches histochimiques sur les acétylcholine et choline estérases, *Acta Anat.*, 1953, **19**, 366-79.
- Granit, R., *Receptors and sensory perception*, New Haven, Yale University Press, and London, Oxford University Press, 1955.
- Hines, M., Nerve and muscle, *Quart. Rev. Biol.*, 1927, **2**, 149-80.
- Hines, M., and Tower, S., Studies on the innervation of skeletal muscles. II. Of muscle spindles in certain muscles of the kitten, *Johns Hopkins Hosp. Bull.*, 1928, **42**, 264-95.
- Hinsey, J. C., Some observations on the innervation of skeletal muscle of the cat, *J. Comp. Neurol.*, 1927-28, **44**, 87-195.
- Howarth, J. V., The effect of hypertonic solutions on the velocity of shortening of the frog's sartorius, *J. Physiol.*, 1957, **137**, 23P.
- Hunt, C. C., Diameter and function of afferent nerves from muscle, *Proc. 19th Internat. Physiol. Congr.*, Montreal, 1953, Abstracts of communications, 485-6.
- Hunt, C. C., Relation of function to diameter in afferent fibers of muscle nerves, *J. Gen. Physiol.*, 1954, **38**, 117-31.
- Hunt, C. C., and Kuffler, S. W., Further study of efferent small-nerve fibers to mammalian muscle spindles. Multiple spindle innervation and activity during contraction, *J. Physiol.*, 1951 a, **113**, 283-97.
- Hunt, C. C., and Kuffler, S. W., Stretch receptor discharges during muscle contraction, *J. Physiol.*, 1951 b, **113**, 298-315.
- Knoll, P., Über helle und trübe, weisse und rothe quergestreifte Muskulatur, *Sitzungsber. k. Akad. Wissensch., math.-naturwissensch. Cl., Wien*, 1889, **98**.
- Knoll, P., Über protoplasmaarme und protoplasma-reiche Muskulatur, *Denkschr. Akad. Wissensch. Wien, math.-naturwissensch. Cl.*, 1891, **58**, 633-700.
- Koelle, G. B., and Friedenwald, J. S., A histochemical method for localizing choline-esterase activity, *Proc. Soc. Exp. Biol.*, 1949, **70**, 617-22.
- Kölliker, A., On the termination of nerves in muscles as observed in the frog; and on the disposition of the nerves in the frog's heart, *Proc. Roy. Soc.*, 1862-63, **12**, 65-84.
- Krause, W., *Die Anatomie des Kaninchens*, Leipzig, 1864, cited by Bullard, 1912-13.
- Krnjević, K., The distribution of Na and K in cat nerves, *J. Physiol.*, 1955, **128**, 473-88.
- Kuffler, S. W., and Hunt, C. C., The mammalian small-

- nerve fibers: a system for the efferent nervous regulation of muscle spindle discharge, *Res. Publ. Assn. Nerv. and Ment. Dis.*, 1952, **30**, 24-47.
- Kuffler, S. W., Hunt, C. C., and Quilliam, J. P., Function of medullated small-nerve fibers in mammalian ventral roots: efferent muscle spindle innervation, *J. Neurophysiol.*, 1951, **14**, 29-54.
- Kühne, W., Über die Endigung der Nerven in den Muskeln, *Virchows Arch. path. Anat.*, 1863 a, **27**, 508-33.
- Kühne, W., Die Muskelspindeln, *Virchows Arch. path. Anat.*, 1863 b, **28**, 528-38.
- Leksell, L., The action potentials and the excitatory effects of the small ventral root fibers to skeletal muscle, *Acta Physiol. scand.*, 1945, **10**, suppl. 31.
- Lloyd, D. P. C., Neuron patterns controlling transmission of ipsilateral hind limb reflexes in cat, *J. Neurophysiol.*, 1943, **6**, 293-315.
- Matthews, B. H. C., Specific nerve impulses, *J. Physiol.*, 1929, **67**, 169-90.
- Matthews, B. H. C., The response of a single end organ, *J. Physiol.*, 1931 a, **71**, 64-110.
- Matthews, B. H. C., The response of a muscle spindle during active contraction of a muscle, *J. Physiol.*, 1931 b, **72**, 153-74.
- Matthews, B. H. C., Nerve endings in mammalian muscle, *J. Physiol.*, 1933, **78**, 1-53.
- Michaelis, L., Der Azetat-Veronal-Puffer, *Biochem. Z.*, 1931, **234**, 139-41.
- Palade, G. E., A study of fixation for electron microscopy, *J. Exp. Med.*, 1952, **96**, 285-97.
- Palade, G. E., Electron microscope observations of interneuronal and neuromuscular synapses, *Anat. Rec.*, 1954, **118**, 335-36 (Abstract).
- Palade, G. E., Studies on the endoplasmic reticulum. II. Simple disposition of cells *in situ*, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 567-82.
- Palade, G. E., An electronmicrograph, in Ham's Histology, 3rd edition, Philadelphia and Montreal, Lippincott, 1957, 405; and in Maximow and Bloom's A Textbook of Histology, 7th edition, Philadelphia and London, Saunders, 1957, 199.
- Pease, D. C., and Quilliam, T. A., Electron microscopy of the Pacinian corpuscle, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 331-42.
- Porter, K. R., The myotendinal junction in larval forms of *Amblystoma punctatum*, *Anat. Rec.*, 1954, **118**, 342 (Abstract).
- Porter, K. R., and Palade, G. E., Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 269-99.
- Reger, J. F., The ultrastructure of normal and denervated neuromuscular synapses in mouse gastrocnemius muscle, *Exp. Cell Research*, 1957, **12**, 662-63.
- Reger, J. F., The fine structure of neuromuscular synapses of gastrocnemii from mouse and frog, *Anat. Rec.*, 1958, **130**, 7-24.
- Richter, G. W., A study of hemosiderosis with the aid of electron microscopy. With observations on the relationship between hemosiderin and ferritin, *J. Exp. Med.*, 1957, **106**, 203-17.
- Robertson, J. D., The ultrastructure of a reptilian myoneuronal junction, *J. Biophysic. and Biochem. Cytol.*, 1956 a, **2**, 381-93.
- Robertson, J. D., Preliminary observations on the ultrastructure of a frog muscle spindle, in Proceedings of the Stockholm Conference on Electron Microscopy, New York, Academic Press Inc., 1956 b, 197-200.
- Ross, M. H., Pappas, G. D., Lanman, J. T., and Lind, J., Electron microscope observations on the endoplasmic reticulum in the human fetal adrenal, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 659-61.
- Ruffini, A., On the minute anatomy of the neuromuscular spindles of the cat, and on their physiological significance, *J. Physiol.*, 1898-99, **23**, 190-208.
- Sager, R., and Palade, G. E., Structure and development of the chloroplast in *Chlamydomonas*. I. The normal green cell, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 463-87.
- Sherrington, C. S., On the anatomical constitution of nerves of skeletal muscles; with remarks on recurrent fibers in the ventral spinal nerve-root, *J. Physiol.*, 1894-95, **17**, 211-58.
- Tiegs, O. W., Innervation of voluntary muscle, *Physiol. Rev.*, 1953, **33**, 90-144.
- Veratti, E., Ricerche sulla fine struttura della fibra muscolare striata, *Mem. r. Ist. Lombardo sc. e lettere*, 1902, **19**, 87-133.
- Yamada, E., The fine structure of the renal glomerulus of the mouse, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 551-65.
- Yoffey, J. M., and Courtice, F. C., Lymphatics, Lymph, and Lymphoid Tissue, Cambridge, Massachusetts, Harvard University Press, and London, Edward Arnold and Co., 1956.

EXPLANATION OF PLATES

The legends of the figures contain numbers identifying the spindle from which each figure was taken. Thus Figs. 1, 4, 6, and others were taken from spindle No. 21.1.1. The first number indicates that the spindle came from animal No. 21; the second number refers to a group of muscles, in this case group No. 1; the last number identifies the block containing a lumbrical trimmed for a single spindle, in this case lumbrical or block No. 1. Spindles Nos. 21.2.1 and 21.2.2 also came from rat No. 21 but came from the lumbricals of the other forepaw and from blocks 1 and 2 respectively.

PLATE 366

FIG. 1. Spindle No. 21.1.1. Fixed by vascular perfusion.

An oblique section through the myotube zone of a spindle cut at an angle of between 10° and 15° to the long axis.

Three relaxed intrafusal fibres (*IF*) of different diameters lie in a complex sheath of endomysium. Myelinated and unmyelinated nerve fibres (*N*) lie in a delicate Henle sheath. The myelinated nerve is cut through a node of Ranvier (*R*) and has an irregular outline. The endomysial sheath (*E*) and the Henle sheath (*H*) lie in the periaxial space (*PS*), which is enclosed by the capsule (*C*). A capillary lies between sheets of the capsule wall. In the upper left corner lies a small extrafusal muscle fibre. There is a marked difference in texture between the extrafusal and intrafusal fibres. The myofibrils of the extrafusal fibre are separated by coarse reticulum associated with mitochondria, whereas the investment of the fibrils of the intrafusal fibres is difficult to see at this magnification.

There is considerably more flocculent precipitate in the periaxial space than there is within the endomysial and Henle sheaths and outside the capsule.

Sensory nerve endings (*SE*), which are conspicuous because of their mitochondria, lie on the intrafusal fibres. Portion of the ending *SEI*, taken from a neighbouring section, appears in Figs. 17 to 19. A portion of the myotube of the intrafusal fibre towards the right of the figure, taken from another section, is shown in Fig. 11. $\times 4,000$.

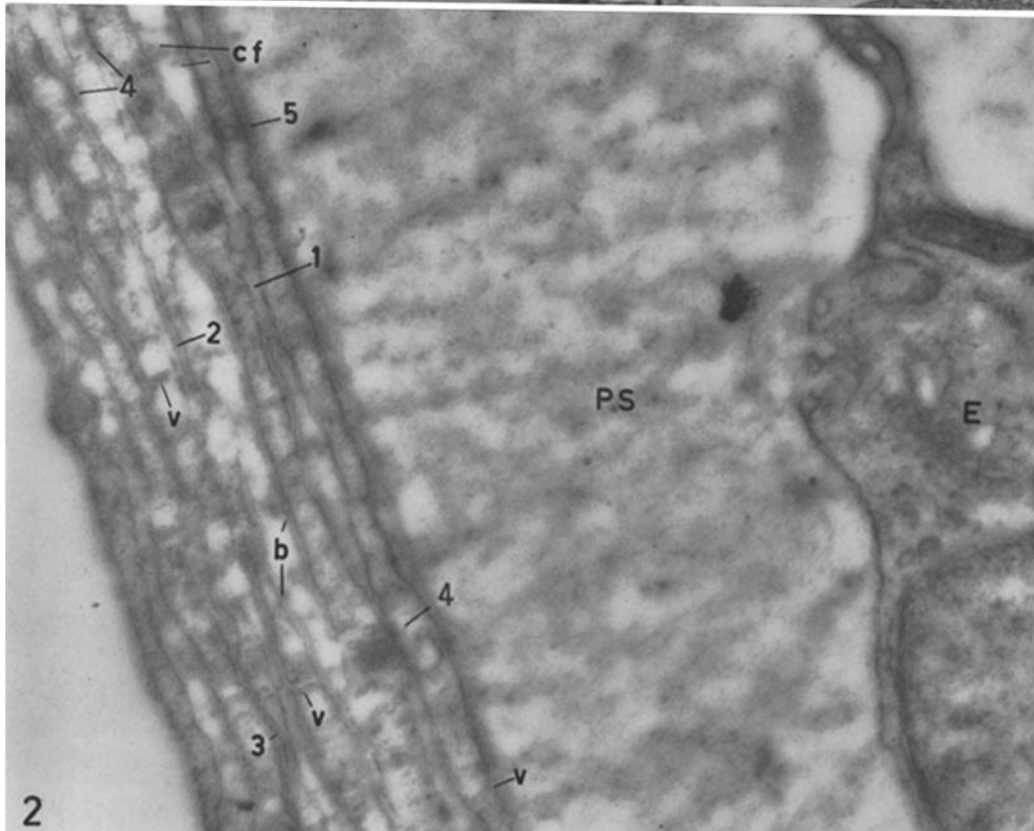
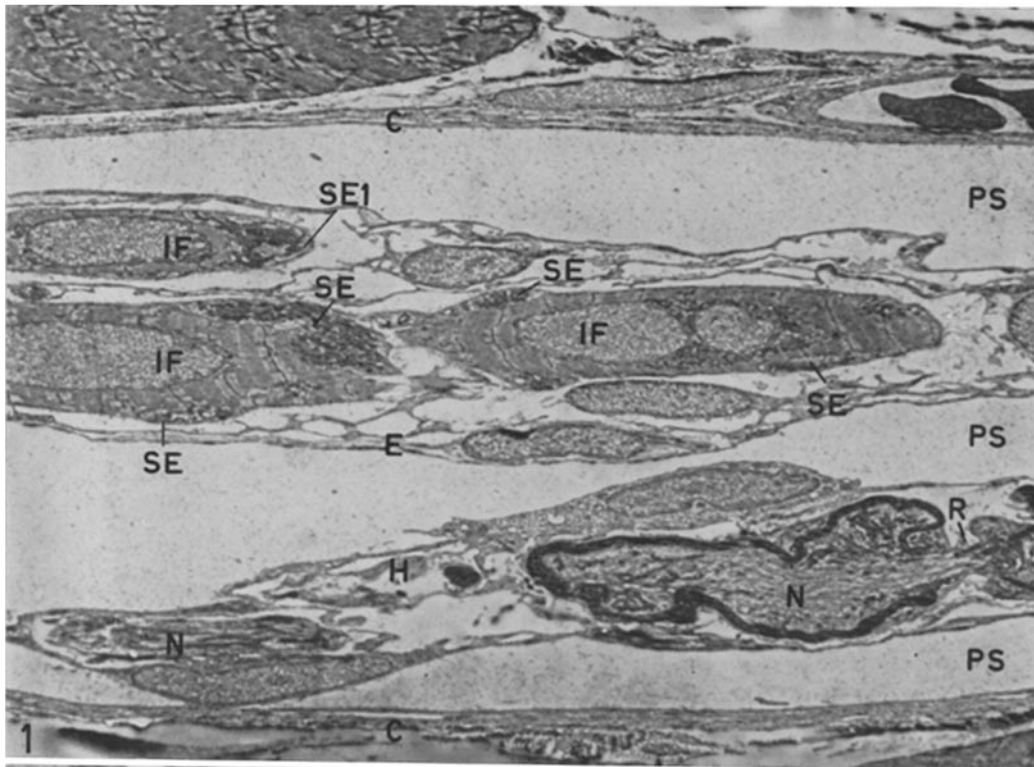
FIG. 2. Spindle No. 21.2.1. Fixed by vascular perfusion.

An oblique longitudinal section through portion of the periaxial space (*PS*).

The capsule, on the left, is made up of six layers of capsular-sheet cells. On the right is portion of an endomysial cell (*E*). Each sheet of capsular-sheet cell cytoplasm is limited by a plasma membrane on both surfaces, and in most places is separated from its neighbours by intercellular basement-membrane material. A few transversely cut collagen fibrils (*cf*) lie between the sheets. Where the sheets are widely separated the intercellular material is condensed into a basement membrane (*b*) close to each sheet. The ragged appearance of the free surface of the basement membranes in these areas of separation suggests that some of the separation is artefact. Where the sheets lie close together the intercellular material either is of uniform density as at 1 or contains a single membranous condensation midway between the two sheets as at 2. In some places (3) the plasma membranes of neighbouring cells are about 200 Å apart, and in other places (4) adjacent cells are attached by dense desmosomes or attachment plaques. A condensation of basement-membrane-like material (5) is very marked within the periaxial space against the inner surface of the innermost capsular sheet.

The cytoplasm of the capsular-sheet cells contains many vesicles and caveolae intracellulares (*v*) elongated perpendicular to the surface of the cell. The endomysial cell cytoplasm contains fewer small vesicles and caveolae.

The fluid in the periaxial space contains a large quantity of solid material that appears as an amorphous precipitate. The uneven distribution of the precipitated material is due to the flow of the embedding plastic before a blunt knife. The direction of cutting was parallel to the portion of capsule shown, and was probably from top to bottom of the figure. Material has been transported and dumped in that direction. $\times 30,000$.



(Merrillees: Fine structure of muscle spindles)

PLATE 367

FIG. 3. Spindle No. 21.2.1. Fixed by vascular perfusion.

A portion of spindle capsule taken from the same block as was that shown in Fig. 2. This section was thinner than that in Fig. 2. Structural detail is poorly preserved and there appears to be considerable separation of the sheets and ragged tearing of the basement membranes. This poor preservation of the capsule was common in the specimens of this series. Neighbouring capsular-sheet cells in the same sheet make close contact with each other (*X*) \times 29,000.

FIG. 4. Spindle No. 21.1.1. Fixed by vascular perfusion.

This low power view contains two nerve fibres (*N*) that are seen at higher magnification in Fig. 6. Immediately surrounding the nerve fibres are small bundles of collagen fibrils (*cf*). Each of the nerve fibres lies in its own very thin investment of Schwann-cell cytoplasm. The extremely attenuated cell (*C*) is a detached capsular-sheet cell lying in the periaxial space. \times 4,000.

FIG. 5. Spindle No. 21.2.1. Fixed by vascular perfusion.

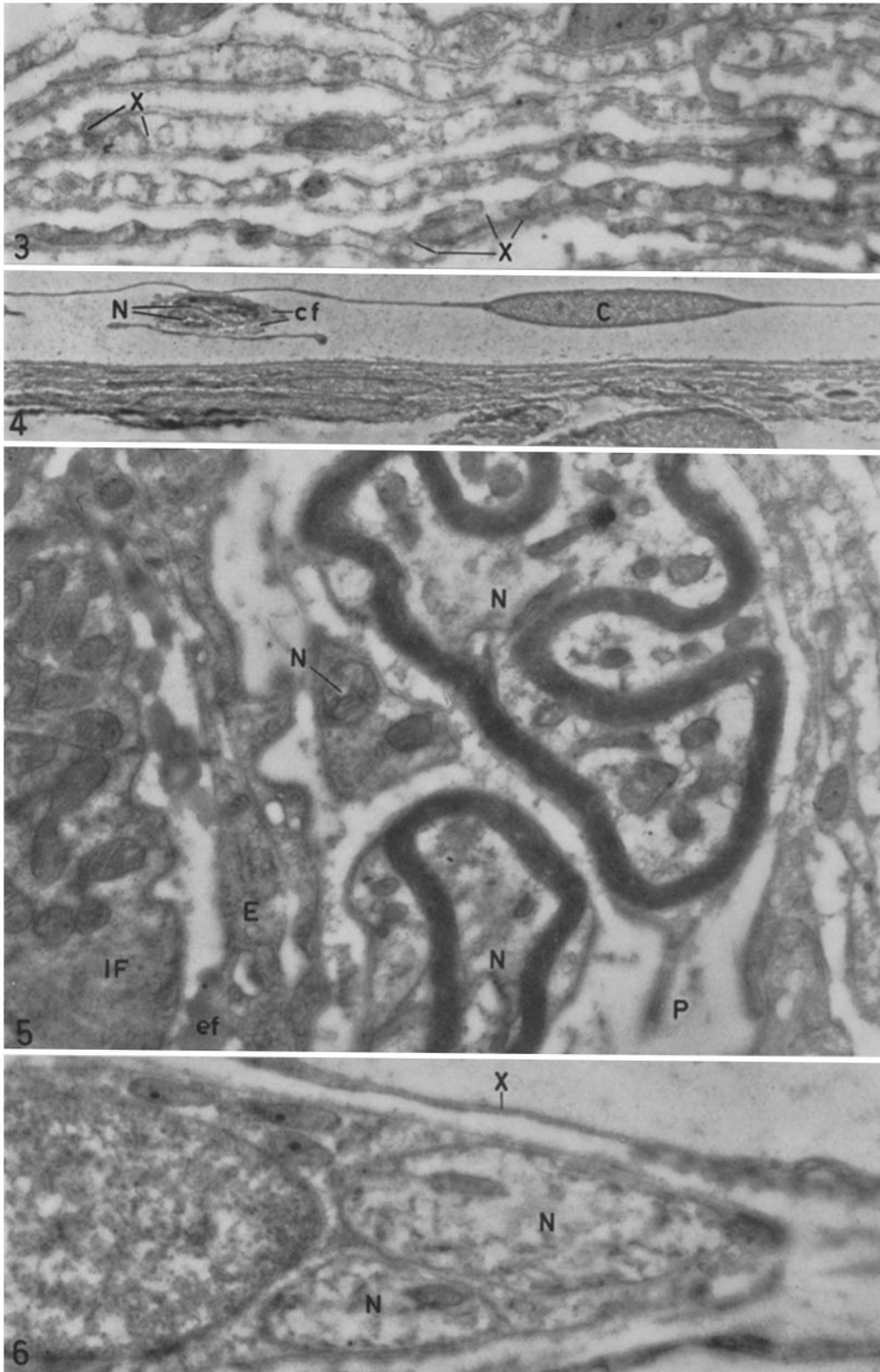
An oblique longitudinal section through the edge of a spindle at about the level of the transition region between polar and myotube zones.

On the left is the edge of an intrafusal muscle fibre (*IF*). Fragments of amorphous material (*ef*) are tentatively identified as elastic fibre. The uneven density of the amorphous material is due to a cutting artefact. Immediately to the right of the elastic fibre is a sheet of endomysial cell cytoplasm (*E*), which is closely associated with extensions of cells of the spindle capsule. The capsule contains myelinated and unmyelinated nerve fibres (*N*). The Henle sheath of nerves traversing the capsule is derived from the capsule. The larger myelinated nerve has a very irregular outline within its Schwann cell. The unmyelinated axon is enclosed in Schwann-cell cytoplasm. *P* is a process of Schwann cell. \times 16,000.

FIG. 6. Spindle No. 21.1.1. Fixed by vascular perfusion.

Two small unmyelinated nerve fibres (*N*) within Schwann-cell cytoplasm accompanied by two portions of capsular-sheet cell cytoplasm.

These fibres were traversing the periaxial space, apart from but close to the nerve trunk seen in Fig. 1, which was taken from a nearby section, and they are seen in low power in Fig. 4. The thickness of the cytoplasmic sheet at *X* is about 300 A. The three mitochondria in the Schwann cell contain unusually dense granules. \times 30,000.



(Merrillees: Fine structure of muscle spindles)

FIG. 7. Spindle No. 25.2.1. Fixed and partly dehydrated *in situ*.

An oblique longitudinal section through an edge of the core and the neighbouring myofibrillar wall of a portion of the myotube.

A "triad" (*t*) of sarcoplasmic reticulum is seen near the junction of A and I bands. The low density of the contents of the dilated peripheral vesicles ("er2" of Porter and Palade (1957)), which sandwich the "intermediary vesicles" (not resolved) between them, is conspicuous. The two groups of vesicles, *I1* and *I2*, are probably portions of triads lying in the periphery of the myotube core. A blunt knife has caused the section to be of varying thickness in narrow vertical bands across the figure. $\times 30,000$.

FIG. 8. Spindle No. 21.2.1. Fixed by vascular perfusion.

A low power micrograph showing one end of an equatorial region in a longitudinal section cut at a very small angle to the long axis of the fibre.

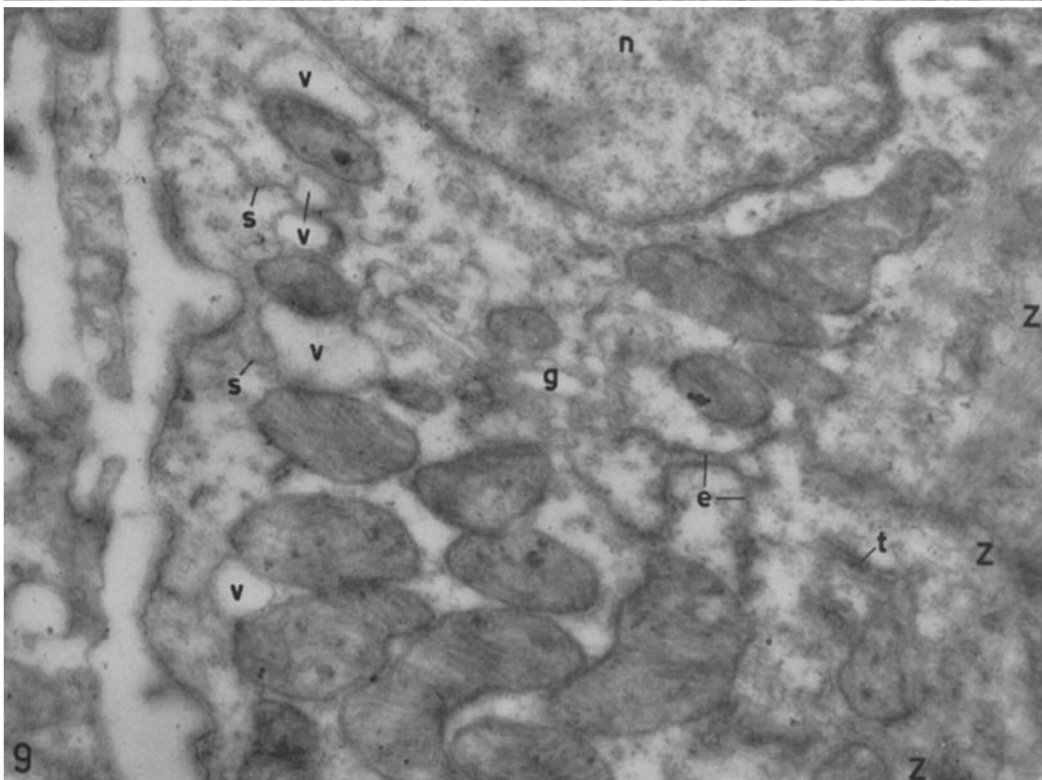
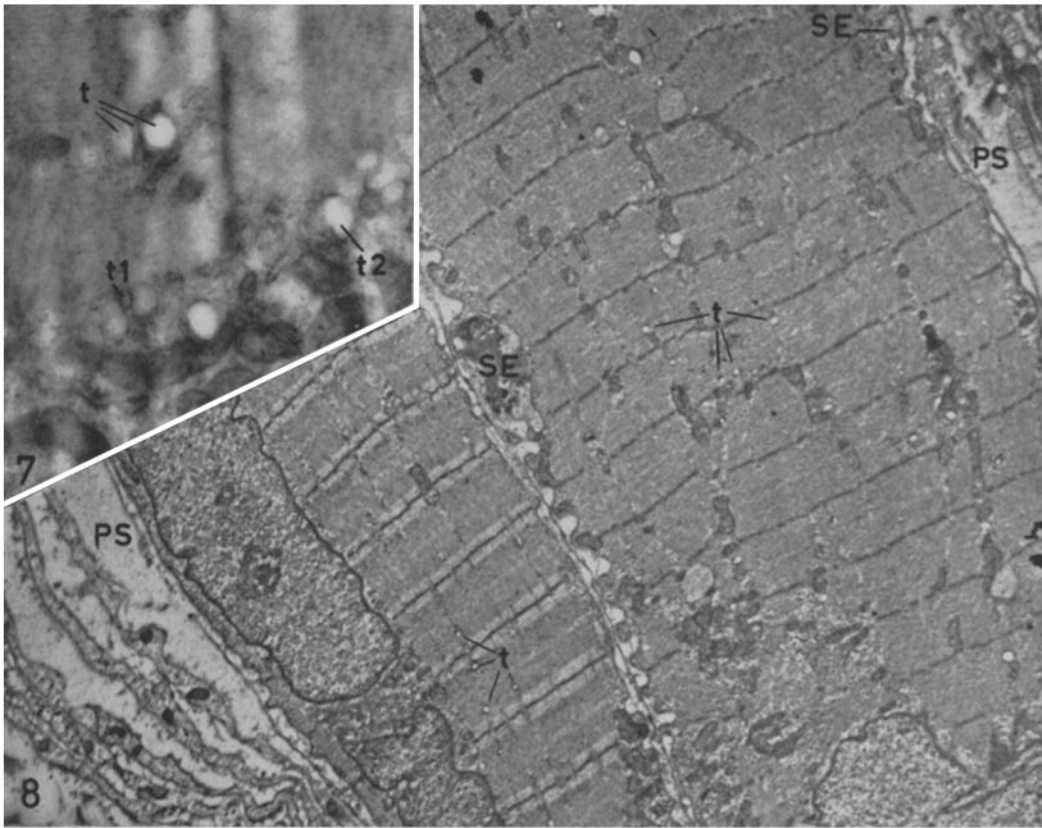
On the left the spindle capsule comes into contact with the endomysium and, therefore, the periaxial space (*PS*) ends. Two intrafusal fibres of different sizes are seen. The small fibre is relaxed, with conspicuous I bands. The fibre on the right is without I bands and is, therefore, contracted. The fibre on the right has so little reticulum that myofibrils are difficult to distinguish. The "triads" (*t*) are conspicuous. In the fibre on the left the triads lie well within the limits of the A bands. Two small sensory endings (*SE*) are seen on the right hand fibre. The peripheral position of the nuclei in the small fibre is unusual but by no means unique. $\times 7,000$.

FIG. 9. Spindle No. 21.2.1. Fixed by vascular perfusion.

An oblique longitudinal section through the edge of an intrafusal fibre in the polar region.

On the left are portions of the innermost layers of the spindle capsule. There is no periaxial space. In this area the endomysial sheath of the fibre was indistinguishable from the capsular-sheet cells in contact with it. The section of fibre seen in this figure was very close to the motor end-plate shown in Fig. 20. In contrast with the structure of the intrafusal fibres seen in the polar regions of other spindles studied in this series, the portions of the polar region of the fibre in Figs. 9 and 20 show an unusually large quantity of reticulum. The several myofibrils seen on the right of this figure are so delicate and are surrounded by so much interfibrillar reticulum that it is difficult to see their details. In this trimmed print even the Z lines are difficult to see but they are in the positions indicated (*Z*). The three elements of the "triad" (*t*) lie in an interfibrillar plane. Two portions of rough surfaced ergastoplasm (*e*) are associated with the myofibrils and merge into a group of smooth surfaced vesicles (*g*), which are probably portion of a Golgi body; the neighbouring large vesicles (*v*) increase that probability. Some of these large vesicles have slit-like extensions (*s*) running towards the surface of the fibre. Connections between these slits and the surface of the fibre have not been resolved. A nucleus (*n*) in the muscle fibre, large mitochondria, and many small particles of medium density occupy much of the remainder of the sarcoplasm.

Although the perinuclear regions of intrafusal fibres are richly supplied with organelles, this frame is unusually well endowed in comparison with many perinuclear regions of polar zones. $\times 33,500$.



(Merrillees: Fine structure of muscle spindles)

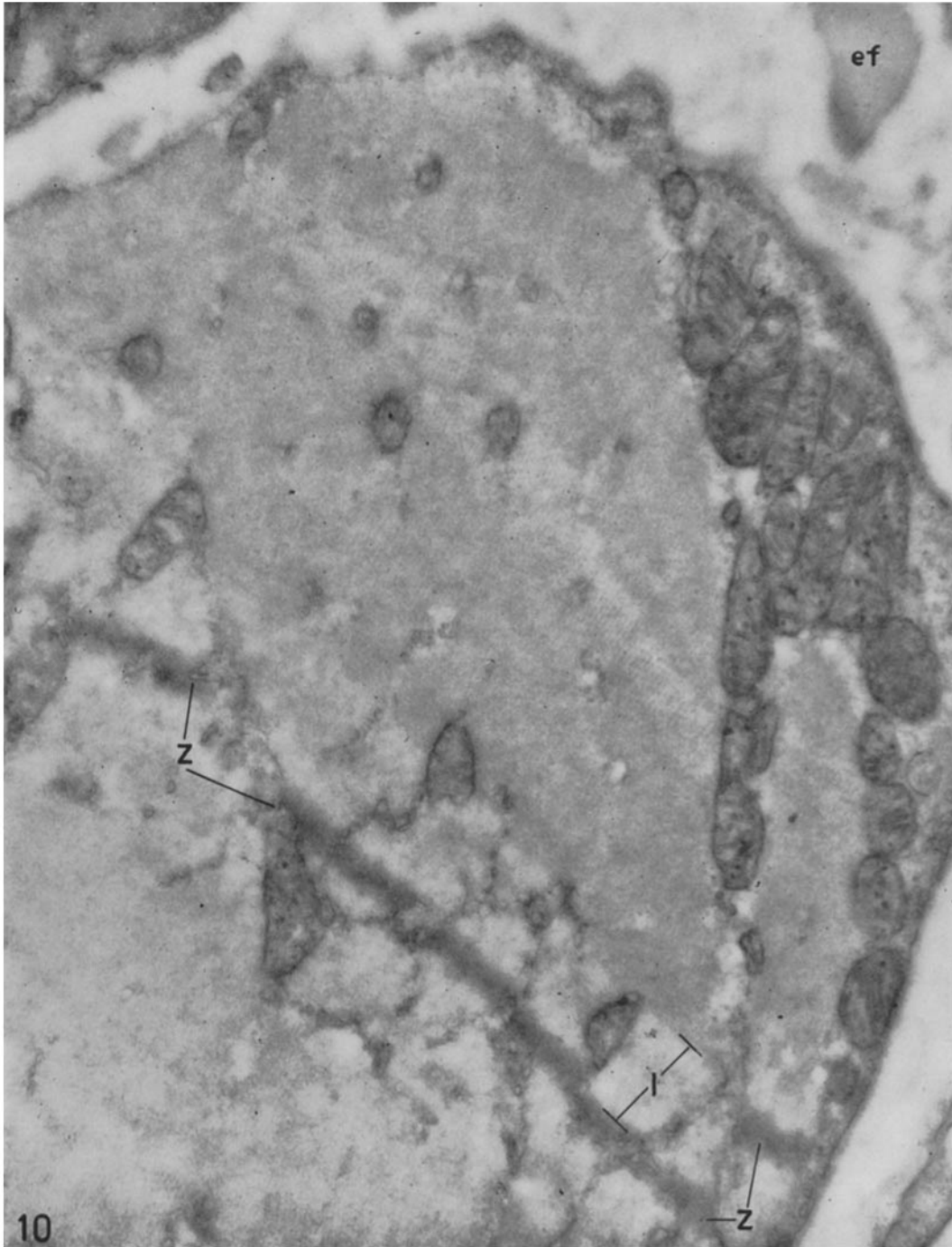
PLATE 369

FIG. 10. Spindle No. 21.2.2. Fixed by vascular perfusion.

An oblique section through the polar zone of an intrafusal muscle fibre.

The plane of section, which was at a considerable angle to the long axis of the fibre, has passed out of the fibre within an A band. Portions of two sarcomeres are seen. The dark Z band (*Z*) is not continuous across the figure but is interrupted in two places indicating that there are at least three myofibrils slightly out of register with each other. The small myofibril on the right is easily seen because of the mitochondria that almost surround it. In the I band (*I*) there is a quantity of fine reticulum enclosing small bundles of filaments. In the two A bands there is little evidence of myofibril formation. Nevertheless, the arrangement of the isolated small mitochondria and vesicles in the A band above the Z line suggests that bundles of a similar size to those seen in the I band also exist in the A band.

This section was very close to the motor-end plate shown in Fig. 29. There was no noticeable difference in the structure of the three intrafusal fibres in this spindle at this level. The structure of the fibre is in marked contrast to that in Fig. 9. In the upper right corner of the figure there is some material of medium density (*ef*) tentatively identified as elastic fibre. $\times 30,000$.



(Merrillees: Fine structure of muscle spindles)

PLATE 370

FIG. 11. Spindle No. 21.1.1. Fixed by vascular perfusion.

A section through the axis of a myotube at a small angle to the long axis of the fibre.

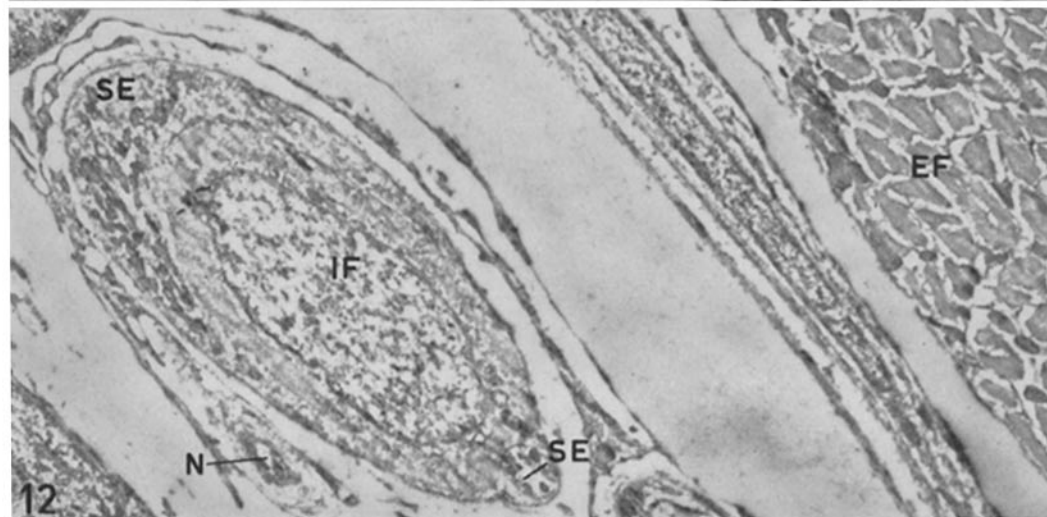
This portion of the myotube is closer to the polar end than to the nuclear-bag end. In this situation the myofilaments are gathered into true myofibrils. There are two layers of myofibrils surrounding the core of sarcoplasm, and the exposed myofibrils are interrupted by the oblique plane of section. The edge of a nucleus (*n*) lies in the core at the extreme left. The core is crowded with mitochondria and vesicles. Several of the mitochondria contain large dense particles (compare with Figs. 6 and 17, which were taken from the same spindle). The small smooth surfaced vesicles of the endoplasmic reticulum are very crowded throughout the myotube core. A stack of flattened vesicles (*g*) is probably a portion of a Golgi body, which are numerous in the core. Short lengths of granular ergastoplasm (*e*) are sometimes seen.

There are a number of large vesicles in the core. In the center of the figure are four large vesicles (*1* to *4*) of a uniform size and shape. They are compound vesicles containing smaller vesicles. Vesicle No. *4* has considerable internal density. Another large vesicle (*5*) contains no small vesicles but does contain many fine particles of high density (compare with similar vesicles in Fig. 21). $\times 30,000$.

FIG. 12. Spindle No. 17.5.2. Fixed by vascular perfusion.

A section through a myotube at an angle of about 45° .

The core of the myotube of an intrafusal fibre is filled by a nucleus. Surrounding the core is a thin continuous shell of myofilaments. To the right is an extrafusal fibre with well marked myofibrils. Two portions of sensory ending (*SE*) lie on the intrafusal fibre. *N* is a small unmyelinated axon in the endomysium. $\times 7,000$.



(Merrillees: Fine structure of muscle spindles)

FIG. 13. Spindle No. 21.2.1. Fixed by vascular perfusion.

A section within the nuclear-bag zone of an intrafusal fibre at a small angle to the long axis.

At the left of the figure the plane of section is passing away from the axis towards the surface of the fibre. Towards the left, therefore, the peripheral shell of myofilaments appears thicker than it actually is. Nevertheless, there is some increase in the number of myofilaments towards the left because the transition from nuclear bag to myotube begins there. This fibre is probably the smaller of the two seen in Fig. 8. Unusual features of this nuclear bag are that small bundles of myofilaments lie between the nuclei (small Z bands (*Z*) can be seen in these bundles), and it contains less vesicular sarcoplasm than was seen in other nuclear bags.

Two portions of the nuclear-bag sensory ending indent the upper border of the fibre. Mitochondria make them conspicuous. In both size and shape the larger (*SE*) of the two is typical of the majority of sections through nuclear-bag endings. On the lower border of the fibre, towards the right, is a fragment of Schwann-cell cytoplasm (*S*) containing a very small section of unmyelinated nerve fibre. Three arrows indicate sections through what appear to be small portions of nerve ending. $\times 9,000$.

FIG. 14. Spindle No. 21.2.1. Fixed by vascular perfusion.

The large section of nuclear bag ending (*SE*) in Fig. 13 at higher magnification.

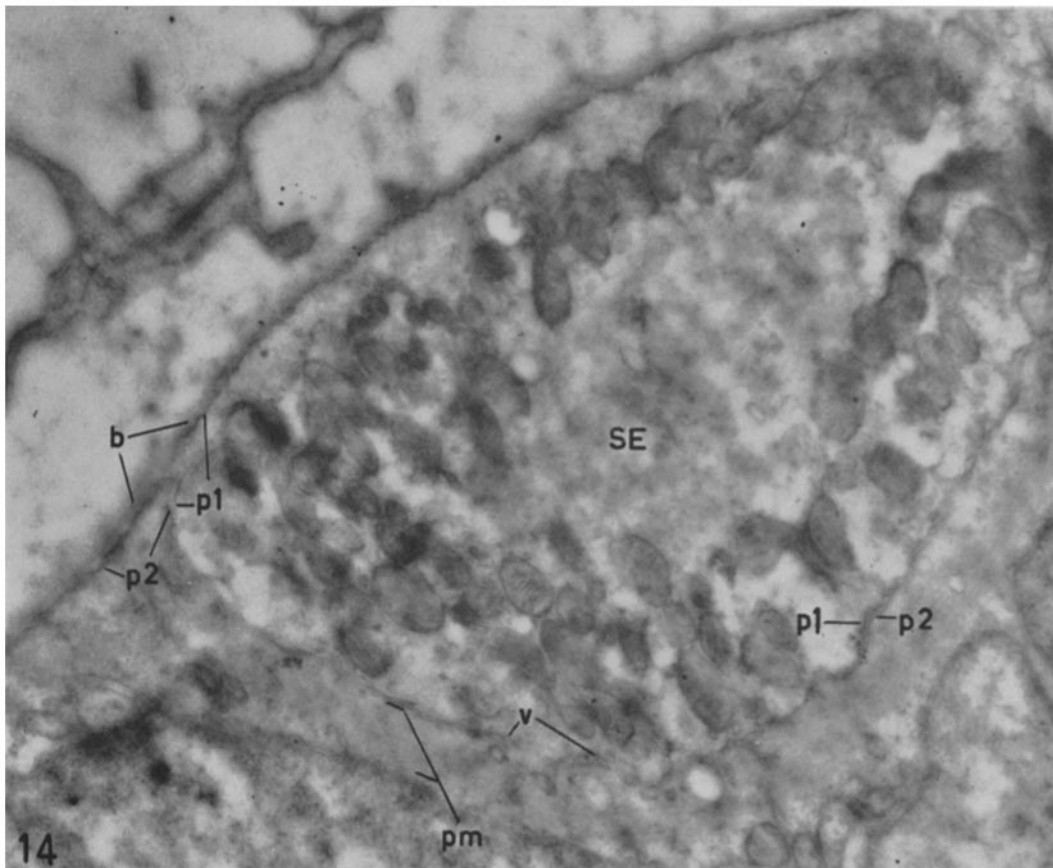
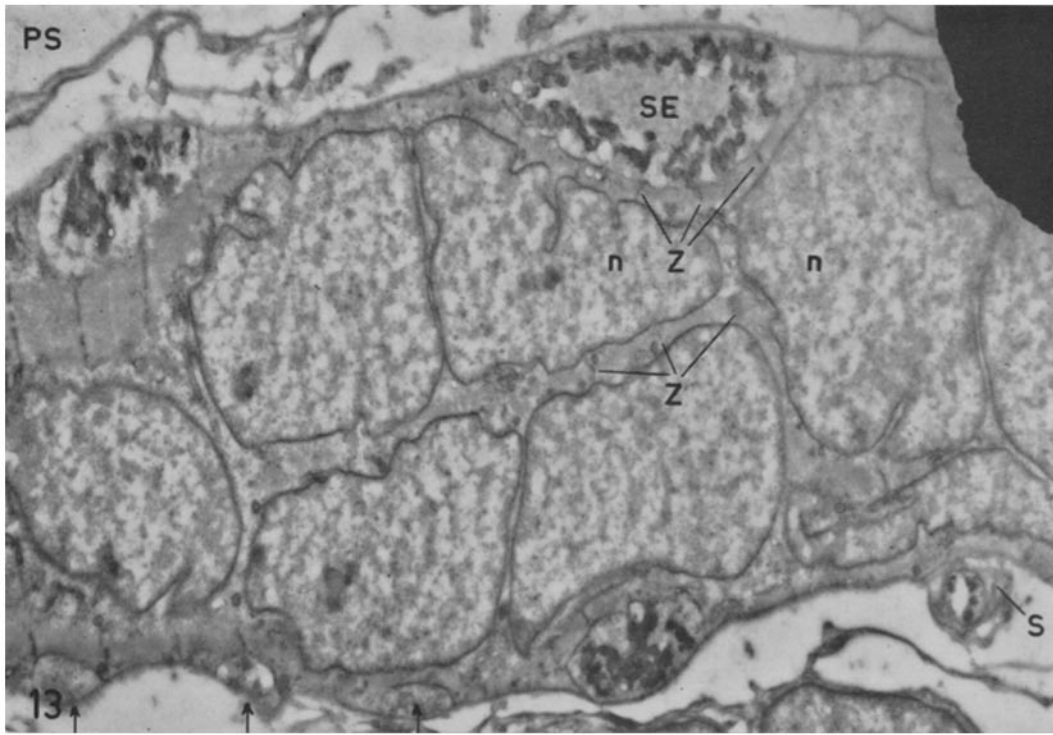
This micrograph is from a thinner section than that seen in Fig. 13, and there is some compression and flow of plastic in a direction parallel to a line joining the lower left and upper right corners.

The transversely cut sensory ending (*SE*), enclosed in its plasma membrane (*p1*), depresses the plasma membrane (*p2*) of the longitudinally cut muscle. Both plasma membranes have fine folds, and because the plane of section is tangential to portions of these folds the continuity of the membranes is difficult to see. The basement membrane (*b*) of the sarcolemma continues over the free surface of the sensory ending (compare with Fig. 16). The distance between the plasma membranes of nerve ending and muscle is difficult to measure because of the corrugated nature of the membranes. At their closest approach the plasma membranes are about 200 Å apart. Small vesicles lie in the cytoplasm of nerve ending and muscle. Some of these vesicles (*v*) lie close to the plasma membranes and tend to obscure portions of obliquely cut membrane. The resolution of detail in this micrograph is inadequate to support an argument for the existence of pores through the plasma membranes.

The nerve ending contains many mitochondria, a number of vesicles of various sizes, and much amorphous material of medium density.

A narrow peripheral zone of myofilaments (*pm*) lies in the muscle between the nuclei and the plasma membrane.

In this section the filaments are seen with difficulty, and transverse striations are lost. In the thicker section in Fig. 13, however, there are delicate Z lines in the same region that is included in this figure. In this figure particles and vesicles are scattered amongst the filaments, which would, therefore, seem to be in small bundles that are less closely packed than they are in an ordinary myofibril. $\times 30,000$.



(Merrillees: Fine structure of muscle spindles)

PLATE 372

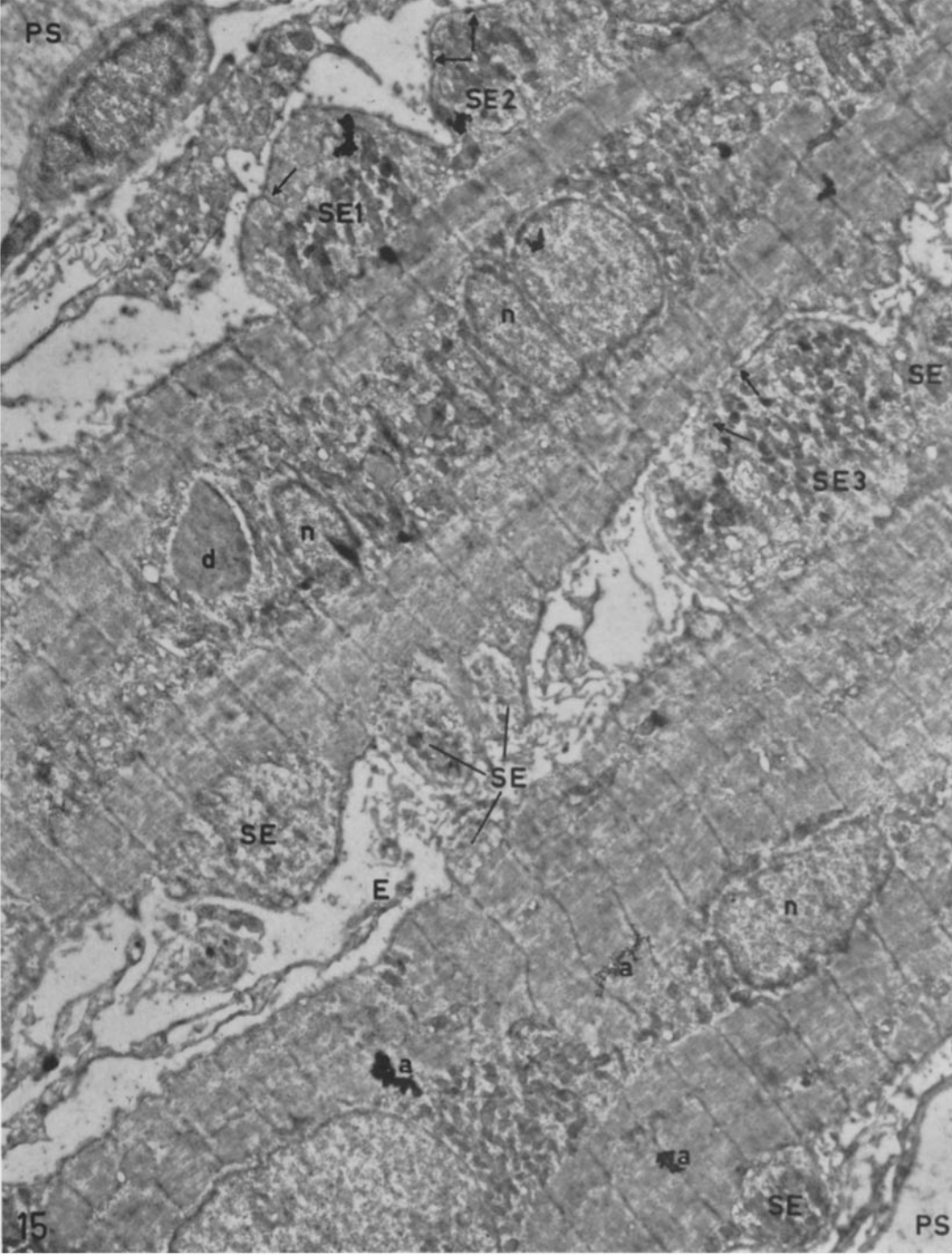
FIG. 15. Spindle No. 21.2.1. Fixed by vascular perfusion.

A longitudinal section within the myotube region of two intrafusal fibres cut almost parallel to their long axes.

A blunt knife has caused some compression and plastic flow in the direction of the long axes of the fibres. The dense black flakes and particles (*e.g. a*) are artefacts. In the extreme upper left and lower right corners of the figure are portions of the periaxial space. The section has grazed the cores of the myotubes and contains little core material and only small portions of the central nuclei. A section through the axes of the fibres would have shown more sarcoplasm and less contractile filaments.

Myofibrils are ill defined. The I bands are narrow, indicating that the fibres are partly contracted. A granule (*d*) in the upper fibre is of unknown composition. The granule may be a lipid droplet, but its relative density seems rather low for lipid. Portions of sensory nerve ending (*SE*) are applied to the surface of the fibres. The wide range of sizes of the sections of sensory ending and their irregular distribution would be compatible with a "flower-spray" distribution on the surface of the myotubes. The surface of the muscle is raised on each side of most of the sections of ending to form a raised gutter in which the ending lies. In the case of *SE1* the lips of the gutter have met (arrow) over the top of the ending. The lips of the gutter supporting *SE2* do not meet (arrows). The large portion of ending *SE3* has a large area of contact with the lower myotube; it is also in contact with the upper myotube (arrows). In neighbouring sections the contact with the upper fibre was more extensive.

The sheet of endomysium (*E*) is deficient towards the right and, therefore, the two intrafusal fibres are not isolated from each other in this region. Such a large deficiency in the endomysial partition between two intrafusal fibres is unusual. $\times 9,000$.



(Merrillees: Fine structure of muscle spindles)

PLATE 373

FIG. 16. Spindle No. 25.2.1. Fixed and partly dehydrated *in situ*.

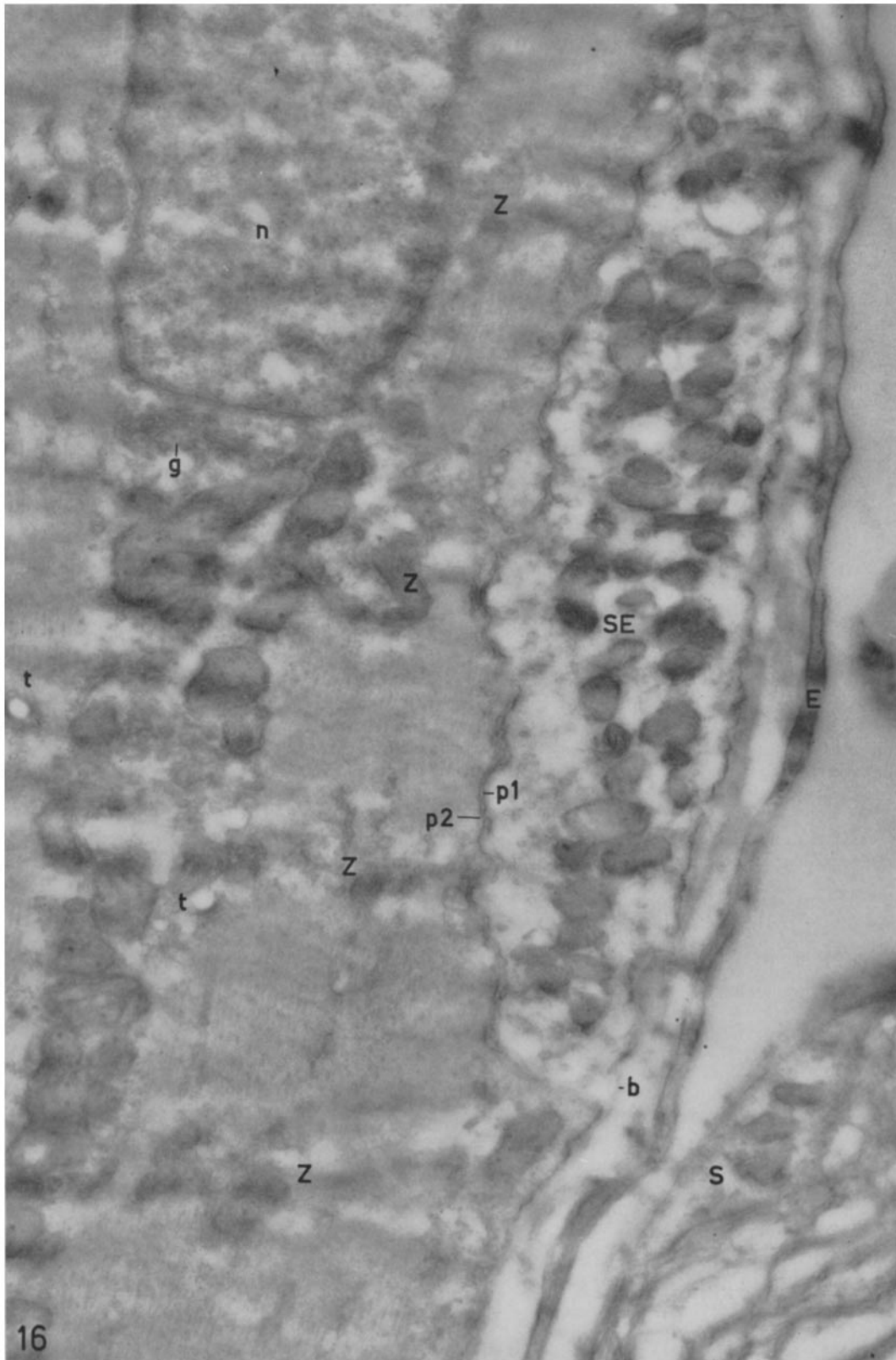
A section within the transition zone between the myotube and polar regions of an intrafusal fibre cut at an angle of about 15° to the long axis.

In this figure the horizontal ripples with the periodicity of about 1 μ m. are due to alternating thick and thin bands in the section, and these are the result of "knife chatter." These bands tend to obscure the transverse striations of the muscle. Z lines are indicated (*Z*). The muscle is partly contracted.

A long and low segment of a sensory nerve ending (*SE*) lies on the surface of the muscle. The plasma membranes of the ending (*p1*) and of the muscle (*p2*) can be followed for considerable distances, but details are obscured where corrugations of the plasma membranes are cut obliquely and where cytoplasmic vesicles lie close to the membranes. The basement membrane (*b*) passes from the surface of the muscle to cover the free surface of the ending. There is no Schwann-cell cytoplasm associated with the ending. There are a number of small compact mitochondria and small vesicles within the ending. Generally the myofilaments lie close to the muscle plasma membrane under the ending.

Some clumps of smooth vesicles (*g*), which are probably portions of Golgi bodies, and some mitochondria are associated with the muscle nucleus (*n*) in the long axis of the fibre. There are several triads (*t*) of the sarcoplasmic reticulum in the section.

A portion of the endomysial sheath (*E*) contains a few small vesicles. In the lower right corner of the figure is a portion of a Schwann cell (*S*) containing some exploded myelin. $\times 35,000$.



(Merrillees: Fine structure of muscle spindles)

PLATE 374

FIGS. 17 to 19 are overlapping serial micrographs. The right hand end of Fig. 17 is continuous with the left hand end of Fig. 18, and, similarly, Fig. 18 is continuous with Fig. 19.

Spindle No. 21.1.1. Fixed by vascular perfusion.

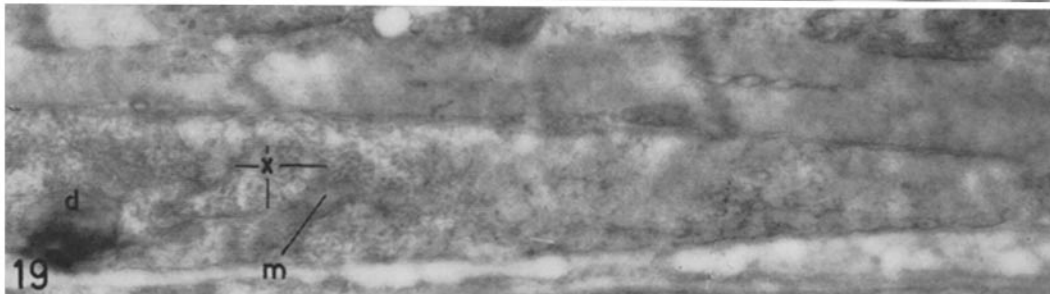
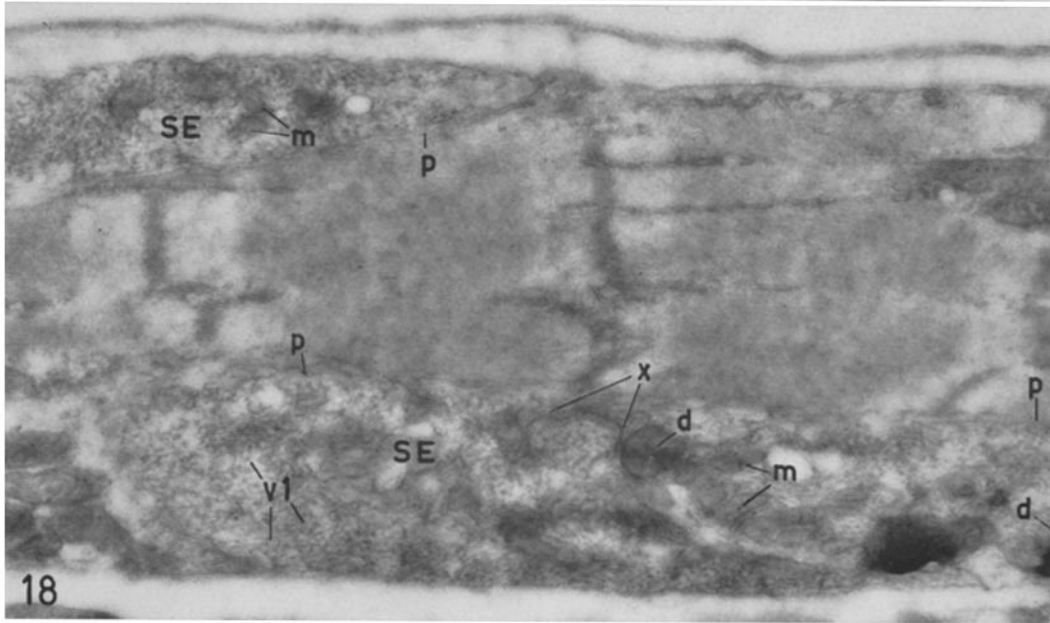
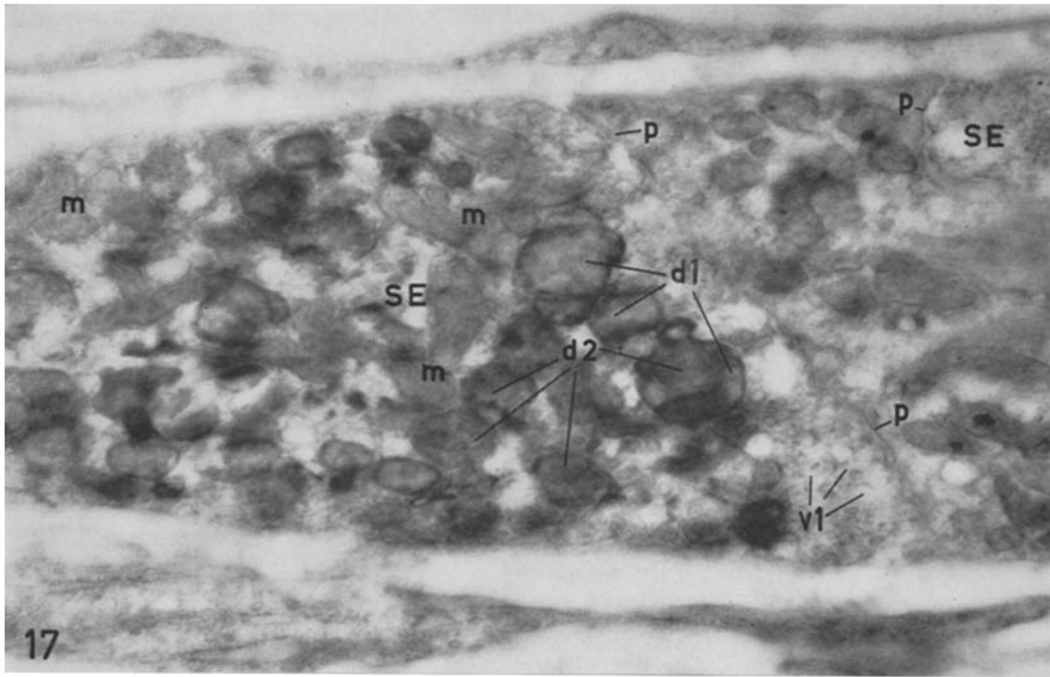
A section within the polar end of a myotube region of an intrafusal fibre cut at a small angle to the long axis. The portions of nerve ending (*SE*) seen in these figures were probably continuous with that marked *SEI* in Fig. 1.

FIG. 17. Towards the left of the figure the plane of section has entered the sensory nerve ending, and towards the right, enters the underlying muscle fibre and another portion of nerve ending that is indenting the muscle. The plasma membrane (*p*) of the muscle is indicated. The major portion of nerve ending contains many mitochondria (*m*) and large amorphous bodies. The latter are difficult to classify; some of them resemble lipid droplets (*d1*) but others of them could equally well be damaged or otherwise altered mitochondria (*d2*). The sensory ending contains many small vesicles of the endoplasmic reticulum, and in the lower right corner there is a group of very small flattened vesicles (*v1*) similar to those seen in profusion in *SE* in Figs. 18 and 19.

Several of the mitochondria in the muscle fibre contain large dense granules similar to those seen in Figs. 6 and 11, which were taken from the same spindle. $\times 30,000$.

FIG. 18. The plane of section has passed close to the surface of the muscle fibre and has passed through the overlying sensory ending (*SE*) in two places. It is not possible to demonstrate the continuity of the plasma membranes (*p*). In both portions of nerve ending there are a few small sections of poorly preserved mitochondria (*m*), and in the lower portion are several dense bodies (*d*) similar to those seen in Fig. 17. Both portions of the ending contain a tightly packed mass of the fine tubular reticulum (*v1*) similar to that in Fig. 17. These tubular profiles resemble delicate mitochondrial cristae and, therefore, by reason of the low resolution of this micrograph, it is difficult to identify mitochondria with obliquely cut membranes lying amongst the reticulum. At *X* a double membrane partly encloses a group of "tubules" but the structure is probably not a mitochondrion. A similar group of enclosed tubules is seen at *X* in Fig. 19. $\times 30,000$.

FIG. 19. The lower sensory ending seen in Fig. 18 is continued in this figure. Towards the right of the ending there is much less reticulum and the background consists of an amorphous material of medium density. At *X* a double membrane encloses some of the reticulum, and a mitochondrion (*m*) lies against the membrane. $\times 30,000$.



(Merrillees: Fine structure of muscle spindles)

PLATE 375

FIG. 20. Spindle No. 21.2.1. Fixed by vascular perfusion.

A section through an intrafusal motor end-plate at a small angle to the axis of the fibre.

The section of intrafusal fibre seen in Fig. 9 was very close to this end-plate.

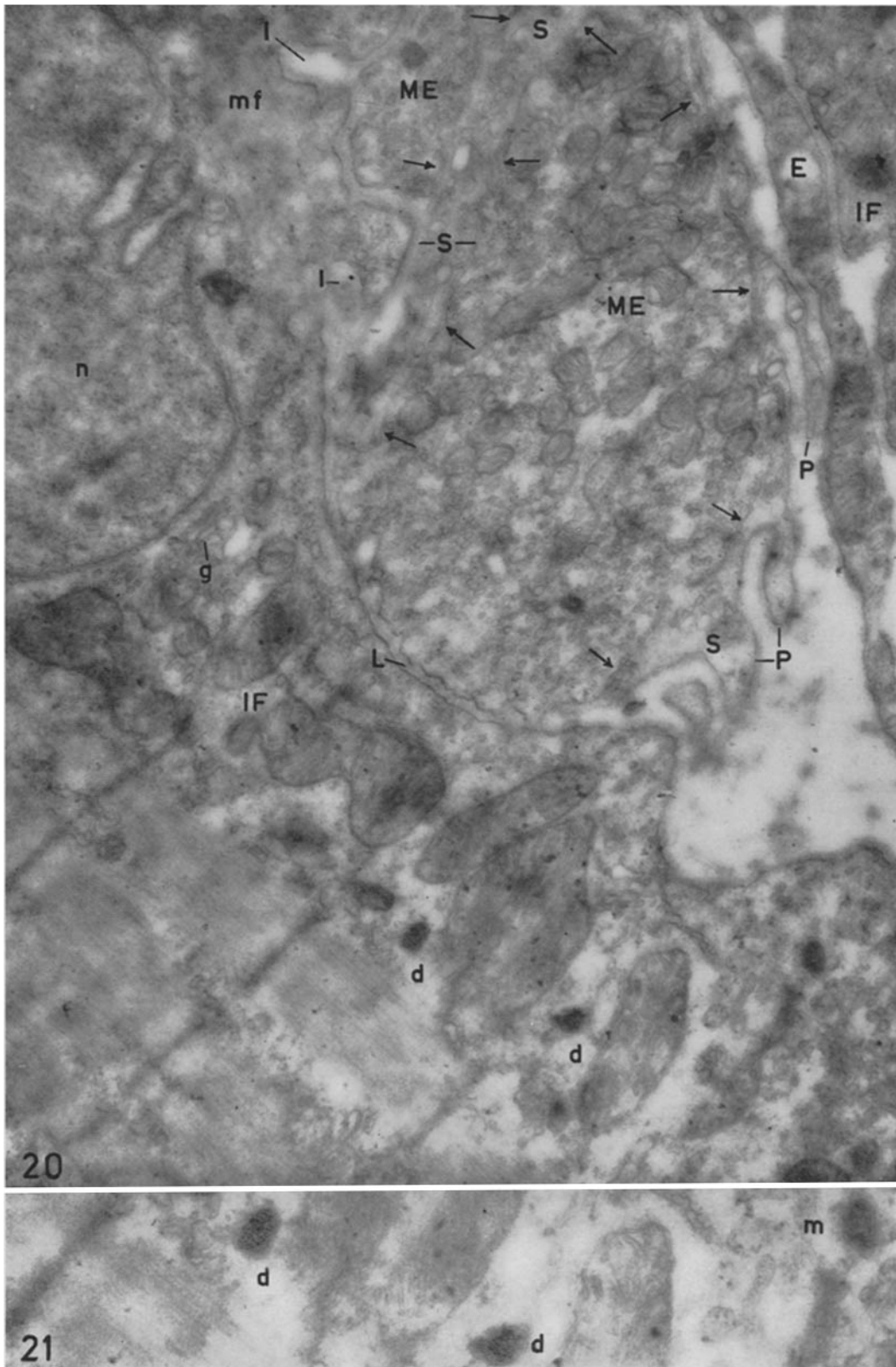
Except for the absence of long and complex invaginations of sarcolemma beneath the motor ending, the fine structure of intrafusal and extrafusal end plates is similar. A very thin layer of Schwann-cell cytoplasm (*S*) forms a cover over two portions of motor ending (*ME*), and the latter are applied to the intrafusal fibre (*IF*). The free surface of the Schwann cell is thrown up into several projections (*P*). In this plane of section the underlying muscle and the cover of Schwann-cell cytoplasm completely invest the motor ending. The plasma membrane of the ending is indicated at intervals by arrows. The plasma membranes of Schwann cell and motor ending lie about 200 Å apart. Within the area of contact between motor ending and muscle fibre, the plasma membranes of ending and muscle are separated by the basement membranes of both nerve and muscle, and lie about 500 Å apart. In this area each basement membrane closely follows the contours of its own cell. A faint line (*L*) corresponding to the position of the apposed surfaces of the basement membranes may be seen midway between the plasma membranes of nerve and muscle. There are two widely dilated invaginations (*I*) of sarcolemma beneath the motor ending. Sarcolemmal basement membrane lines the cavity of the upper invagination and fills the lower one. Compare the relatively simple contours of the sarcolemma in Figs. 20 and 22 with the complex invaginations under the extrafusal motor ending in Fig. 23.

The nerve ending contains a number of small mitochondria and many small vesicles.

The small layer of sarcoplasm that separates the myofibrils from the motor ending contains the edge of a nucleus (*n*), stacks of vesicles suggesting Golgi bodies (*g*), large mitochondria containing small dense granules, and some small vesicles and granular material. Several dense bodies (*d*) lie among the mitochondria (see Fig. 21). An isolated bundle of myofilaments (*mf*) lies between the nucleus and the sarcolemma under the motor ending.

Towards the upper right corner of the figure a sheet of endomysial-cell cytoplasm (*E*) separates the motor end-plate from a neighbouring intrafusal muscle fibre (*IF*). Where the endomysium and muscle cell lie in contact their basement membranes have fused and given rise to a fine dense line midway between the plasma membranes, which are separated by the basement membranes to a distance of about 500 Å. The relationships of this membrane complex are, therefore, similar to those found between a muscle fibre and its motor ending. $\times 30,000$.

FIG. 21. A photographic enlargement of the dense bodies (*d*) in Fig. 20. In this low-density print some structures resembling mitochondrial cristae can be seen in the body (*m*) to the extreme right. The two dense bodies (*d*) are limited by at least one membrane and contain many fine and extremely dense particles. The density of the particles suggests a high metallic content, e.g. iron in ferritin. $\times 45,000$.



(Merrillees: Fine structure of muscle spindles)

PLATE 376

FIG. 22. Spindle No. 21.2.2. Fixed by vascular perfusion.

A section through portion of an intrafusal motor end-plate at a considerable angle to the long axis of the fibre. The section of intrafusal fibre seen in Fig. 10 was very close to this end-plate.

Portion of an intrafusal fibre occupies the lower part of the figure, and a motor ending occupies the upper part. Portions of the investing Schwann cell (*S*) lie at either side. The small vesicles (*v*) in the ending are clustered near the nerve plasma membrane (*p1*). The three invaginations of the sarcolemma are very short and simple, and somewhat dilated.

The amorphous material *ef* is tentatively identified as elastic fibre. $\times 30,000$.

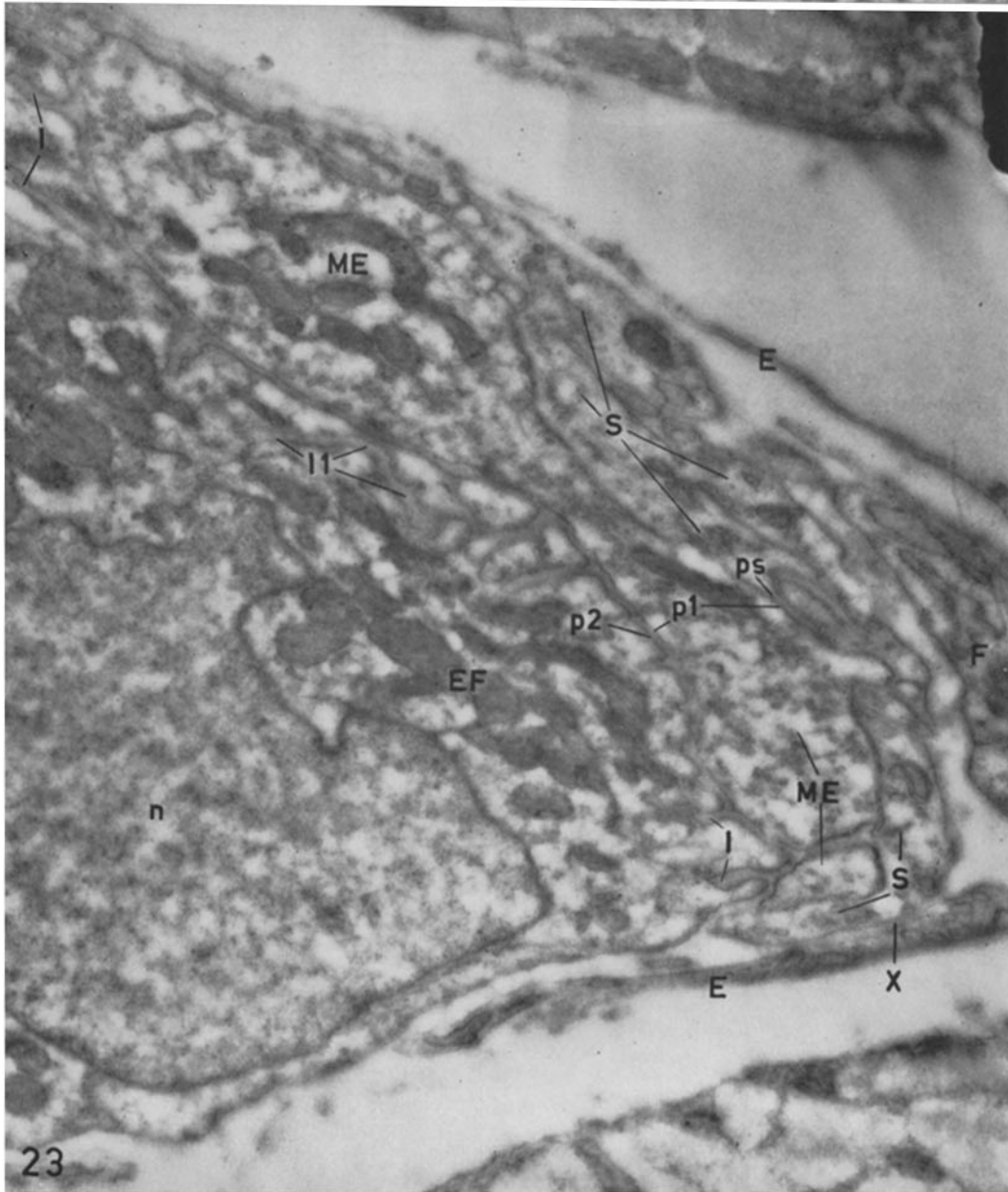
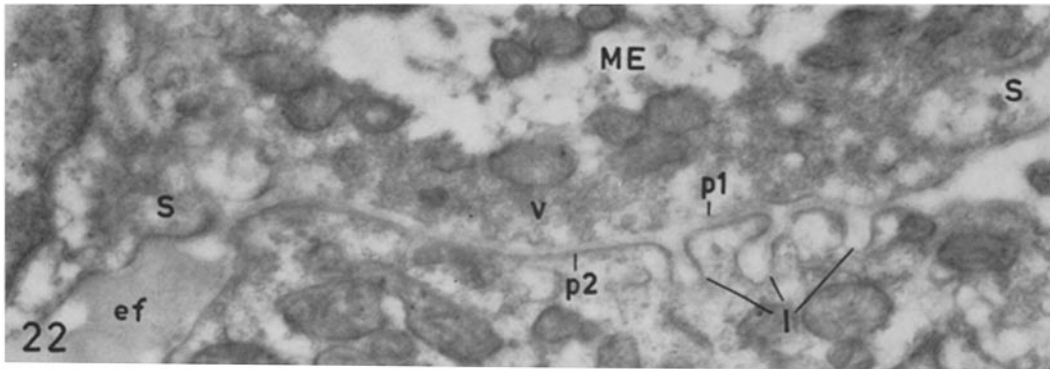
FIG. 23. Block No. 25.2.1. Fixed and partly dehydrated *in situ*.

A section through portion of an extrafusal motor end-plate at a considerable angle to the long axis of the muscle fibre.

This end-plate was found on a muscle fibre close to a spindle. In comparison with many other extrafusal end-plates found in the lumbricals and other skeletal muscles of the rat, this ending is relatively simple. The motor ending (*ME*) lies on the sole plate of the extrafusal muscle fibre (*EF*) and is completely covered by a thin sheet of Schwann-cell cytoplasm (*S*). In this plane of section the continuity of the Schwann-cell cytoplasm is interrupted by numerous folds. The plasma membranes of nerve (*p1*) and Schwann cell (*ps*) are about 200 A apart. The nerve ending contains some mitochondria and a large number of small vesicles. The plasma membranes of nerve ending (*p1*) and muscle (*p2*) are separated to a distance of more than 500 A by the interposed basement membranes of both nerve ending and muscle.

The invaginations of sarcolemma (*I*) under the nerve ending are typical of a motor end-plate. This specimen was described above as simple because the invaginations are few in number, and with the exception of the one marked *II* the invaginations are short and simple. In many end-plates the invaginations are crowded, long, and complex ridges, which, in a favourable plane of section, are seen to fuse with one another.

Portion of an endomysial cell (*E*) forms a thin cover over the end-plate. At *X* the endomysial cell and Schwann cell are in close contact. Their plasma membranes are separated only by their closely apposed basement membranes. At the upper and lower right borders of the figure are portions of neighbouring extrafusal fibres. *F* indicates a fibroblast. $\times 27,000$.



(Merrillees: Fine structure of muscle spindles)