THE TERMINATIONS OF THE AFFERENT NERVE FIBRE IN THE MUSCLE SPINDLE OF THE FROG

By B. KATZ, F.R.S.

Department of Biophysics, University College London

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[Plates 26 to 61]

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1. The sensory nerve contacts in the muscle spindle of the frog were examined in the electron microscope.

2. The terminal branches of the sensory axon form long beaded chains, i.e. bulbous expansions up to 2 to 3 μ thick connected in series by thin cylinders of as little as 0·15 μ diameter. Many nerve bulbs are seated in cup-like depressions of the intrafusal muscle fibres forming close contact with them. There is a residual gap between nerve and muscle surfaces of about 150 Å, but the gap is bridged here and there by fine filaments or processes of one cell closely approaching or touching the other.

3. The region of sensory contacts along the intrafusal fibres extends over several hundred microns and is divided into two morphologically distinct types of zones: (*a*) two 'compact' zones at either end, each about 300 μ long, in which the fibre retains approximately the same size and number of myofilaments as in the remote, extracapsular, region; (*b*) a 'reticular' zone in the centre, about 100 μ long, in which the fibre loses some 85 % of its content of filaments and splays out into several fins and branches held together by slender membrane connexions. The interstices between the splayed-out parts are filled with a dense network of fine connective tissue fibrils (about 50 Å thick). A minority of the intrafusal fibres does not show this differentiation in the sensory region and retains most of its myofilaments throughout.

4. Several characteristic differences are described between motor and sensory nerve endings on intrafusal muscle fibres. Among them are (*a*) that the motor terminal forms an 'epectolemmal', the sensory ending a 'hypectolemmal' contact (referring to the external basement membrane of the cells as the 'ectolemma'); (*b*) the motor ending remains invested by a covering Schwann cell layer, while the sensory endings are not closely associated with satellite cells; (*c*) the cytoplasm of motor endings is characterized by an accumulation of 500 Å vesicles near the synaptic surface, that of sensory endings by an accumulation of small mitochondria.

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5. A structure of unusual periodicity (a longitudinal 'micro-ladder' with rungs about 1600 Å apart) was observed in the interior of intrafusal muscle fibres, located generally in the neighbourhood of sensory nerve contacts.

6. The functional significance of some of the observed morphological features is discussed. It is suggested that mechanical stimulation and depolarization of the sensory nerve endings occurs at the points of adhesion between the intrafusal muscle fibre and the terminal nerve bulbs. The differentiation between 'dynamic' and 'static' components of the sensory stretch response may arise from different visco-elastic properties of the 'compact' and 'reticular' zones. Motor activation of the intrafusal muscle fibres would lead to intense stimulation of the sensory endings mainly within the 'reticular' zone. This zone is protected against overstretching by a feltwork of connective tissue fibrils.

Introduction

The muscle spindle of the frog is a greatly simplified version of its mammalian counterpart, and therefore of relatively little interest when one is concerned with the mechanisms of muscular reflex control in higher animals. But for certain purposes, for example, when exploring the initiation of impulses in a mechano-receptor structure, the spindles of the frog offer advantages, not only because they are easier to handle experimentally, but because their neuronal connexions are less complicated. Thus, in the frog there is only a single type of sensory ending (Cajal 1888; Dogiel 1890; Matthews 1931*a*; Katz 1950*a*; figure 1, plate 26), and the motor supply to the intrafusal muscle fibres consists of branches of extrafusal axons (Katz 1949*a*; Eyzaguirre 1957; Gray 1957).

The physiological behaviour of the amphibian spindle has been described by several authors (Adrian & Zotterman 1926; Matthews 1931*a*, *b*; Katz 1949*a*, 1950*a*, *b*). Sensory impulses are initiated by stretch applied to the whole muscle or, at a fixed muscle length, by intrafusal contraction (Katz 1949*a*; Eyzaguirre 1957, 1958). The latter can be elicited in the form of either a propagated twitch or a slower non-propagated contraction, depending on whether large or small axons are selected for stimulation.

The response to applied stretch depends on two main factors: the velocity and the amplitude of the imposed increase of muscle length (figure 1*B*, plate 26). The response can be recorded in the form of a graded local depolarization of the terminal part of the sensory axon. This local potential change causes the discharge of repetitive nerve impulses whose frequency varies in direct proportion to the primary depolarization of the terminals. The velocity, or 'dynamic', component of the response was found to be half-maximal when the muscle (ext. l. dig. IV; Katz, 1950*b*) was extended at a rate of 0·25 % per ms, and to reach a maximum at a rate of lengthening of about 1 % per ms. The displacement, or 'static', component is half-maximal when the muscle is lengthened about 10 % and reaches a maximum when the increment of length is approximately 25 %.

As in the case of other sense organs, the nature of the primary 'transducer' effect by which the energy of the specific stimulus is transformed into a membrane depolarization remains unknown. Various possible mechanisms have been considered and some of them rejected (see Katz 1950*b*; Gray 1959), but no positive evidence is available. It is natural to suppose that a mechano-receptor such as the spindle owes its high and specific sensitivity, at least in part, to the way in which the mechanical stimulus is brought to bear on the membrane of the sensory nerve terminal. It seemed possible, therefore, that a study of the fine structure of the intrafusal muscle fibres and of their connexions with the afferent axon might provide useful clues to the sensory mechanisms.

Methods

The preparation was the same as that used in earlier physiological work (Katz 1949*a*, 1950*a*, *b*), the M.ext.l.dig.IV of English *Rana temporaria*. The muscle was dissected together with its nerve; in a few cases, the muscle was reduced further until only a spindle bundle and a few extrafusal fibres remained. The preparation was kept either in Ringer's solution (NaCl 116 mM, KCl 2 mM, $CaCl_2$ 1·8 mM) or was transferred to 95 mM K_2SO_4 at 5 °C for about 12 h before fixation. In this latter solution, the muscle stayed in a depolarized condition; it relaxed after an initial transient contraction, and the contracture produced by the subsequent application of the fixative was much reduced, though not completely abolished. The muscle was subjected to varying degrees of stretch just before fixation, and it did appear, from longitudinal examination of sarcomeres, that the K_2SO_4-treated fibres were in a rather more uniform state than those which had been transferred directly from ordinary Ringer to the fixative and which clearly had undergone irregular local shortening.

The preparations were cooled to 0 °C, fixed in ice-cold buffered osmic acid (Palade 1952), stained after dehydration in 1 % phosphotungstic acid in alcohol (P.T.A.) and embedded in Araldite (Glauert & Glauert 1958) after allowing the plastic mixture to infiltrate for a few days at room temperature. Although all preparations were treated with OsO_4 and P.T.A. in this way, there was considerable variation in the degree of staining and contrast achieved in the electron micrographs. As has been mentioned previously (Birks, Huxley & Katz 1960; Birks, Katz & Miledi 1959), the density of the external 'basement membranes' of nerve and muscle fibres and of the associated fine connective tissue filaments are particularly subject to such variability. In two exceptional blocks (e.g. figure 6*A*, *B*, plate 31), the contours of cell surfaces, nuclei and mitochondria were well defined, but other structures such as myofilaments and connective tissue fibrils could only be seen very faintly, and over large areas of the muscle the sarcomeres appeared in 'inverted' low contrast. It may be that, for some accidental reason, precipitation of P.T.A. had occurred in the spaces between the myofilaments; but whatever the reason, these results were included here because the cell membranes and their contact relations showed up satisfactorily.

In the muscles used for this work, several spindles are arranged in series along one or two parallel bundles which run the whole length from tendon to tendon. One or two of the most proximal spindles were embedded for longitudinal or transverse sectioning. Sections less than 0·1 μ thick were examined in a Siemens Elmiskop I operating at 60 kV. The magnification was varied by adjusting the intermediate lens current. The lowest magnification was subject to variation of as much as 15 %, while the higher settings were considerably more accurate.

Results

General description

Figure 1, plate 26, shows a low-power picture of a fresh, isolated spindle and the classical histological drawings by Cajal (1888) and Dogiel (1890). The spindle shape arises from the formation of a bulging capsule (figures 6*B*, 15, 16, 18, plates 31, 40, 41 and 43; Robertson 1956) which is itself a continuation of the endothelial sheath of the sensory axon (sheath

of Henle) and extends for several hundred microns along the intrafusal bundle, gradually tapering off and losing its several layers of cells. The lymph space within the spindle communicates, through the open ends of the capsular envelope, with the interstitial fluid spaces of the whole muscle.

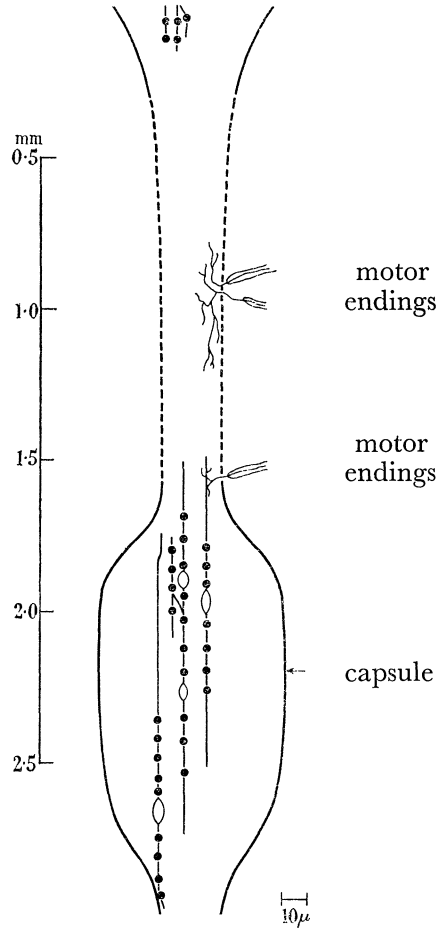

FIGURE 37. Schematic presentation of the distribution of nerve endings along an intrafusal bundle. M. ext. l. dig. IV, slackened before fixation. The most proximal sensory region (cut off at the top) had been used for longitudinal examination, the remainder of the specimen for transverse sectioning. The heavy outlines show the external capsules of the sensory portions, while the broken lines indicate the size of the fibre bundle in the extracapsular (motor) region. The outer diameter varied between about 23 μ in the motor region and 70 μ in the capsular region. The extent of sensory nerve contacts on different muscle fibres is shown by the black beads; a point of branching and recombining between two fibres is also indicated. The location of 'reticular' zones is shown by the hollow segments interrupting the beaded lines (which represent the 'compact' zones).

A bundle of intrafusal muscle fibres passes through the capsule and establishes numerous sensory nerve contacts within it. A 'fluid cushion' is provided not only for the intrafusal bundle as a whole, separating it from the rest of the muscle, but on a smaller scale for each of its muscle fibres individually. As one follows the bundle through its region of sensory contacts, the fibres are seen to separate from one another (figures 14 to 18, plates 39 to 43). The capsule subdivides forming several septa which enclose lymph spaces surrounding

the individual muscle fibres, as well as a separate space for the myelinated portion of the sensory axon (figure 16, plate 41). By comparison, the extracapsular portions of the same fibres are packed fairly close together and separated only by bundles of collagen from the rest of the muscle (figure 14, plate 39).

The disposition of the capsule is shown schematically in figure 37. This result was obtained by a serial examination of a muscle which had been slackened off before fixation. Its most proximal spindle had been removed for longitudinal sectioning. The remainder of the muscle was examined in cross-section at various levels, usually not more than 50 μ apart, over a length of 2·8 mm. The individual intrafusal fibres (10 to 11 in number) could be followed over the greater part of this length, though uncertainties of identification arose at two levels. Certain special properties, such as junctions and recombination between fibres (cf. Barker 1959) will be mentioned below. The only apparently unusual feature about this muscle was that its intrafusal components were grouped together into one fairly large bundle, instead of running in two parallel smaller bundles.

The shape of the sensory endings

According to Cajal (1888) and Dogiel (1890), confirmed by Huber & de Witt (1897) and Gray (1957), the myelinated sensory axon after entering the capsular space breaks up into numerous non-myelinated terminal branches which run alongside the intrafusal muscle fibres and are characterized by varicose swellings which give them the appearance of beaded chains (figure 1 *C*, *D*, plate 26).

This histological picture was readily confirmed when the spindle was studied in longitudinal section in the electron microscope. As soon as the level of the section passed into the interior of the capsule, the varicose threads described by Cajal and Dogiel were seen in the form of chains of 'micro-spindles' (figures 2 to 4, plates 27 to 29), some running freely through the capsular space at that level, while others formed characteristic contacts with the surface of intrafusal muscle fibres (figures 5 to 13 *D*, plates 30 to 38).

'Varicose swellings' of various sizes (up to 2 or 3 μ in diameter) were connected in series by thin cylindrical tubes (about 0·15 μ thick). Usually only one or two links of any one chain could be followed in a single section, though occasionally as many as 5 or 6 were seen connected in a row.

An interesting feature of these structures is that they run separately and, unlike motor endings, do not form close association with satellite cells (see also Robertson 1956, 1960). They are further characterized by the presence of many, often masses of, small mitochondria inside the bulbous expansions (figures 2, 7, 8, plates 27, 32 and 33). In addition, a tubular reticulum is often seen (figures 3, 5, plates 28 and 30) and occasionally vesicles are present but, again by contrast with the motor terminal, these are not a regular or striking feature (cf. figures 32, 33, plates 57 and 58).

Sensory contacts formed on intrafusal muscle fibres

On the present evidence, there can be little doubt that the chains of 'micro-spindles' shown in figures 2 to 4, plates 27 to 29, represent the 'varicose' terminal branches of the sensory nerve described by Cajal and Dogiel. Many of the 'nerve bulbs' (i.e. the varicose

parts of the chain) are anchored to the intrafusal muscle fibres. As shown in figures 5 to 13, plates 30 to 38, the surface of the muscle fibre contains numerous sockets in which the nerve bulbs are seated in close-fitting contact.

The structural details of these contacts are clearly of great importance for an interpretation of the receptor mechanism, but they are also technically the most difficult to elucidate. The difficulties arise because of the close proximity and often irregular curvature of the articulating membranes which means that most sections will contain oblique and, therefore, ill-defined and possibly overlapping aspects of these membranes. Nevertheless, by examining those regions where the membrane contours are clearly defined, a certain amount of pertinent information may be obtained.

At many regions of contact, the only feature that stands out clearly is that the two cell membranes approach to within 100 to 200 Å and retain this separation over an appreciable area. This is a much narrower gap than found at the motor end-plate (Robertson 1960; Birks, Huxley & Katz 1960), and in contrast with the latter, the external 'basement membrane' of the nerve and muscle fibre does not extend into the sensory joint. This can be appreciated clearly in those specimens in which the stain was sufficiently intense to show up the basement membrane well (figures 11 to 13, plates 36 to 38; compare with 32 and 33).

For the purpose of the further discussion, it may be convenient to use a somewhat simplified terminology in describing the synaptic membrane relations. It has been shown by Birks, Katz & Miledi (1959) that the sarcolemmal 'basement membrane' (i.e. the external diffuse layer of the surface membrane complex of the muscle fibre, Robertson (1959); see also figure 36, plate 61) is a separate structure of relatively stable properties which can be dissociated from the cell membrane by certain procedures, for example, by inducing cellular atrophy. In the following this external layer will be referred to as the 'ectolemma'. Using the term in this sense, the position of the nerve ending at the motor end-plate may be described as 'epectolemmal' (the ectolemma being interposed in the articulation of the two cells), while the sensory nerve ending in the muscle spindle is clearly 'hypectolemmal'.

Although there is a residual gap of about 150 Å between nerve and muscle over most of the sensory contact area, one finds on further examination that there are points at which the gap is crossed by fine filaments, and other places at which the gap is bridged by a short process of one cell closely approaching or touching the other surface. There were many indications of such local adhesions, but in only few instances was the picture sufficiently clear (see figure 13, plate 38). It must be emphasized that adhesions between closely adjacent cells are not confined to the sensory contacts; similar features can be seen if one carefully explores the narrow clefts between nucleated satellite cells and the muscle fibre. It is, nevertheless, important to remember that the gap between sensory axon and muscle is not an amorphous or empty space, but that there are localized structural connexions through which the mechanical stimulus could conceivably be transmitted directly to the terminal nerve membrane (see Katz 1960, and p. 236 below).

In figure 38*a* a sensory contact is shown schematically in longitudinal section. Tangential and transverse aspects are seen in figures 38*b*, *c*. The picture varies a good deal, depending not only on the plane and level of the section, but on natural variations in the size of the nerve 'bulb' and the depth and extent of the 'ball-and-socket' joint which they form with

the muscle fibre. Often, the surfaces are crenated and show interdigitating notches (cf. figure 9, plate 34), but such irregularities could, of course, be produced by shrinkage artifacts.

An unsuccessful attempt was made to produce significant changes in the sensory contacts by stretching or relaxing the muscle before fixation. What one finds, in the intrafusal fibres as elsewhere, are the well-known changes in length and internal arrangement of the sarcomeres (H. E. Huxley 1957; also figure 35, plate 60); there were also obvious differences in the longitudinal appearance of the sensory nerve chains (e.g. figures 3, 4, plates 28 and 29)

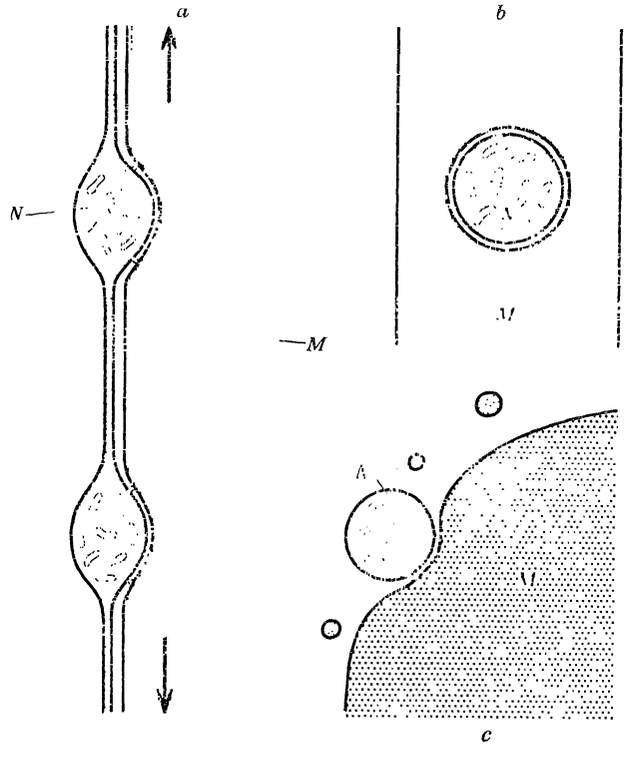

FIGURE 38. Diagram illustrating sensory nerve–muscle contacts in longitudinal (*a*), tangential (*b*), and transverse (*c*) aspects. *N*, terminal nerve chain, showing the bulbous expansions and its small mitochondria. *M*, 'compact' region of muscle fibre. Several 'bridges' across the nerve–muscle gap are also shown.

but no instructive differences were observed in the details of the sensory contacts. This is perhaps not surprising in view of the variable aspects which one obtains at a given length, but it means presumably that the mechanical stimulus does not produce gross changes, such as an extensive separation or retraction of the cell membranes in the sensory articulation.

Differentiation between sensory and other contacts on the intrafusal muscle fibres

The identification of the 'chains of microspindles' as the terminal branches of the sensory nerve depends on several observations, one of which is their close correspondence to the histological descriptions of Cajal, Dogiel and others. It is important to differentiate them from two other types of surface contacts which are found on muscle fibres: (*a*) Schwann and other nucleated 'satellite' cells; (*b*) motor nerve endings.

It has already been pointed out that there are nucleated cells closely associated with the surface of muscle fibres (e.g. figures 30, 31, plates 55 and 56), and these are found particularly frequently in the capsular region of intrafusal fibres. Some of these satellites may well be derived from Schwann cells which have separated from the axon terminals, as suggested by Robertson (1956). All these cells are in 'hypectolemmal' contact, like the sensory nerve bulbs. They differ from the sensory endings in that (i) they do not form chains of microspindles, (ii) they have nuclei and their cytoplasm contains a great variety of granular and vesicular bodies, but not the characteristic accumulation of small mitochondria, (iii) unlike sensory terminals, nucleated satellites survive denervation (figure 28, plate 51). It is possible to distinguish the majority of the contacting structures on one or other of these criteria, especially in longitudinal section, but there are doubtful instances, particularly in transverse sections which often fail to reveal the nuclei of the satellite cells.

Figures 28, 29, plates 51 and 52, were obtained from a spindle 6 weeks after severing the sciatic nerve. The microspindles had disappeared, and all that was left were a few empty 'sockets' in the surface of the intrafusal muscle fibres such as those shown in longitudinal and tangential section in figure 28. Nucleated satellite cells, however, were still present.

To discriminate sensory from the ordinary ('twitch-type') motor nerve endings may seem quite redundant because (i) the motor innervation is known to take place in the extra-capsular region (Cajal 1888); (ii) the contact between motor ending and muscle fibre is 'epi'- and not 'hypo'-ectolemmal (p. 226; Birks, Huxley & Katz 1960); (iii) the motor ending is covered by a terminal Schwann cell which the sensory endings lack; (iv) the cytoplasm of the motor endings is characterized by an accumulation of 500 Å vesicles in the neighbourhood of the synaptic surface, very different from the accumulation of small mitochondria in the sensory 'nerve bulbs'; and (v) the muscle membrane at the motor end-plate is thrown into an array of junctional folds which is not seen in the sensory region.

However, it is not known whether all or any of these criteria can be applied to the terminals of small motor axons on the 'slow' muscle fibres of the frog, and there is evidence that a small proportion of intrafusal fibres belong to this slow system (Katz 1949*a*; Eyzaguirre 1957, 1958; Gray 1957). Histologically these endings have been described as of the 'en grappe' type, i.e. consisting of varicose filaments; they apparently lack the 'subneural' structures which correspond to the junctional folds of the muscle membrane (Couteaux 1958), and their spatial distribution along the muscle fibres is much more extensive than that of the 'twitch' endings. Hence, on purely histological grounds, a differentiation between slow-motor and sensory terminals might not be very easy.

The evidence on this point obtained with the electron-microscope was as follows: in the cross-sectional examination summarized schematically in figure 37, motor nerve junctions were observed on 6 out of 10 or 11 intrafusal muscle fibres, at the levels indicated in the diagram. There was no clear evidence on which one could distinguish slow from twitch fibres, but there were reasons for segregating 2 of the fibres from the rest (see p. 230), and possibly they belonged to the slow system.

Motor junctions of intrafusal fibres are shown in figures 32, 33, plates 57 and 58; that in figure 33 belonging to one of the two 'odd' fibres. The absence of junctional folds in these pictures is probably of no special significance; in the frog, cross-sections are apt to by-pass the folds which are arranged in regular transverse alinement (Couteaux 1958; Birks, Huxley

& Katz 1960; Birks, Katz & Miledi 1960). The other distinctive criteria mentioned above were clearly fulfilled by all the intrafusal motor junctions which have been seen. The observations were incomplete in that end-plates on five fibres were missed, but it should be noted that each of these fibres was joined at some point to one of the other six (see p. 234), and it is therefore very unlikely that an unusual *type* of motor junction could have been overlooked. It may thus be concluded that the *sensory* contacts, which were substantially similar on all intrafusal fibres, could not possibly have been confused with motor nerve endings.

A distinction between sensory nerve endings and capsule cells presents no problem in cross-section, but in their longitudinal aspect the outlines of the thin endothelial cells might at times be confused with a connecting tube of a 'free' nerve chain. The ambiguity can usually be resolved by following the structure along several microns, when the absence of the characteristic 'nerve bulbs', the longitudinal extent of the thin cell body and, occasionally, the presence of a nucleus serve as identification marks. In addition, the cytoplasm of capsule cells has a less dense appearance than that of the nerve tubes (see figures 2, 5, 7, plates 27, 30 and 32). Capsule cells survive nerve degeneration as shown in figures 28, 29, plates 53 and 54.

Differentiation of the muscle fibre in the intracapsular region

One of the problems of spindle function concerns the mechanism by which contraction of the intrafusal fibres stimulates the sensory nerve endings. It used to be thought that the striated myofibrils—and therefore the contractility of the fibre—terminated on either side of the region of sensory contacts. More recently, it has been found (Cooper & Daniel 1956; Robertson 1956) that some striated filaments do run through the whole of the capsular portion, but that they are greatly reduced in number. It is of some importance to get quantitative information on this point, and in order to do this, it was necessary to count the myofilaments of individual fibres in an extensive series of cross-sections. This was done on seven fibres of the bundle shown in figures 14 to 16, plates 39 to 41.

The procedure was to select a suitable, usually circular, area containing several hundred thick (*A*-band) filaments and to measure this as well as the total cross-sectional area of the fibre, not including large 'extra-fibrillar' regions (e.g. nuclei, mitochondria). When the diameter of the fibre was very small, and in all cases where the section was made through the 'reticular zone' (see below), a *total* count of the *A*-filaments was made. Occasionally, partial *and* total counts were checked on the same section. The process was aided by the fact that this muscle had been slackened before fixation, with the result that *I*-bands were generally very short and the greater part of the cross-sections contained the more easily discernible thick *A*-filaments (cf. figure 35, plate 60).

It will be clear from the pictures already examined that many sensory contacts occur on regions of the muscle fibre which possess normal striations and whose internal structure does not differ from ordinary muscle. Other parts, however, have an entirely different appearance: in longitudinal section, the muscle fibre seems 'fenestrated', i.e. split up into a complicated framework with interstitial spaces which are filled with a dense network of fine connective-tissue fibrils (figures 6, 22, 24 and 25, plates 31, 47, 49 and 50).

Examination of serial cross-sections revealed that on most fibres there are two morphologically quite distinct types of zones inside the capsule, indicated schematically in

figure 37. The length of the fibre covered by sensory contacts extends for about 600 μ. The majority of nerve bulbs are situated in the two 'compact' zones, each about 250 μ long and possessing much the same cross-sectional area and number of myofilaments as the extracapsular parts of the fibre. Flanked by the compact zones is a relatively short 'reticular' zone in the centre, approximately 50 to 100 μ long. In this region, the fibre loses some 85 % of its content of myofilaments and divides into many fins and branches which are held together by slender membrane connexions. Some fibres, after passing through a reticulated region were found to have split into two separate fibres or to recombine with other fibres in the following compact region. One fibre (see figure 37) passed through two reticular zones in series within the same spindle capsule. The transition between the zones is shown in figure 6, plate 31; it often occurs abruptly within a length of less than 10 μ.

Various aspects of the reticular zone are shown in figures 18 to 25, plates 43 to 50. The wide interstices between the fins and branches of the fibre are filled with an extracellular meshwork of filaments of about 50 Å diameter. This network, together with its embedded intrafusal muscle fibre and with the associated sensory terminals and satellite cells, makes up a cylinder whose outer circumference is about 50 % greater than that of the fibre in the 'compact' zone, and which remains separated by a lymph space, about 0·2 to 0·5 μ wide, from the fibre capsule. The cross-sectional area of the muscle fibre, not including the centrally situated row of nuclei, was reduced to about 40 % of that in the compact zone while the perimeter of the fibre was greatly enlarged, making its surface/volume ratio in the reticular zone larger by a factor of 5 to 6.

The mass of extracellular fibrils is clearly a local proliferation of very similar connective tissue filaments which are found, much more sparsely, on the surface of ordinary muscle fibres and associated with their ectolemma (see figure 36, plate 61). Indeed the dense network of filaments can be seen to be continuous with the ectolemma of the intrafusal muscle fibre and to replace it in the reticular zone (figure 25, plate 50). The fine filaments appear to be attached at many points to the reticulating surface of the muscle fibre. The contour of the fibre membrane shows up in this zone in discrete dense patches which usually curve outward (figures 19 to 21, plates 44 to 46). This may be due to traction by the attached connective tissue filaments. It is possible that the same dense areas of the fibre membrane provide also attachments internally, for myofilaments which terminate in this region.

While the behaviour just described applies to the majority of the intrafusal fibres, there are exceptions. Two of the ten or eleven fibres examined serially in cross-section (fibres no. 1, 7) were found to show little differentiation in the 'reticular' zone, and their myofilaments were only slightly reduced in number (to about 70 %). Their diameters were 2·5 and 5 μ, while the intrafusal fibre sizes ranged from 2 to 9 μ; this may be significant in connexion with Eyzaguirre's (1957) suggestion that the 'slow' components of the spindle bundle are probably to be found among the smaller fibres.

Distribution of sensory endings

The portion of the fibres which is covered with sensory contacts is indicated in figure 37. The fibres within this capsule could be divided into 3 or 4 groups according to the level of their innervation zones.

To obtain an approximate estimate of the number of 'contacts' and their spatial distribution, the various 'satellite' structures which surround an intrafusal fibre were counted in a cross-sectional series and plotted as in figures 39 and 40. The upper curve represents *all* satellites (i.e. the sensory nerve chains seen in a variety of diameters as well as Schwann and similar nucleated cells); the lower curve shows the number of nerve bulbs which articulated with the intrafusal fibre at that level. In the compact zones, the ratio between the two ordinates was usually a little less than 10:1.

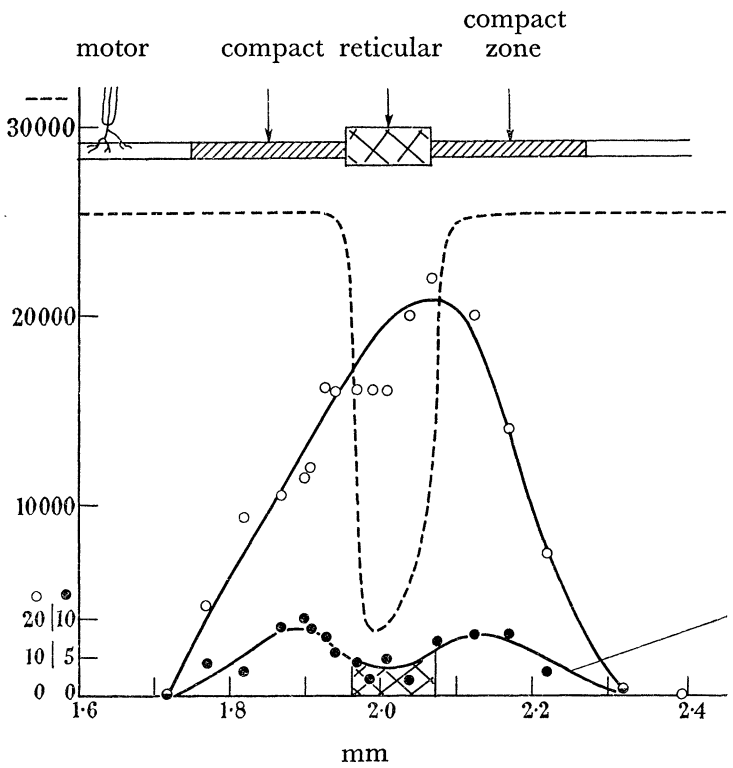

FIGURE 39. Distribution of sensory 'satellites' and number of myofilaments in the sensory region of an intrafusal fibre (fibre no. 2, size approx. 7·5 μ; cf. figure 19, plate 44). Top diagram shows extent of compact and reticular zones. – – –, *A*-filaments: this is a smoothed curve, showing the sudden reduction in the reticular zone. ○, total number of 'satellites', including nucleated cells and non-contacting portions of nerve chains. ●, number of nerve bulbs found in contact with muscle fibre.

The curves showed a peak in the middle part of the innervated region, either within the reticular zone itself, or more frequently a double peak at the edges of this zone with a dip in its centre. The number of satellites diminishes towards the periphery in an approximately exponential fashion covering a total length of about 0·5 mm. It is clear that the majority of contacts take place in the compact zones, and only a relatively small fraction is situated within the reticular region. On the other hand, in this reticular region the mechanical stimulus could be imparted to the nerve bulbs, not only through their connexions with the muscle fibre, but also through connexions with the extracellular network of fibrils.

From these observations, together with the evidence obtained by longitudinal sectioning, a very rough estimate can be made of the total number of sensory contacts, and of their

parallel and series connexions with the afferent axon. The estimate is bound to be rough because two somewhat arbitrary assumptions had to be made. These were: (*a*) that of all the cross-sectioned satellites surrounding an intrafusal fibre, one-quarter belong to the chains of 'microspindles' which are activated by this fibre, and (*b*) that the nerve bulbs along these chains repeated at intervals of 4 μ. The latter value is of the right order for a

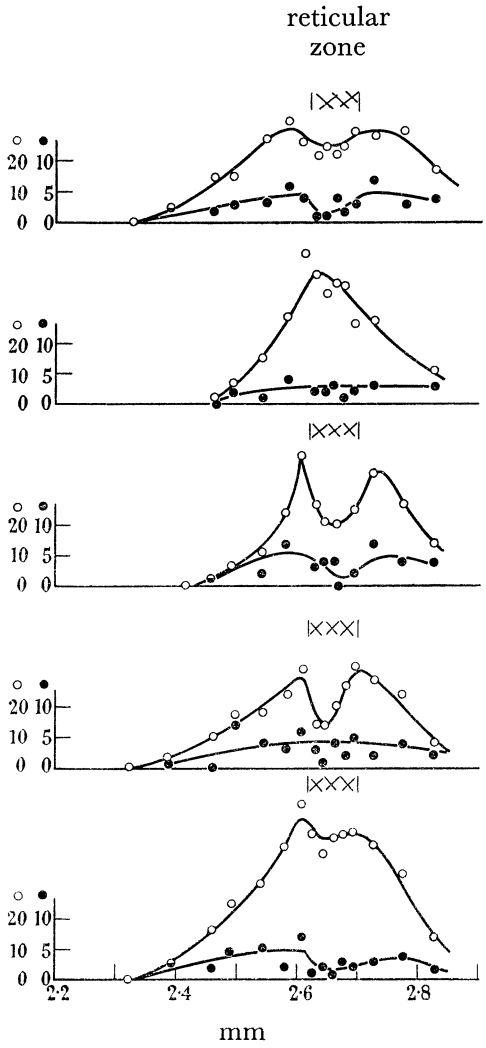

FIGURE 40. Distribution of sensory 'satellites' (○) and nerve contacts (●), in the group of five fibres shown in figures 17 to 18, plates 42 and 43. The results correspond, by serial number, to the following fibres (successively from above): 3, 7, 6, 5 and 4. Position of reticular zone is indicated by hatched area for each fibre: fibre 7 did not reticulate.

'slack' muscle. The former value was chosen on the supposition that the fraction of contacting chains must be larger than (probably at least twice) the ratio of 1:10, between sensory contacts and total satellites in any one cross-section; this must be so because of the longitudinal 'staggering' between the thin 'tubes' and thick 'bulbs' of adjacent chains.

These assumptions, though inaccurate, cannot be seriously misleading if one is concerned with the general order of magnitude. They lead to the following results: the number of *chains* of microspindles contacting each fibre in a given region is on the average

about 8, making a total of 100 chains (or 100 series of chains) in parallel, for all the fibres in this capsule. The number of sensory contacts in the 'compact zones' varies between 300 on a small fibre and 1500 on a large fibre. The number of sensory bulbs contacting the fibre or suspended in the network of the 'reticular zone' varies between 100 and 500, respectively, the average ratio between the two zones being about 3·5:1. The grand total of these terminal structures in the capsule was estimated to be about 10000, i.e. an average of 100 in series along 100 parallel lines, all of which are ultimately connected to one afferent axon.

A structure of submicroscopic periodicity in intrafusal muscle fibres

One of the most puzzling findings in the course of this study was the 'ladder-like' structure illustrated in figures 26, 27, plates 41 and 42. It was seen exclusively in the interior of intrafusal fibres, within their region of sensory contacts. The structure consisted of long 'ladders' whose 'rungs' appeared as dense lines (presumably disks) about 0·2 to 0·8 μ wide and repeating at longitudinal intervals of approximately 1600 Å. The rungs were connected by a material whose filamentous composition can just be recognized in several sections. In stretched muscle, the ladders were straight with the rungs arranged transversely and parallel, though occasionally a local 'misalinement' was observed (cf. the odd longitudinal rungs in figure 27*A*, plate 42).

The ladders were situated near the surface of the fibre and were found both in the compact and reticular zones. Their distribution is much less frequent than that of, say, the sensory contacts; in some specimens, very few ladders were seen. This in itself makes it difficult to suggest what the role of these structures may be in the process of mechanical stimulation. In some sections, the ladders appeared to terminate at the surface of the muscle fibre close to a sensory articulation. A possible interpretation is that the ladders form intracellular 'micro-tendons', i.e. an intermediary structure between myofilaments and certain sensory contacts.

The length of the 'ladders' and the number of 'rungs' may be very considerable. In one section, over 50 rungs were counted, and these continued in a longitudinal serial section to make up over a hundred, covering a length of about 20 μ.

When comparing stretched and relaxed (short) muscles, the characteristic period of this structure was found to be less variable than the average length of the sarcomeres. This is illustrated in figures 26, 27, also 35, plates 51, 52 and 60) and summarized in table 1. The values shown in this table are averages for different specimens examined in longitudinal section. There was, in addition, a good deal of variation within the sections of each block; thus, the extreme range of all the ladders was 1150 to 2500 Å, that of the sarcomeres 0·9 to 3·1 μ. In considering these values, some allowance should be made for compression artifacts, for all these sections were made with the knife cutting in the axial direction along the muscle fibres. This will introduce a systematic shortening of all observed periods as well as variability within a given specimen block. The random variation will be greatly reduced if one only considers the average figures as given in table 1; a comparison between the range of these mean values and the observed extreme range indicates that up to 1·4 to 1·5 fold changes may have arisen from variations in the degree of knife compression. The 'systematic compression factor' may be arrived at by supposing that the value for the sarcomeres in the

most highly extended muscles (2·7 μ) was in reality 3·5 μ (see A. F. Huxley 1957). This gives a correction factor of 1·3 times for the averages, i.e. the grand mean values in table 1 would be 2100 Å (instead of 1600 Å) for the characteristic period, and 2·5 μ (instead of 1·95 μ) for the sarcomere.

TABLE 1. LONGITUDINAL SPACING IN 'MICRO-LADDERS'

Average values from nine different specimens, fixed in various states of shortening or extension.

period (Å)	1700	1600	1450	1400	1400	1600	1350	1800	2000
sarcomere (μ)	2·5	2·1	1·5	1·1	1	2·7	1·5	2·5	2·7

Mean: Period, 1600 Å; Sarcomere, 1·95 μ. Assuming average compression factor of 1·3, the true period of the ladders would be 2100 Å, and the mean sarcomere length 2·5 μ.

The fact that the period of the 'ladder' changes less than the length of the muscle, or of its sarcomeres, presumably means that the rungs are connected by relatively inextensible filaments. At the shortest muscle length the ladders are no longer straight and the rungs tilted at various angles, but the distance between them is not greatly diminished.

Although in the present work 'micro-ladders' were only encountered at the characteristic intrafusal sites mentioned, the same kind of structure has been described on occasions elsewhere, for example, in an avian skeletal muscle (Ruska & Edwards 1957) and in the heart (see Thoenes & Ruska 1960).

Other observations made on intrafusal muscle fibres

In the course of the serial study a number of unexpected observations were made which may be of more general interest. On passing through a zone of reticulation, a given fibre did not always reconstitute itself individually, but on occasions split into two parts, one of which might combine with an adjacent fibre, or form an additional fibre in a separate capsular compartment.

In the extracapsular regions, there were several places where two fibres contacted each other and their membranes appeared to join. The pictures were not entirely clear, but suggested the presence of localized communication through narrow and rather tortuous channels (cf. figure 34, plate 59). Occasionally larger parts of the surface seemed to coalesce, which has already been observed histologically by Forster (1894) and Barker (1959). Protoplasmic junctions of this kind are not confined to the spindle bundles; they were also seen occasionally in small extrafusal fibres.

As previously mentioned, the surface of many muscle fibres is invested here and there with hypectolemmal satellite cells (e.g. figures 30, 31, plates 55 and 56) even in places where they are clearly not related to either motor or sensory nerves. Their function is unknown; they are perhaps concerned with some stage of the development and growth of the fibre.

Finally, it should be mentioned that quite apart from changes in cross-sectional area which may occur in individual fibres at points of partial fusion, or within the sensory regions, the diameter and content of small muscle fibres shows significant, and sometimes considerable variation at different points of their length. Thus, fibres no. 5 and 6 increased in their content of *A*-filaments by 78 % between two levels 200 μ apart. This again is a phenomenon on which further light could be shed by studying the development of myofibrils.

Discussion

The local depolarization which a mechanical stimulus produces in the afferent nerve must originate within the terminal chains of 'microspindles'. By analogy to what has been found in other receptors, in particular at the Pacinian corpuscle (Gray 1959; Loewenstein & Rathkamp 1958; see also Eyzaguirre & Kuffler 1955), it is probable that the terminal chains serve a special 'transducer' function, but do not themselves give a propagating spike which arises higher up, possibly in the myelinated portion of the axon.

The first point to consider is whether the transducer action is more likely to occur in the 'bulbs', many of which are in contact with the muscle fibres, or in the 'thin tubes' by which the bulbs are connected in series. On the former view, the potential change is generated at the site of contact between muscle and nerve terminal, and the thin tubes merely serve as passive cables to transmit the electrical change up the chain towards the axon. On the latter view, the nerve bulbs might be considered merely as points of anchorage between which the thin tubes are stretched and thereby stimulated.*

There are at present no means of deciding between these suggestions, and the rest of the discussion is, therefore, speculative. On the former view, one encounters the difficulty that some nerve chains appear to lie in the free lymph space and their bulbs lack direct contact with the muscle surface, though the chain is presumably attached at some remote point. Are these 'free-space' bulbs redundant, or should one discard the first hypothesis on their account?

On the other hand, one can give several reasons for preferring it to the second hypothesis. Thus, it is difficult to see how the effect of stretching a cylindrical fibre by 10 to 20 % could account for the half-maximal to maximal depolarization at the sensory terminals (Katz 1950*b*; Gray 1959). It is true that the thin connecting tubes would undergo a proportionally greater extension than the whole chain, but this 'mechanical advantage' amounts to no more than a factor of two and is by itself far too small to explain the strong depolarizing action of stretching. But even supposing that a p.d. is generated by a membrane change along the thin connecting tubes, the inert bulbous parts of the chain whose diameter is about ten times larger than that of the tubes would act as low-impedance membrane shunts to the 'generator potential' and probably make it less effective.

On the other hand, if the depolarization originates in the surface membrane of the nerve bulbs, the situation is more favourable, for now the source of the potential has a low impedance. It is fed into a high-impedance line which does not act as a short-circuit though it will attenuate the voltage signal if it is to be conducted passively over appreciable distances. It is of some interest to speculate about the design of the dimensions of bulbs and tubes along the chain on the basis of this hypothesis. The size of the bulbs should be governed by the requirements (*a*) for a sufficiently large contact surface at which stimulation occurs and (*b*) for a volume accommodating a sufficient number of mitochondria, which are presumably concerned with recovery metabolism and the restoration of ionic concentration gradients.

* 'Stimulation' is used synonymous with terminal transducer action; 'excitation' denotes the initiation of an impulse.

The size of the passive connecting links would have to be determined by a compromise between two conflicting requirements: (*a*) to keep the inert membrane surface small and so avoid excessive shunting of the 'generator potential', (*b*) to avoid excessive attenuation in the cable transmission along the chain. The last factor is difficult to evaluate, as we do not know the total length of individual terminal chains. They might arise from a bifurcation of the axon in the centre of the reticular zone, running towards the periphery for distances up to 300 to 400 μ. Alternatively, there may be several points of bifurcation along the sensory region, with several shorter pieces of chains arranged in series.

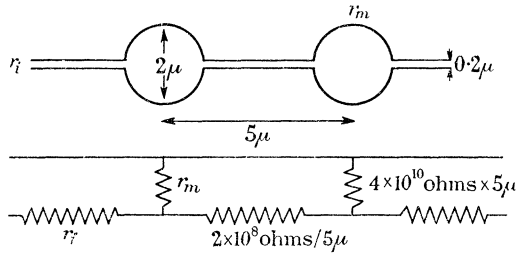

FIGURE 41. Diagram of part of a terminal nerve chain (above) and approximate electric circuit-equivalent (below). r_m and r_i are 'lump values', for a bulb and a tubular segment, respectively, assuming resistivities of 200 ohms cm for the cytoplasm and 5000 ohms cm^2 for the membrane.

If we consider a chain of the properties shown in figure 41 and, for simplicity, treat it as though its longitudinal resistance resided entirely in the thin tubes, and its membrane conductance in the spherical bulbs, then the length constant of such a chain comes to approximately 70 μ. It is clear that in such a chain, potential changes which are set up more than 100 μ distant from the supplying nerve branch contribute little to the initiation of an impulse. This suggests that there may be several terminal branches arranged in series along the sensory region of each fibre, a point which will have to be studied further.

If one favours the hypothesis of 'stimulation at the cell contacts', the next question concerns the mechanism by which a stretch applied to the ends of the muscle, or a contraction of intrafusal muscle fibres, exerts its influence on the sensory nerve bulbs and causes them to become depolarized. In some way, the stimulus is transmitted through the intrafusal muscle fibre to the nerve terminals connected to it. It might be an indirect mechanism involving, for instance, the release of a substance from the stretched part of the muscle fibre which then chemically depolarizes the nerve ending. Or it might be a direct mechanism, that is a mechanical deformation of the nerve bulb causing it to become 'leaky' to previously non-permeant ions and so to lose its resting potential. There is no direct information to distinguish between these possibilities, nevertheless, the former suggestion seems unattractive because it invokes without necessity an additional step, namely, an intermediate physico-chemical change in the muscle fibre, instead of direct mechanical transmission through it to the nerve terminal.

That there is a possible structural basis for such direct transmission can hardly be denied, for there are points of adhesion between the nerve bulbs and the muscle fibre, both at the sites of sensory contacts and via the network of extracellular filaments in the reticular zone. Unfortunately, the resolution obtained in the present pictures does not suffice to

show the detailed structure of these adhesions, nor any differences between shortened and stretched conditions. There seems to be at present no obvious way of improving this; moreover, one has to reflect that the 'holes' in the membrane, which one would need to explain the depolarization, are not much larger than the diameter of a sodium ion. Hence, the structural alterations which are associated with the transducer effect may not be detectable by present methods.

A matter of considerable interest is the differentiation of the sensory contact region into 'compact' and 'reticulated' zones. The most striking feature of the reticular zone is that the muscle fibre loses the majority of its myofilaments and instead becomes 'reinforced' by a dense extracellular network of connective tissue fibrils. Now, a sensory response can be elicited, either through stretch applied to the resting muscle fibres, or through excitation of the intrafusal muscle fibres. In the latter case stimulation must be caused, as has frequently been pointed out (Matthews 1931*b*; Katz 1949*a*; Eyzaguirre 1957), by a localized stretch of the sensory region owing to the contraction of the rest of the intrafusal fibre. Many intrafusal fibres give propagated action potentials (Eyzaguirre 1957; Katz 1949*a*) and twitch-like contractions, and the only part of the sensory region likely to be subjected to stretch under these conditions is the reticular zone which is almost devoid of contractile filaments. It follows, therefore, that the sensory terminations within the reticular zone are most probably the ones that are stimulated by intrafusal contraction.

The function, or at least one of the functions, of the extracellular network is similarly easy to guess. An active muscle 'gives' when it is subjected to a force greater than twice its isometric tension (Hill 1938; Katz 1939); hence a local reduction in the number of myofilaments to less than one-half would mean that this region of the fibre cannot sustain the full force developed by the rest of the fibre. In the absence of some elastic support, the reticulated portion of the fibre, lying freely in the fluid space of its capsule, would probably be overstretched and torn during intrafusal contraction, by the tension developed in the compact zones. The extracellular network may be supposed to give the necessary 'parallel-elastic' protection. The stress/strain diagram of such a feltwork of fibrils is presumably of the highly non-linear kind found in other muscle preparations (Fenn 1947; Hill 1938), allowing a limited extension to occur, but strongly resisting beyond a certain point.

The structural differences between 'compact' and 'reticular' zones are of interest in another context. In the compact zone we have a mass of interdigitating *A*- and *I*-myofilaments, which are sliding past each other when the muscle is stretched (H. E. Huxley 1957), and relatively little 'parallel elastic' ('ectolemmal') material. The converse is true for the reticular zone which is almost denuded of myofilaments, and whose 'ectolemma' has proliferated into a massive feltwork.

It is conceivable that the viscous resistance of an unstimulated muscle fibre depends mainly on the sliding motion of *A*- and *I*-filaments, while the elastic restoring force resides mostly in the ectolemma and other extracellular fibrils (Ramsey & Street 1940). If this is so, then the differences between compact and reticular zones assume further significance, in that they may be related to the two separate components of the stretch response. The *velocity* effect may arise mainly from the reticular zone which has low viscosity and is therefore subjected to the greatest deformation during the dynamic phase of stretching.

The static *displacement* response may reside mainly in the compact zones, but the argument cannot be pressed very far without knowing the stress/strain relations in the two zones.

It is hardly necessary to emphasize the unsatisfactory aspects of the present predictions. They are based on pictures of fixed material, and there is no immediate prospect of testing them on the living preparation. Also, the proposed scheme does not account for all the known properties of the afferent discharge. Thus, it is difficult to explain the decline of sensory excitation during maintained stimulation of intrafusal twitch fibres, and the inhibitory 'off-effect' at the end of such stimulation (Eyzaguirre 1958). Furthermore, no interpretation at all has been offered for the presence of the 'micro-ladders' inside the intrafusal fibres, and yet they must be suspected to play an important part in the sensory mechanism.

As was mentioned above, two of the fibres in the intrafusal bundle of figure 37 did not 'reticulate' nor did they become deprived of the major part of their myofilaments. This was regarded as a possible indication of their belonging to the 'small-motor' system (Kuffler & Vaughan Williams 1953*a*, *b*), of which some representatives are to be looked for in the spindle (Eyzaguirre 1957; see also, Katz 1949*a*). These muscle fibres do not conduct impulses (Burke & Ginsborg 1956*a*, *b*), and give local contractions spreading electrotonically around the motor junctions (Kuffler & Vaughan Williams 1953*b*); hence the sensory portion would be stretched by local contractions in the motor regions on either side, even without interposing a specialized zone devoid of myofilaments. If our hypothesis (end of p. 237) is correct, it would follow that such non-reticulating fibres do not give a velocity, but only a steady displacement response to passive stretch. This point might be tested in the more highly differentiated mammalian spindle; in the frog, the sensory terminals from all the fibres of the intrafusal muscle bundle are joined within the capsule to a single axon, so that the response to passive stretch will depend on the average contribution from all the affected sensory contacts.

Finally, concerning the intrafusal *twitch* fibres, some earlier experiments gain renewed interest in view of the structural findings. The physiological evidence was (Katz 1949*a*; Eyzaguirre 1957) that many intrafusal fibres receive branches of large motor axons and thus form a part of ordinary motor units. The intrafusal junctions are more resistant to curare than those of extrafusal fibres (Katz 1949*a*; Eyzaguirre 1957; Henatsch & Schulte 1958), a property which, it was suggested, might arise from an unusually large ratio between the diameters of motor endings and muscle fibre (cf. figure 32, plate 57). The intrafusal twitch fibres (identified by their ability to elicit sensory action potentials) propagate impulses from their end-plates; in one experiment, the conduction velocity was measured and found to be 0·64 m/s (slack muscle, 17 °C, Katz 1949*a*, p. 212). This was a residual spike obtained in a critically curarized muscle, and it could be traced over a distance of 9 mm, i.e. it must have travelled well past the next capsular region of the fibre. These observations indicate that the membrane properties of the reticular zone do not necessarily interfere with the propagation of a muscle action potential.

In some critically curarized preparations (Katz 1949*a*, p. 212) the residual intrafusal impulse—recorded with external electrodes—gave a larger spike potential than could reasonably be attributed to a single small fibre, and it was suggested that several muscle fibres must have been discharging simultaneously and could not be separated by curarization.

This behaviour becomes more easily intelligible now, in view of the cross-connexions found between several intrafusal fibres. Thus, in the bundle examined by serial cross-section there were points of local fusion among the following groups of fibres: 3 and 4, 4 and 5, 5 and 6, 8 and 9, 9 and 11, 10 and 11. 'Syncytial' connexions of this kind may be more general and will also have to be looked for among small extrafusal fibres. This and the peculiar longitudinal variations in fibrillar content may be better understood when more has been learnt about development and growth of striated muscle.

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Note on plates 26 to 61

All illustrations apart from figure 1 are electron micrographs of muscles fixed in buffered OsO_4 and stained with phosphotungstic acid in alcohol. Calibration scales are 1 μ, unless otherwise labelled. At the lowest magnification, the calibration is approximate (see Methods).

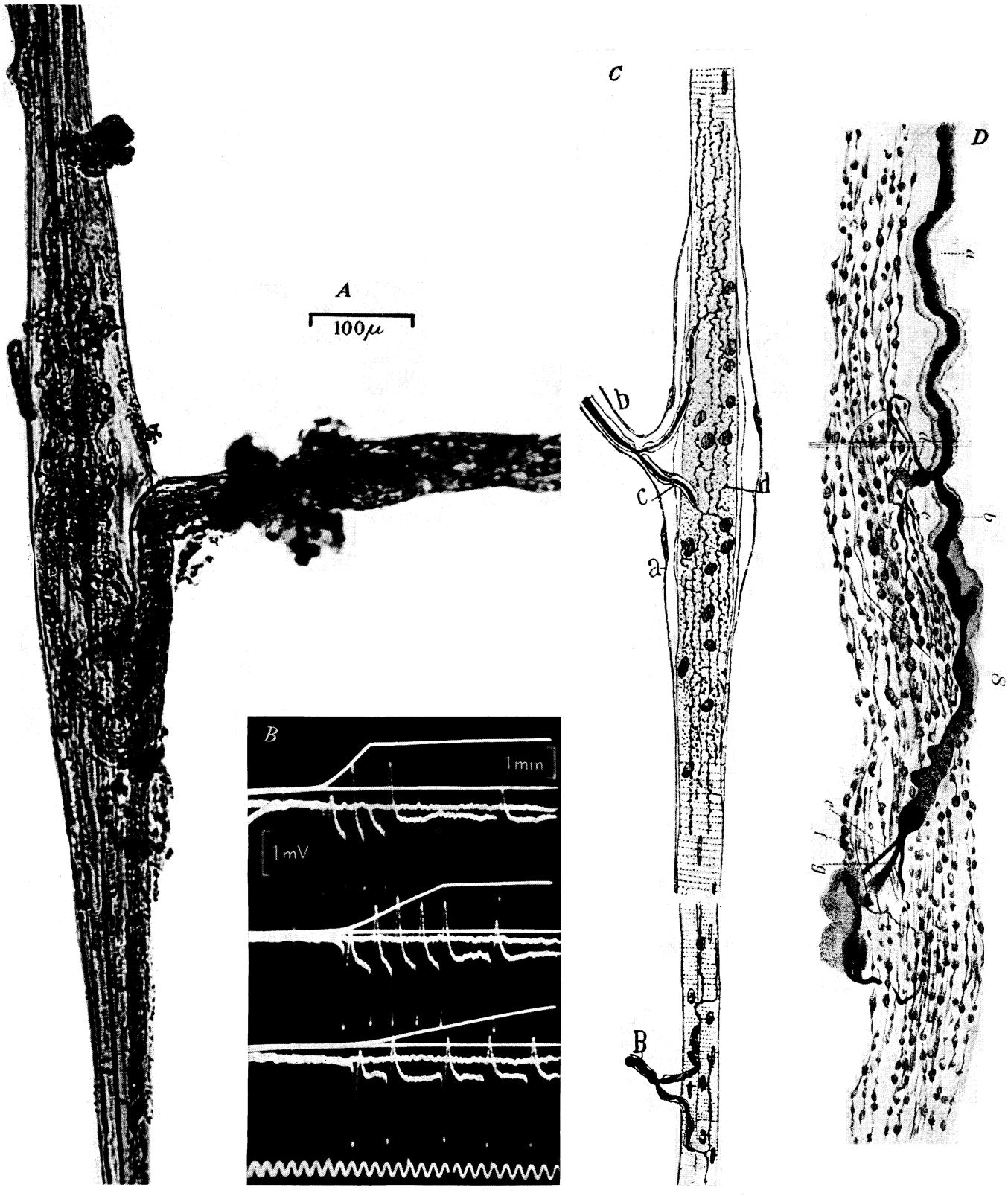

FIGURE 1. *A*. Isolated functioning spindle, from a toe muscle of the frog, photographed while immersed in Ringer solution (from Katz 1950*a*). *B*. Showing the electric responses (lower trace of each pair) in the spindle nerve, during three mechanical stretches applied to the toe muscle at different speeds (upper traces). The response consists of propagating spikes and of a slow local depolarization which depends upon rate and amplitude of stretching. Time signal: 500 c/s (from Katz 1949*b*). *C*. Histological drawing of frog spindle by Cajal (1888). Fixed and stained with methylene blue. *D*. Drawing of similar preparation by Dogiel (1890), showing the termination of the sensory nerve fibre in the form of axial 'varicose threads'.

(*Facing p.* 240)

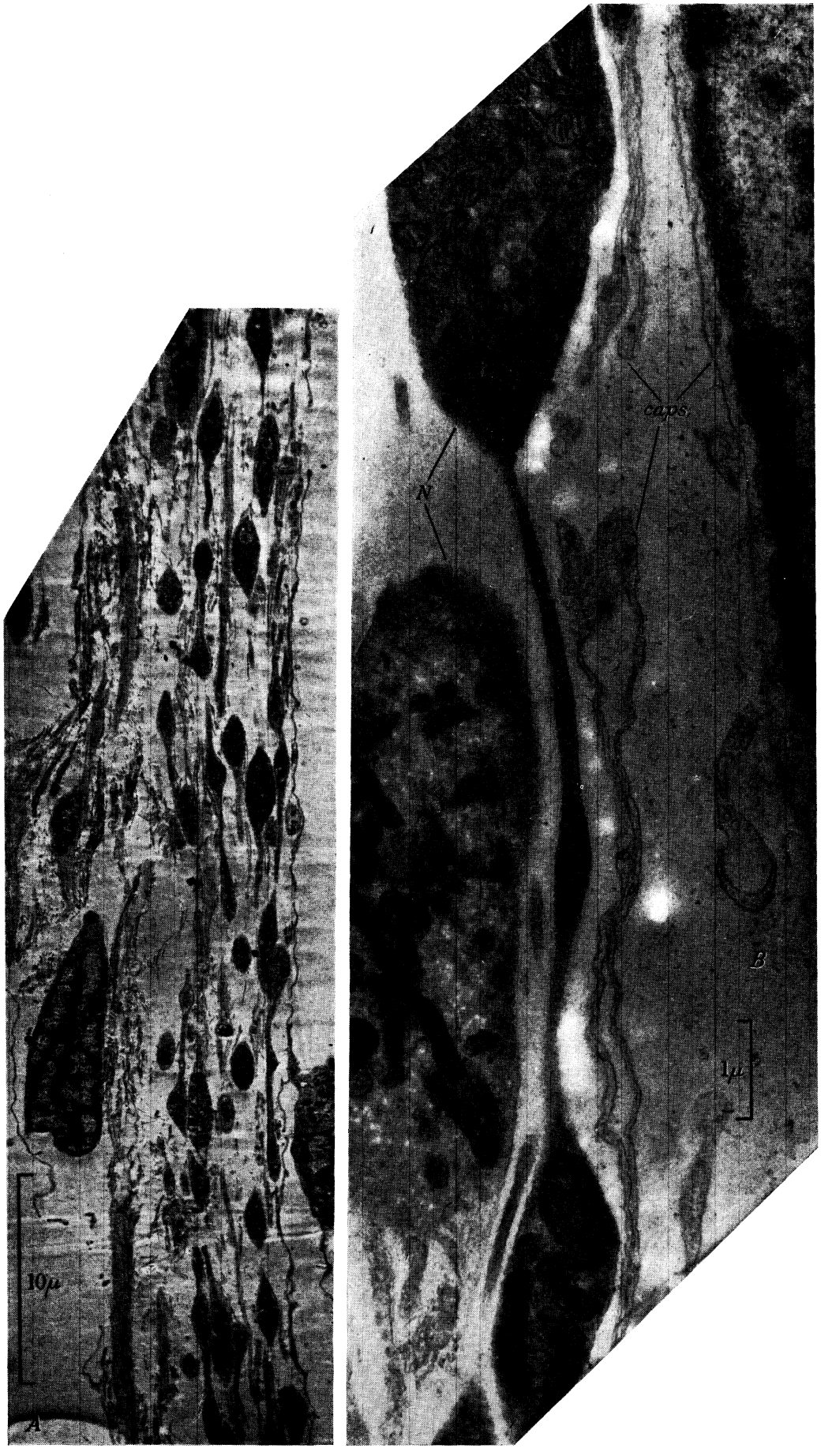

FIGURE 2. Electron micrographs of muscle spindles showing the 'varicose threads' at higher magnifications. These are longitudinal sections through the sensory region, made parallel to, and a few microns away from, the intrafusal muscle elements. Capsular cells and nuclei are also seen (e.g. *caps* in *B*); note the low density of the capsular cytoplasm compared with that of the nerve chains (*N*).

FIGURE 3. Same structures (terminal branches of sensory nerve) at higher magnification.

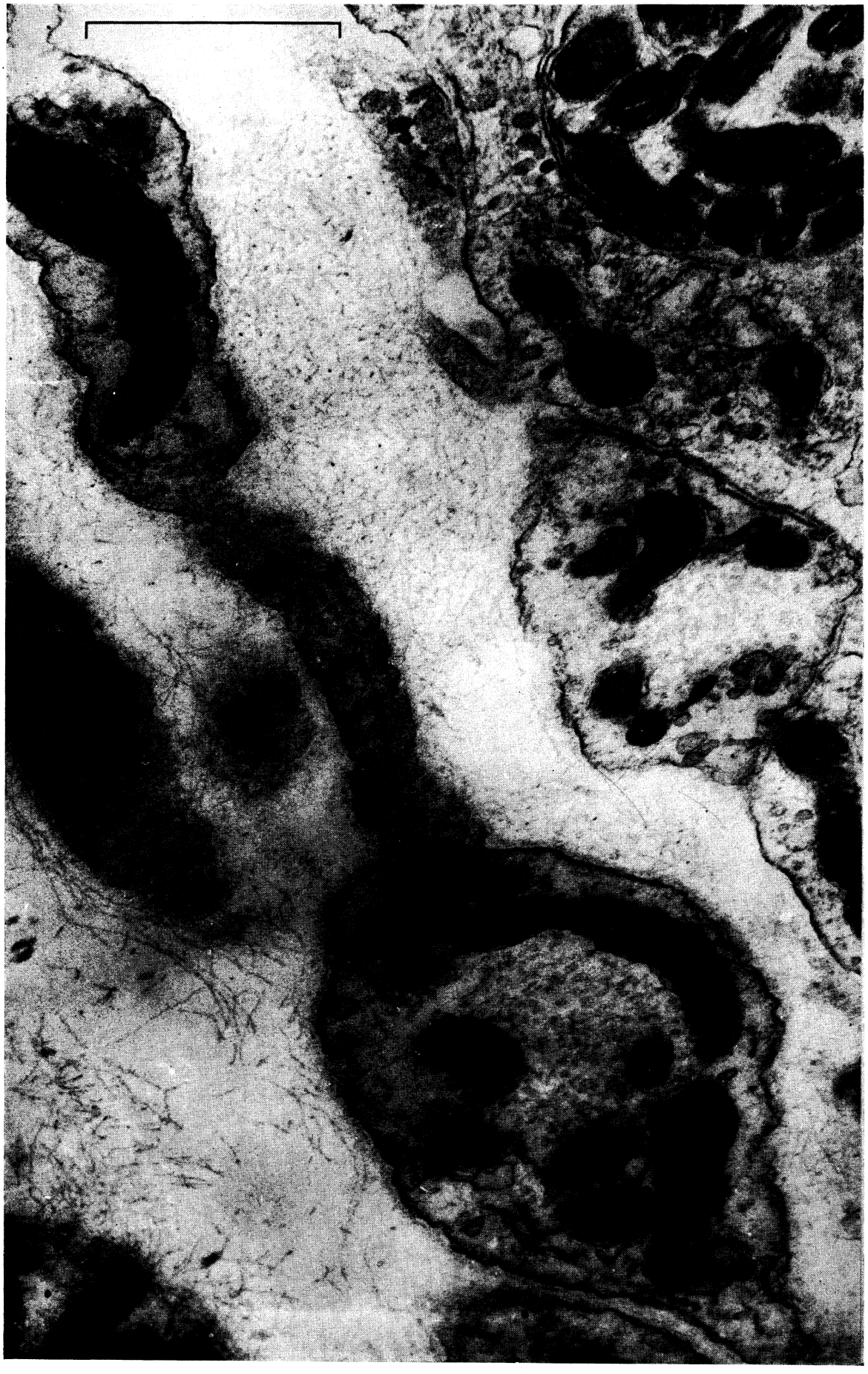

FIGURE 4. While the preceding pictures were from stretched muscles, this is from a slack (short) toe muscle.

FIGURE 5. Showing the relation of nerve 'bulbs' to the surface of the muscle fibre. *N*, Nerve bulbs, two of which are seen in contact with muscle fibre (*M*). Aspects of other nerve chains (including a point of branching) are shown in an adjacent lymphatic compartment, separated by a capsular layer (*C*).

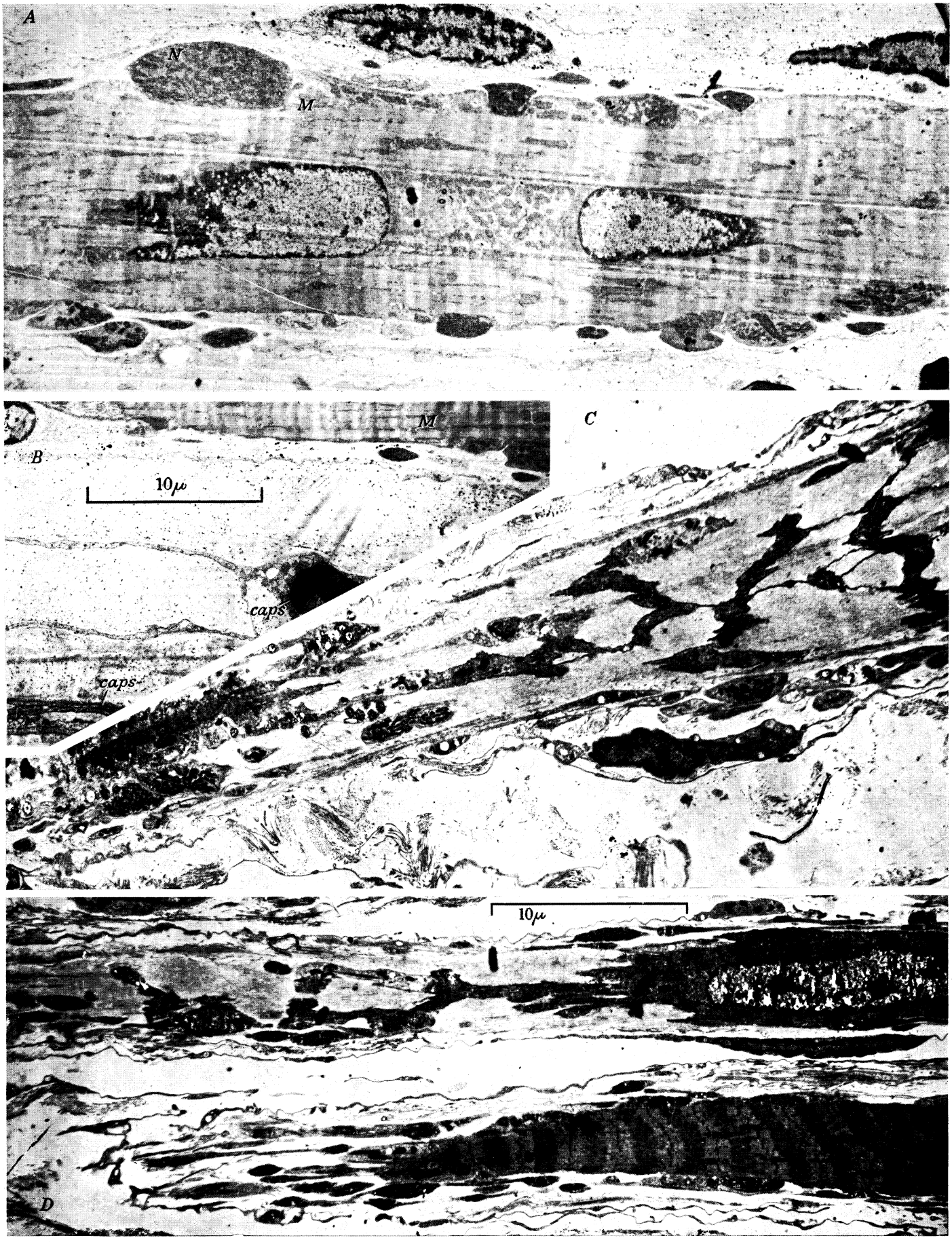

FIGURE 6. Longitudinal 'survey' pictures, showing (*A*) a 'compact' portion of the muscle fibre (*M*) invested with sensory contacts (e.g. *N*); (*B*) space between capsule and fibre; (*C*) a 'reticular' portion of a muscle fibre, its interstices being filled with a dense material (shown at higher magnification in figures 22, 25, plates 47 and 50); (*D*) two stretched intrafusal fibres, the upper being seen at the transition from 'compact' (right-hand portion) to 'reticular' zone.

FIGURE 7. Sensory contacts at higher magnification (scale 1 μ). *C*, capsule; *N*, nerve bulbs; *M*, muscle fibre. In the right-hand part, the sensory nerve bulb labelled *N* in figure 6*A* is shown enlarged. It contains a mass of small mitochondria interspersed with a random network of tubules. This is in striking contrast with the accumulation of vesicles found in motor nerve endings (e.g. figure 32, plate 57).

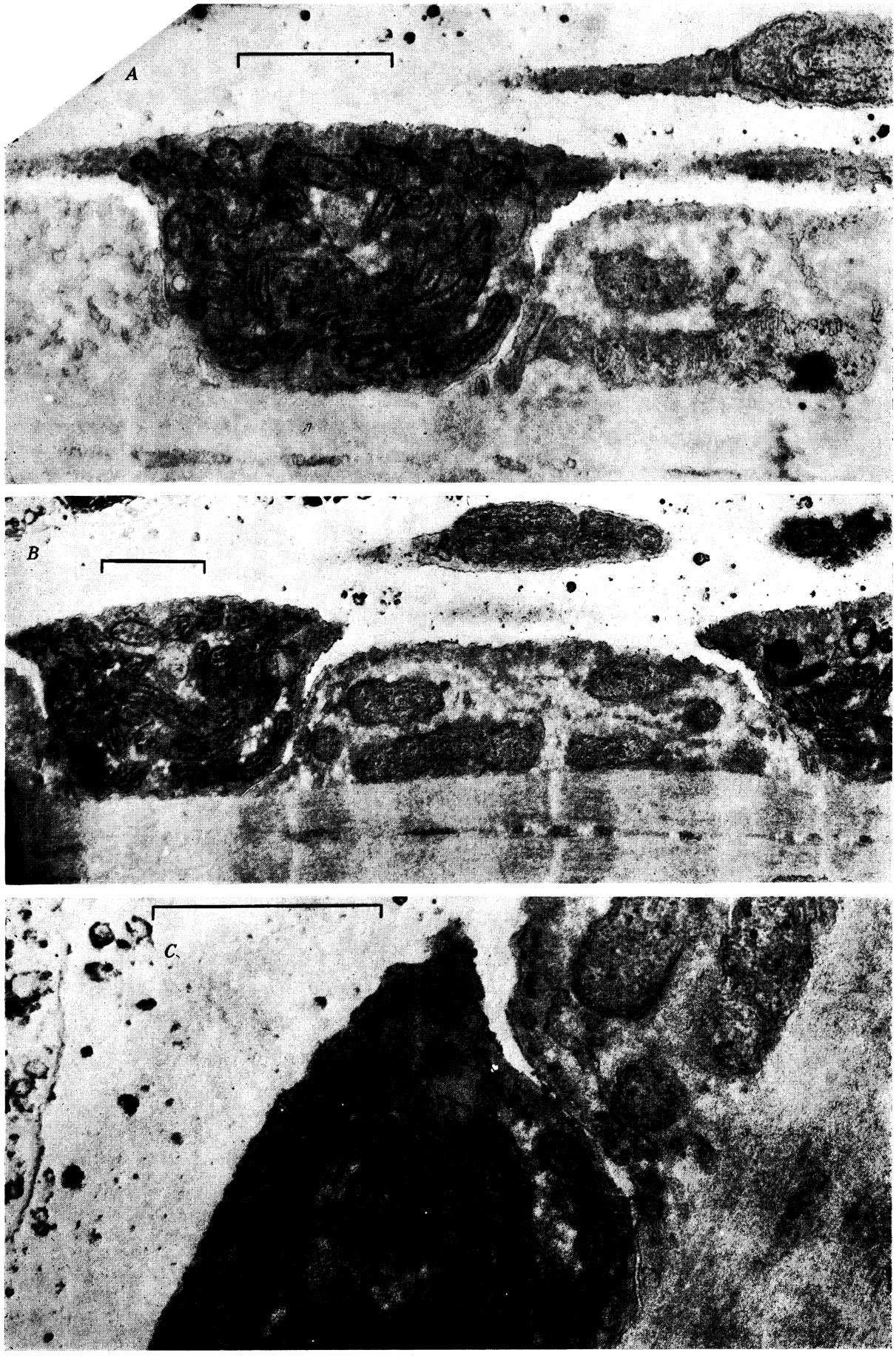

FIGURE 8. More sensory contacts (cf. survey picture in figure 6*A*).
B and *C* are from the same section, but serial with *A*.

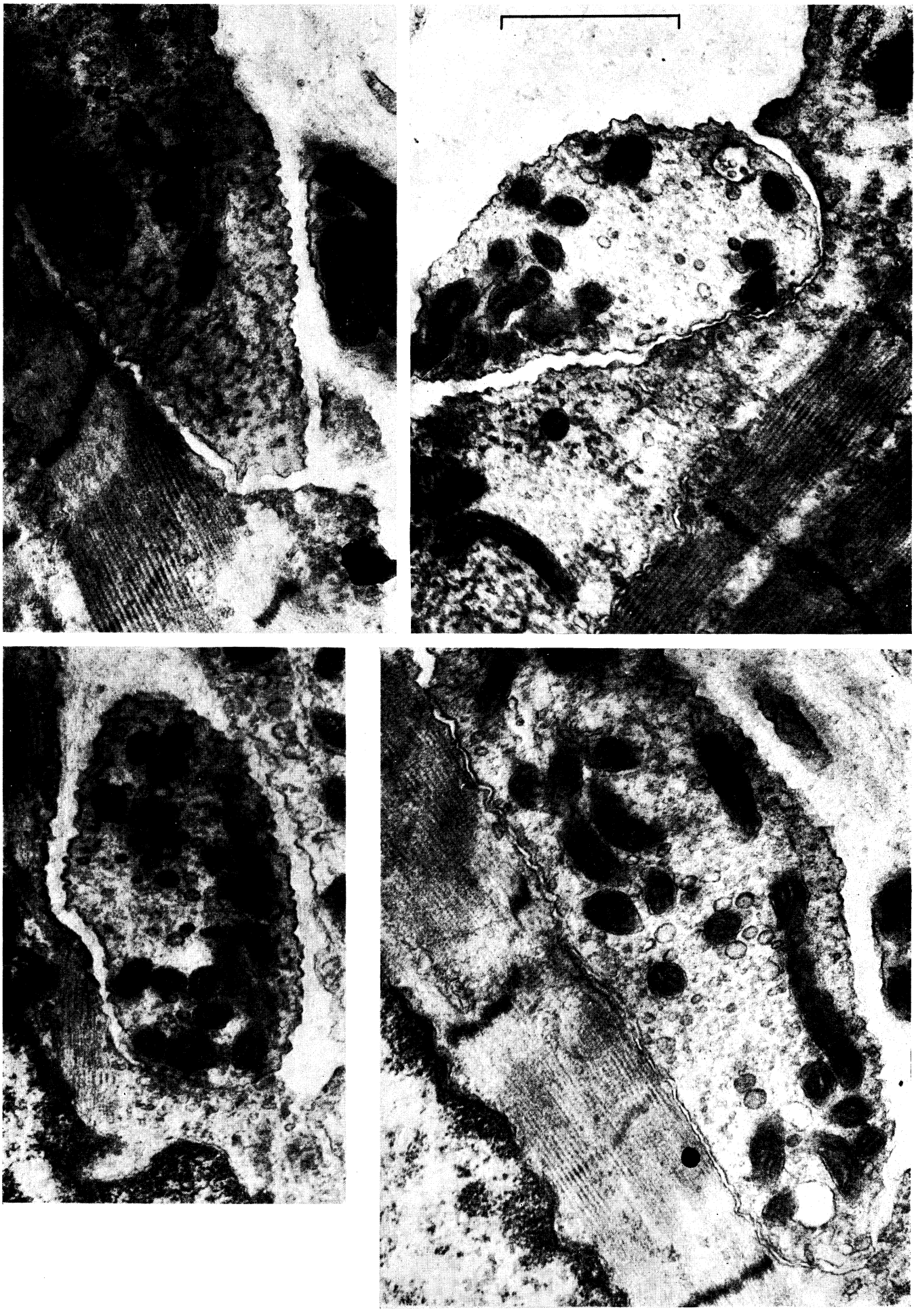

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FIGURE 10. Muscle was slackened off before fixation. Sensory contact. (Longitudinal section, grazing the muscle surface in a reticular zone.)

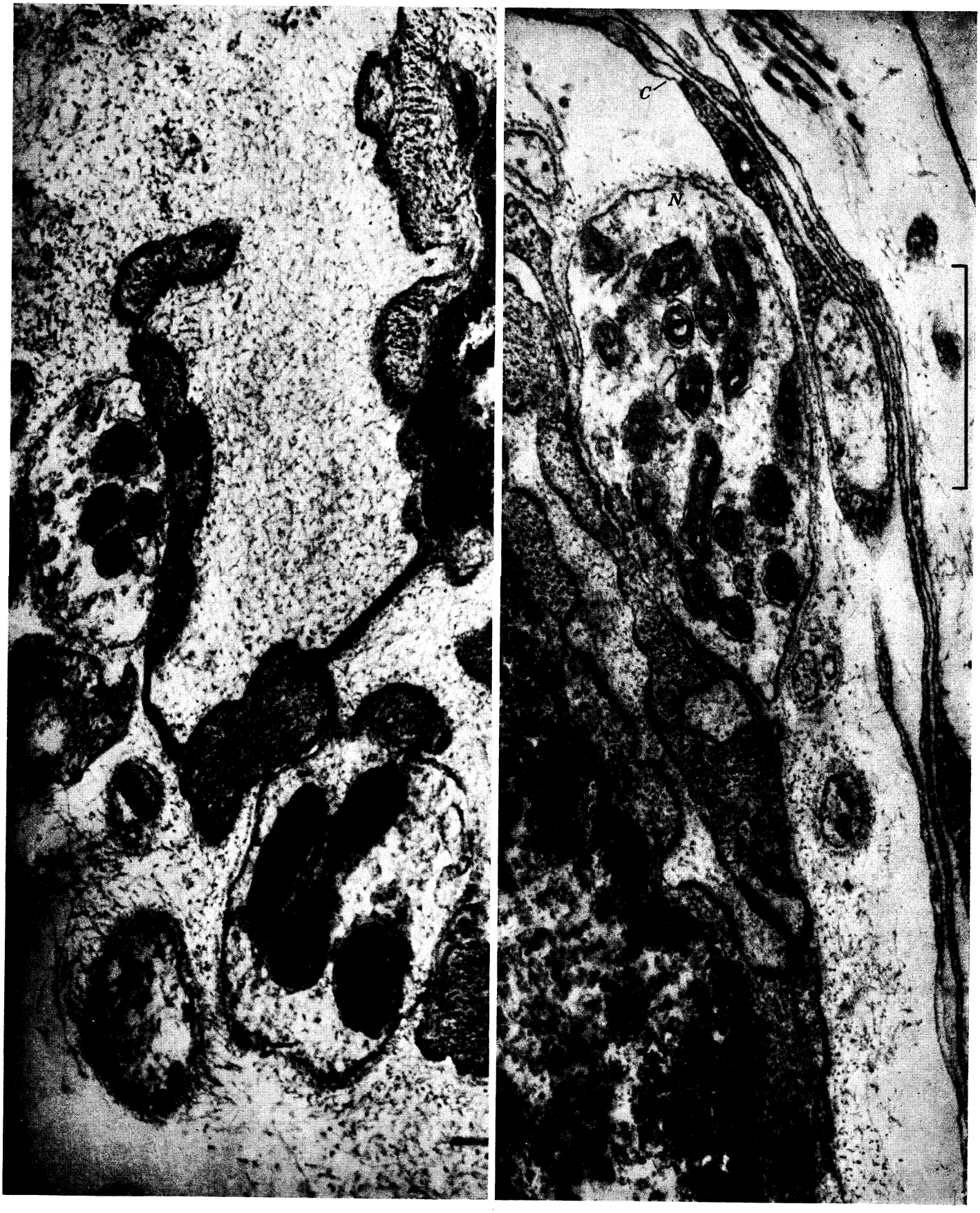


FIGURE 11. Sensory contacts in transverse section through a reticular zone.
C, capsule; *N*, nerve bulb.

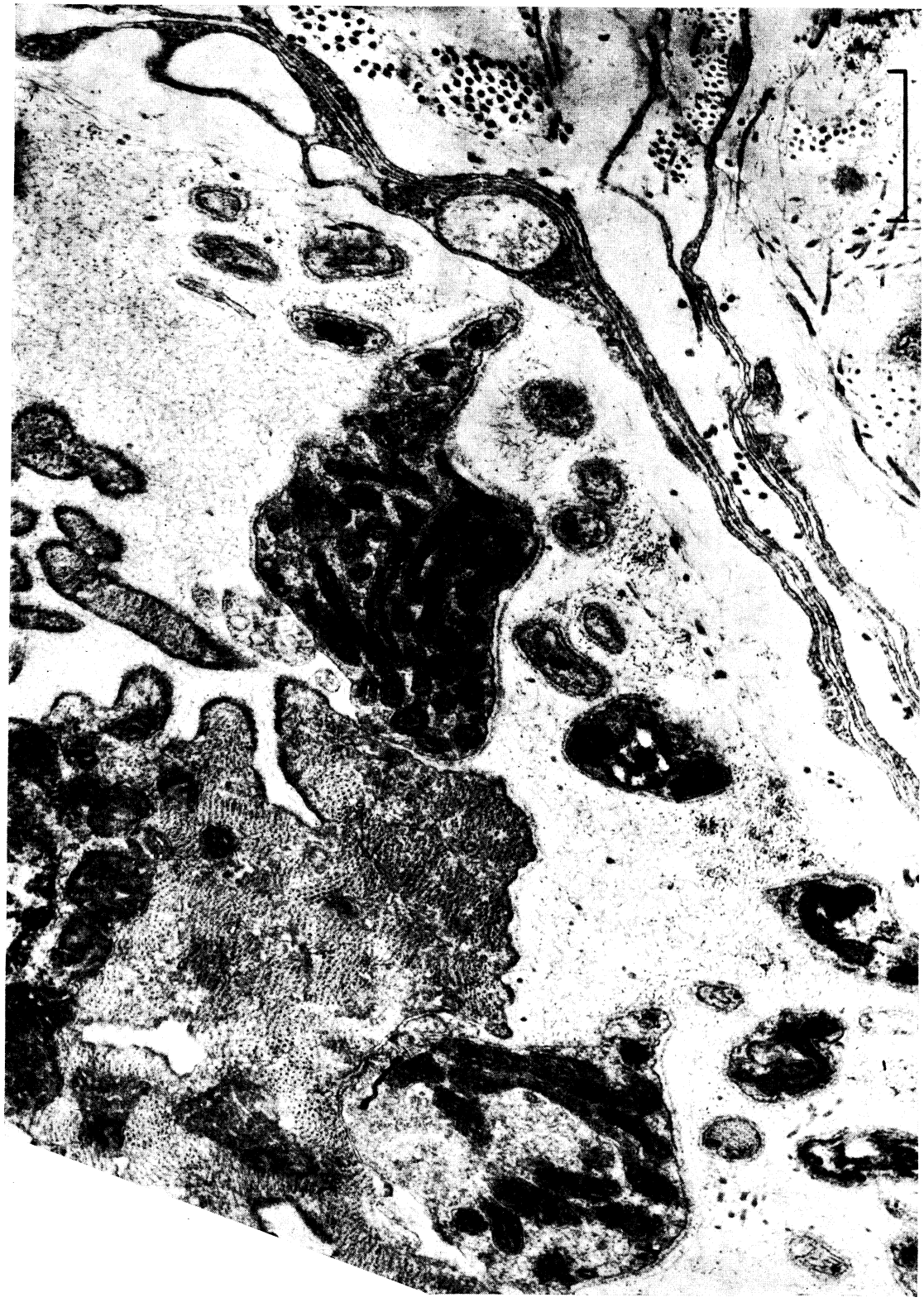


FIGURE 12. Transverse section of sensory contacts.

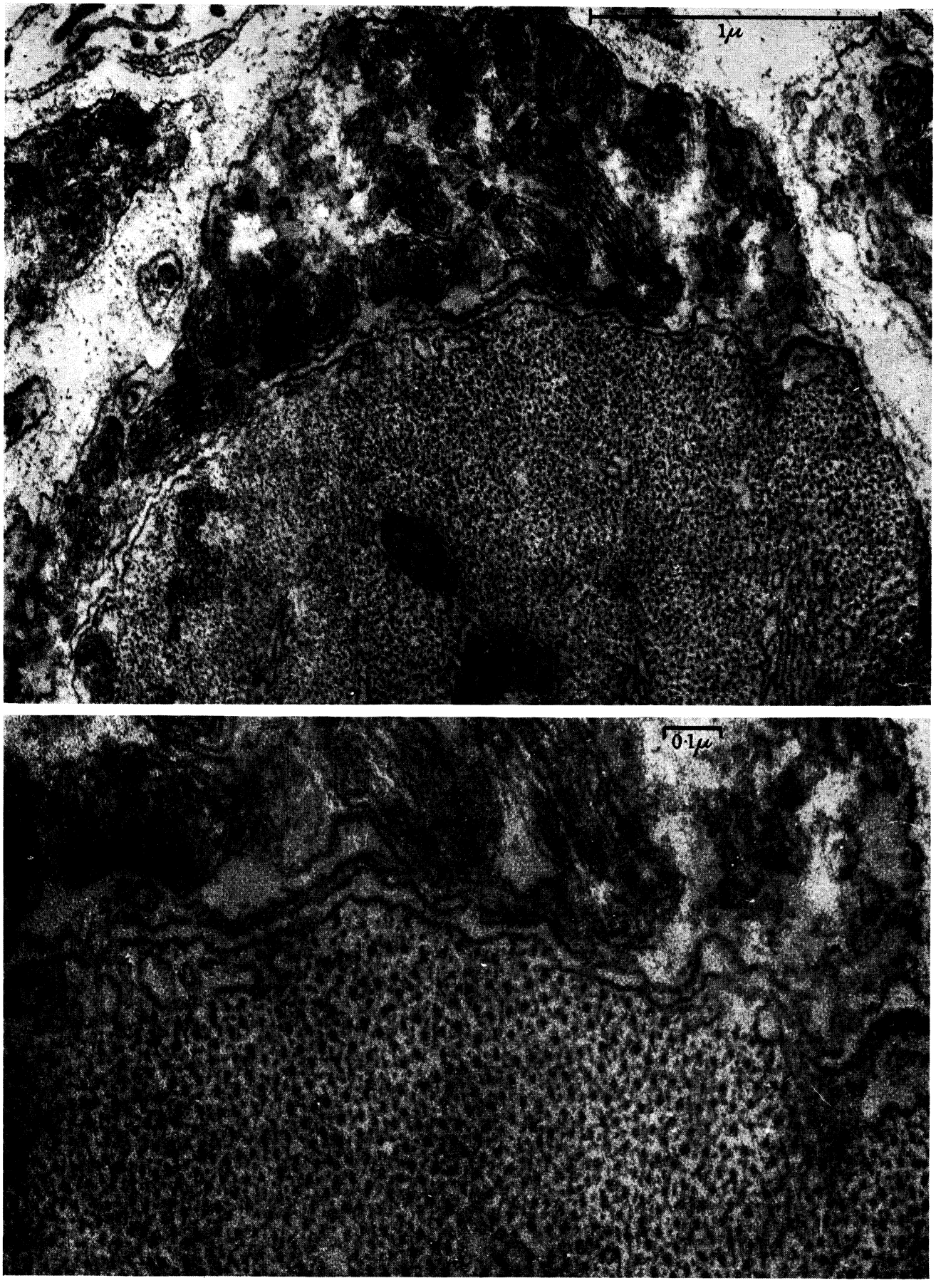


FIGURE 13. Sensory contact, showing 'bridges' across the intercellular gap.

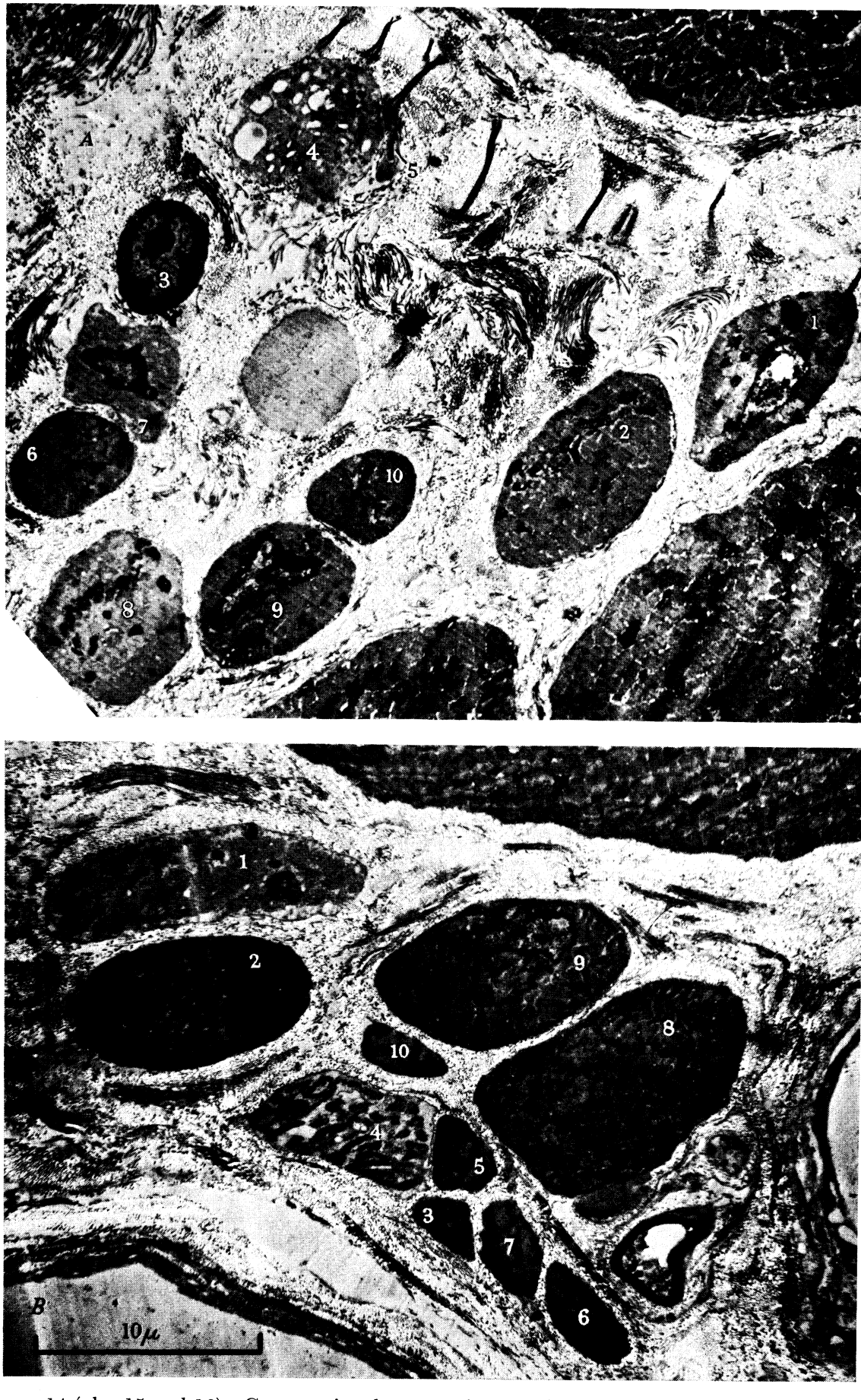


FIGURE 14 (also 15 and 16). Cross-sectional survey pictures, showing group of 10 intrafusal muscle fibres well away from their sensory contact region ('extra-capsular'). Level of section: *A*, 382 μ; *B*, 1120 μ.

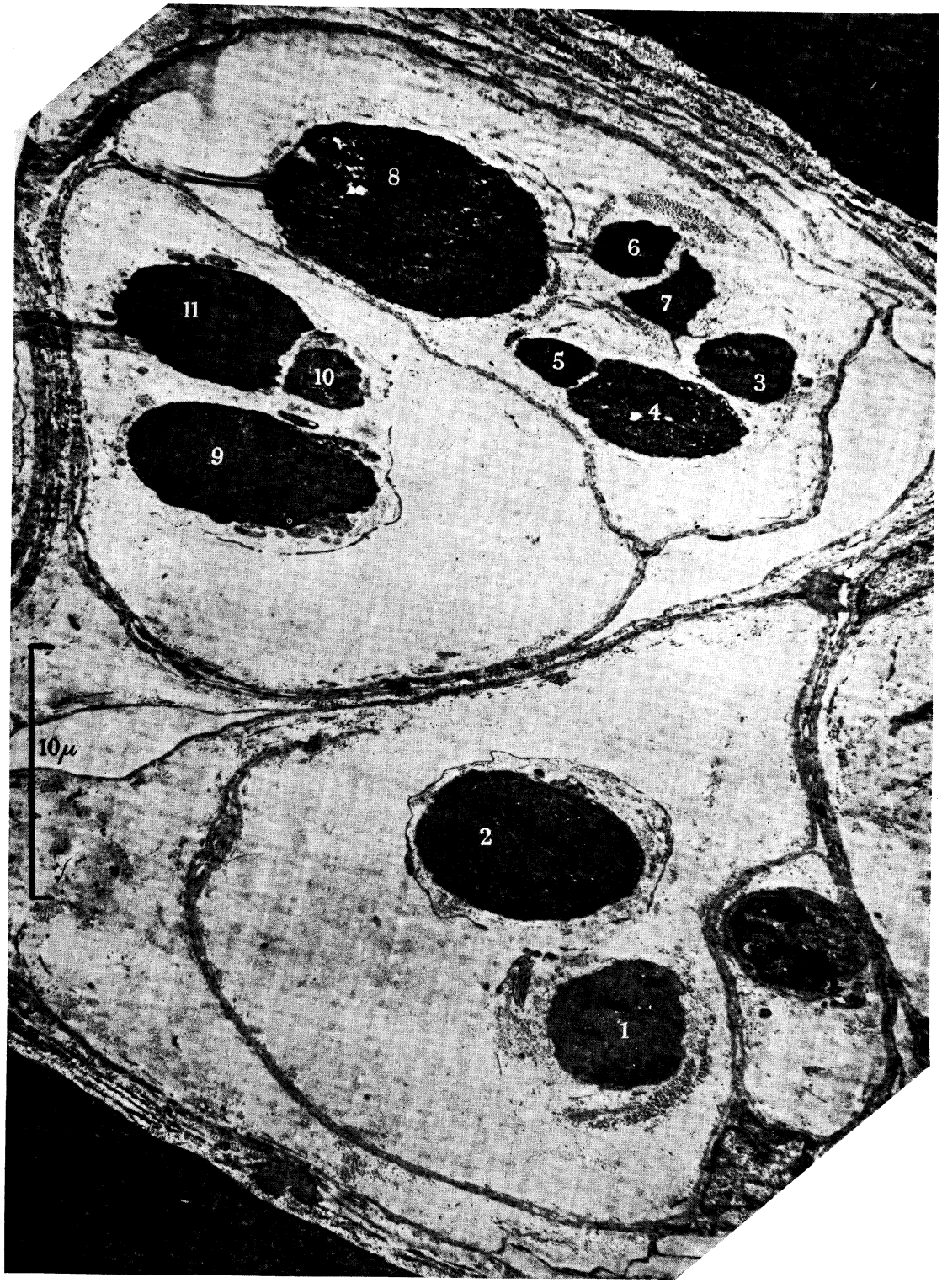


FIGURE 15. Following the group of fibres into the capsular region. Level, 1717 μ. *Note*: uncertainty of identification occurred at a level of 1300 to 1400 μ, so that the numbering of fibres in figures 15 *et seq.* may not entirely correspond to that in figure 14.

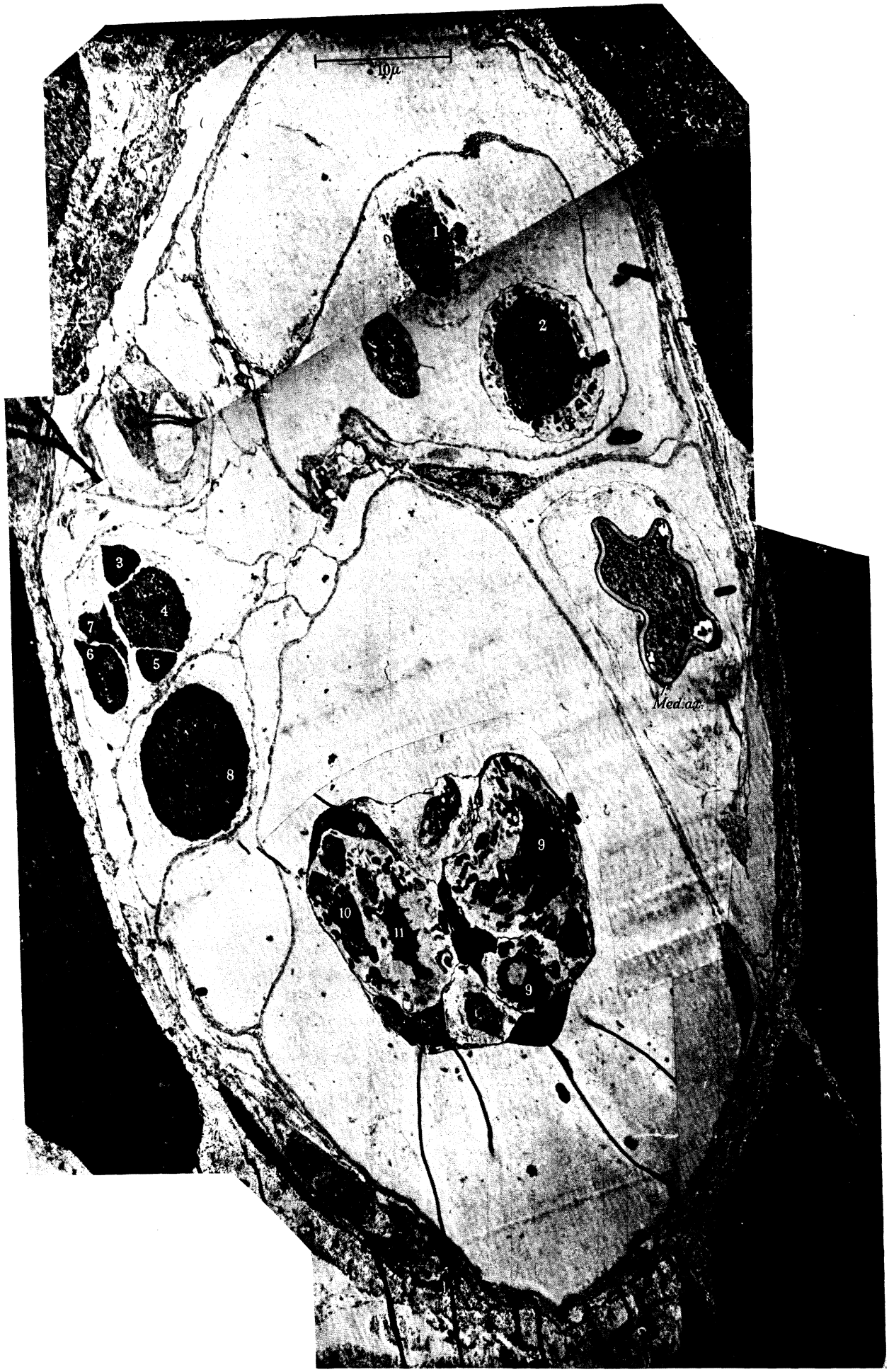


FIGURE 16. Near the centre of the capsular region. Level, 1872 μ. *Med. ax.*, medullated sensory axon.

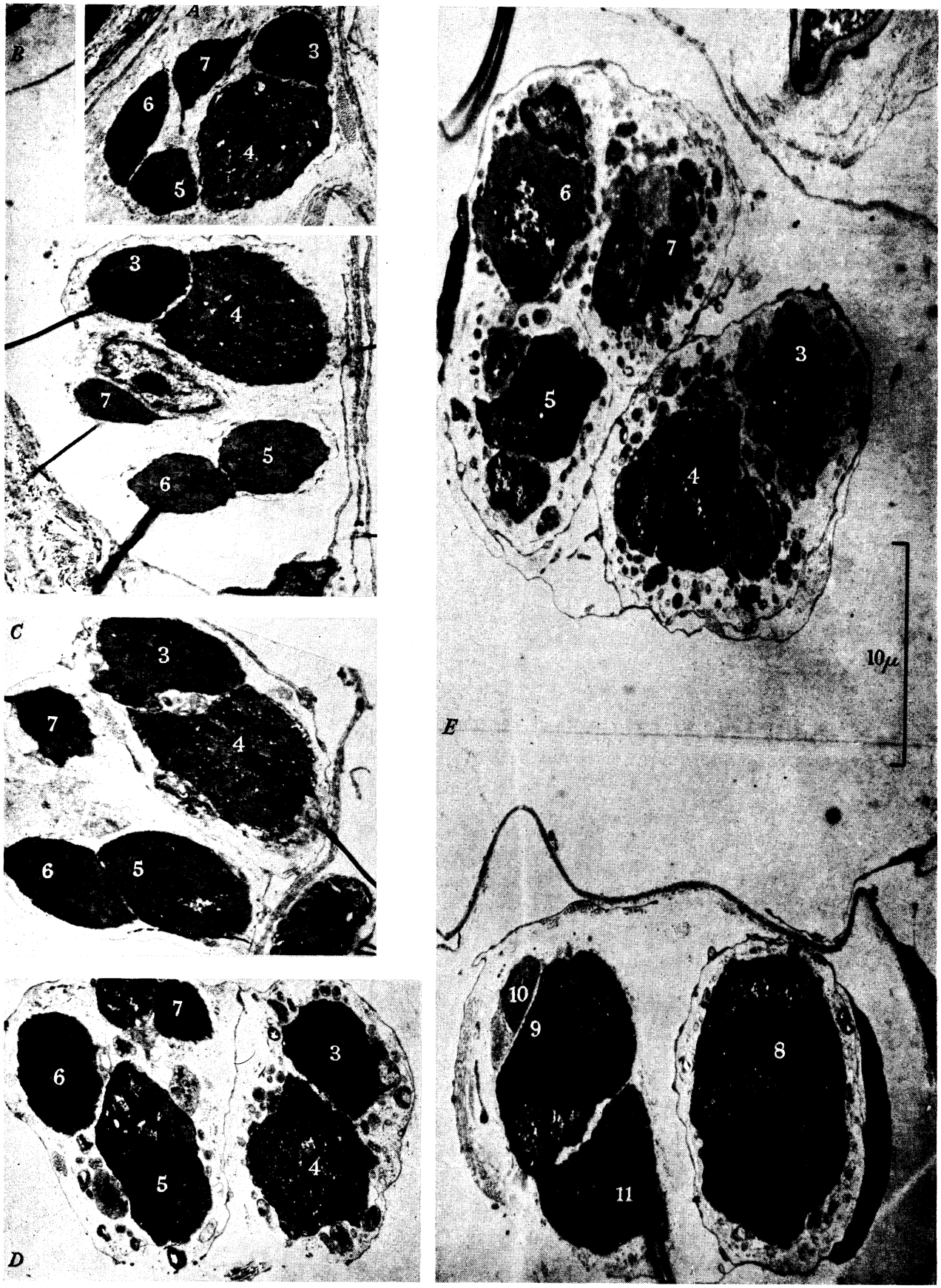


FIGURE 17 (cf. 18). Following one 'subdivision' of this group.
Levels: *A*, 1969 μ; *B*, 2322 μ; *C*, 2387 μ; *D*, 2460 μ; *E*, 2577 μ.

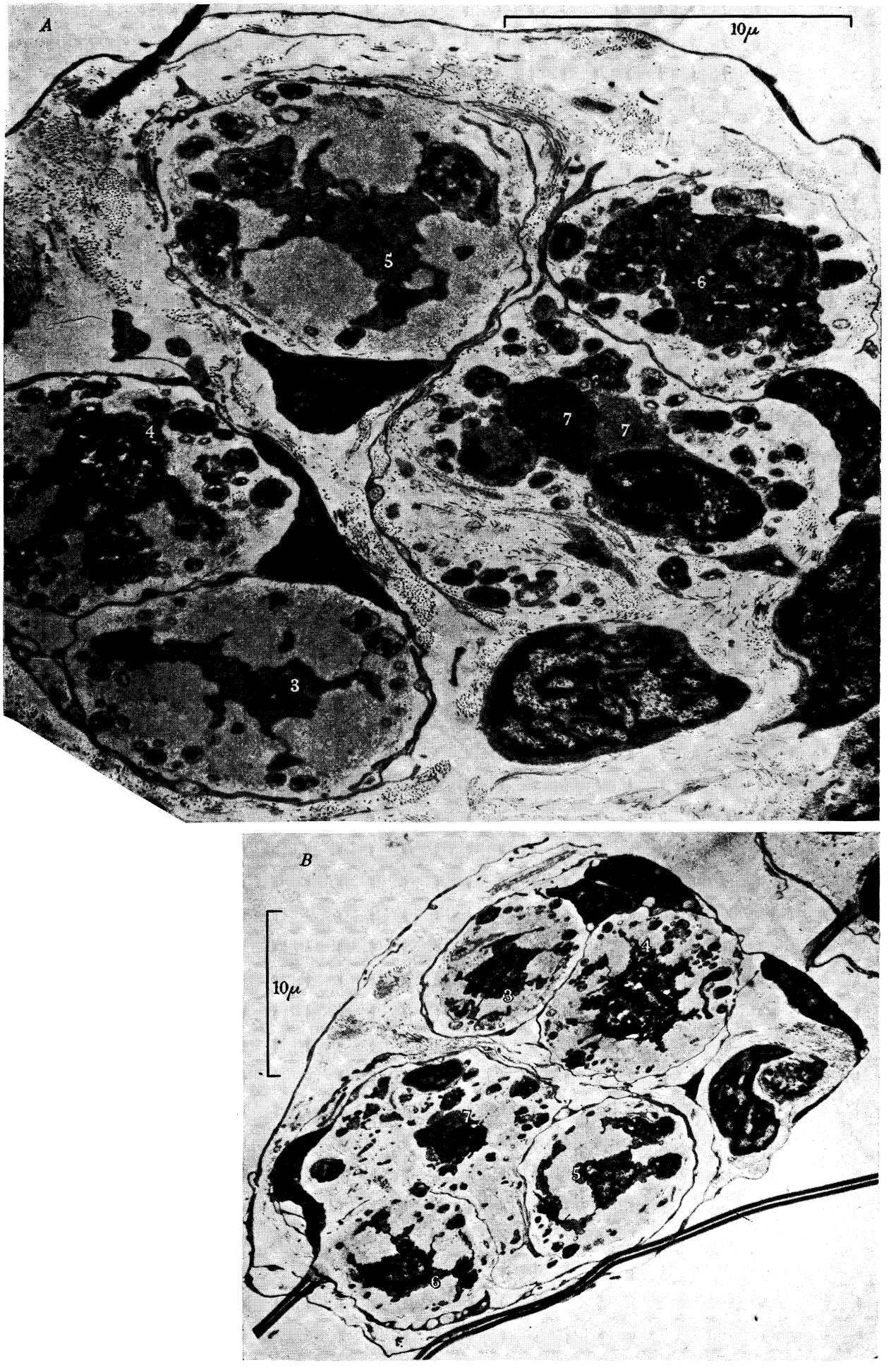


FIGURE 18. Continuing from figure 17. Levels: *A*, 2628 μ; *B*, 2658 μ.

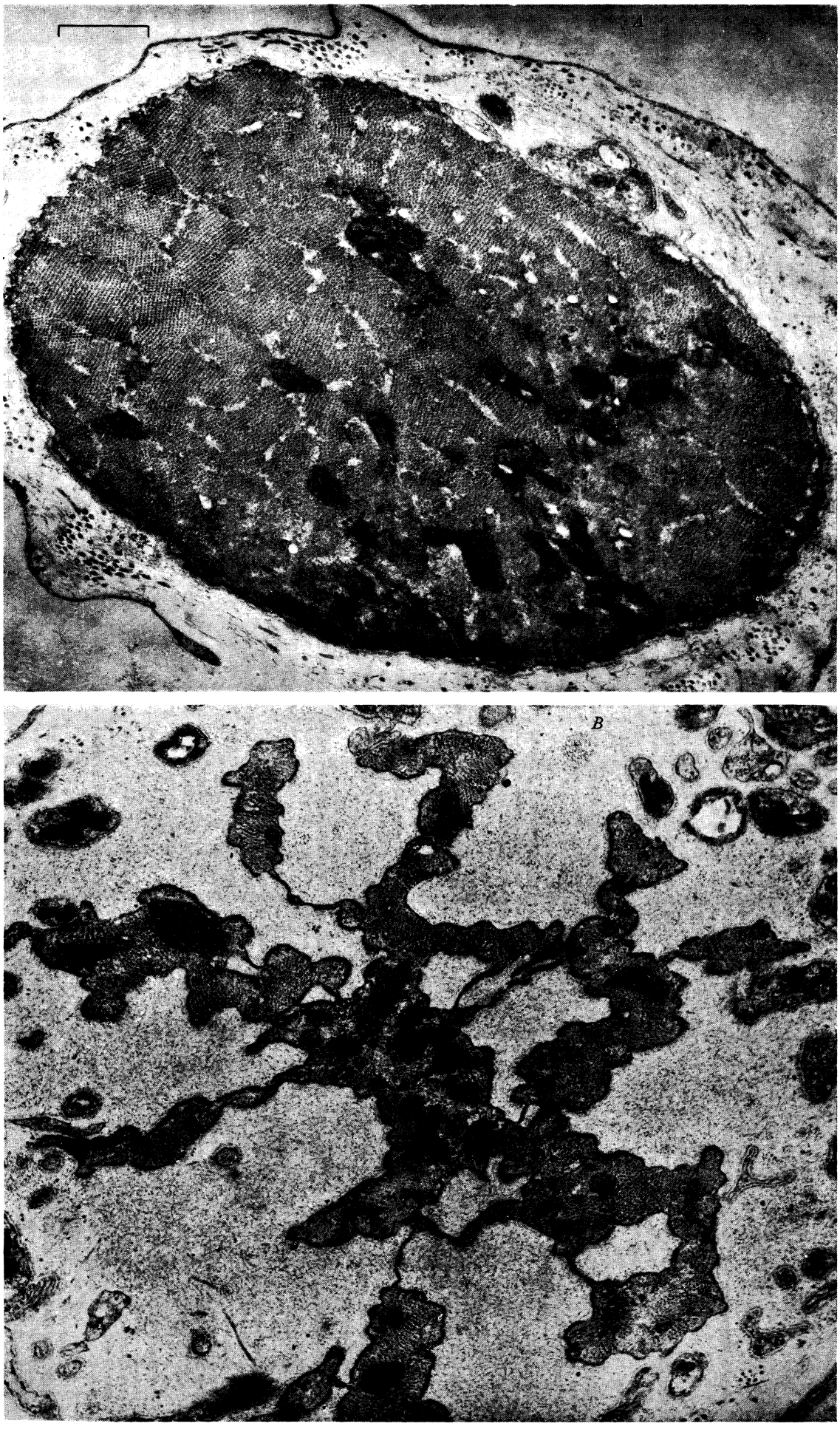


FIGURE 19. Cross-sections of the same intrafusal fibre (no. 2).
A, at entry into capsular region; *B*, in the 'reticular' zone.

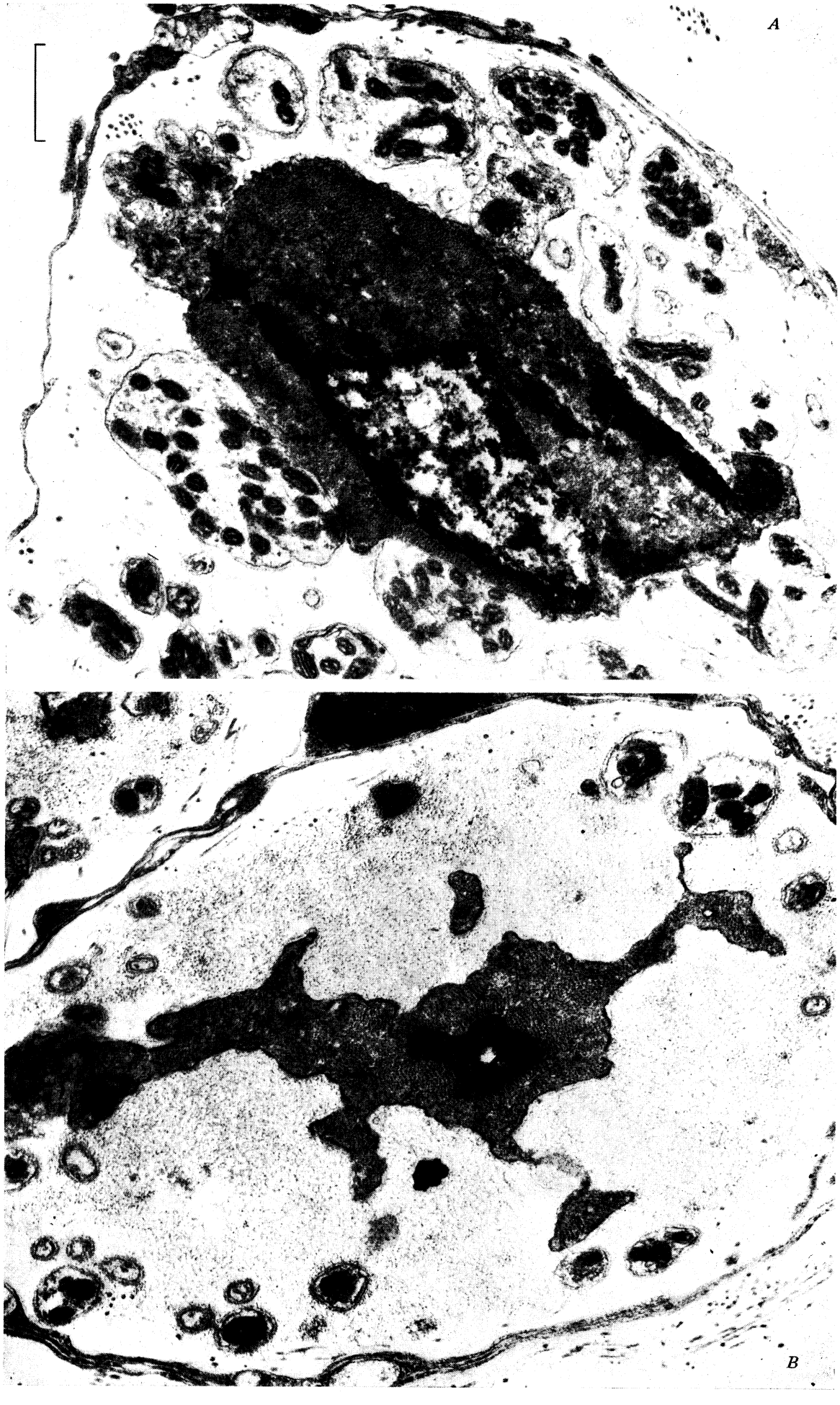


FIGURE 20. Another fibre (no. 3) in the 'compact' (*A*), and 'reticular' (*B*), zones of sensory contacts.

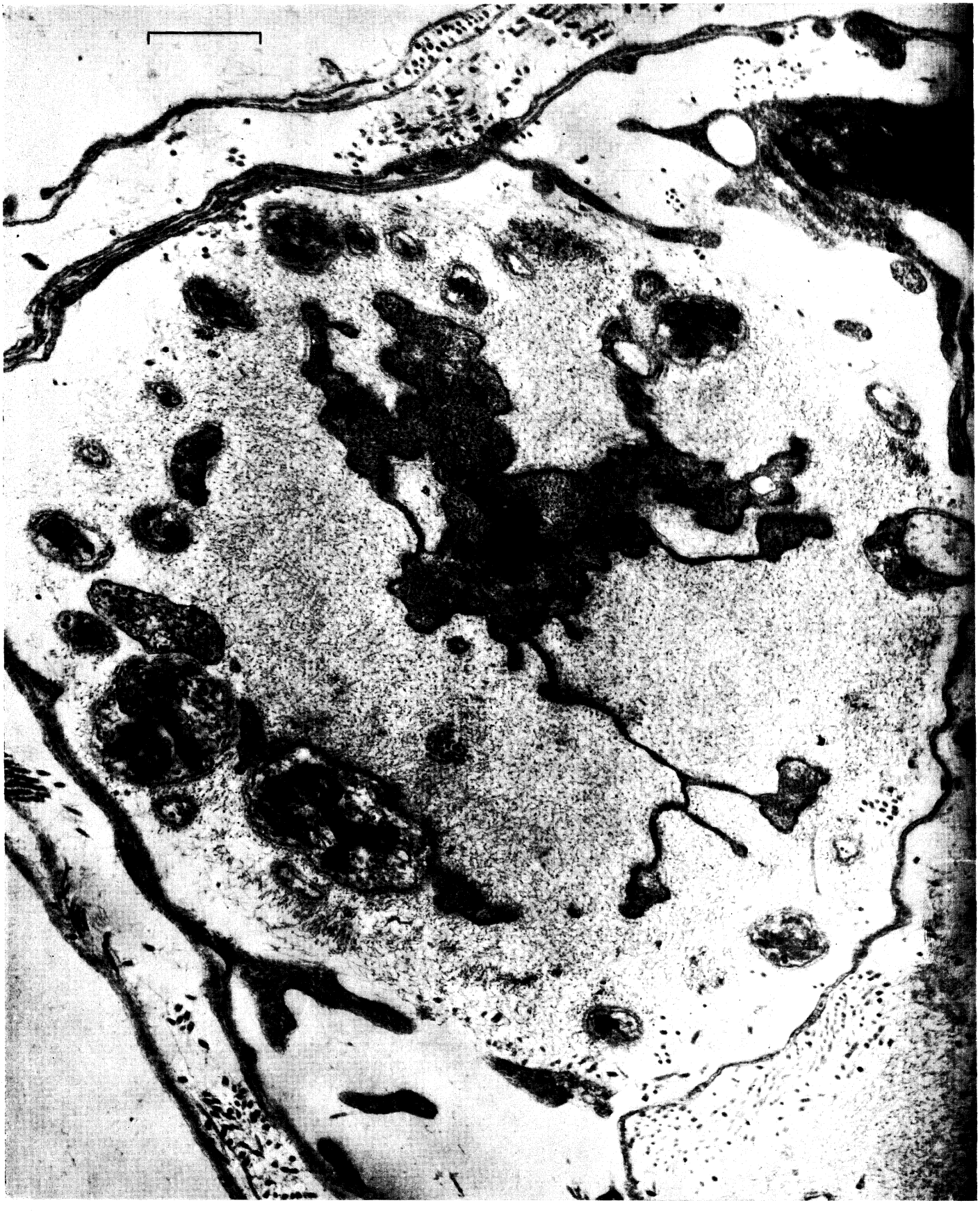


FIGURE 21. Transverse section through 'reticular' zone.

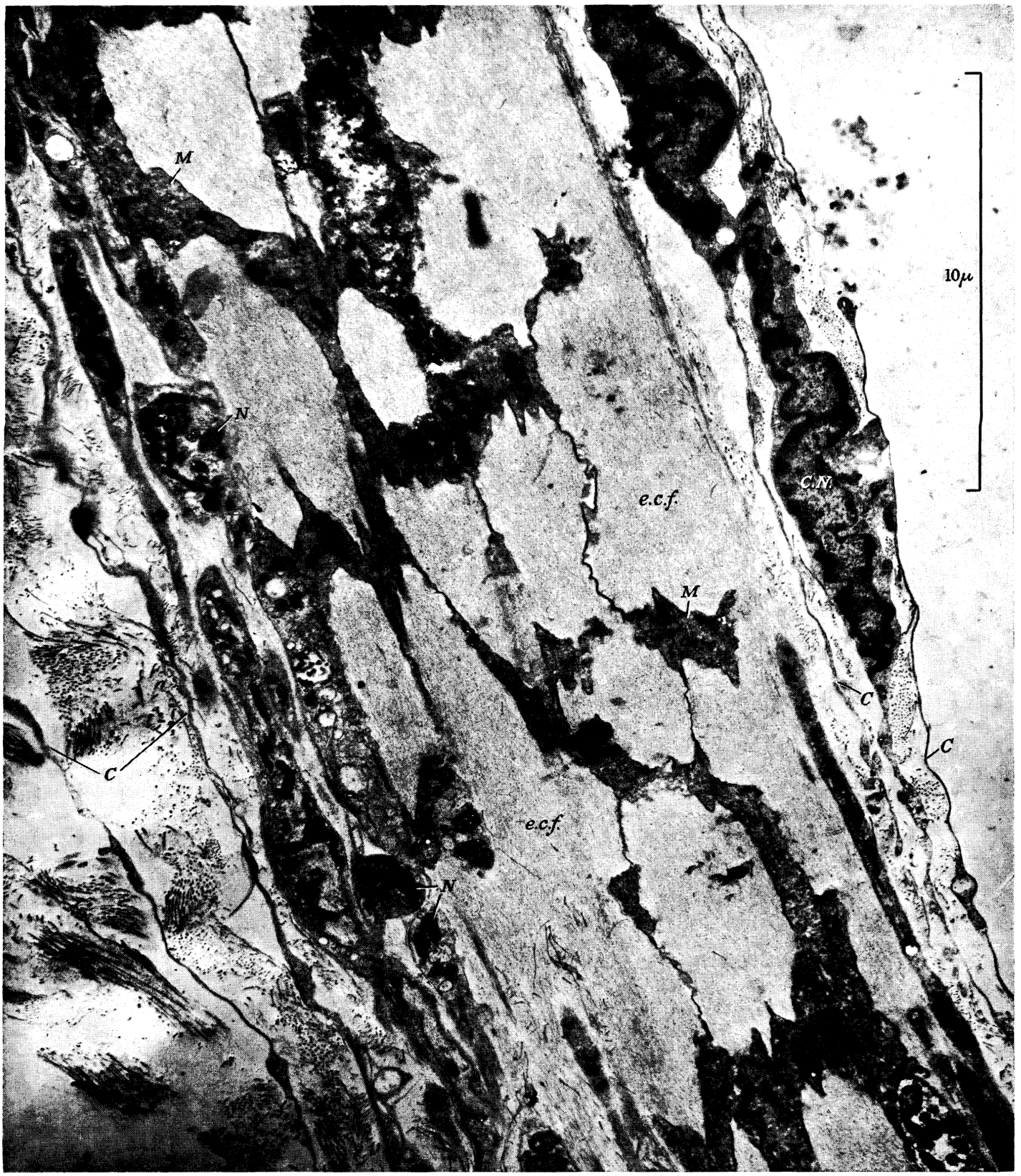


FIGURE 22. Longitudinal section through 'reticular' zone. *C*, capsule cells, with capsular nuclei (*C.N.*). *N*, nerve bulbs; *M*, portions of the fenestrated muscle fibre cut obliquely. The fibre surface forms a complicated framework which is embedded in a dense mesh of extracellular fibrils (*e.c.f.*).

FIGURE 23. Longitudinal section through nuclear part of a 'reticular' zone. *N*, nerve chain; *M*, muscle fibre; *M.N.*, muscle nucleus.

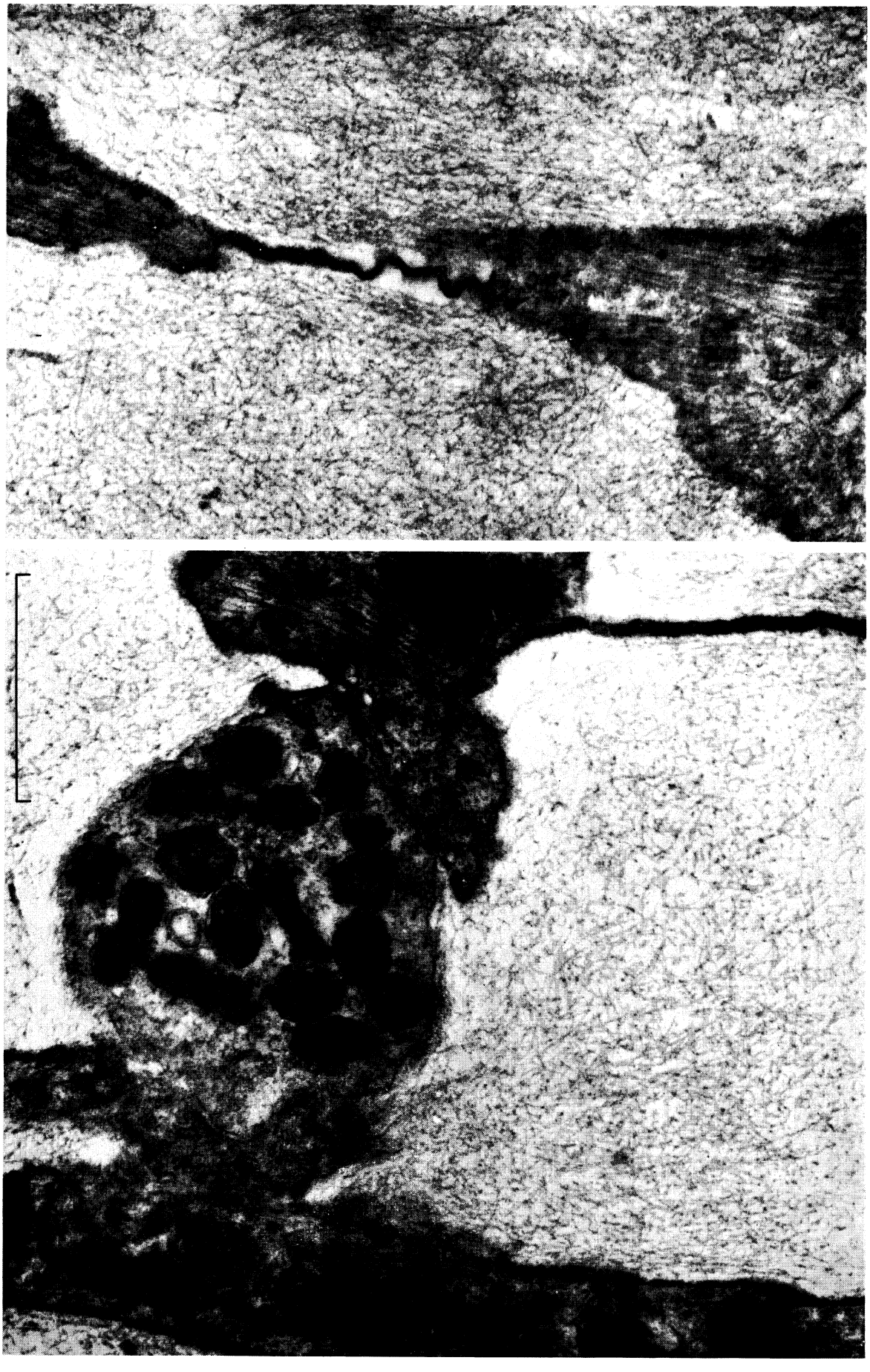


FIGURE 24. Higher magnification of connective tissue network in 'reticular' zone.

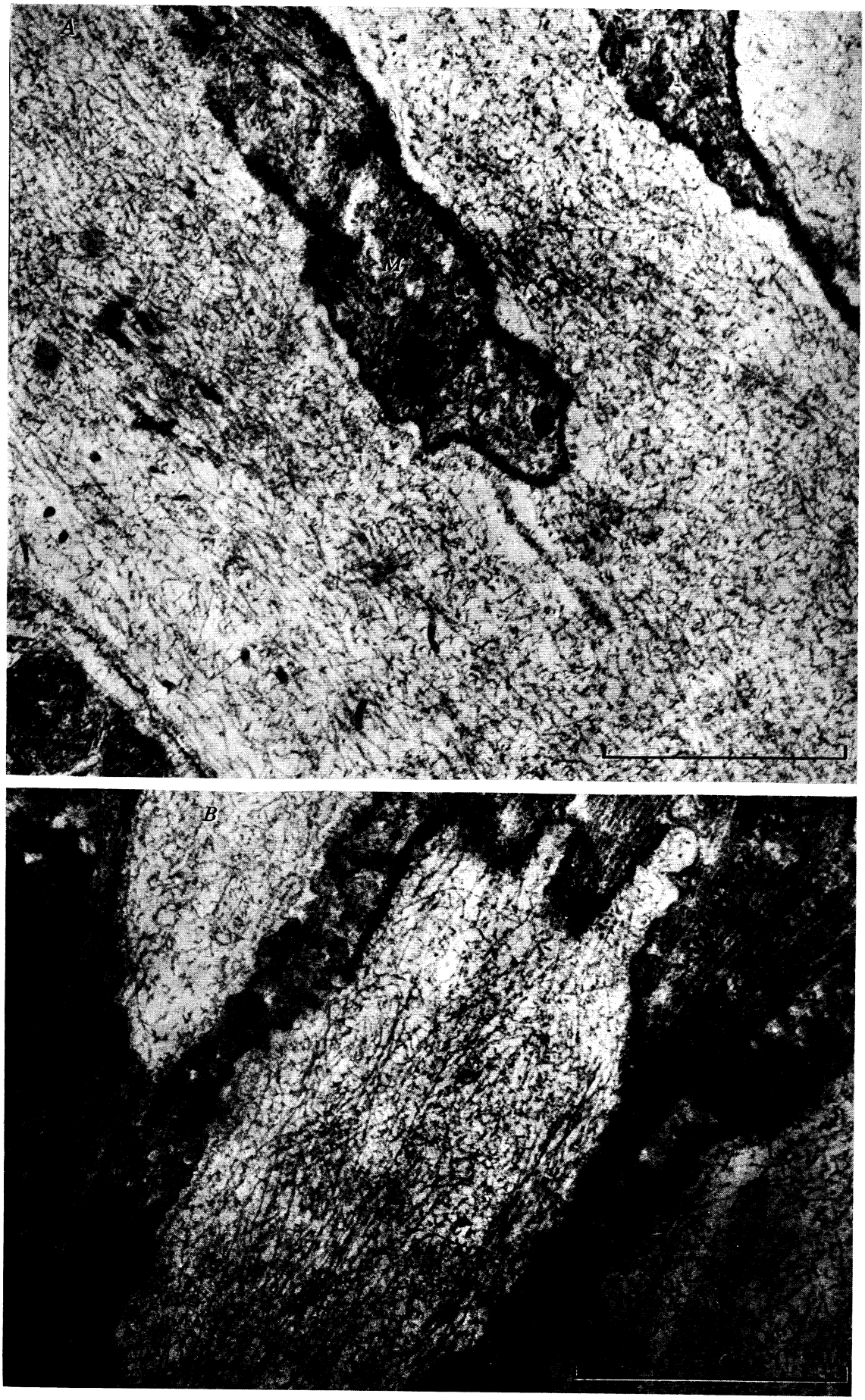


FIGURE 25. Extracellular network in 'reticular' zone. Longitudinal sections. In *A*, note trace of 'ectolemma' at the surface of the muscle fibre (*M*) and its relation to the extracellular network.

FIGURE 26. 'Microladder' in intrafusal fibres. *A*, from a 'reticular' zone. *B*, survey picture from a 'compact' zone.

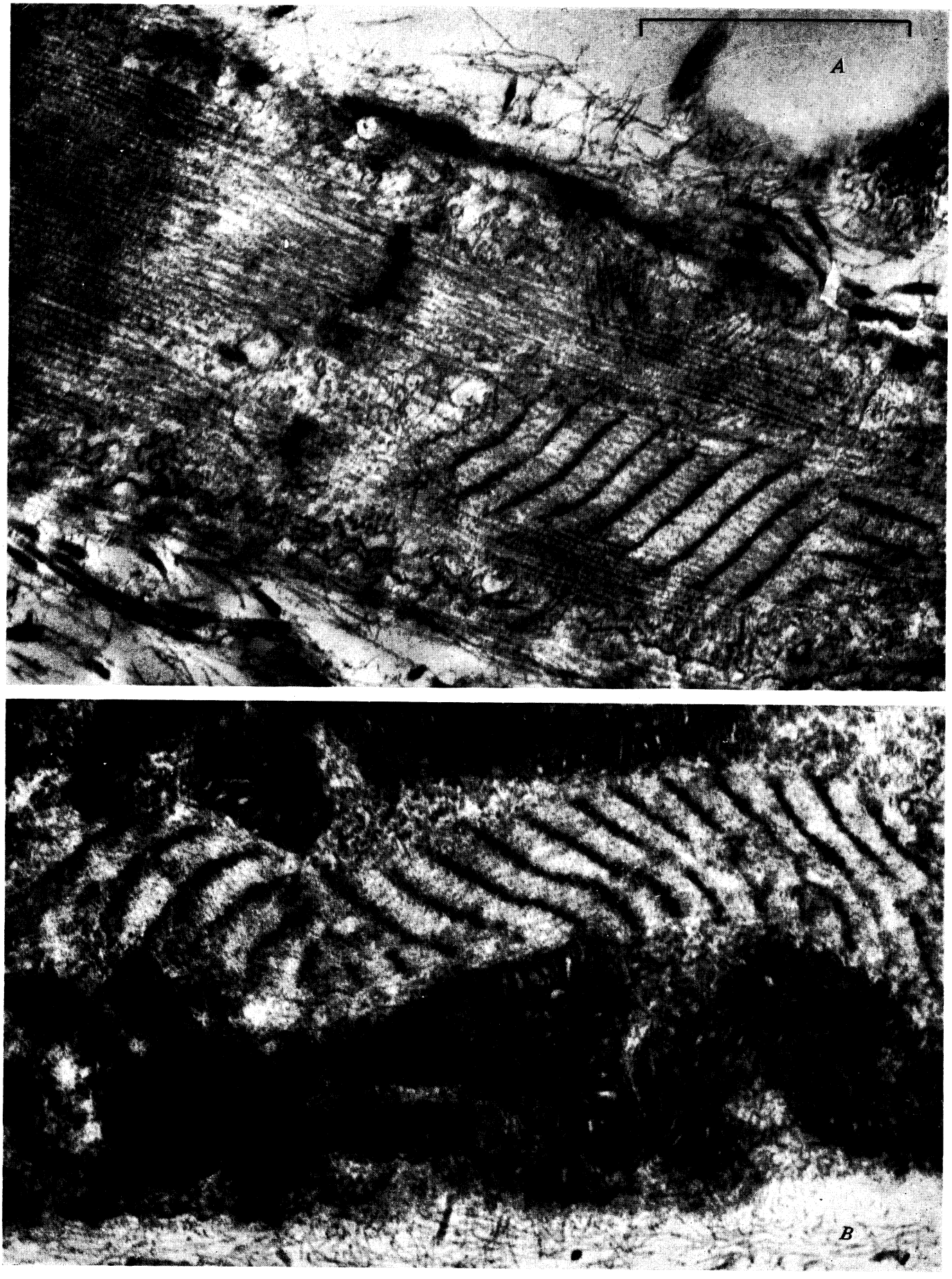


FIGURE 27. 'Ladders' in stretched (*A*) and slack (*B*) muscle.

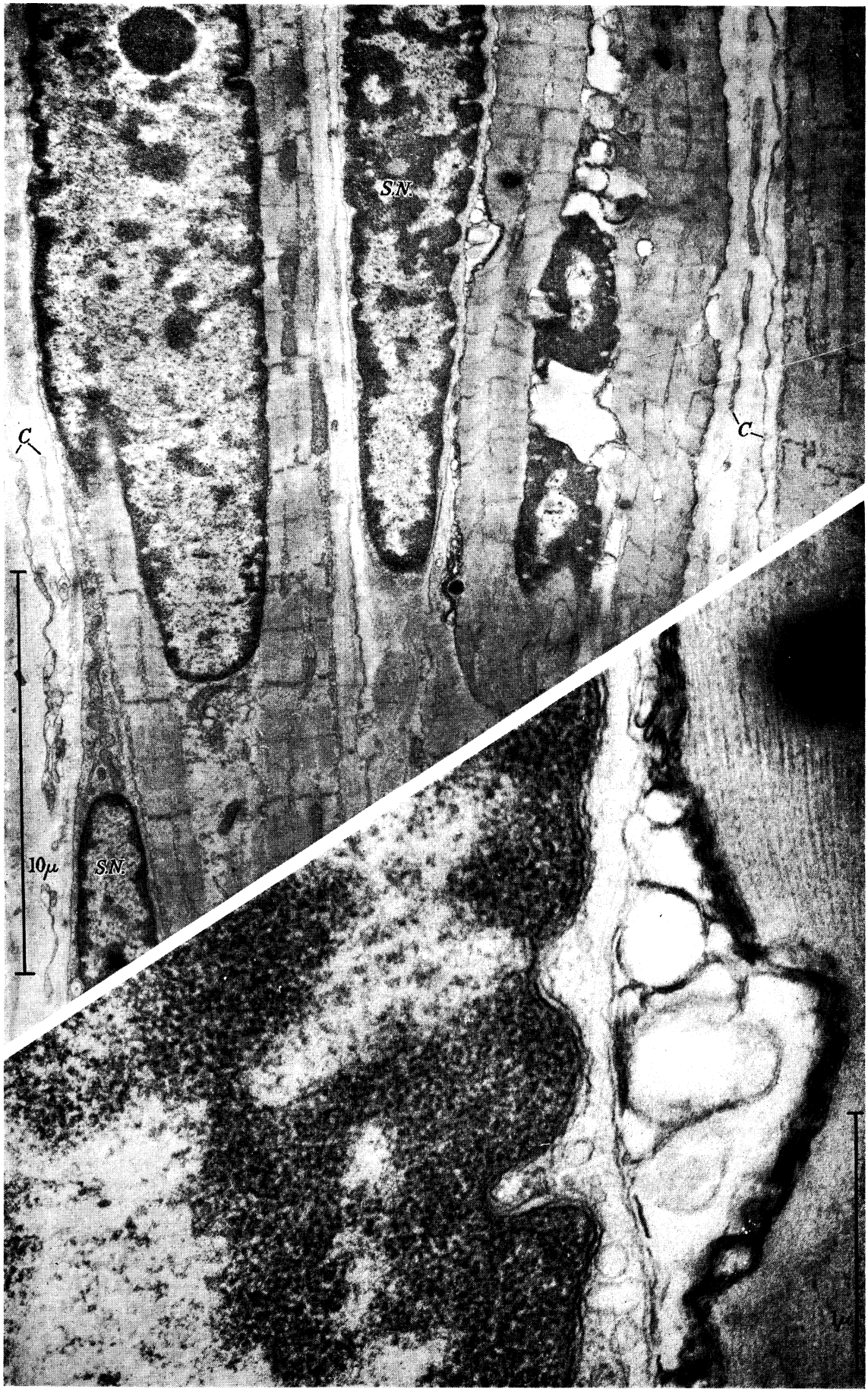


FIGURE 28. Denervated muscle, about 2 months after sciatic section. No sensory contacts are left, only occasional remnants as shown in this picture. *S.N.*, satellite nucleus. *C*, capsule cells.

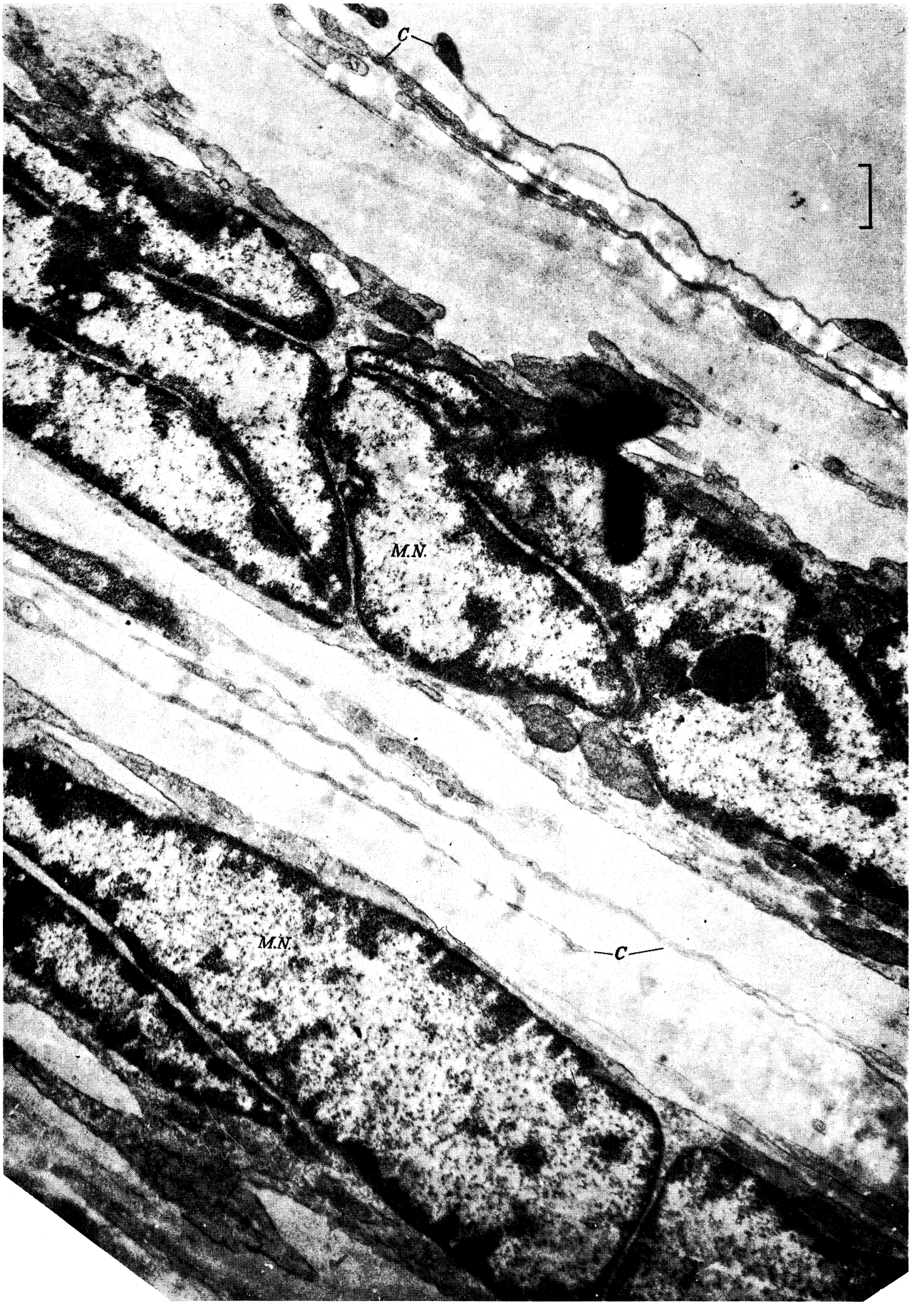


FIGURE 29. Denervated spindle. Nuclear part of 'reticular' zone. No 'micro-spindles' or sensory contacts are found. *C*, capsule cells. *M.N.*, muscle nuclei.

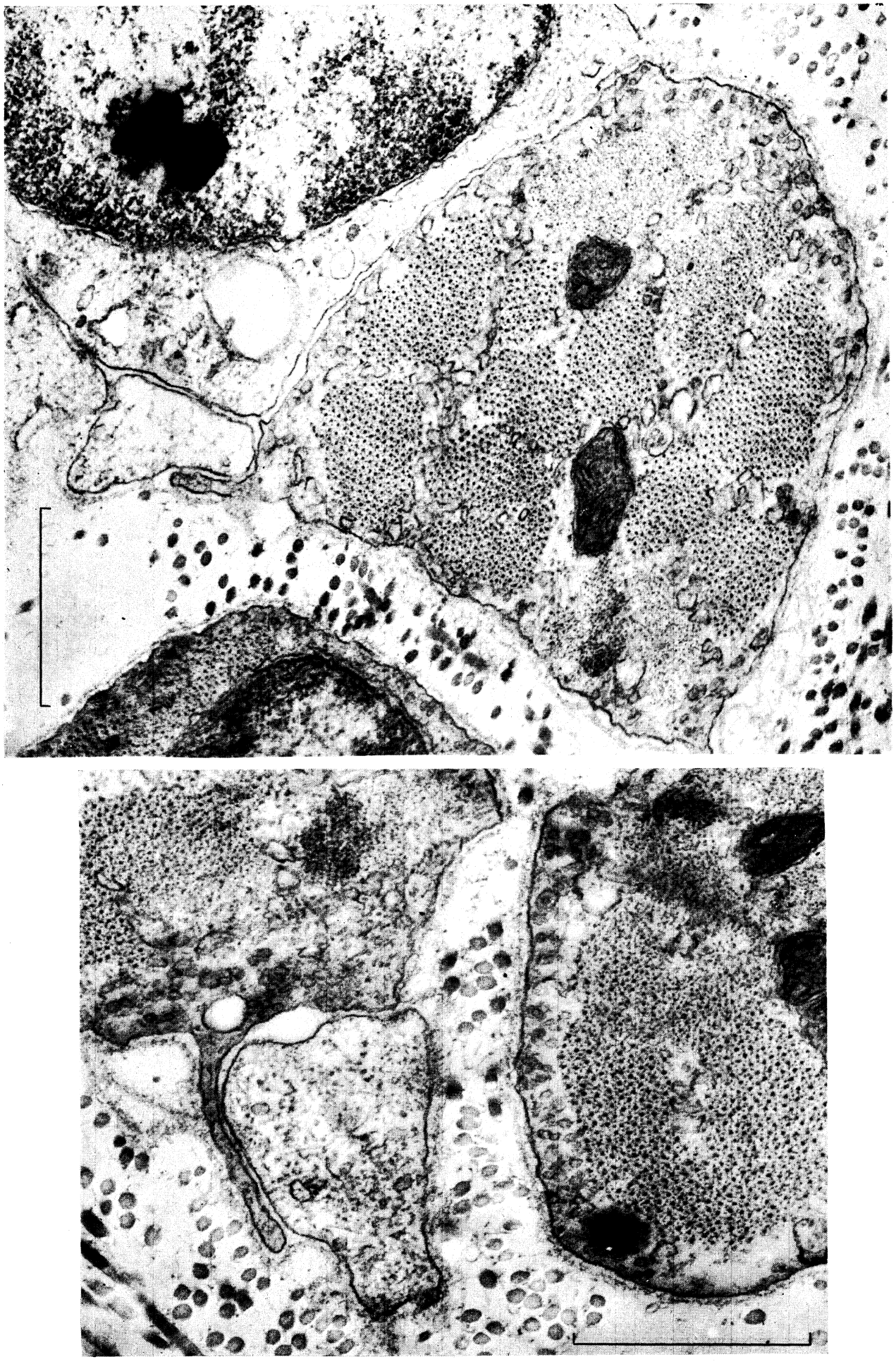


FIGURE 30. Small intrafusal fibres in cross-section, outside sensory region, together with portions of 'hypectolemmal' satellite cells.

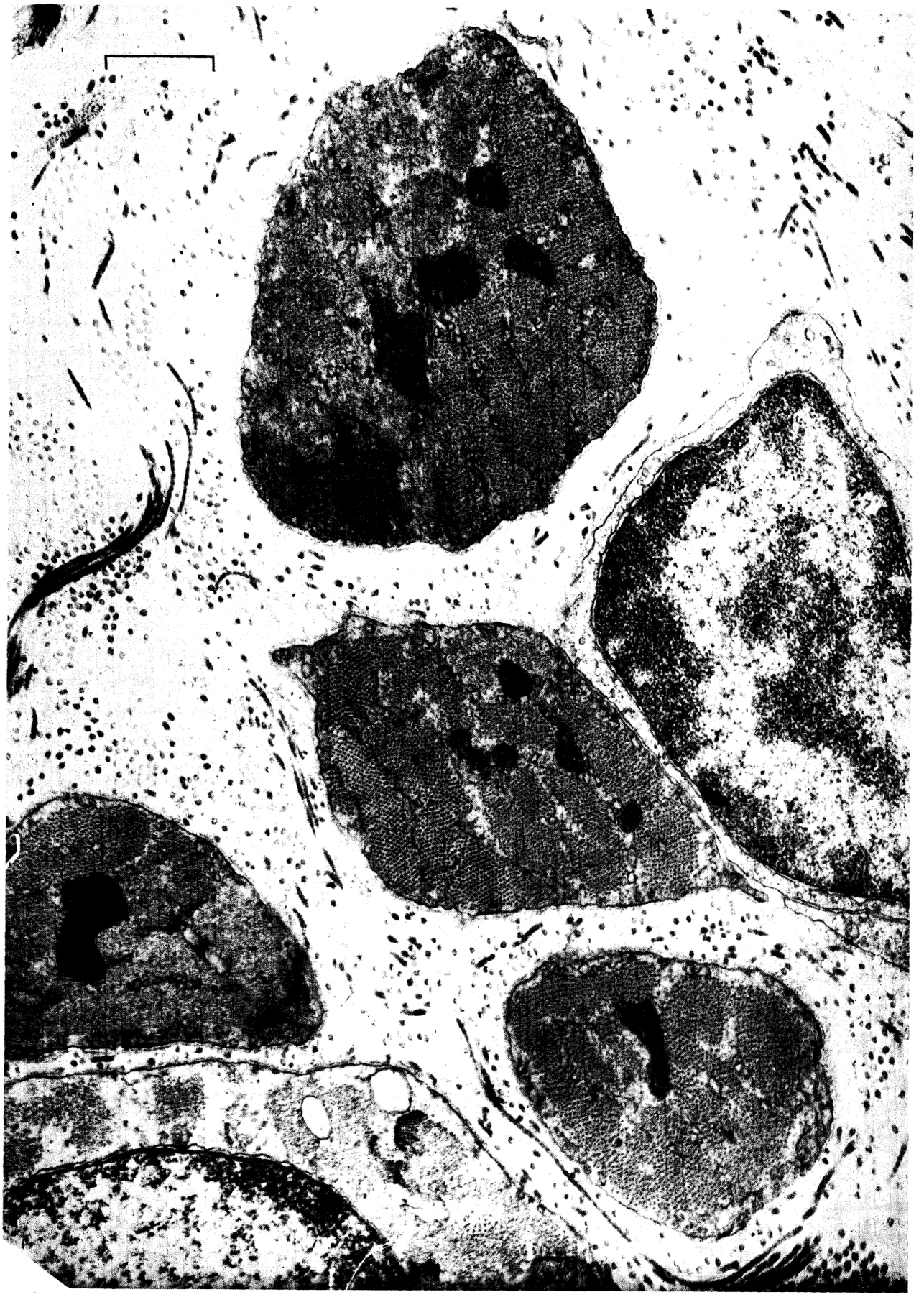


FIGURE 31. Group of small intrafusal fibres, one of them associated with nucleated satellite cell.

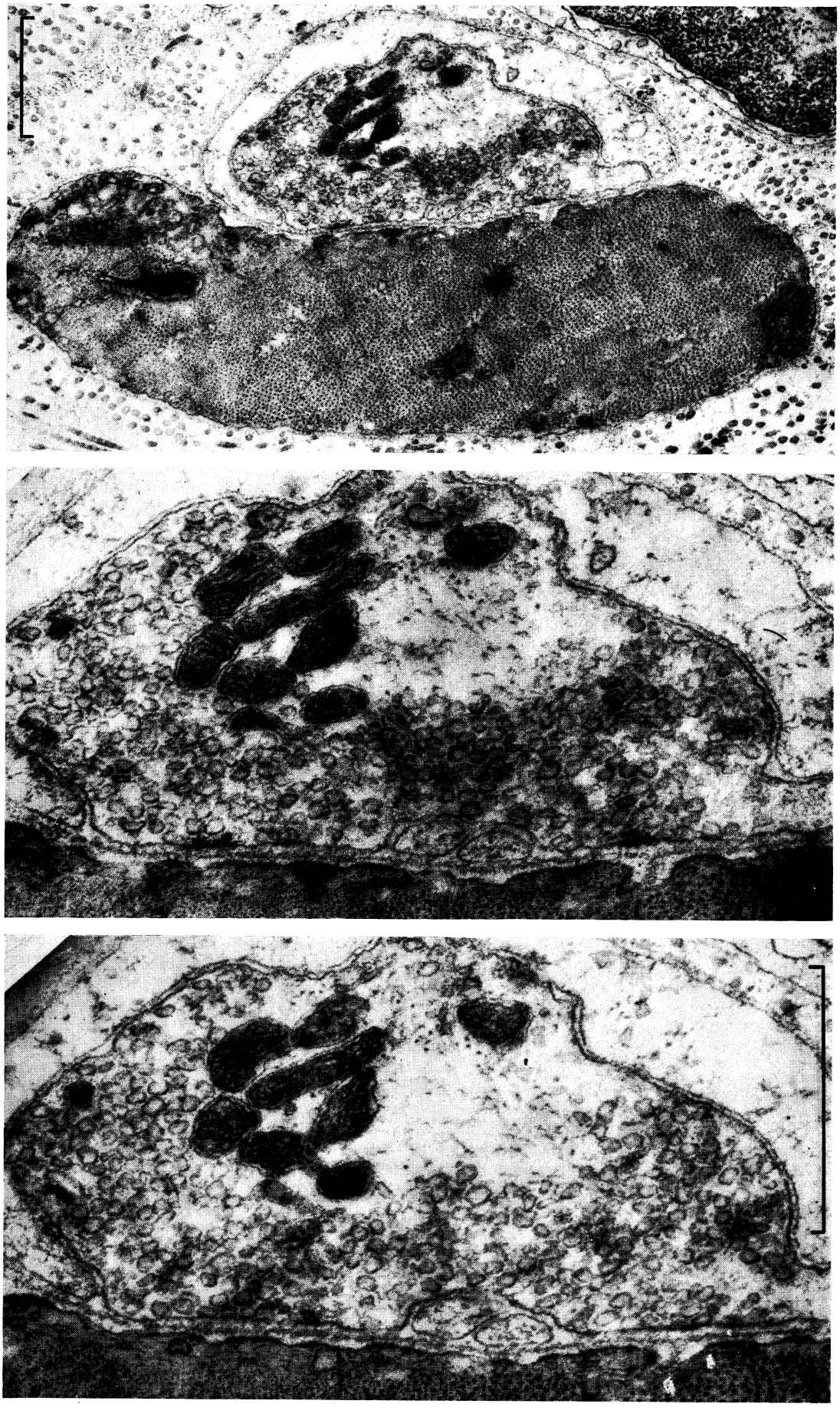


FIGURE 32. Intrafusal fibre with motor nerve ending and 'Schwann cover'. The motor ending is 'epectolemmal', in contrast with the sensory contacts, and contains the characteristic accumulation of vesicles. The two lower pictures are from serial sections, taken at higher magnification than the top picture. Level, 866 μ.

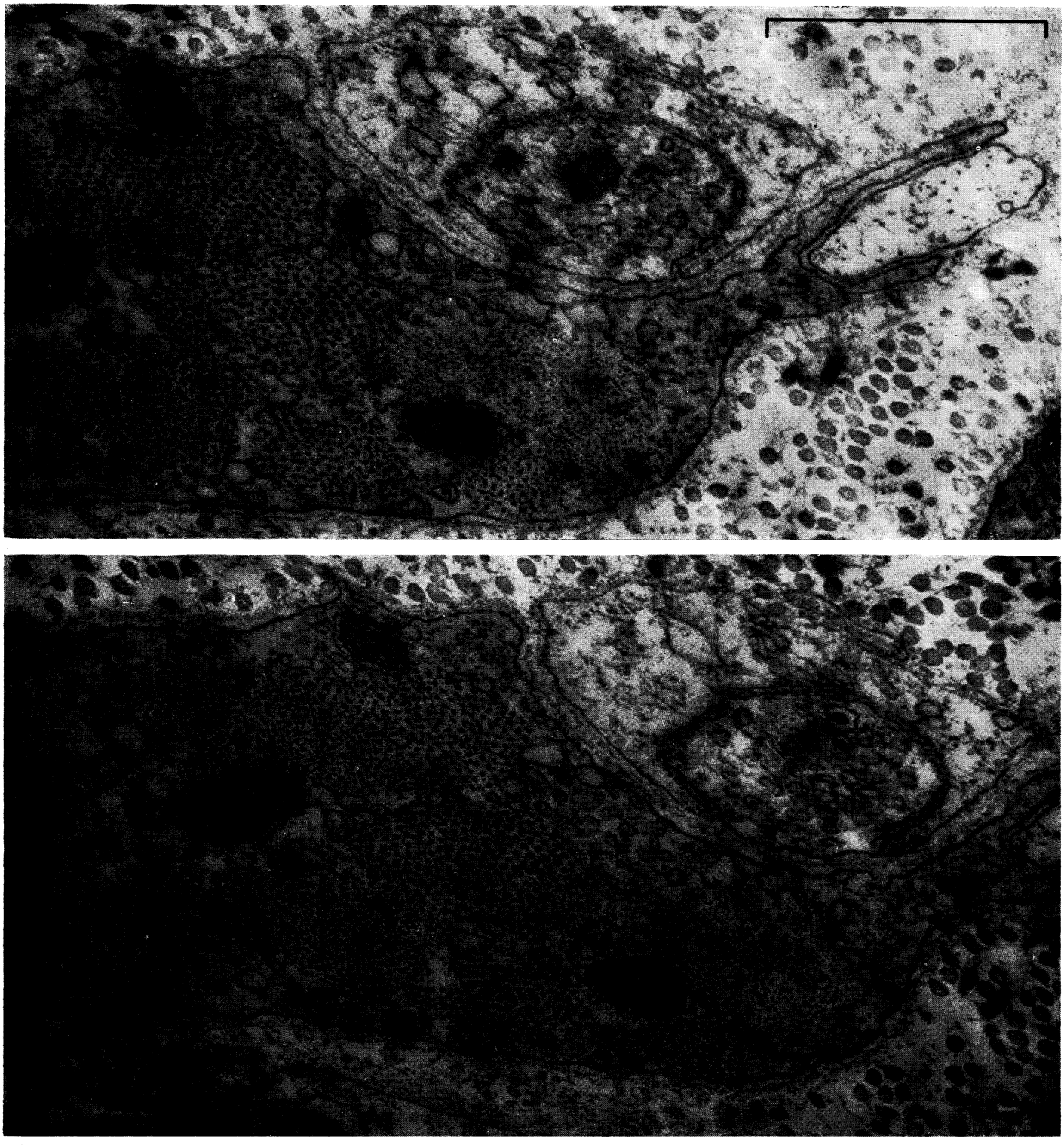


FIGURE 33. Two serial cross-sections of motor ending on another intrafusal fibre. (This one was possibly a 'slow' fibre.) Level, 866 μ.

FIGURE 34. Two intrafusal fibres at a 'junction'. Level, 2387 μ. The lower strip, *B*, from a neighbouring section, shows an enlarged picture of the surface relation of the two fibres.

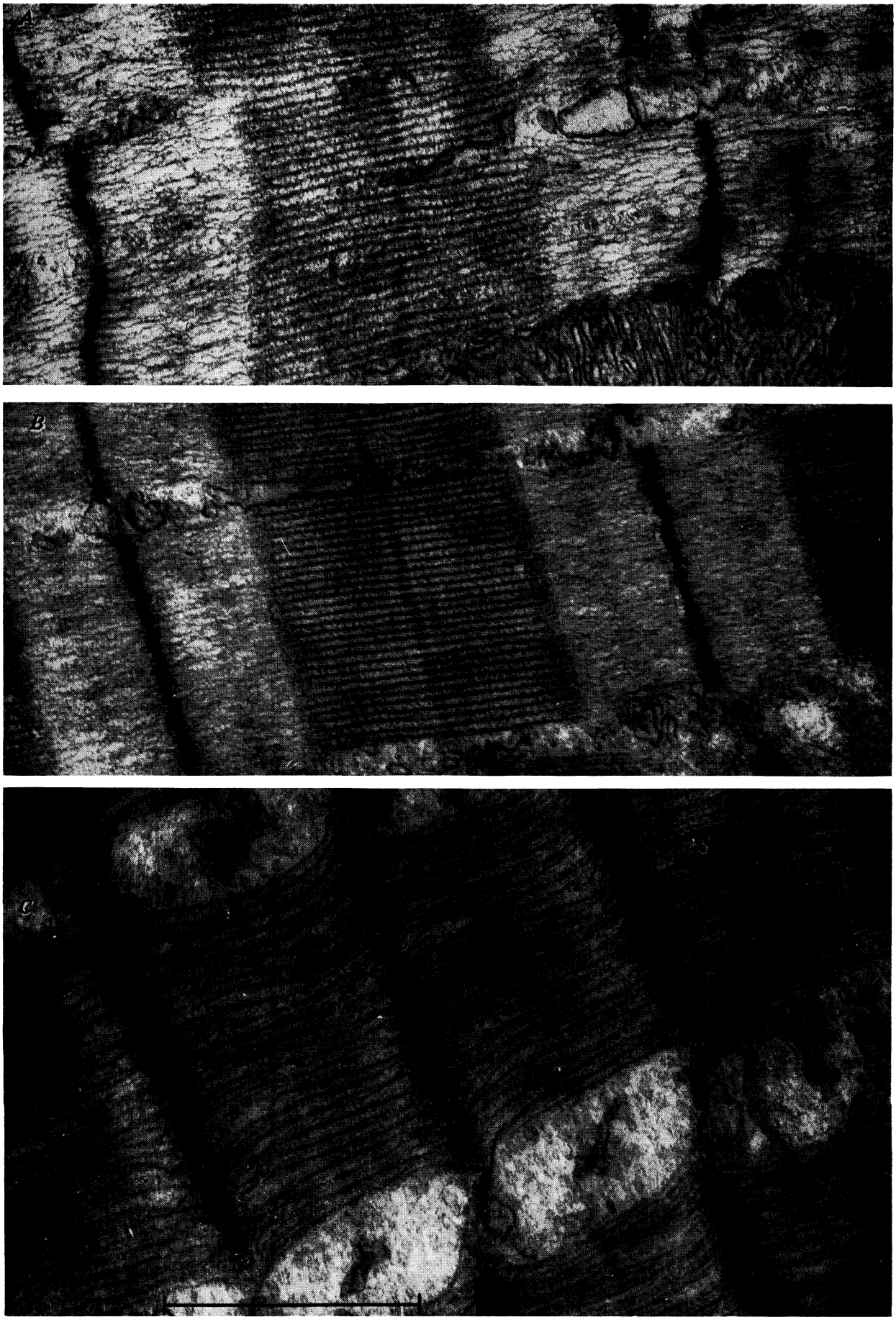


FIGURE 35. Longitudinal sections through ordinary fibres. *A* and *B* from stretched, *C* from short ('slackened') toe muscle. *Note*: sarcomere length in *C* is about half that in *B*, the *I*-bands having vanished, while the *A*-bands remain of about the same length.

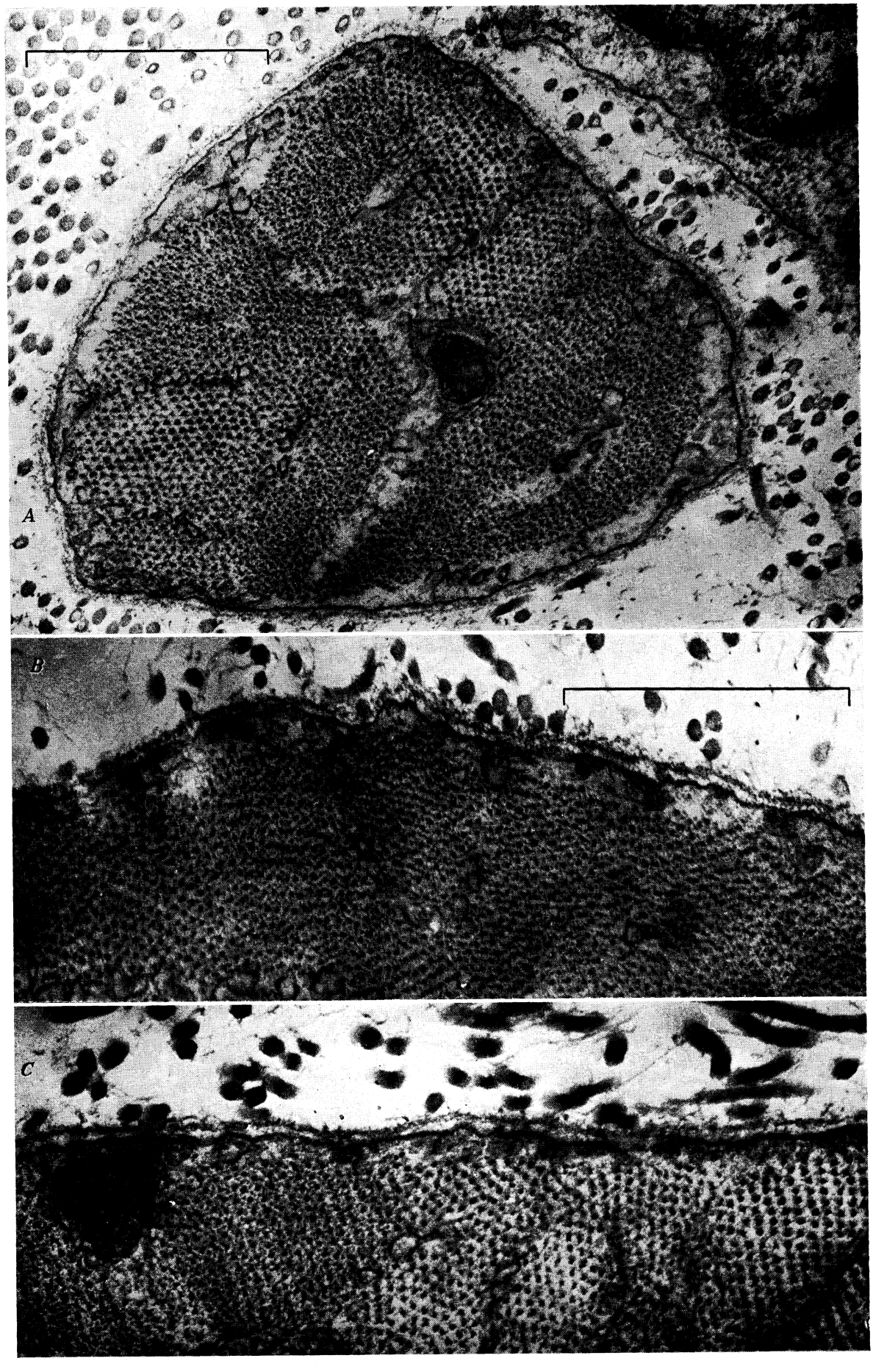
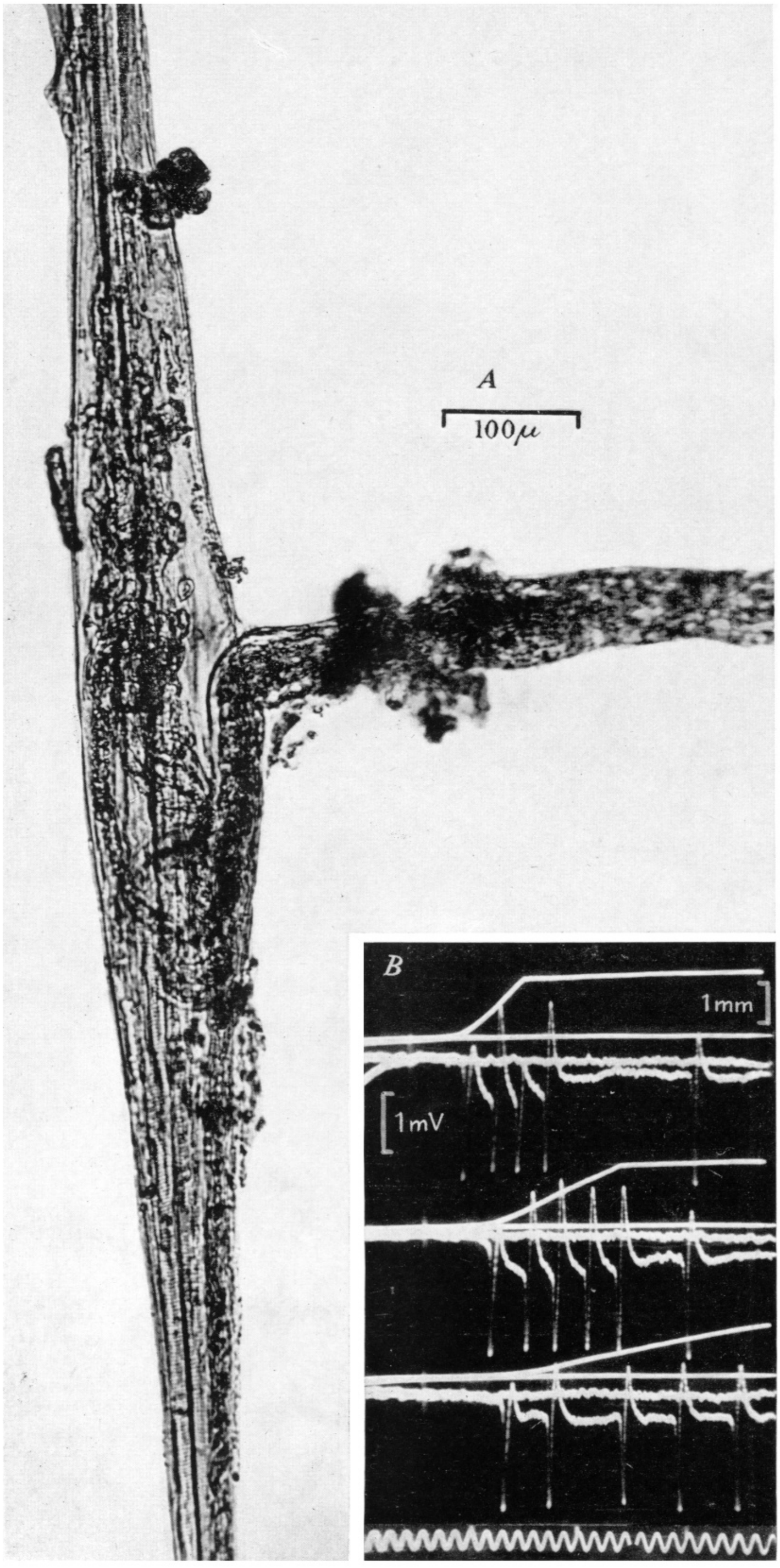
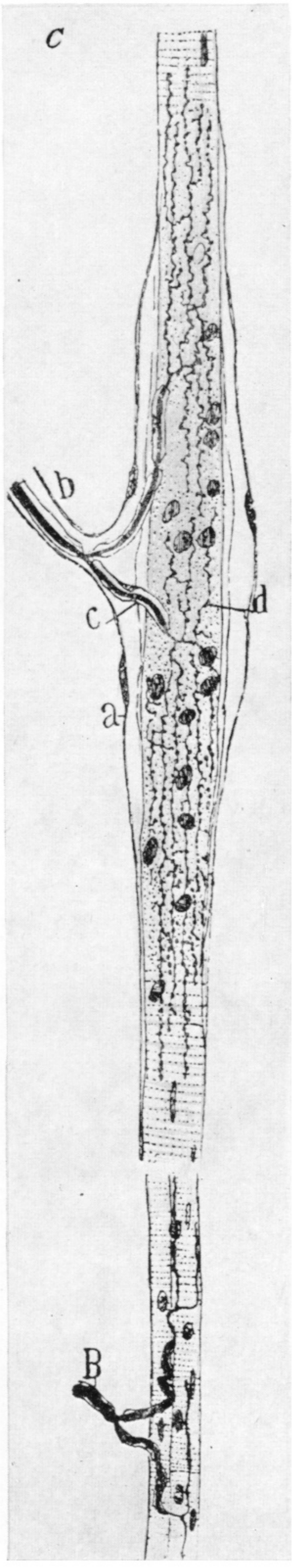


FIGURE 36. Showing surface of muscle fibres in cross-section. *A*, small, intrafusal, fibre; *B* and *C*, larger fibres from other parts of the toe muscle. Note 'ectolemma' and connective tissue fibrils.





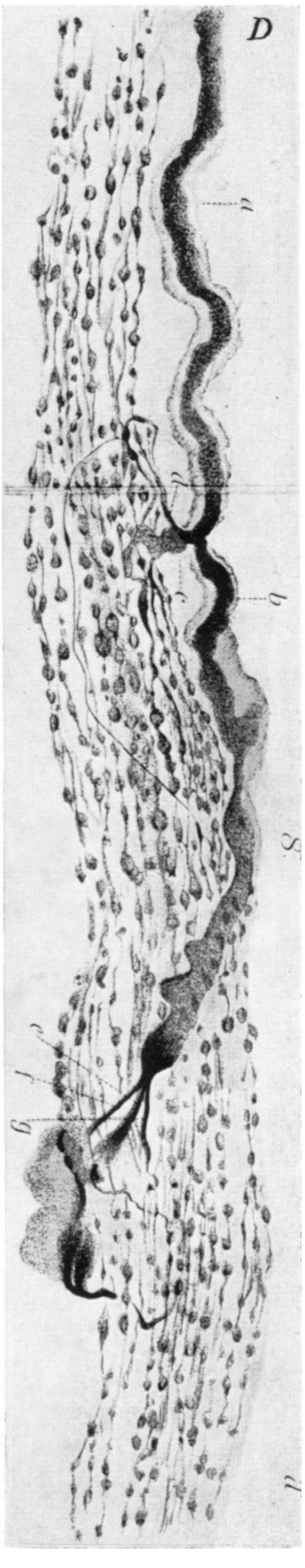
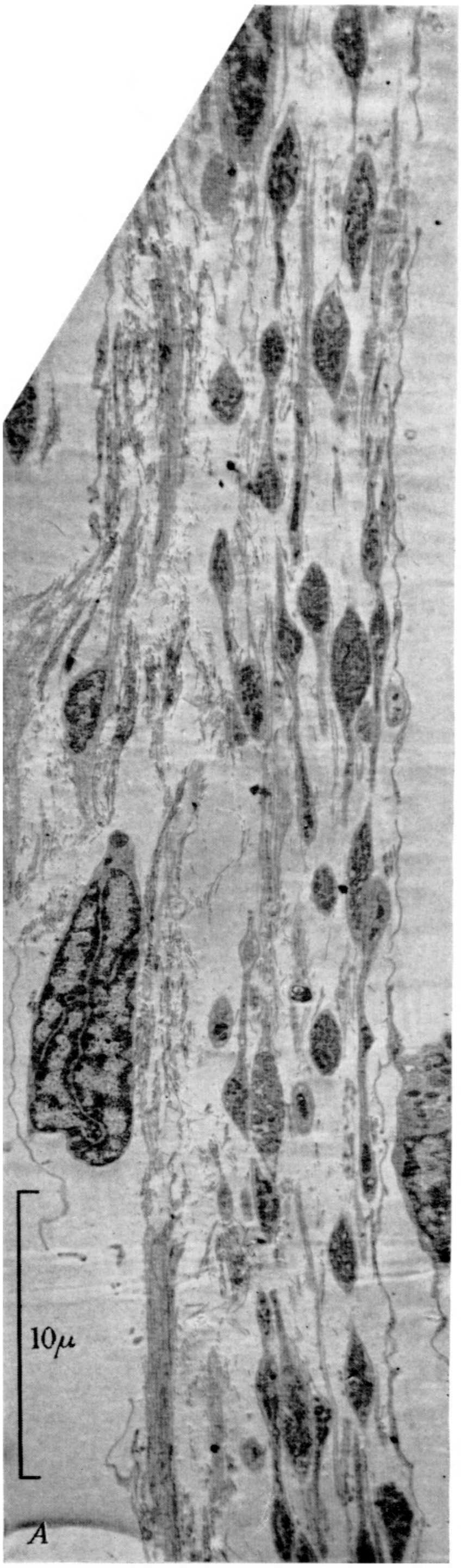


FIGURE 1. *A*. Isolated functioning spindle, from a toe muscle of the frog, photographed while immersed in Ringer solution (from Katz 1950*a*). *B*. Showing the electric responses (lower trace of each pair) in the spindle nerve, during three mechanical stretches applied to the toe muscle at different speeds (upper traces). The response consists of propagating spikes and of a slow local depolarization which depends upon rate and amplitude of stretching. Time signal: 500 c/s (from Katz 1949*b*). *C*. Histological drawing of frog spindle by Cajal (1888). Fixed and stained with methylene blue. *D*. Drawing of similar preparation by Dogiel (1890), showing the termination of the sensory nerve fibre in the form of axial 'varicose threads'.



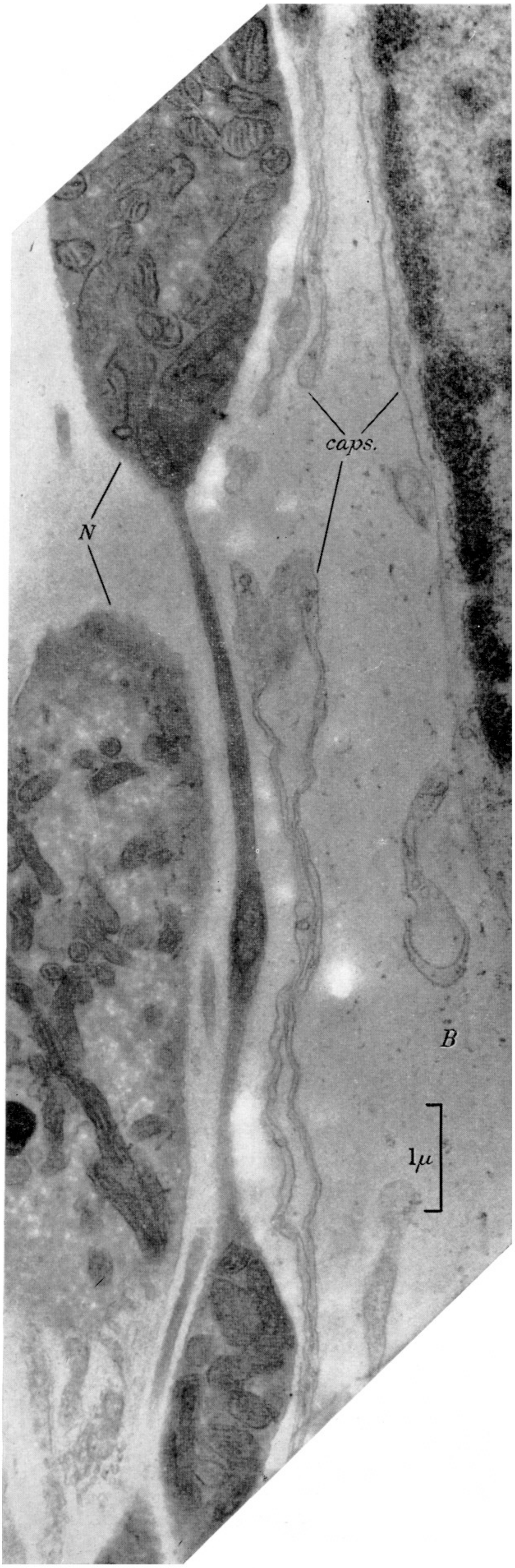


FIGURE 2. Electron micrographs of muscle spindles showing the 'varicose threads' at higher magnifications. These are longitudinal sections through the sensory region, made parallel to, and a few microns away from, the intrafusal muscle elements. Capsular cells and nuclei are also seen (e.g. *caps* in *B*); note the low density of the capsular cytoplasm compared with that of the nerve chains (*N*).

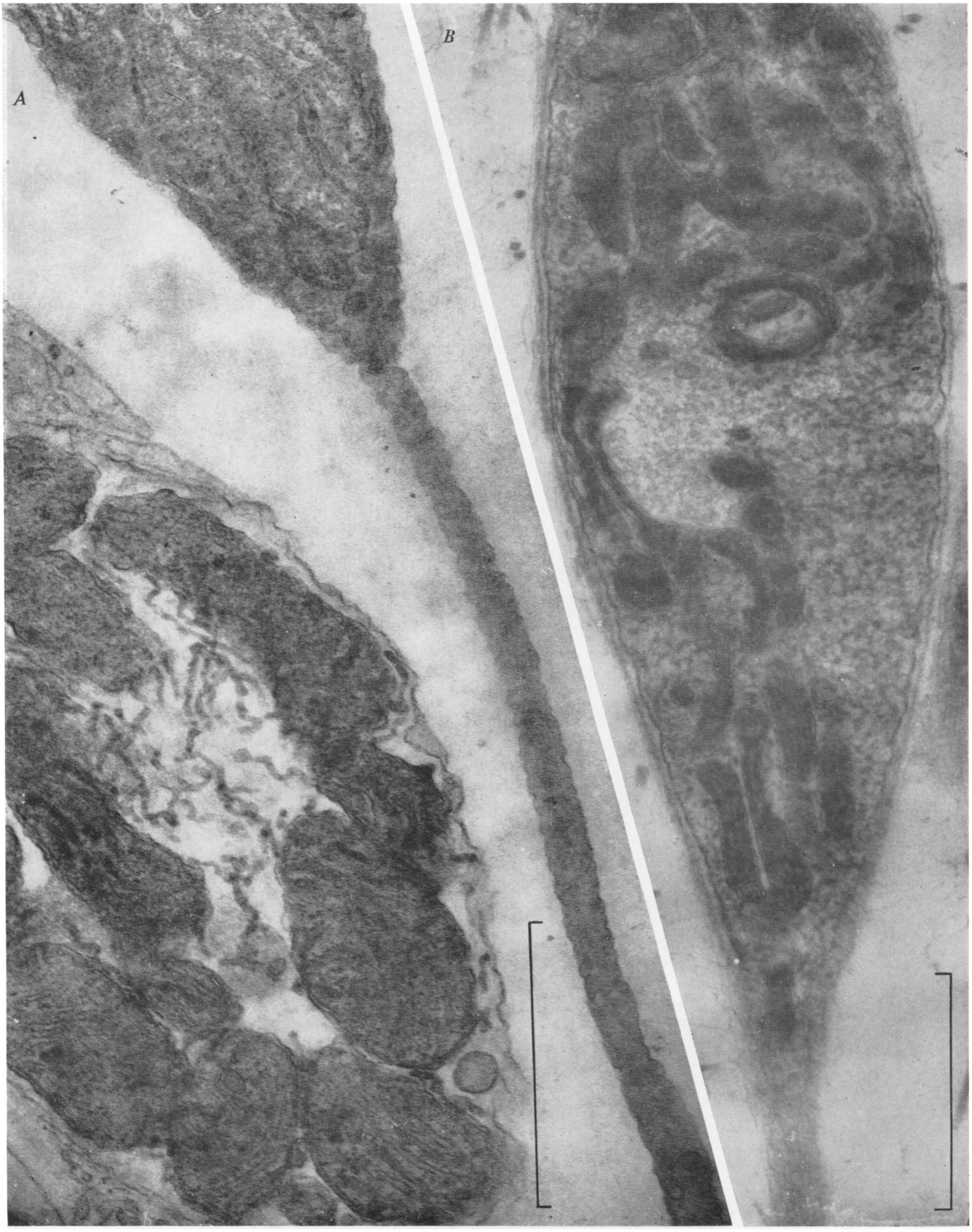


FIGURE 3. Same structures (terminal branches of sensory nerve) at higher magnification.

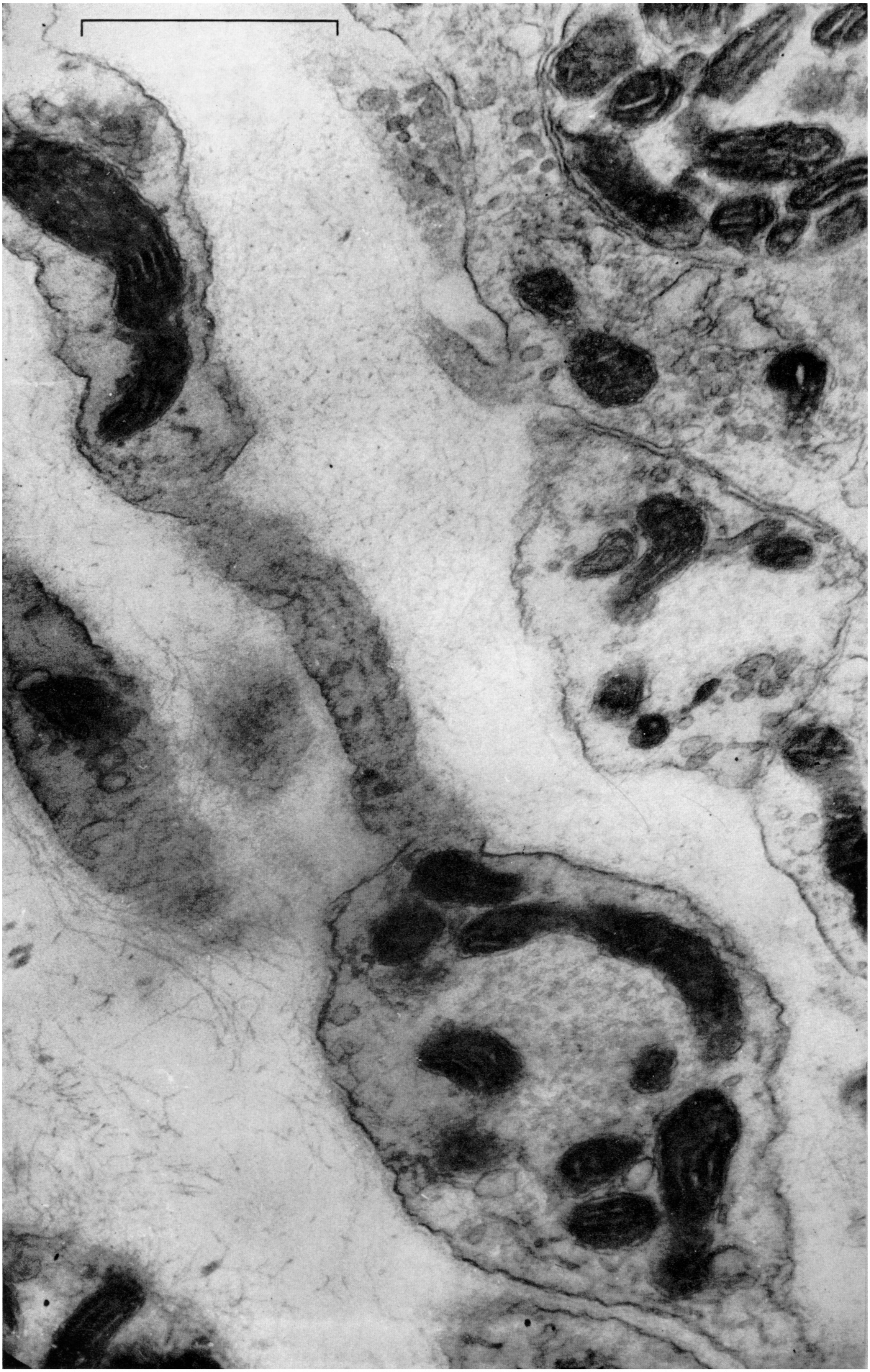


FIGURE 4. While the preceding pictures were from stretched muscles, this is from a slack (short) toe muscle.

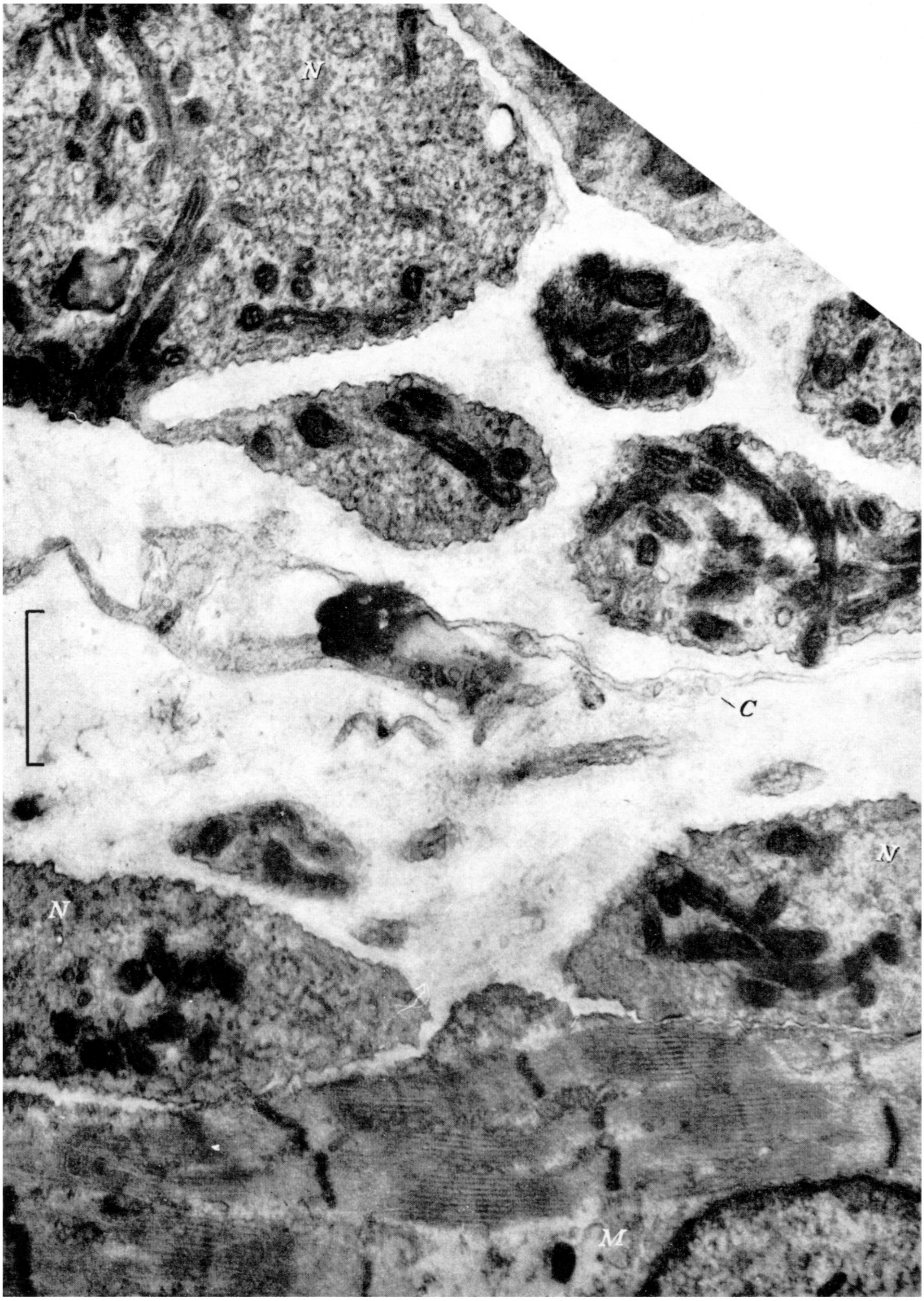
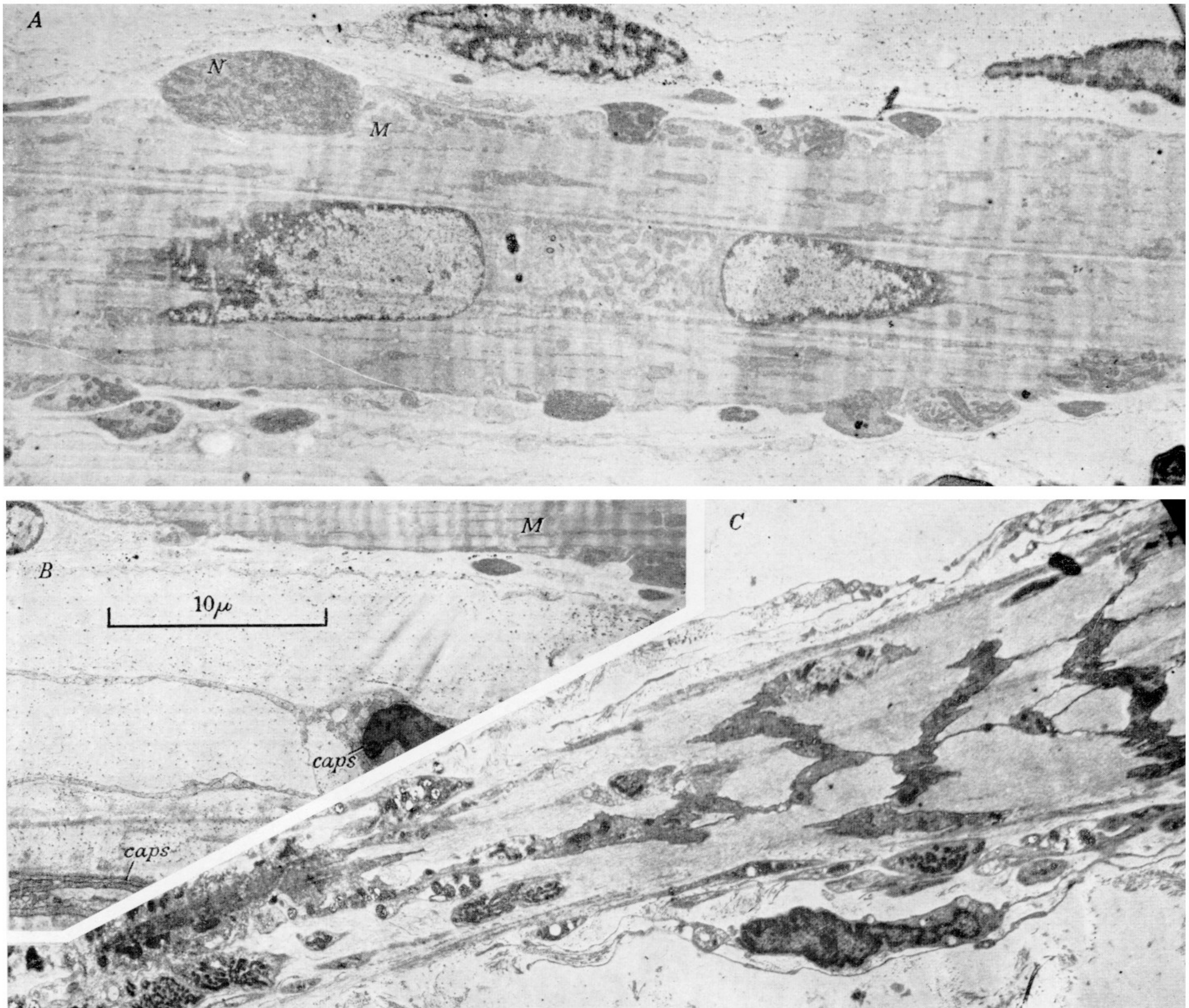


FIGURE 5. Showing the relation of nerve 'bulbs' to the surface of the muscle fibre. *N*, Nerve bulbs, two of which are seen in contact with muscle fibre (*M*). Aspects of other nerve chains (including a point of branching) are shown in an adjacent lymphatic compartment, separated by a capsular layer (*C*).



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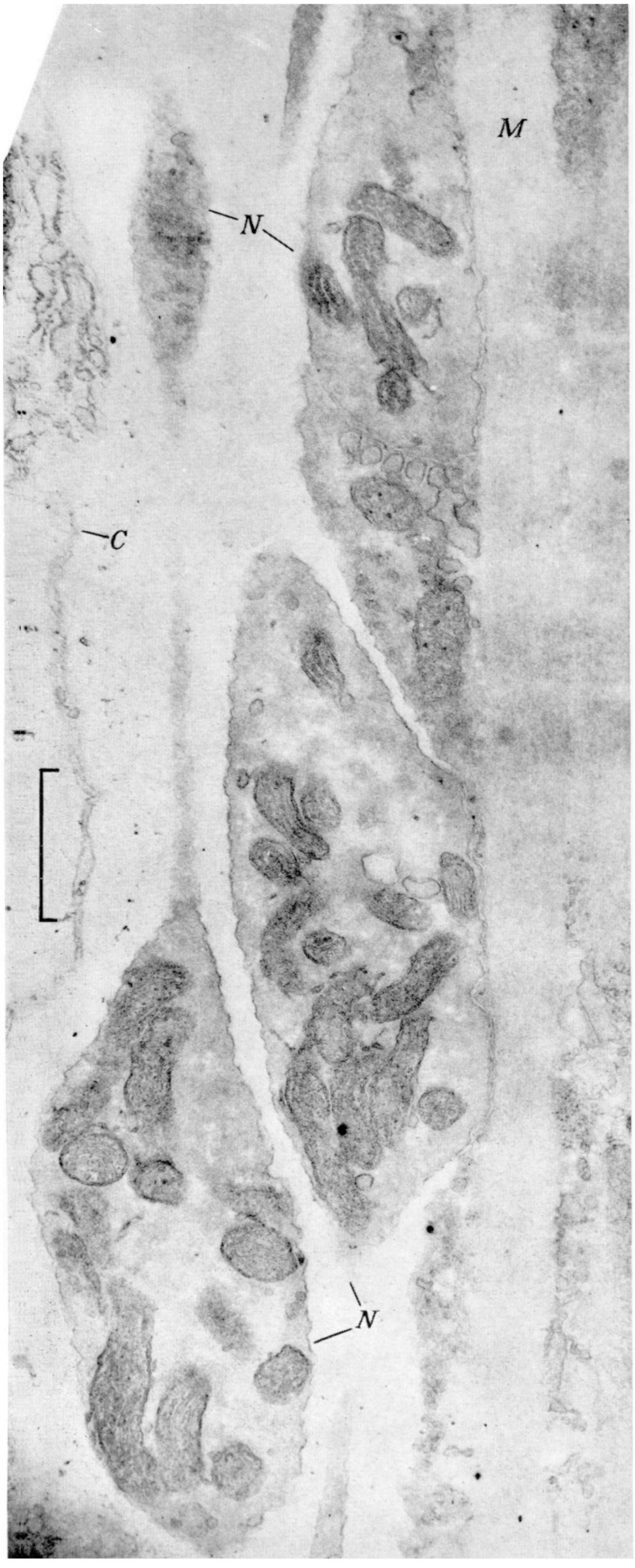


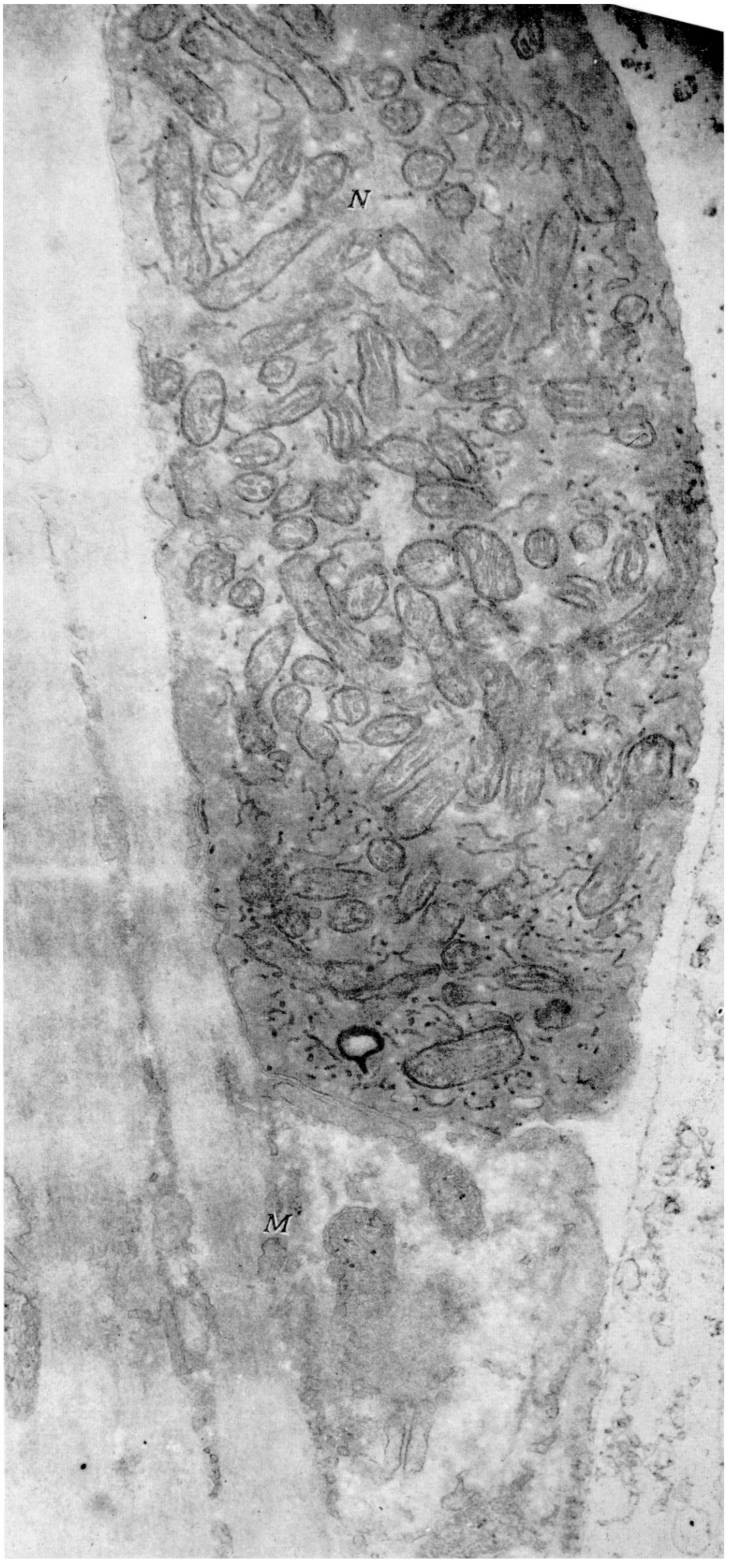
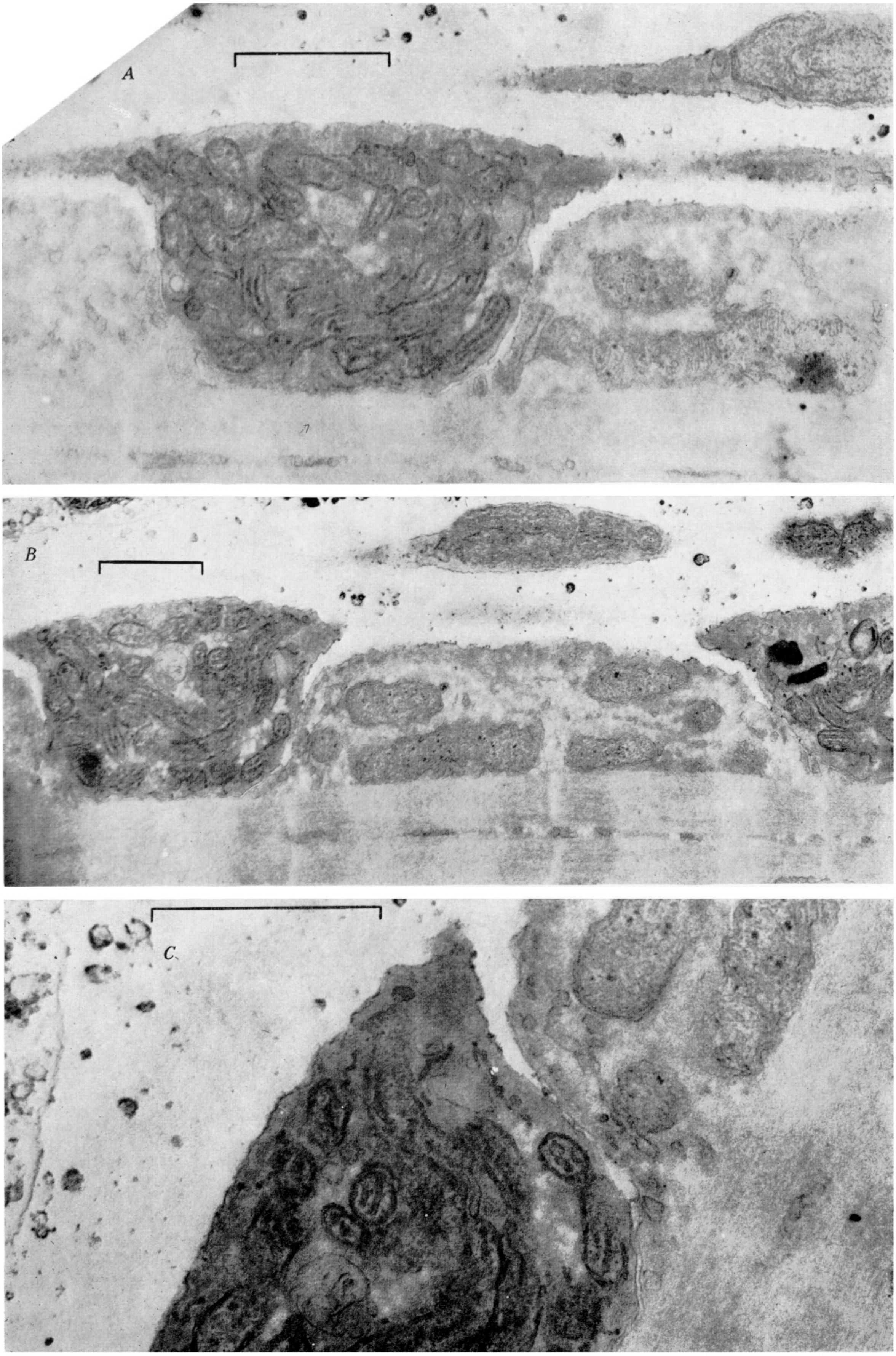


FIGURE 7. Sensory contacts at higher magnification (scale 1 μ). *C*, capsule; *N*, nerve bulbs; *M*, muscle fibre. In the right-hand part, the sensory nerve bulb labelled *N* in figure 6*A* is shown enlarged. It contains a mass of small mitochondria interspersed with a random network of tubules. This is in striking contrast with the accumulation of vesicles found in motor nerve endings (e.g. figure 32, plate 57).



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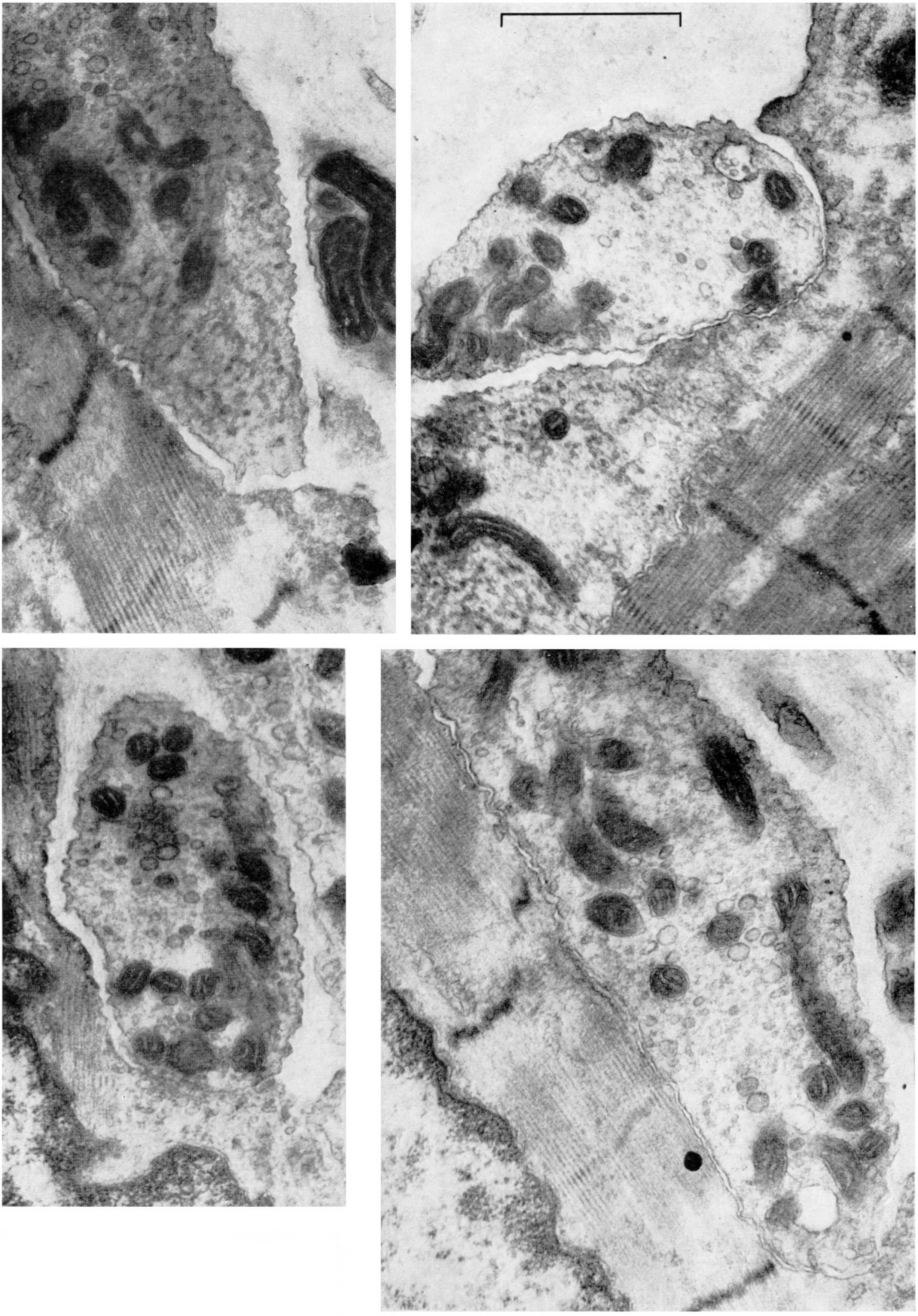


FIGURE 9. Sensory contacts.

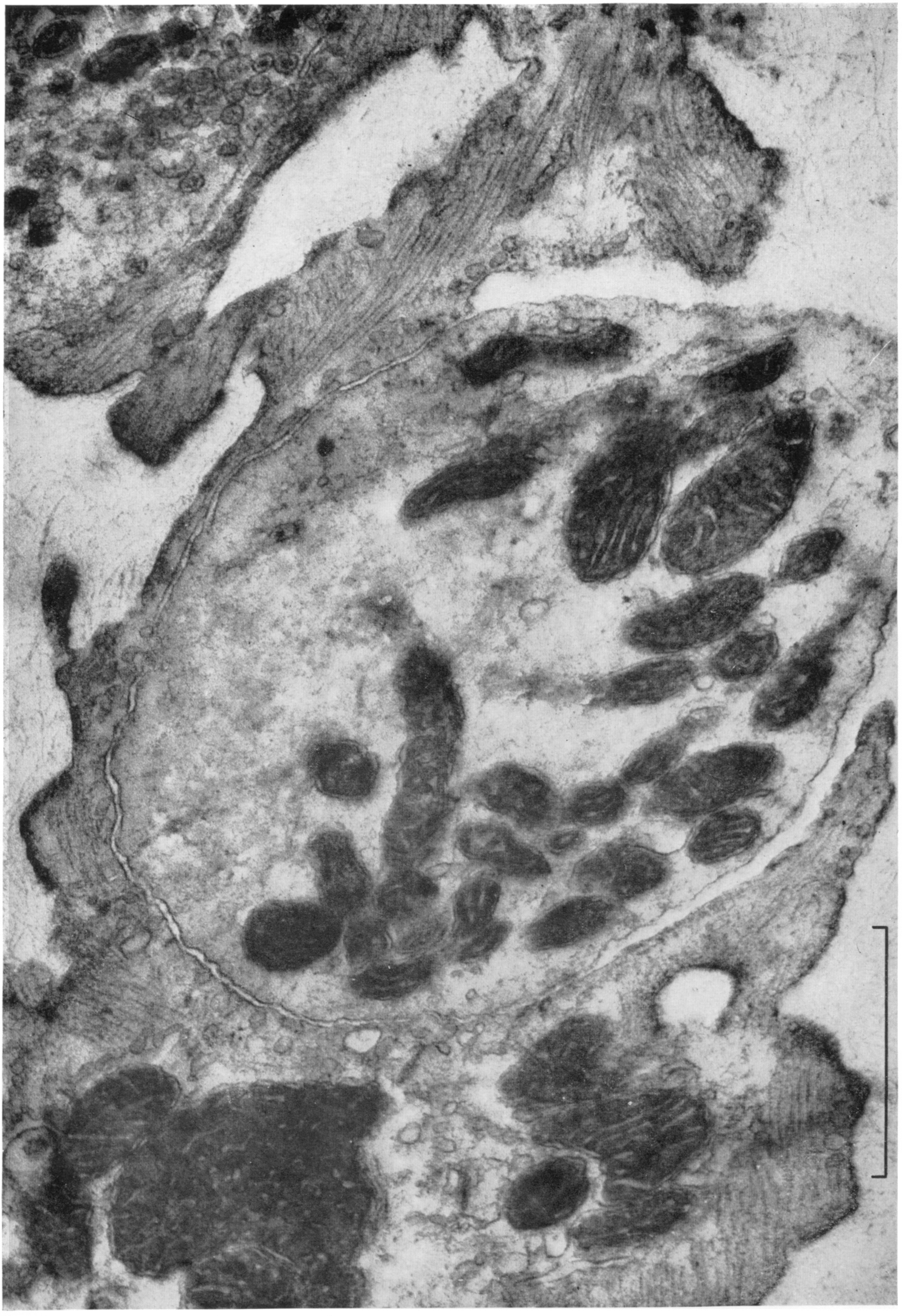


FIGURE 10. Muscle was slackened off before fixation. Sensory contact. (Longitudinal section, grazing the muscle surface in a reticular zone.)

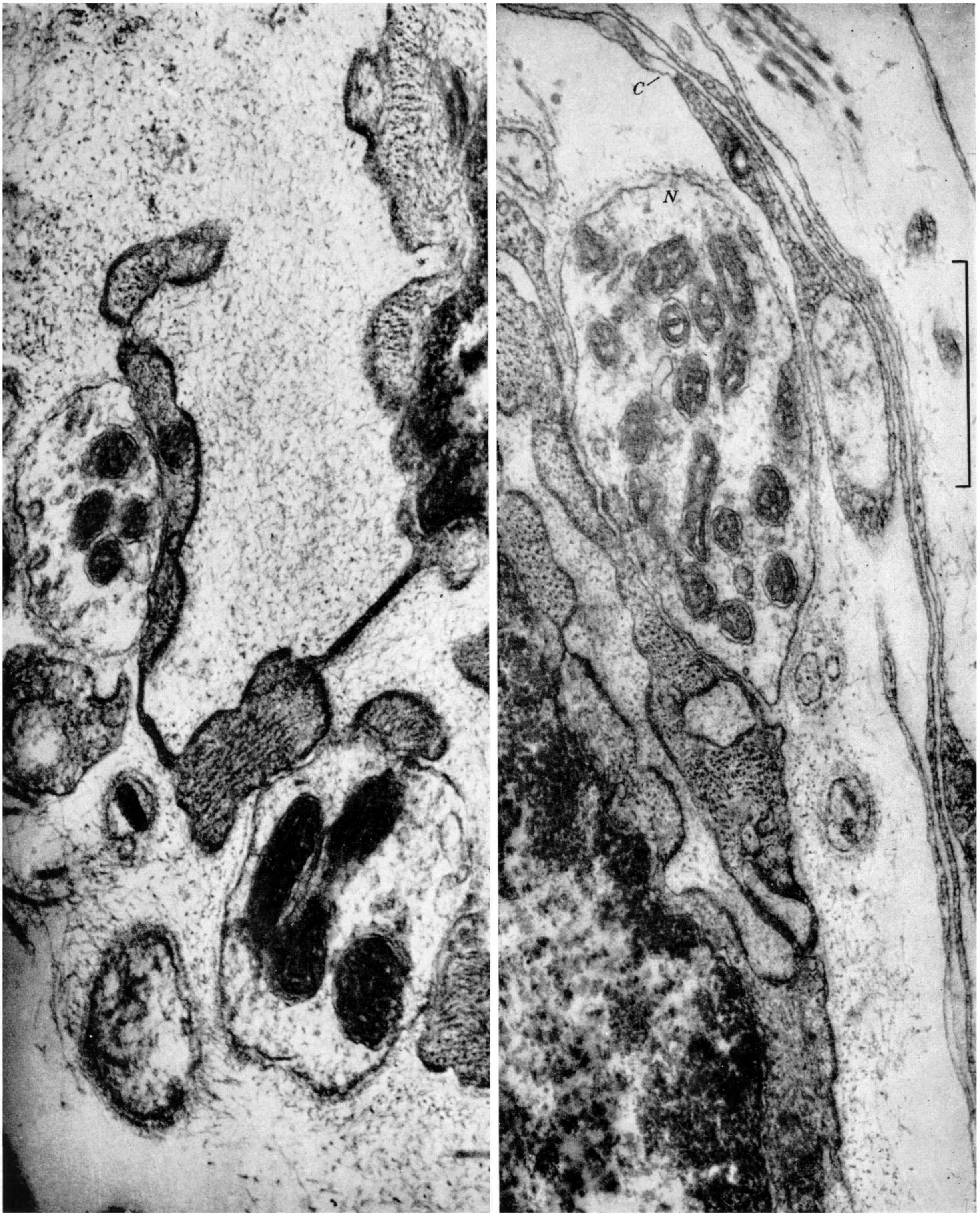


FIGURE 11. Sensory contacts in transverse section through a reticular zone.
C, capsule; *N*, nerve bulb.

FIGURE 12. Transverse section of sensory contacts.

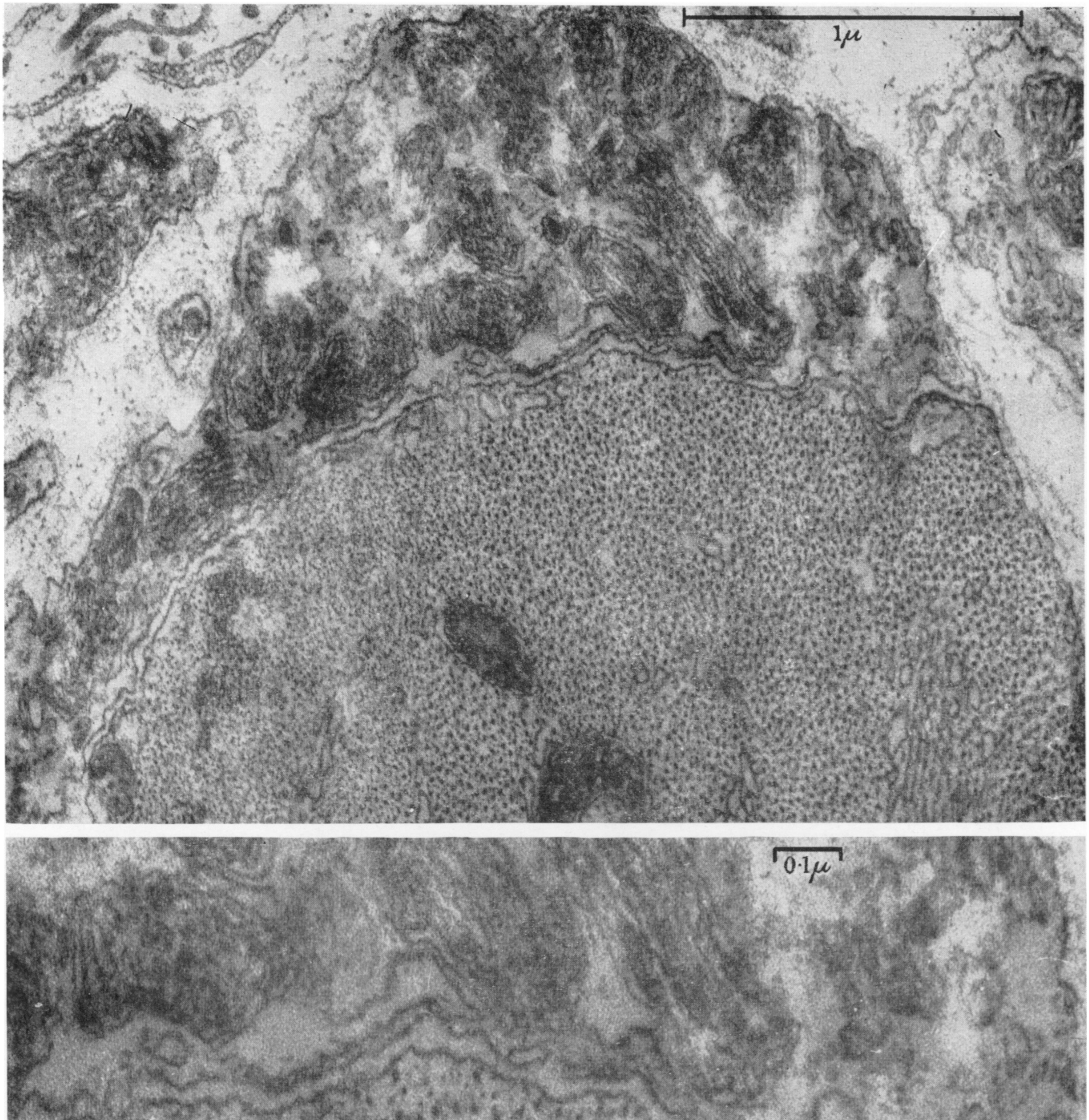


FIGURE 13. Sensory contact, showing 'bridges' across the intercellular gap.

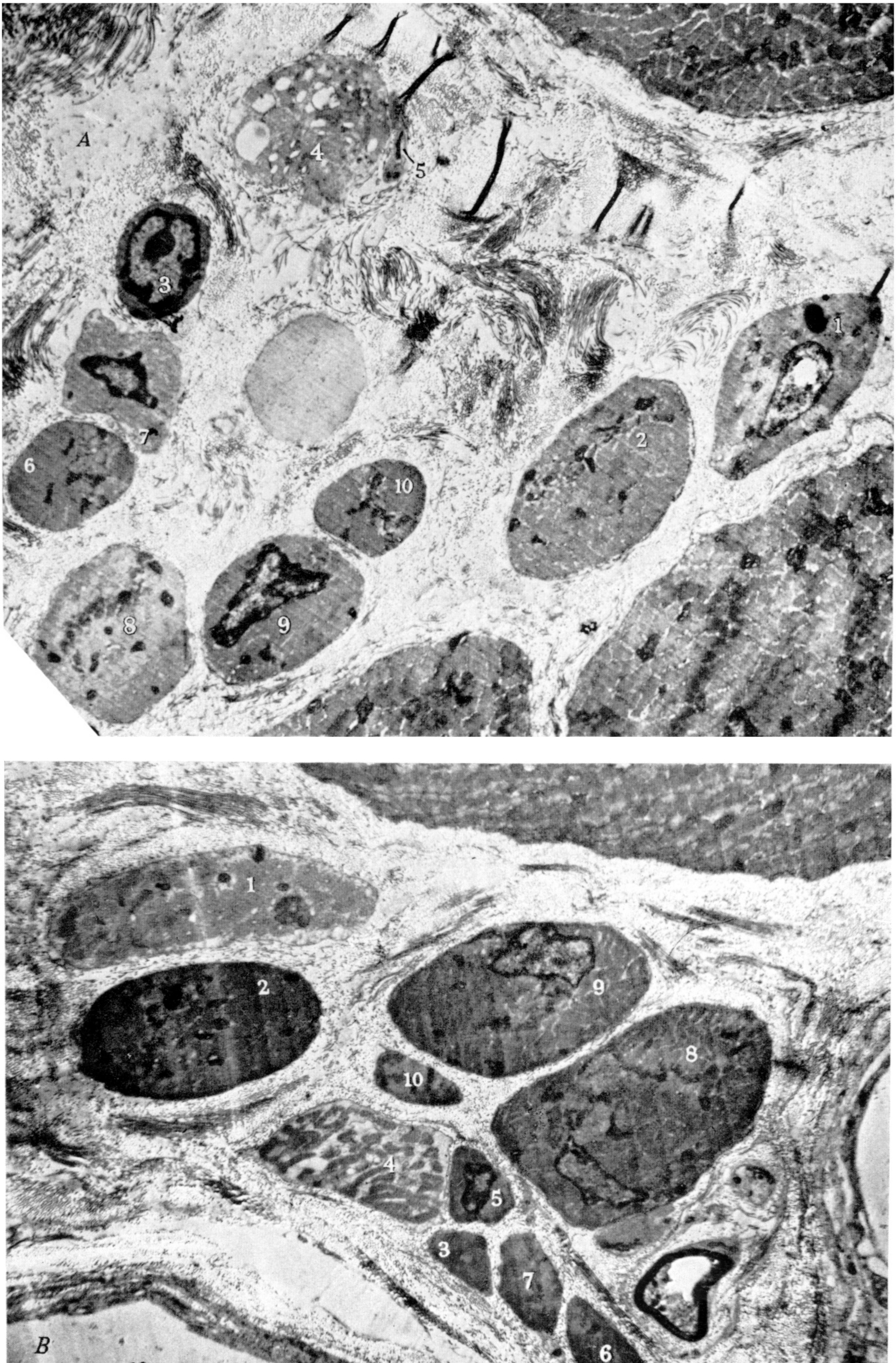


FIGURE 14 (also 15 and 16). Cross-sectional survey pictures, showing group of 10 intrafusal muscle fibres well away from their sensory contact region ('extra-capsular'). Level of section: *A*, 382 μ; *B*, 1120 μ.

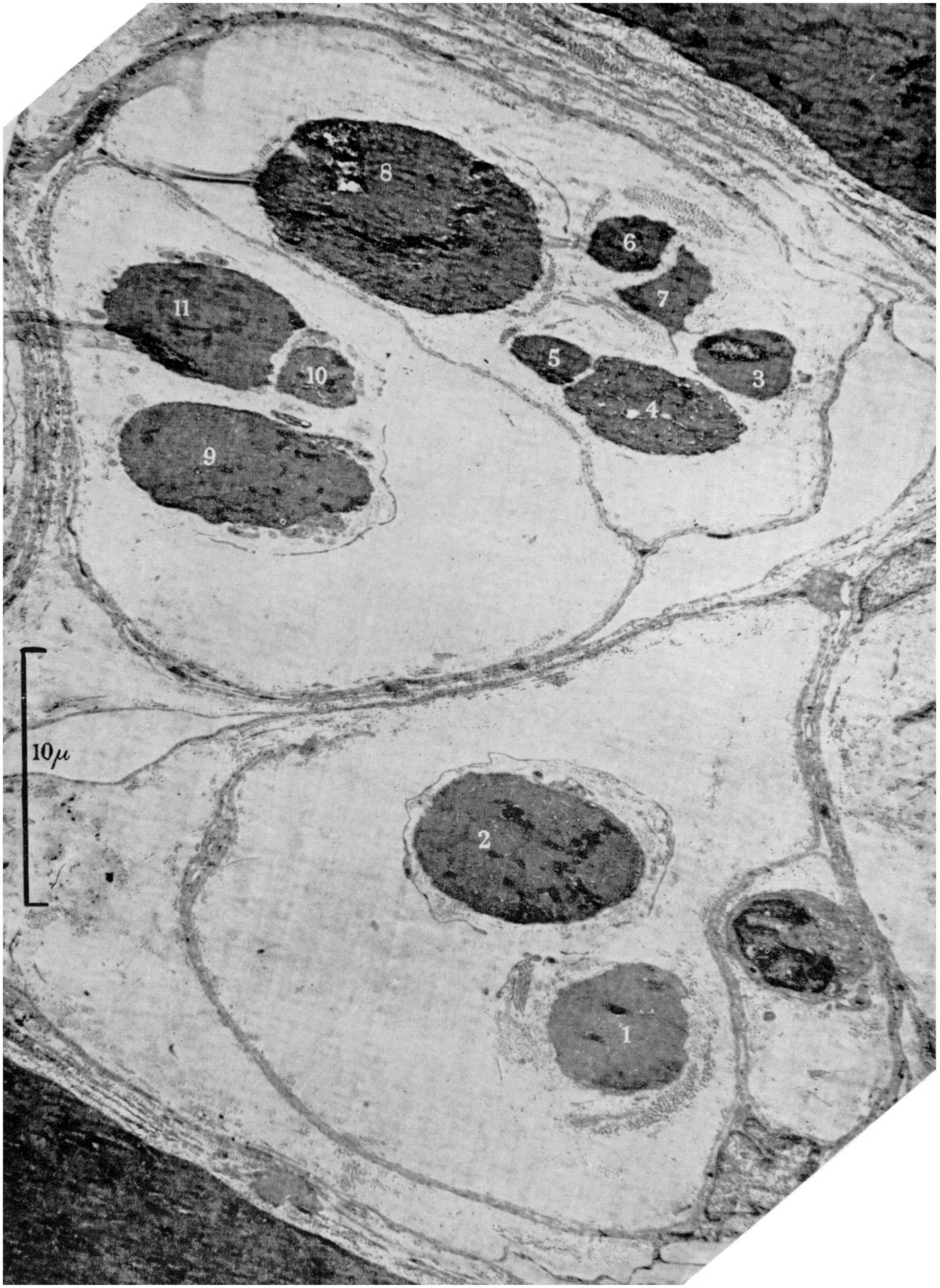


FIGURE 15. Following the group of fibres into the capsular region. Level, 1717 μ. *Note*: uncertainty of identification occurred at a level of 1300 to 1400 μ, so that the numbering of fibres in figures 15 *et seq.* may not entirely correspond to that in figure 14.

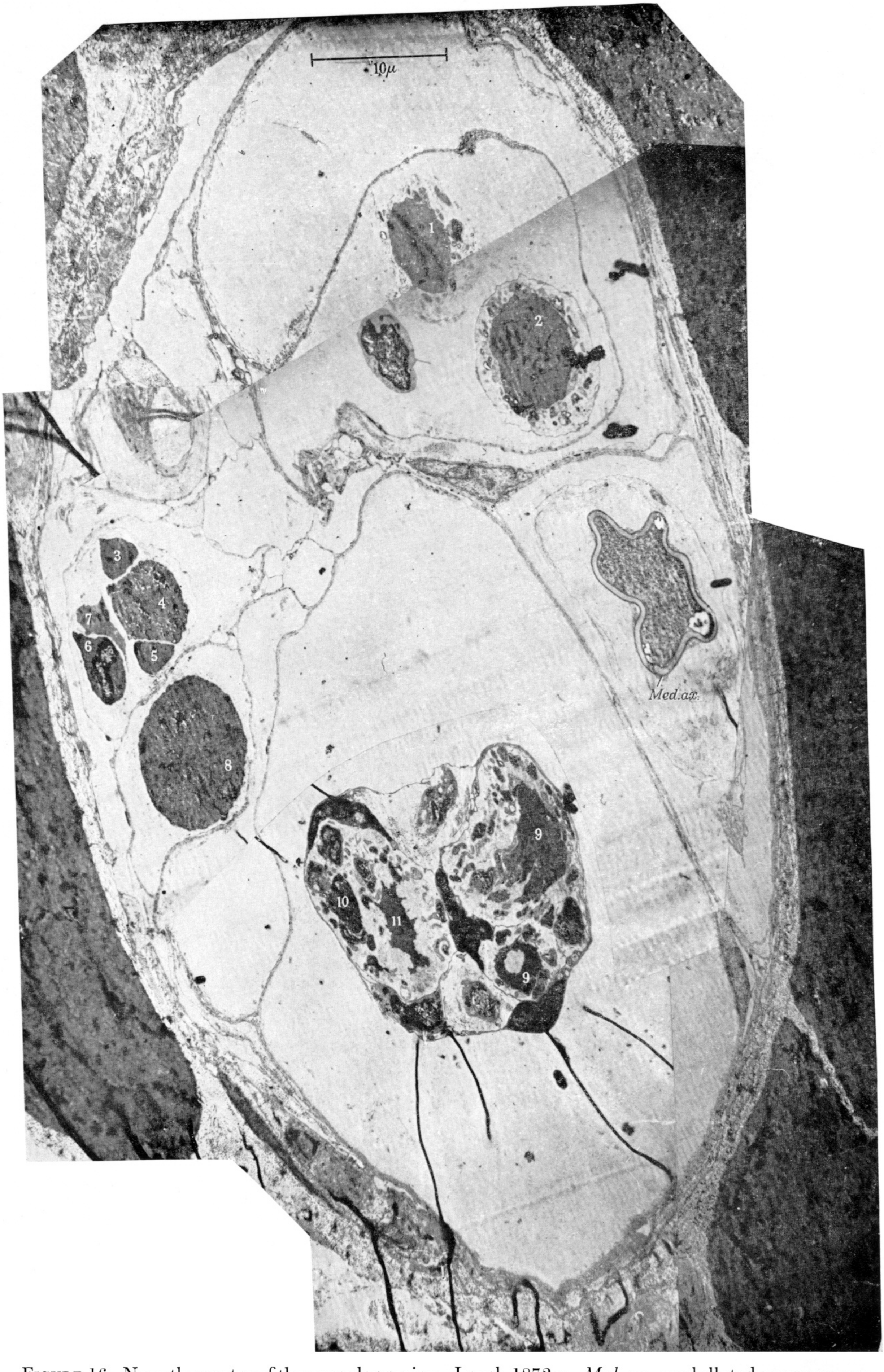


FIGURE 16. Near the centre of the capsular region. Level, 1872 μ. *Med. ax.*, medullated sensory axon.

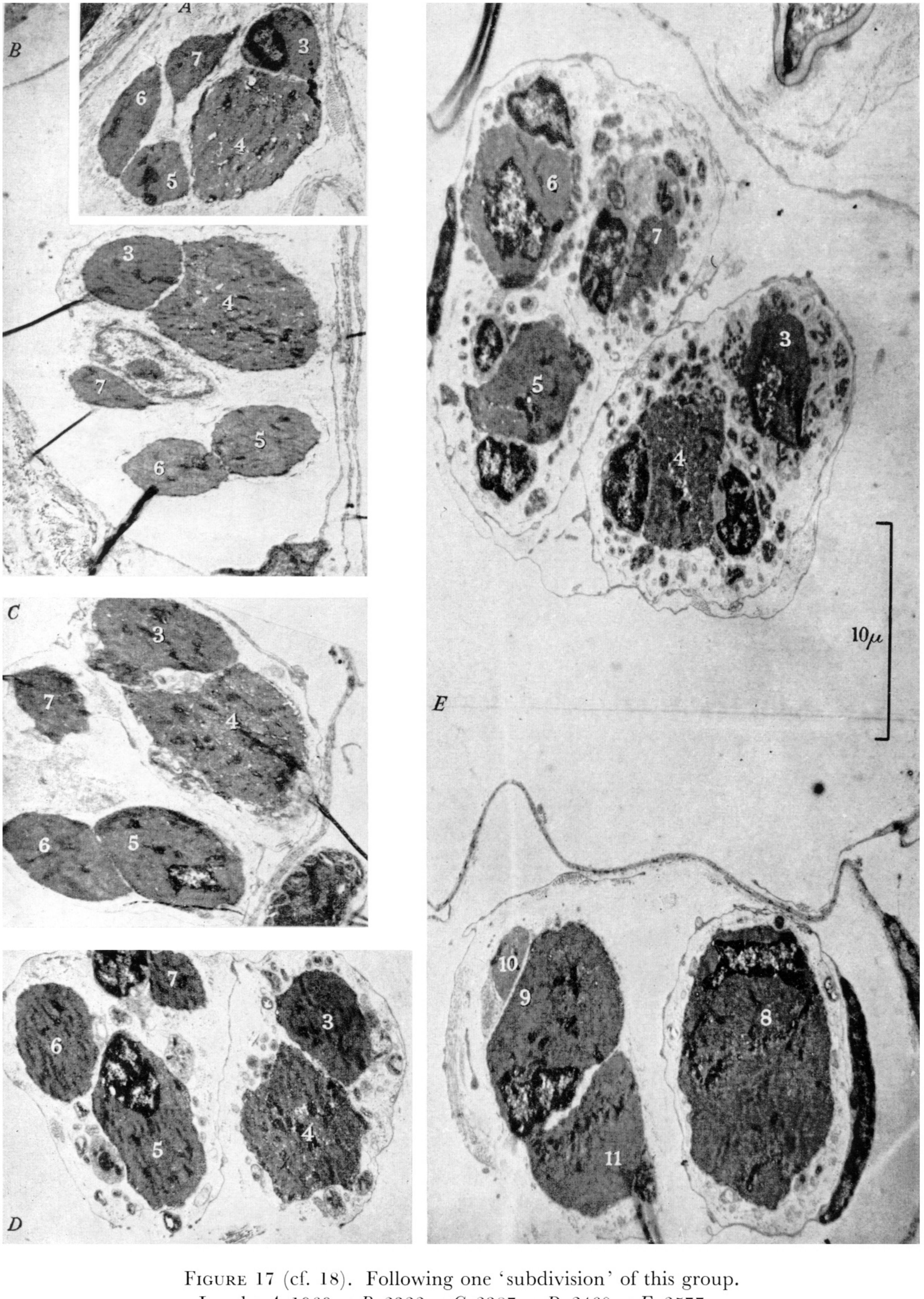


FIGURE 17 (cf. 18). Following one 'subdivision' of this group.
Levels: *A*, 1969 μ; *B*, 2322 μ; *C*, 2387 μ; *D*, 2460 μ; *E*, 2577 μ.

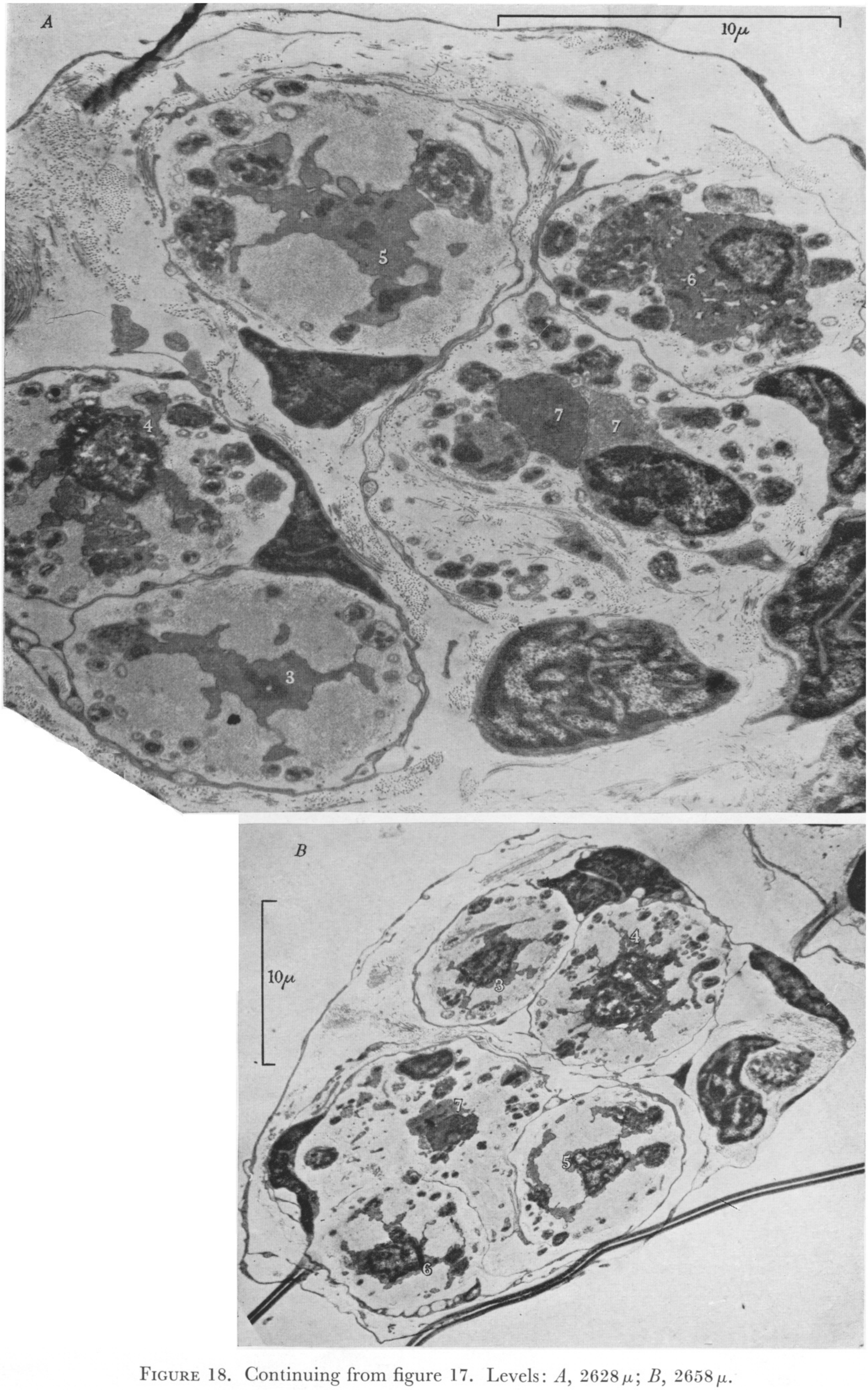


FIGURE 18. Continuing from figure 17. Levels: *A*, 2628 μ; *B*, 2658 μ.

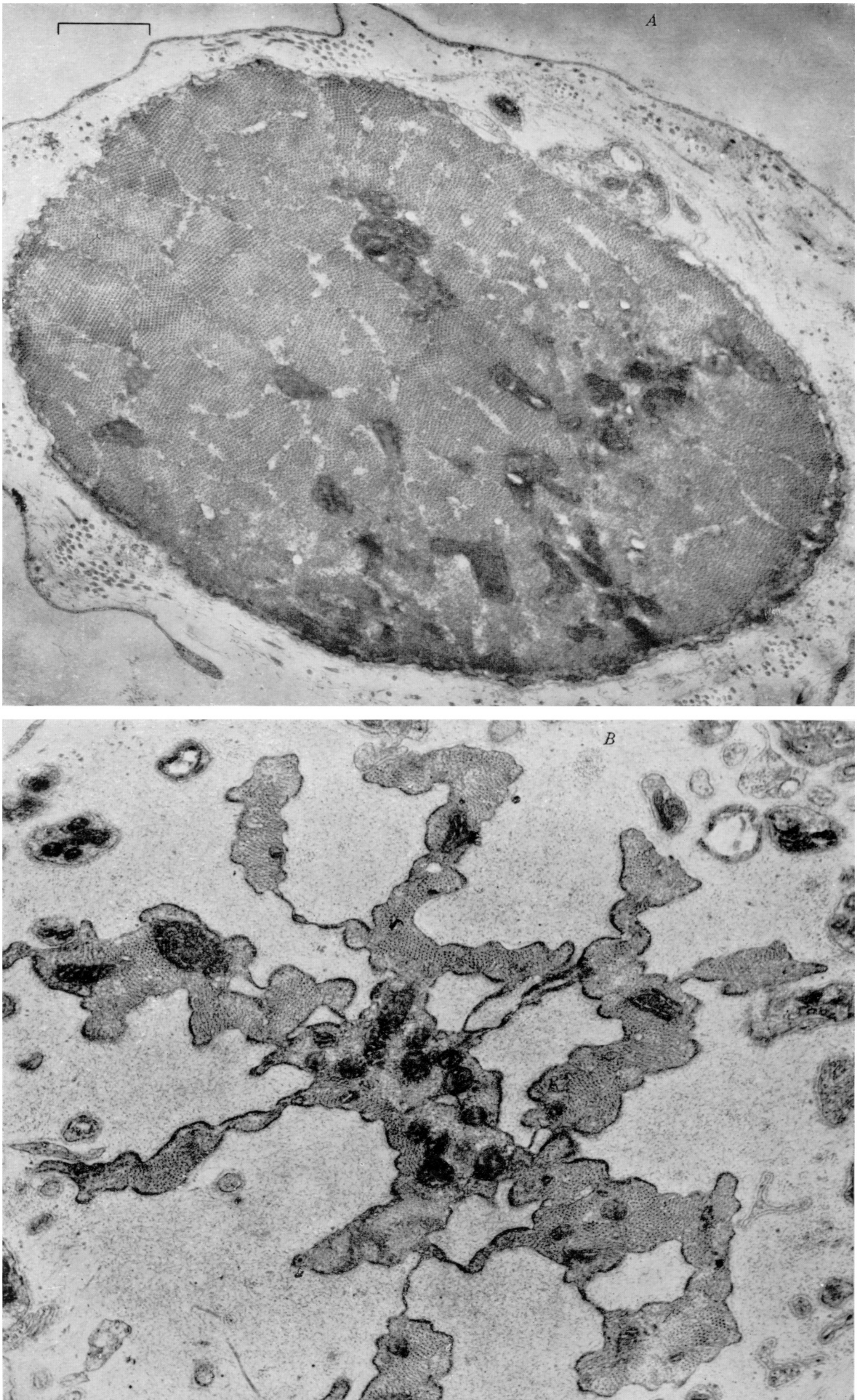


FIGURE 19. Cross-sections of the same intrafusal fibre (no. 2). *A*, at entry into capsular region; *B*, in the 'reticular' zone.

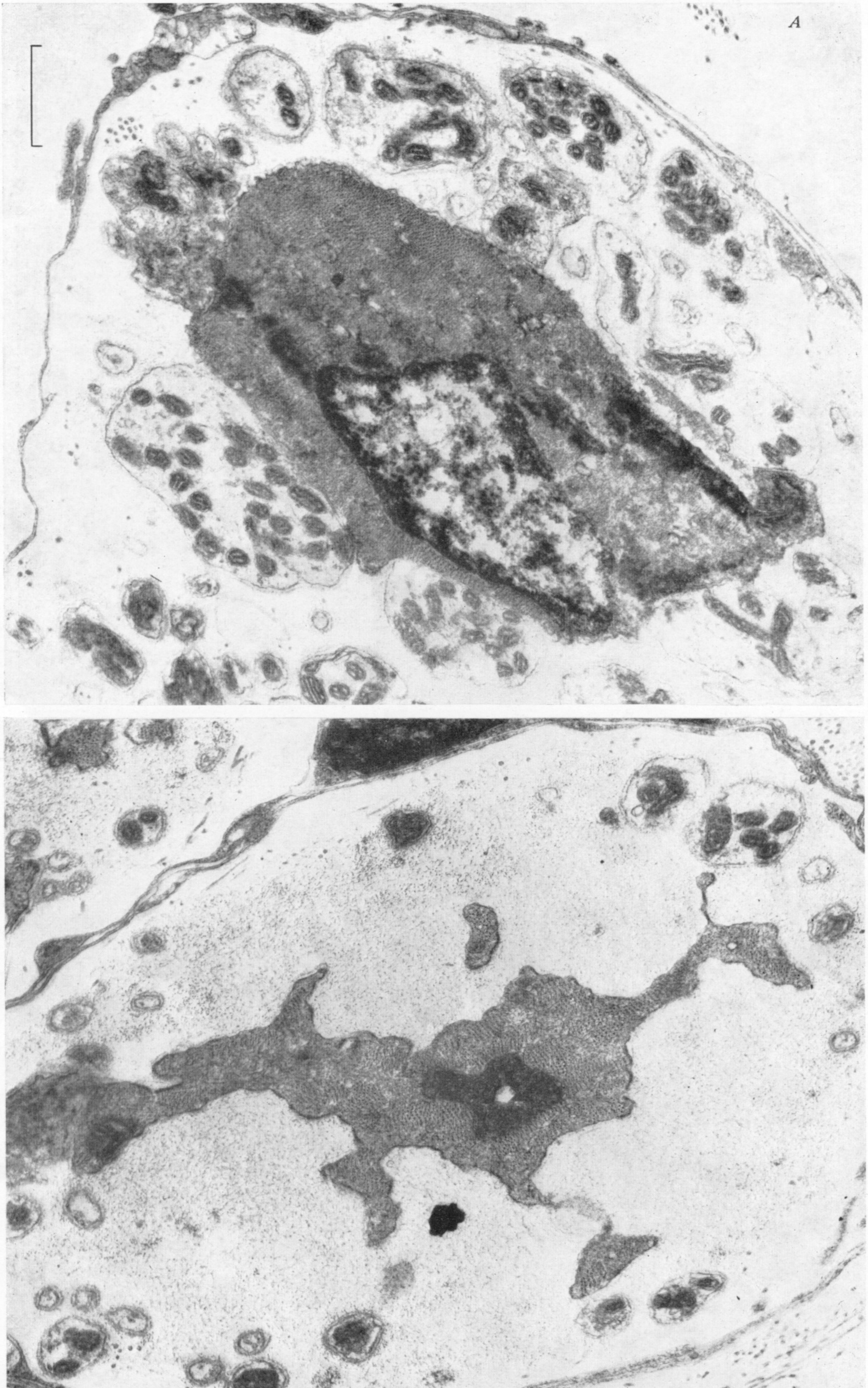


FIGURE 20. Another fibre (no. 3) in the 'compact' (*A*), and 'reticular' (*B*), zones of sensory contacts.

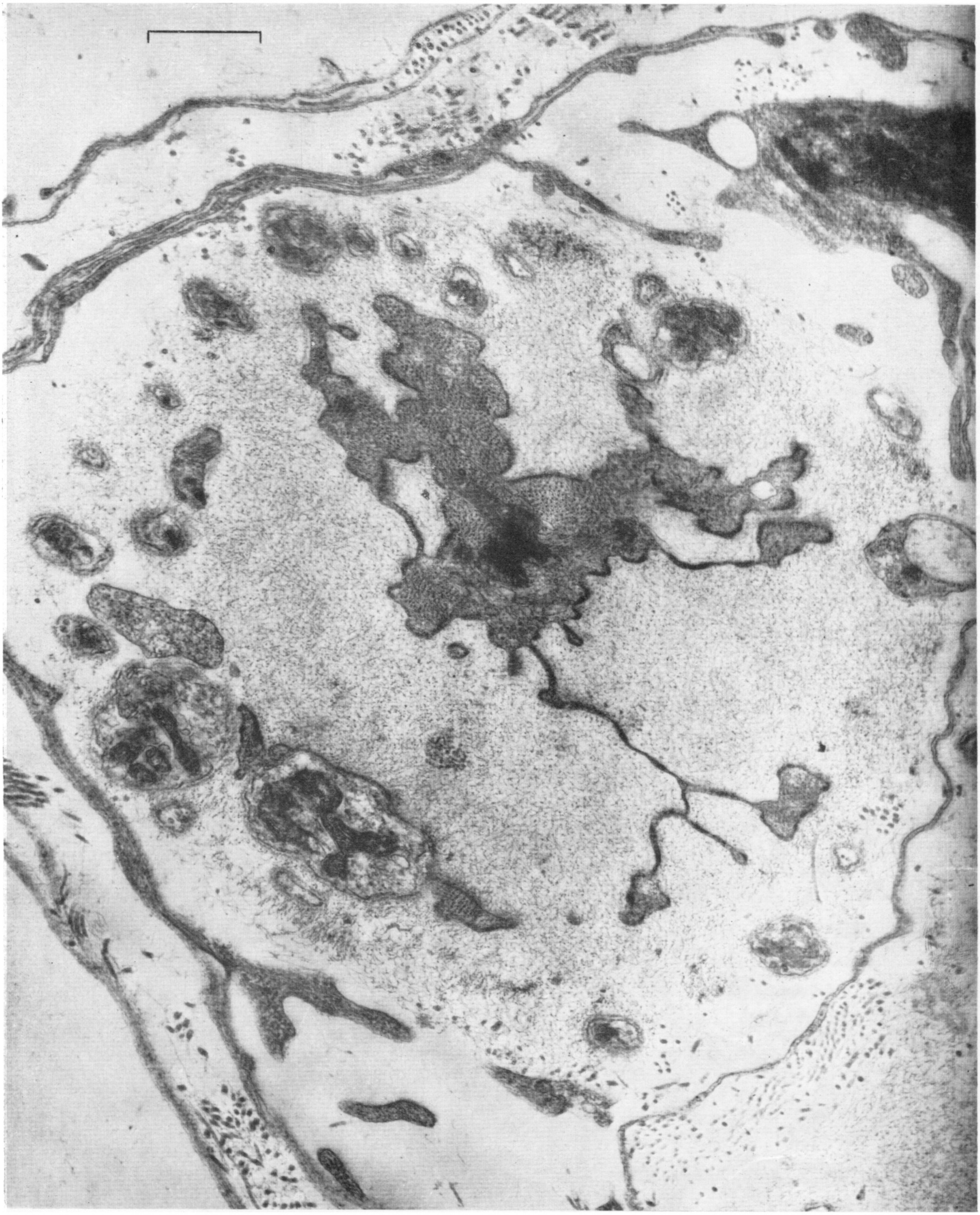


FIGURE 21. Transverse section through 'reticular' zone.

FIGURE 22. Longitudinal section through 'reticular' zone. *C*, capsule cells, with capsular nuclei (*C.N.*). *N*, nerve bulbs; *M*, portions of the fenestrated muscle fibre cut obliquely. The fibre surface forms a complicated framework which is embedded in a dense mesh of extracellular fibrils (*e.c.f.*).

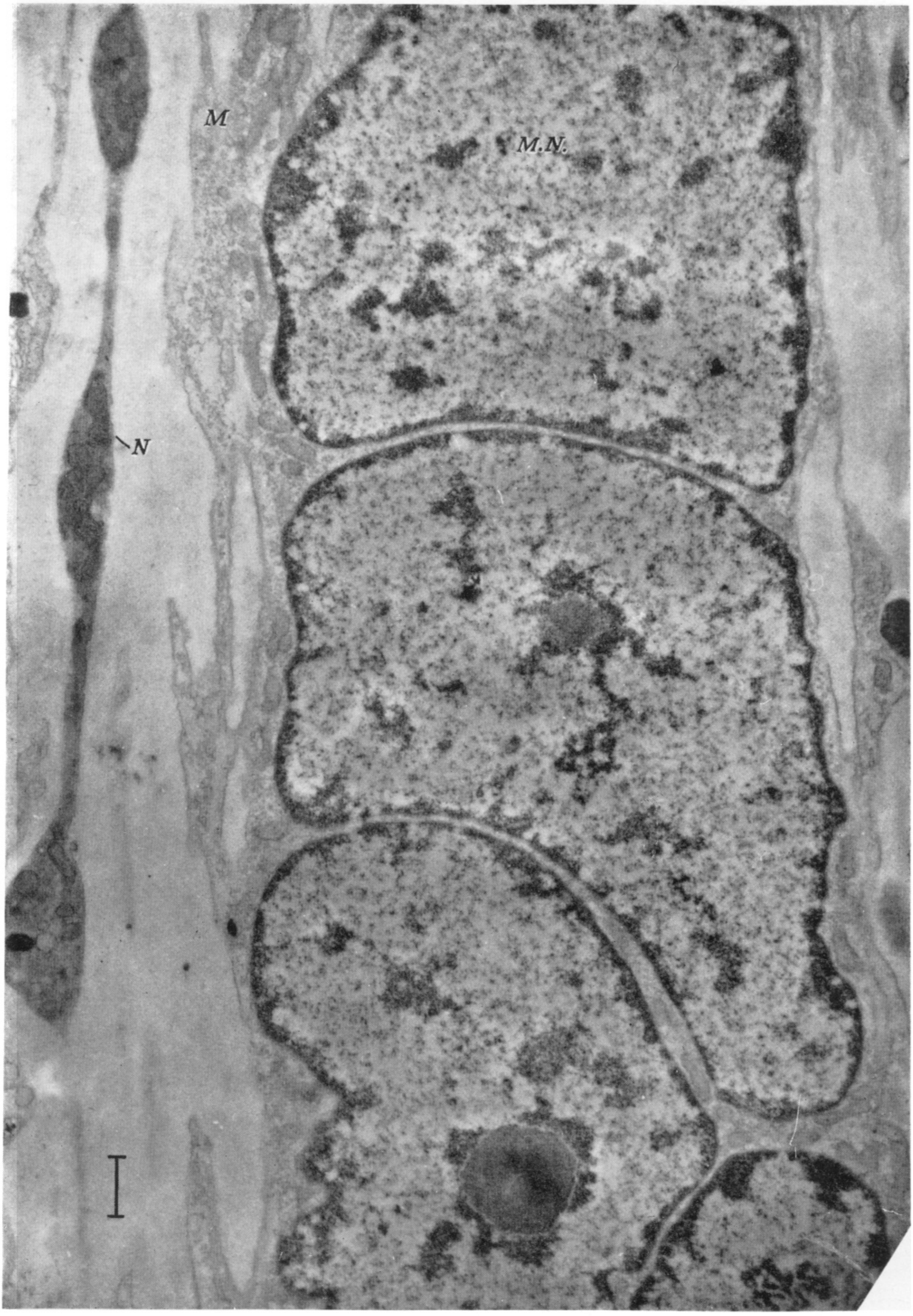


FIGURE 23. Longitudinal section through nuclear part of a 'reticular' zone. *N*, nerve chain; *M*, muscle fibre; *M.N.*, muscle nucleus.

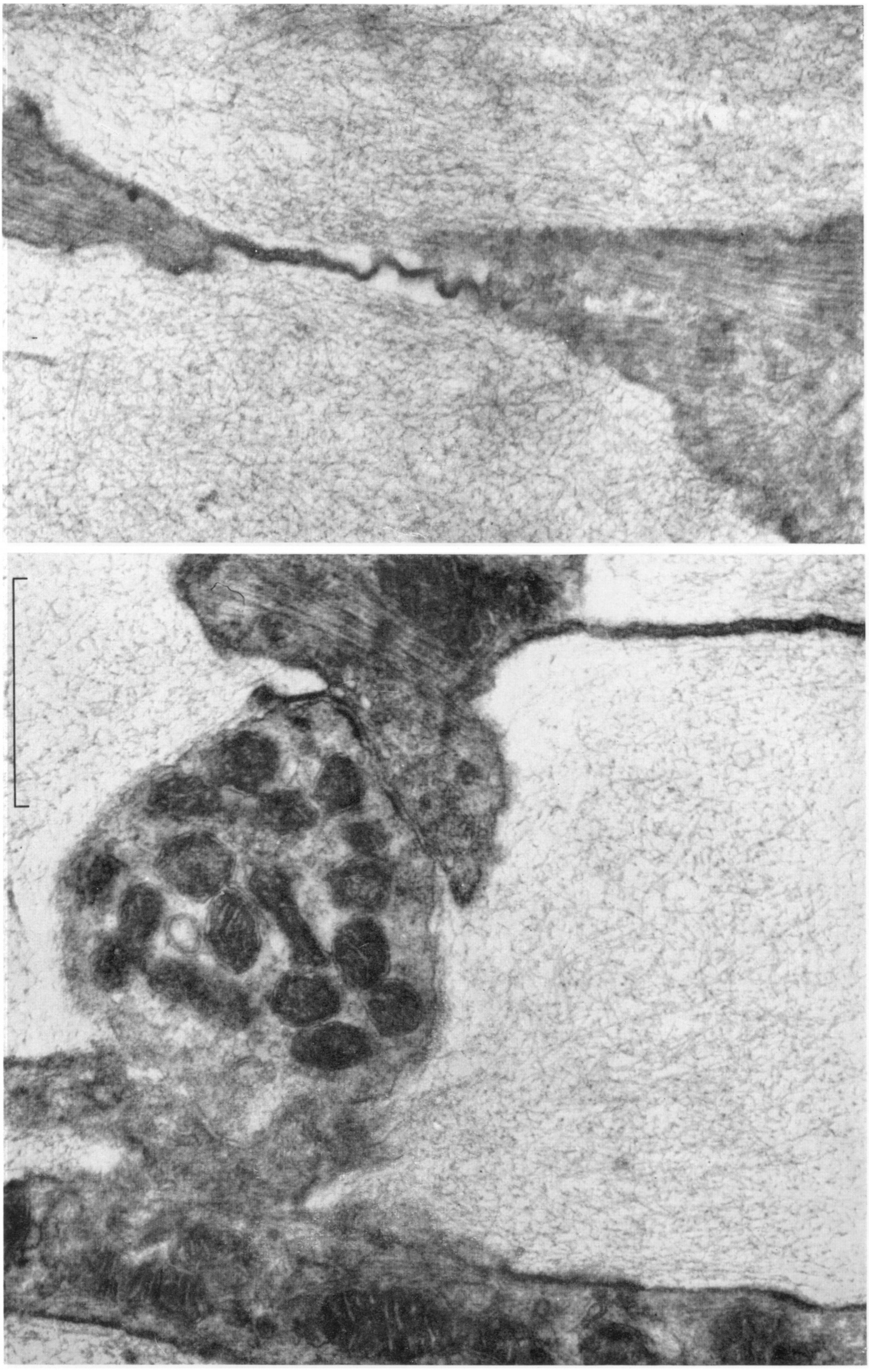
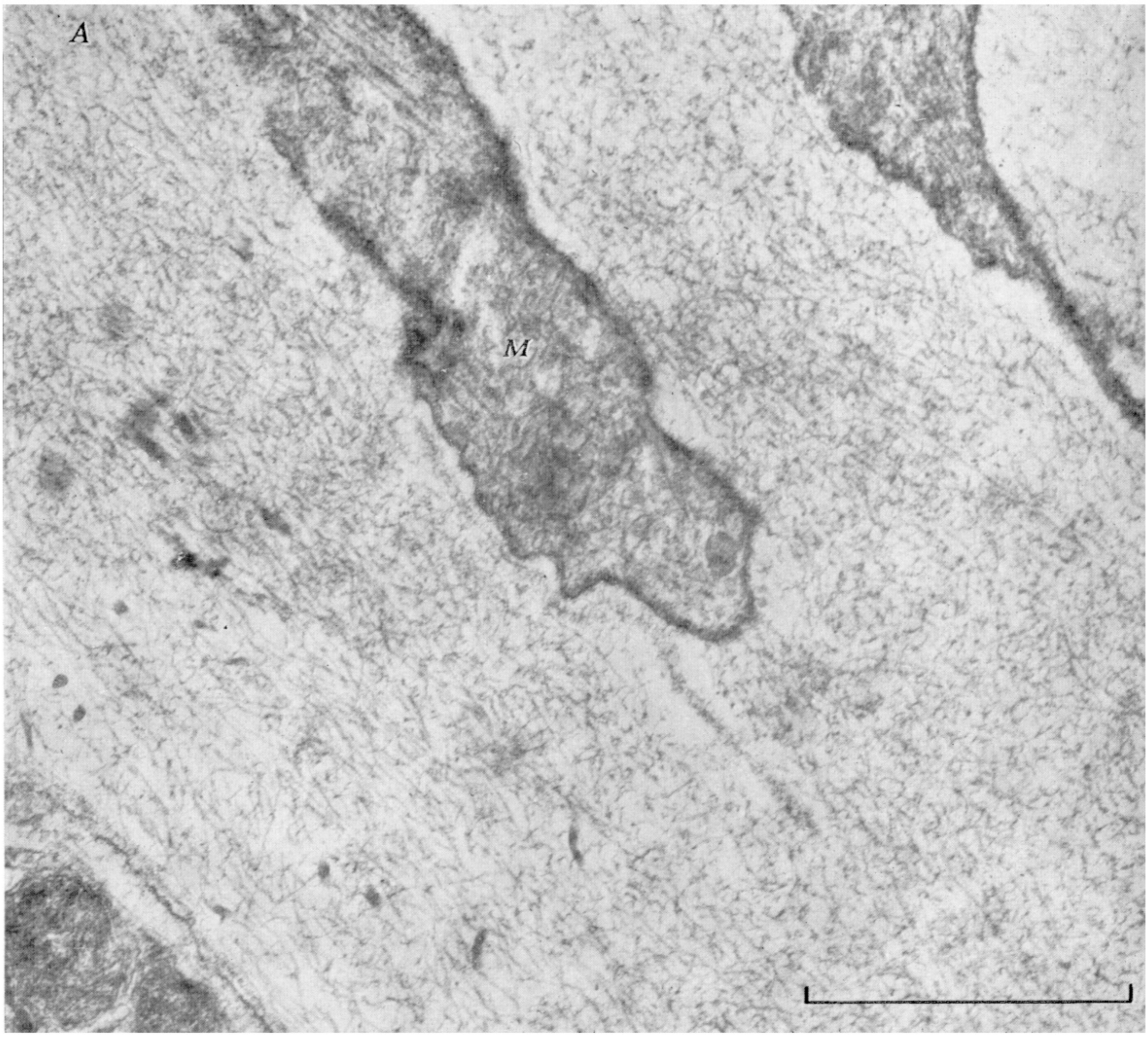


FIGURE 24. Higher magnification of connective tissue network in 'reticular' zone.



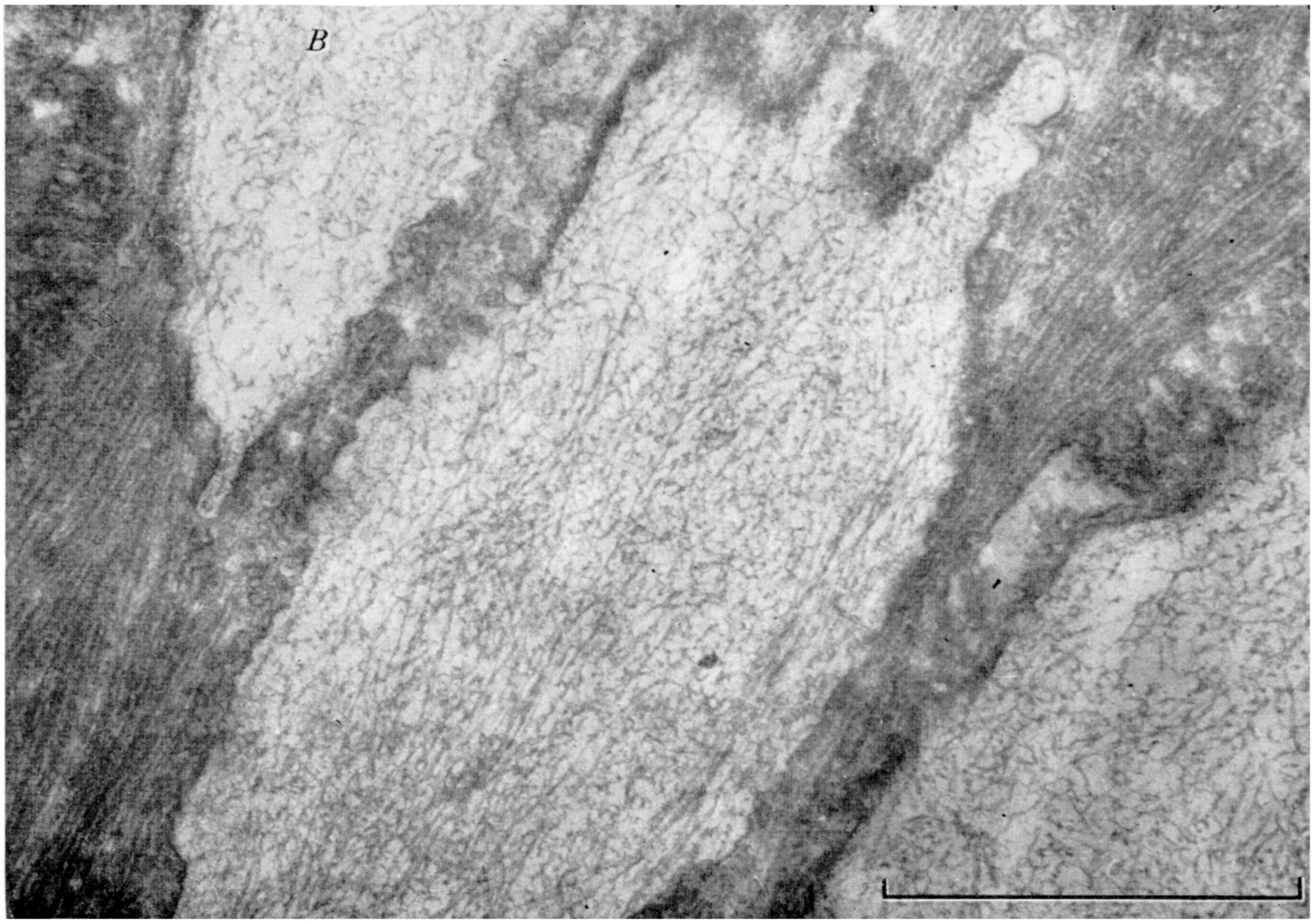


FIGURE 25. Extracellular network in 'reticular' zone. Longitudinal sections. In *A*, note trace of 'ectolemma' at the surface of the muscle fibre (*M*) and its relation to the extracellular network.

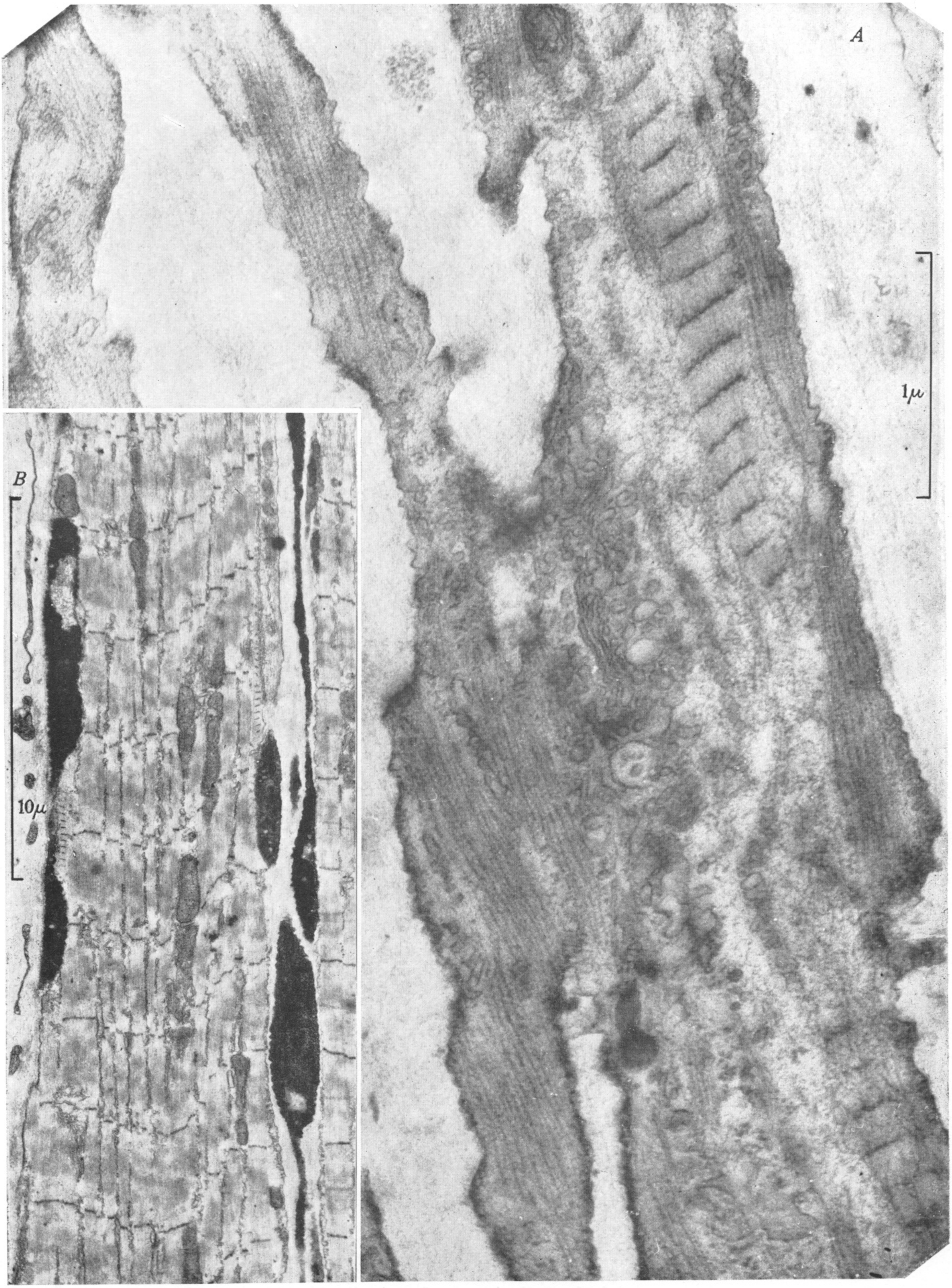


FIGURE 26. 'Microladder' in intrafusal fibres. *A*, from a 'reticular' zone. *B*, survey picture from a 'compact' zone.

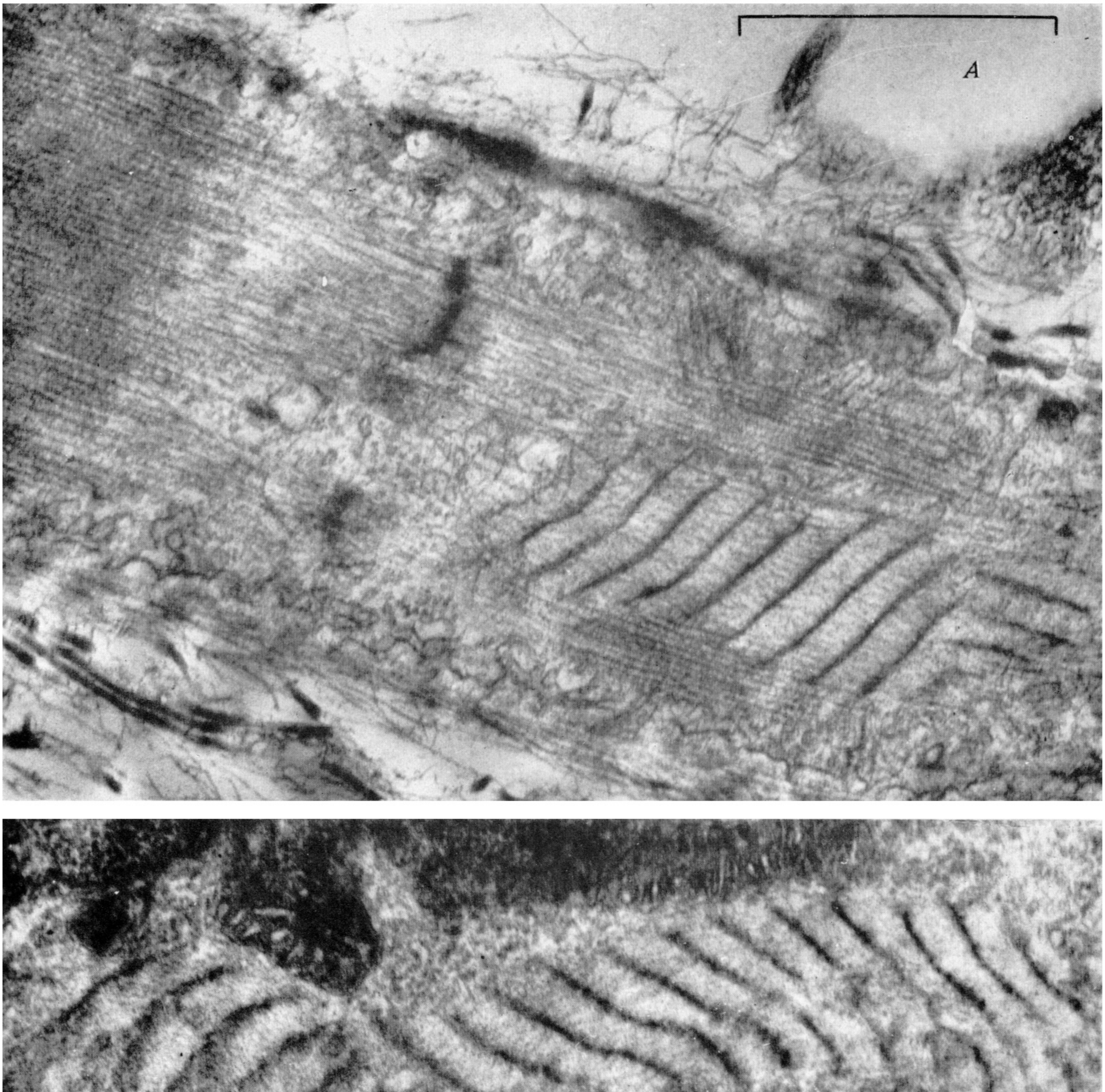


FIGURE 27. 'Ladders' in stretched (*A*) and slack (*B*) muscle.

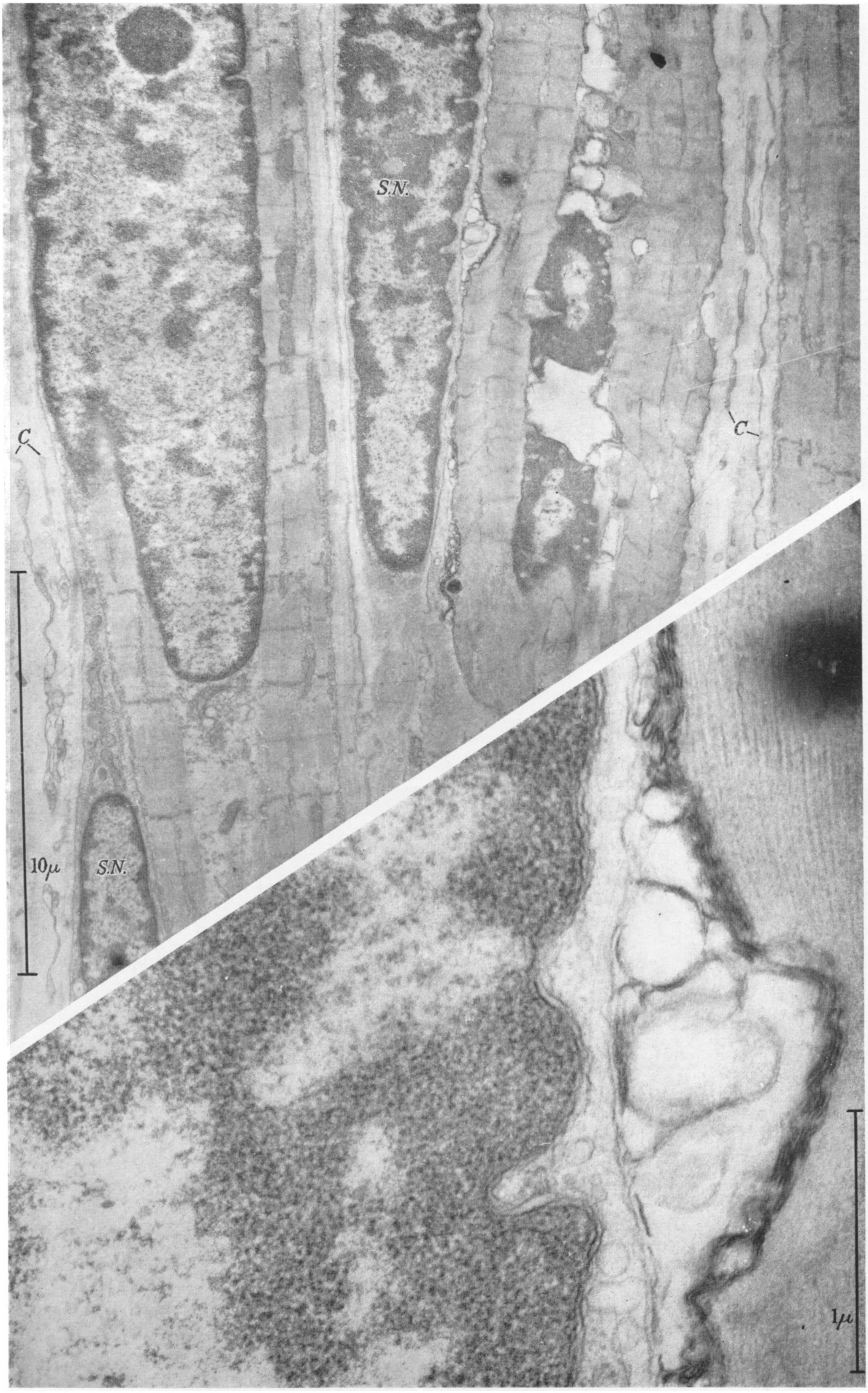


FIGURE 28. Denervated muscle, about 2 months after sciatic section. No sensory contacts are left, only occasional remnants as shown in this picture. *S.N.*, satellite nucleus. *C*, capsule cells.

FIGURE 29. Denervated spindle. Nuclear part of 'reticular' zone. No 'micro-spindles' or sensory contacts are found. *C*, capsule cells. *M.N.*, muscle nuclei.

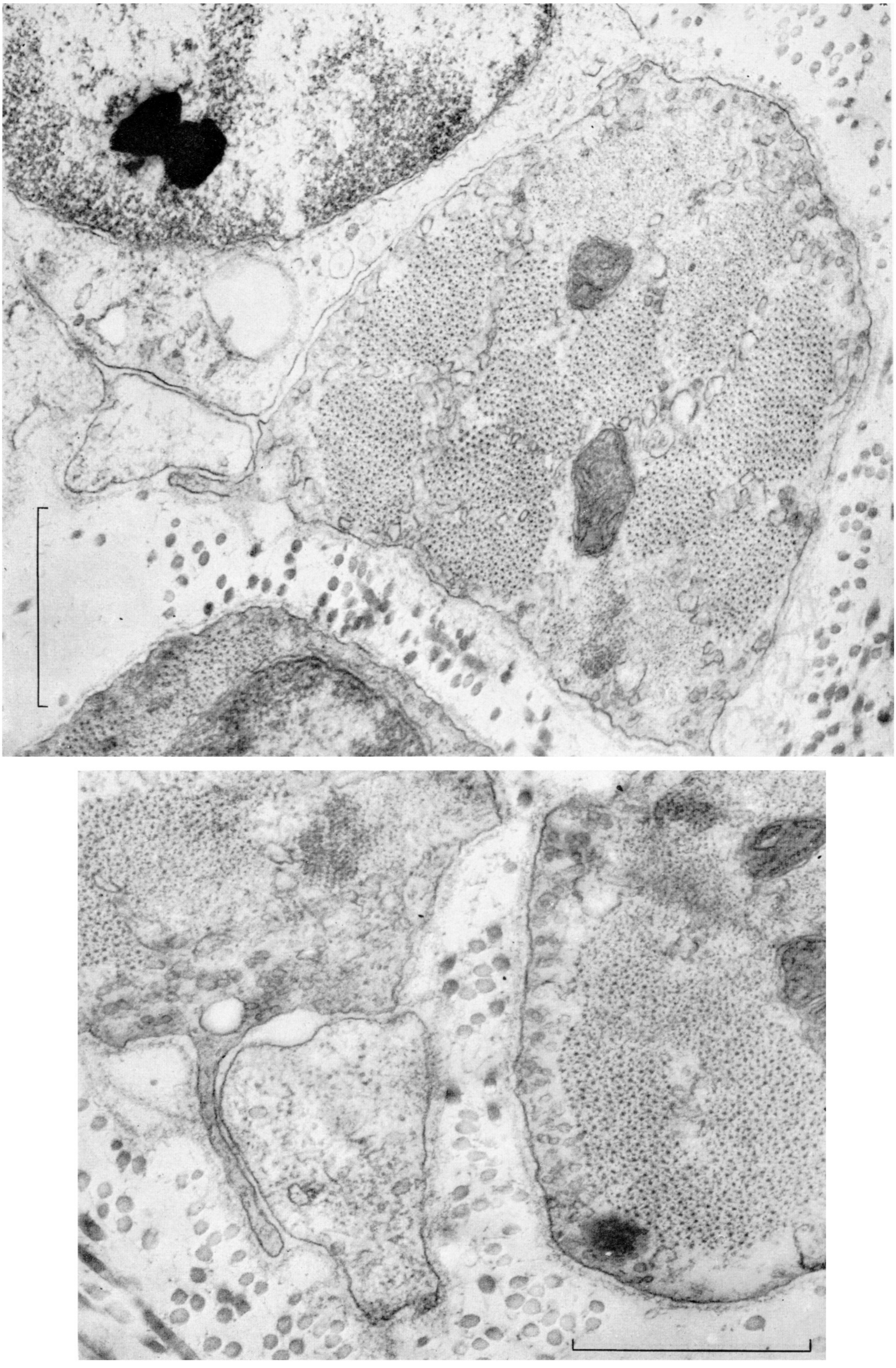


FIGURE 30. Small intrafusal fibres in cross-section, outside sensory region, together with portions of 'hypectolemmal' satellite cells.

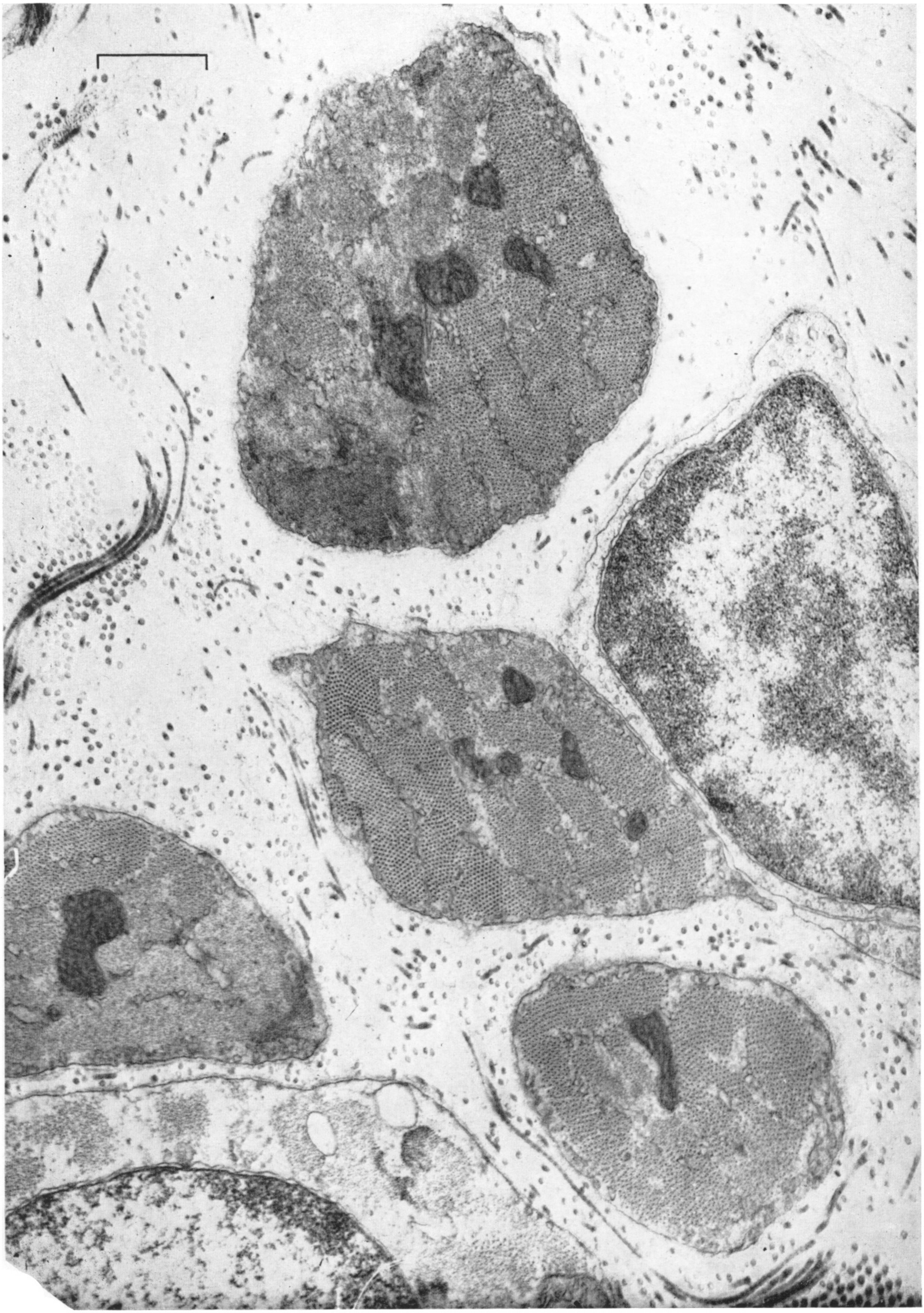


FIGURE 31. Group of small intrafusal fibres, one of them associated with nucleated satellite cell.

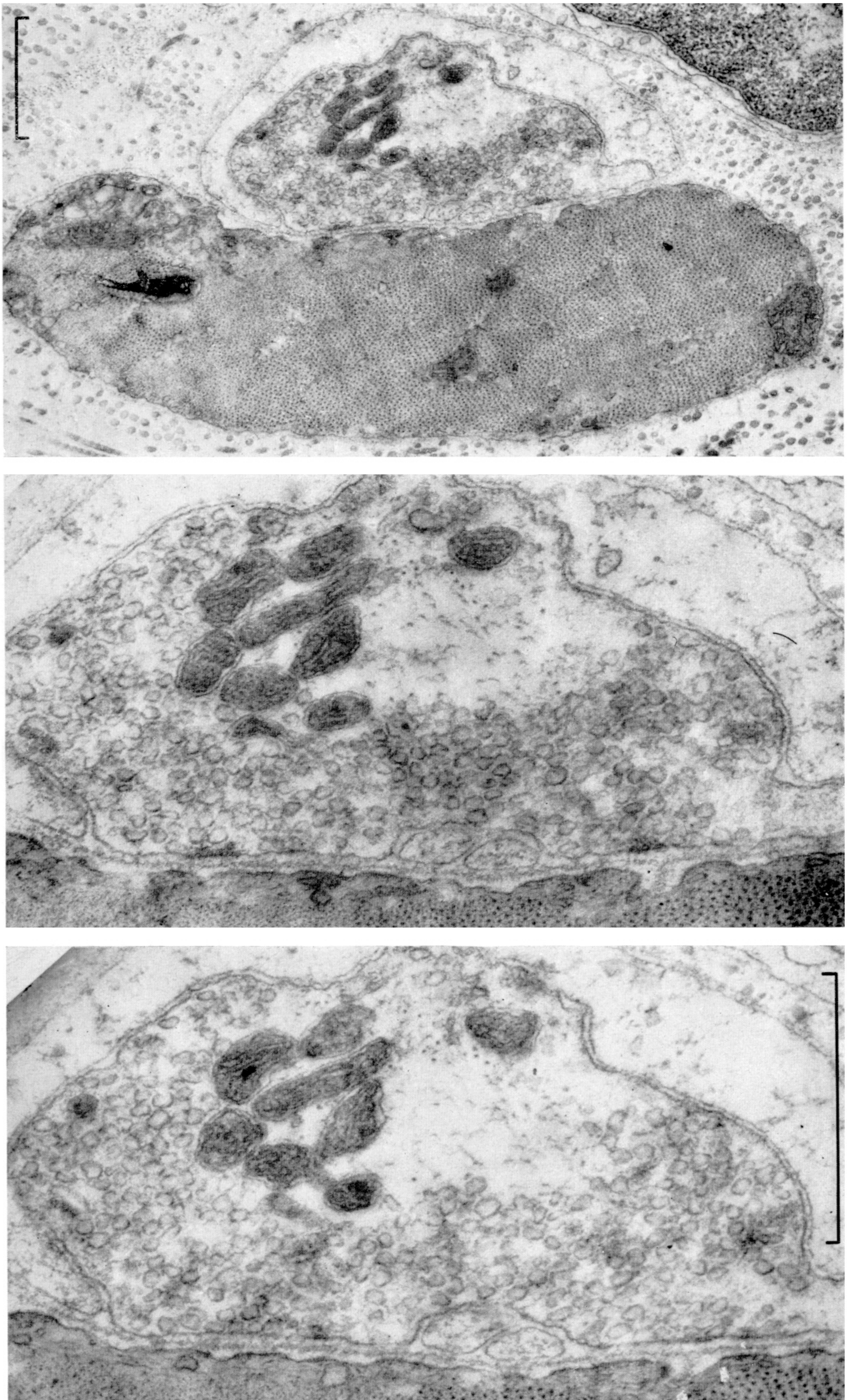


FIGURE 32. Intrafusal fibre with motor nerve ending and 'Schwann cover'. The motor ending is 'epectolemmal', in contrast with the sensory contacts, and contains the characteristic accumulation of vesicles. The two lower pictures are from serial sections, taken at higher magnification than the top picture. Level, 866 μ.

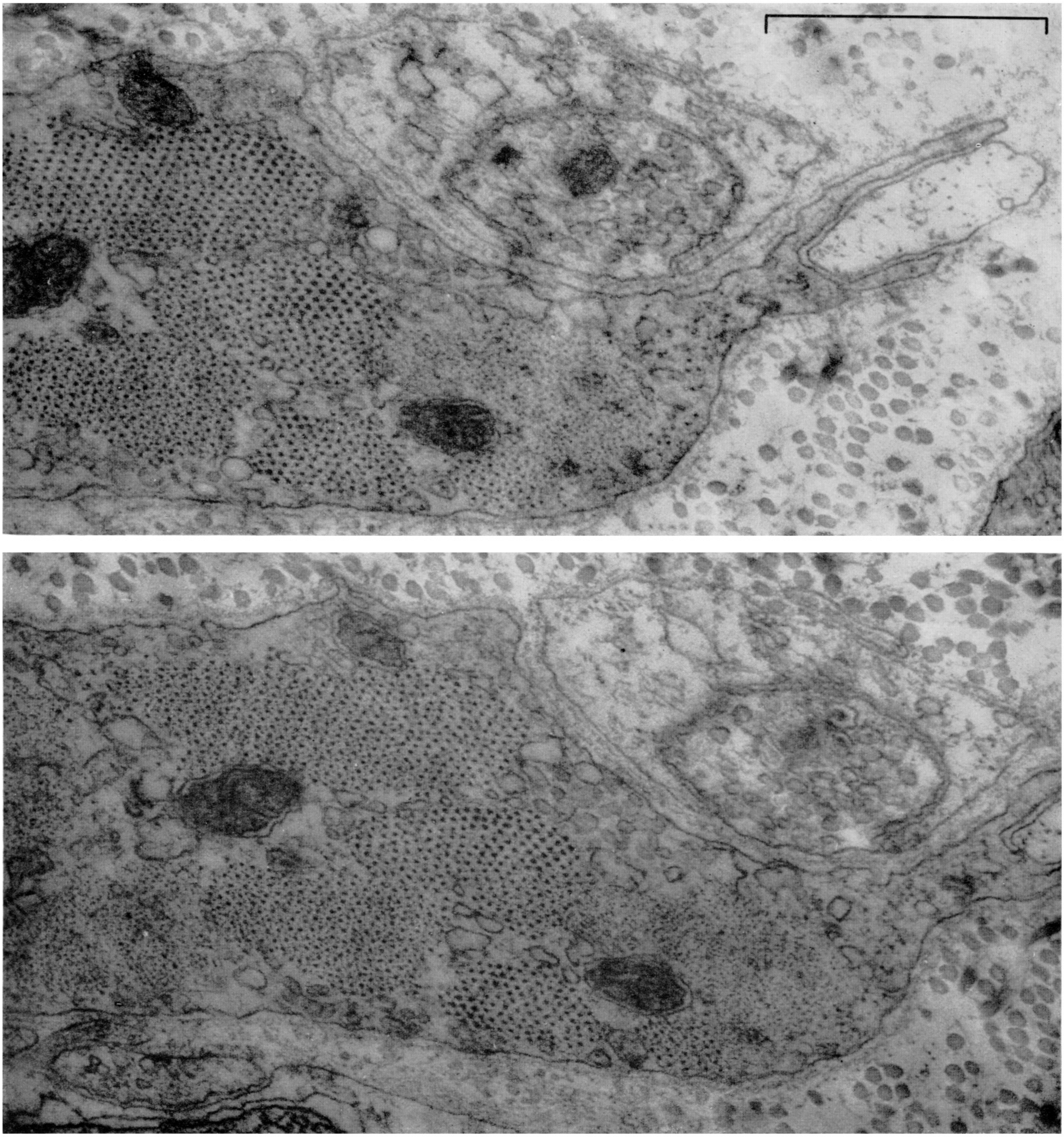


FIGURE 33. Two serial cross-sections of motor ending on another intrafusal fibre. (This one was possibly a 'slow' fibre.) Level, 866 μ.

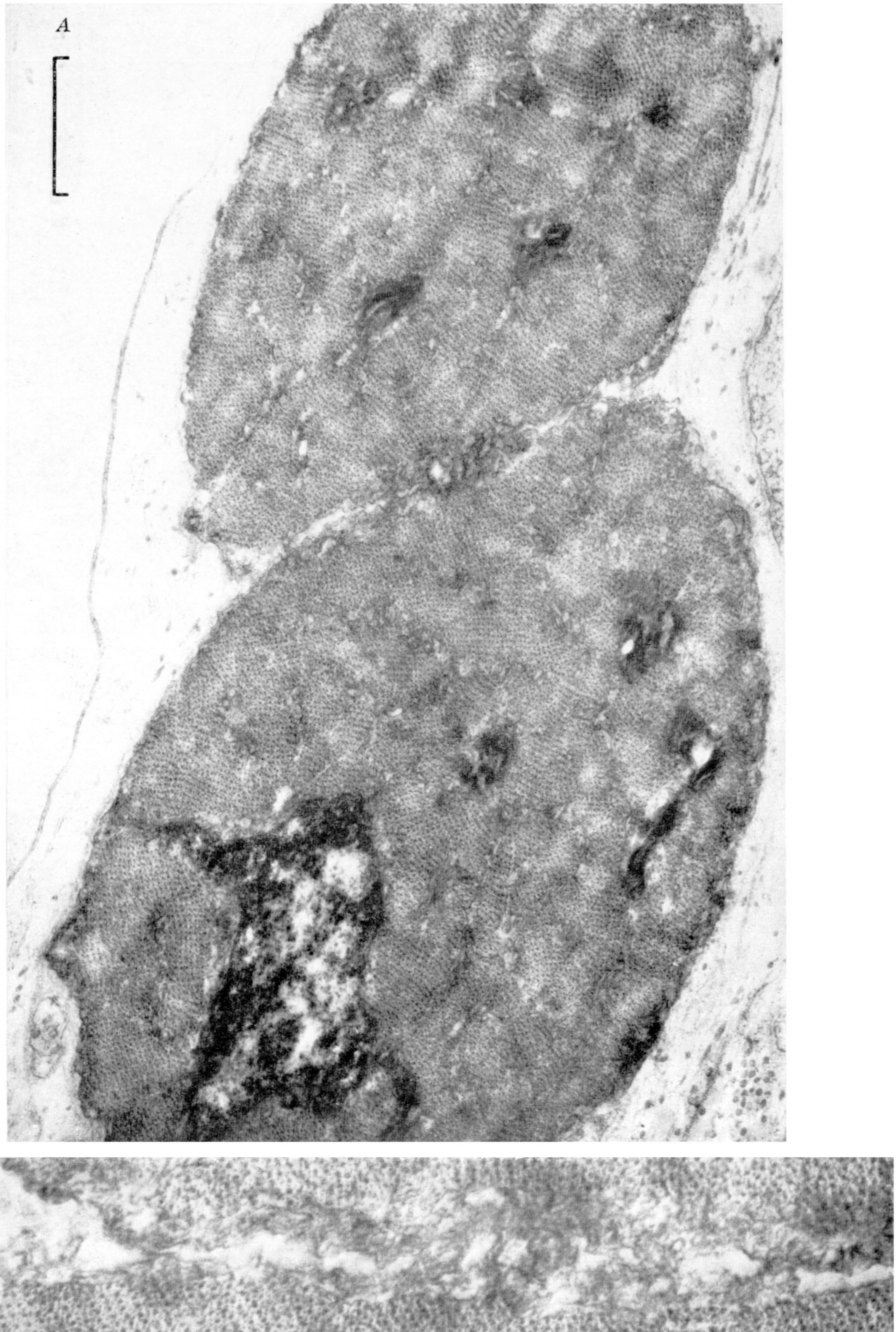
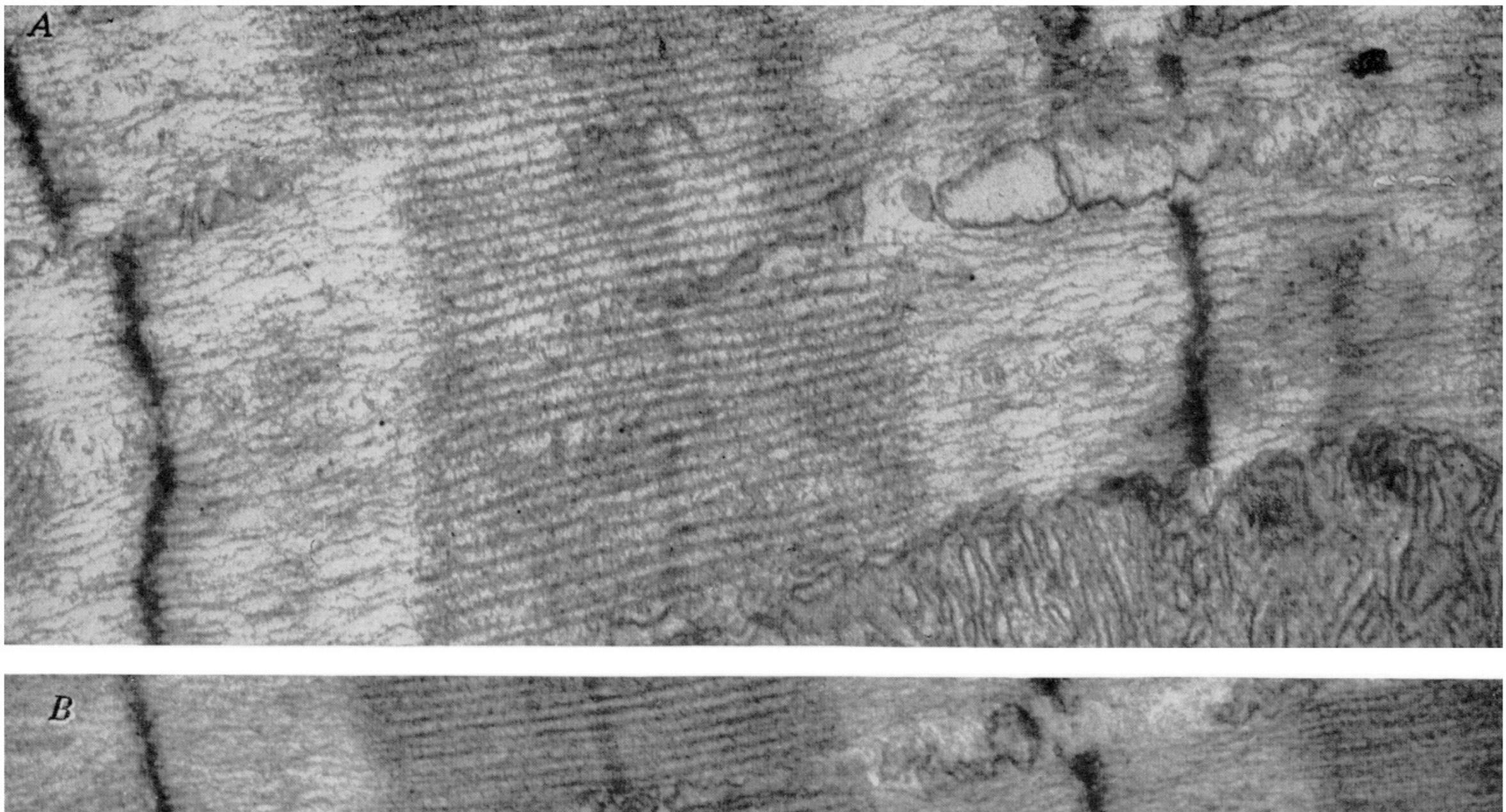


FIGURE 34. Two intrafusal fibres at a 'junction'. Level, 2387 μ. The lower strip, *B*, from a neighbouring section, shows an enlarged picture of the surface relation of the two fibres.



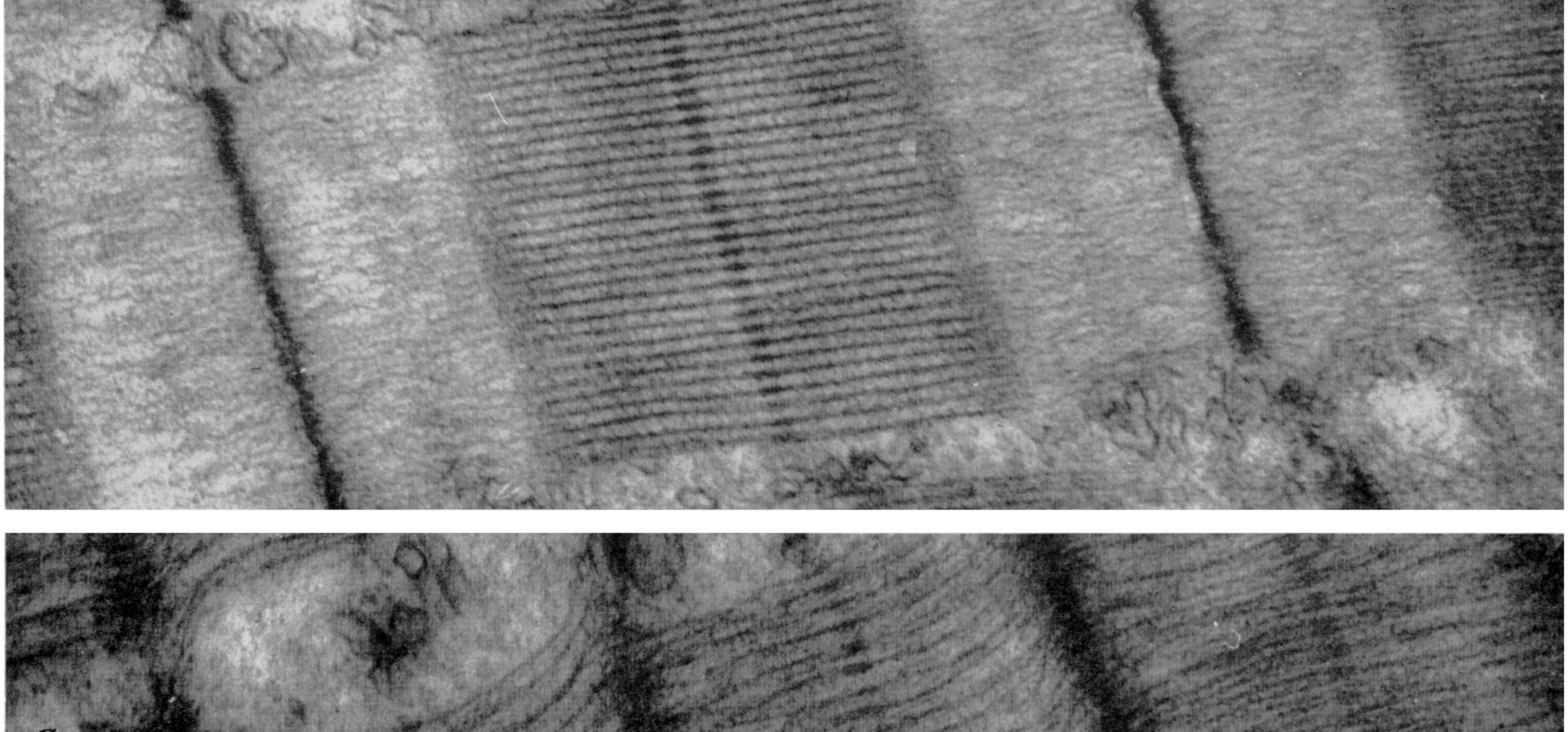


FIGURE 35. Longitudinal sections through ordinary fibres. *A* and *B* from stretched, *C* from short ('slackened') toe muscle. *Note*: sarcomere length in *C* is about half that in *B*, the *I*-bands having vanished, while the *A*-bands remain of about the same length.

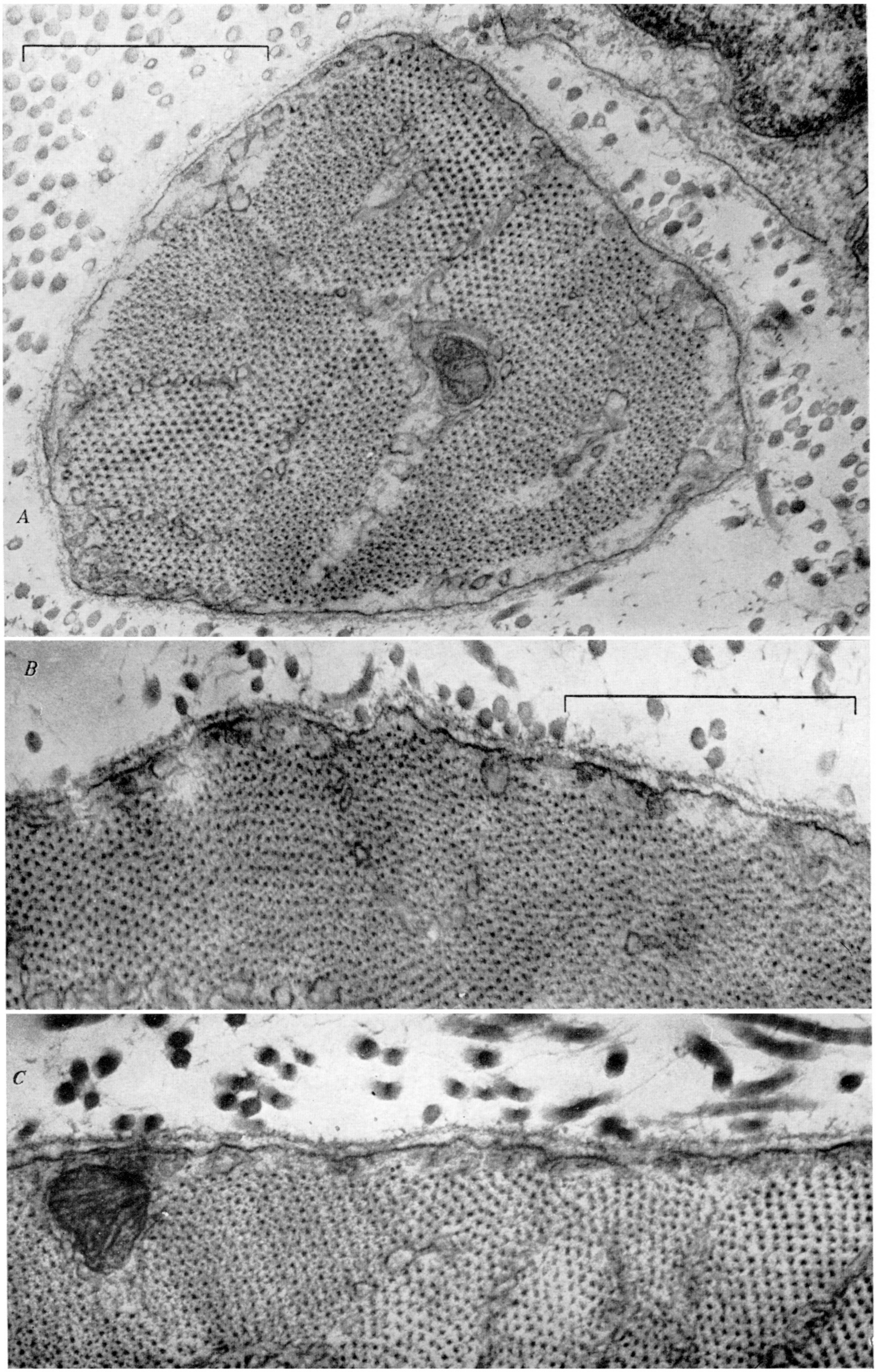


FIGURE 36. Showing surface of muscle fibres in cross-section. *A*, small, intrafusal, fibre; *B* and *C*, larger fibres from other parts of the toe muscle. Note 'ectolemma' and connective tissue fibrils.