FUSIMOTOR INNERVATION IN THE CAT

By D. BARKER, M. J. STACEY and M. N. ADAL†
Department of Zoology, University of Durham

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[Plates 60 to 70]

CONTENTS

The motor innervation of cat spindles was examined in hindlimb muscles using a variety of techniques employed in light and electron microscopy. Observations were made on teased, silver preparations of 267 spindles sampled from the peroneal, flexor hallucis longus, and soleus muscles, hereafter referred to as the PER/FHL/SOL series.

The γ innervation. Trail endings are almost invariably present, and innervate both bag and chain muscle fibres. Trail fibres accounted for 64.6 to 74.8% of the total fusimotor supply to samples of spindle poles in the PER/FHL/SOL series, the mean number of fibres per pole varying from 2.7 to 5.0 in the different muscles, and the mean number of ramifications (areas of synaptic contact) per fibre being 3.7. By contrast, the p_2 innervation of a spindle pole generally consists of a single fibre supplying only

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† Present address: Department of Physiology and Biophysics, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, Missouri 63110, U.S.A.

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one plate. In the above samples p_2 fibres accounted for 4.1 to 28.0 % of the total fusimotor supply, and the mean number of fibres per pole varied from 0.3 to 1.2 in the different muscles. Ninety per cent of p_2 plates innervate bag fibres.

The α innervation. The structure of p_1 plates as seen in both light and electron microscopy compares very closely with that of extrafusal plates. After nerve section p_1 plates degenerate at the same time as extrafusal plates, being the first of the three types of fusimotor ending to disappear. The frequency of the p_1 innervation is similar to that of the p_2 innervation. In the same samples of PER/FHL/SOL spindle poles as above p_1 fibres accounted for 6.0 to 28.8 % of the total fusimotor supply, the mean number of fibres per pole varying from 0.25 to 2.1 in the different muscles. The majority of p_1 fibres enter a pole to terminate in one plate only. Seventy-five per cent of the plates innervate bag fibres.

The three types of fusimotor ending are thus not selectively distributed to the two types of intrafusal muscle fibre. All three types of fusimotor fibre may branch within the spindle so as to innervate both bag and chain fibres. Bag fibres receive both types of plate ending as well as trail endings. Most chain fibres receive trail endings only; the rest receive either a p_1 or a p_2 plate innervation in addition, 25 % of the p_1 and 10 % of the p_2 innervation being distributed to chain fibres. The significance of this non-selective innervation is interpreted as indicating that the type of contraction elicited by stimulating a fusimotor fibre depends upon the type of ending initiating it rather than upon the type of muscle fibre executing it. Reasons are given for concluding that the dynamic response is controlled via the p_1 and p_2 plates, and that the static response is controlled by the trail endings.

The participation of the α fibres in mammalian fusimotor innervation, previously regarded as a vestigial feature, proved to be widespread in the muscles studied and more prevalent in fast muscles (FHL, peroneus digiti quinti) than slow (soleus). A low frequency of p_1 innervation is offset by a high frequency of p_2 (as in peroneus longus), and vice versa (as in FHL). It is unlikely that collaterals from slow α fibres innervating type B muscle fibres are wholly responsible for the high frequency of the p_1 innervation in FHL, and it is suggested that collaterals may also be derived from fast α fibres innervating type C muscle fibres. The possibility of there being some motor fibres of α conduction velocity and with an exclusively fusimotor distribution is also taken into account.

1. INTRODUCTION

The purpose of this paper is to attempt a definitive analysis of the motor innervation of cat muscle spindles, which has been the subject of controversy over the past decade. Differences of opinion arose when it was realized that in addition to the intrafusal motor end-plate, the spindle also receives another more diffuse type of motor ending. This was first detected in cholinesterase preparations, which revealed the presence of diffuse enzymic activity near the equatorial region, in contrast to that shown by the discrete subneural apparatuses of plates located further along the poles (Coërs & Durand 1956; Kupfer 1960; Hess 1961; Coërs 1962). Boyd (1962*b*) maintained that the diffuse ending terminates exclusively on the nuclear-chain muscle fibres, while the motor end-plates are always located on the nuclear-bag fibres. The contrary view, that there is not an exclusively selective innervation of this kind, was stated by Barker & Ip (1965), who named the diffuse ending the 'trail ending'. It then transpired that there are, in fact, three types of motor ending present, the plate endings being of two kinds which degenerate at different rates after nerve section and have different sources of supply, namely, from α fibres (p_1 plates) and from γ fibres (p_2 plates) (Barker 1966, 1967). The present situation is unsatisfactory since no detailed description of this triple innervation has been published, and no full account of the whole problem has appeared since the publication of Boyd's (1962*b*) monograph. Apart from the issue of selective innervation, we have been concerned with the electron microscopy of the three types of ending, their occurrence in different combinations among spindles, and the proportional distribution of these combinations in different hindlimb muscles.

One of the problems we hoped to shed some light on is whether the p_1 plates are supplied exclusively by collaterals of slow α fibres, or whether there are not, in addition, some that are supplied by fusimotor fibres whose diameter and conduction velocity places them within the α range. The α-collateral derivation has been demonstrated histologically by Adal & Barker (1965), and electrophysiologically by Bessou, Emonet-Dénand & Laporte (1963, 1965), who showed that in the cat 1st deep lumbrical muscle the α fibres concerned have slow conduction velocities of 61 to 31 m/sec. It is suggested by Bessou *et al.* (1965) and by Boyd & Davey (1968) that the existence of such α fibres in mammals probably represents a vestigial condition in the evolution from the skeleto-fusimotor disposition that characterizes the motor innervation of spindles in lower vertebrates (see review by Barker 1968*b*). On the other hand, the case for a functionally significant fast and direct fusimotor α pathway to the spindle, initially inconclusive (see review by Matthews 1964), has been consistently urged by Haase and his colleagues (see, for example, Barrios, Haase & Heinrich 1967), and has received further support from Carli, Diete-Spiff & Pompeiano (1966, 1967*a*, *b*). In our approach to the problem we assumed that if p_1 plates are supplied exclusively by collaterals of slow α fibres, they should occur with the greatest frequency among spindles located in slow muscles composed largely of type B muscle fibres. On the other hand, in a fast muscle composed largely of type A muscle fibres and supplied with more fast than slow α fibres, p_1 plates should be less frequent. Moreover, if the dynamic response of the spindle is controlled via its plate innervation, as concluded by Barker (1968*b*), the spindles in fast muscles should have a plate innervation that is predominantly p_2. We have therefore determined the relative frequencies of p_1 and p_2 plate innervation in spindles sampled from the soleus, peroneal, and flexor hallucis longus muscles, comparing the peroneal samples with those from the slow soleus and the fast flexor hallucis longus. We have also determined the ratio of γ fibres to spindles in these muscles, and have correlated this with the relative frequency of the p_1 and p_2 innervation in each one.

2. MATERIALS AND METHODS

Most of our observations have been made on the three peroneal muscles of young adult cats, peroneus longus (PL), peroneus brevis (PB), and peroneus digiti quinti (PDQ). We have also studied spindles sampled from the soleus (SOL), flexor hallucis longus (FHL), and flexor digitorum longus (FDL) muscles, tenuissimus, tibialis anterior and posterior, and the interosseous and lumbrical muscles of the hindfoot, usually taking the muscles from the right hindlimb. The fusimotor innervation has been examined in both normal and de-afferented spindles using a variety of techniques employed in light and electron microscopy. We have, for all purposes, removed normal material from a total of twenty cats, taking this from the normal hindlimbs of operated animals whenever possible. The results described in §3 (*f*) and §3 (*g*), summarized in tables 1 to 3, pp. 339 and 341, are based on observations made upon 267 spindles sampled as follows:

muscle	no. animals used	no. spindles normal	de-afferented	total
PL	6	18	28	46
PB	10	22	43	65
PDQ	8	31	25	56
FHL	5	56	1	57
SOL	4	31	12	43
		totals: 158	109	267

(a) *Operative procedures*

Cats were operated on under pentobarbitone sodium B.P. anaesthesia (dosage 40 mg/kg injected intraperitoneally) for the following purposes: de-afferentation in order to study the motor innervation in spindles with degenerated sensory innervation, and, in some cases, to obtain fibre-diameter histograms of the motor component of muscle nerves; sympathectomy followed by de-afferentation; and nerve section in order to study the progressive degeneration of muscle nerve endings.

(i) *Laminectomy*

In the earlier work carried out by Barker & Ip (1965), it was considered essential to isolate the fusimotor innervation by de-afferentation. Having become familiar with the identity of the three types of motor ending, we have preferred to study them in normal spindles, since these have the advantage of being free from the effects of any damage that might be inflicted on the ventral roots during laminectomy. We have, nevertheless, made use of a series of seven cats de-afferented for the earlier work, studying material from four of these (C159, C167, C169 and C172), the de-afferentation being unsuccessful in the remaining three, and muscles supplied by the sacral roots of cat C169 being excluded because of damage to the S1 ventral root. We have de-afferented a further two cats for both muscle and muscle-nerve study; one of them (C289) yielded successfully de-afferented material. De-afferentation was accomplished by removing the dorsal root ganglia L6 to S2 from the right side by a proximal extradural cut followed by a distal cut at the level of root fusion, great care being taken not to damage the underlying ventral roots. The animals were killed after an interval of 20 to 30 days, usually 26 to 28 days. This period allows sufficient time for sensory degeneration, and is short enough to rule out the possibility of motor fibres growing into the nerves and muscles by regeneration following crush damage to the ventral roots. In rabbits the rate of growth of axons after crushing is just over 1 mm/day faster than after severance and suture, i.e. 4.36 ± 0.24 mm/day as compared with 3.45 ± 0.16 mm/day (Young 1942). In cats the growth rate after severance is 2.5 mm/day (Bentley & Hill 1936), but the rate after crushing is unknown. We have assumed it to be 3.5 mm/day, and have allowed for a reorganization period of 3 days.

A check on the success of each de-afferentation was made by removing the operated roots and fixing them in Bouin's fluid prior to embedding in paraffin wax, longitudinal serial sectioning at 10 μm, and staining by the Holmes (1943) silver method. Apart from the presence of a few cell bodies of sensory fibres inevitably left intact in the ventral roots, or in the proximal part of the ventral ramus of the mixed nerve (see Stacey 1969), de-afferentation was judged to be complete in the animals used for study. There were no other cell bodies of sensory fibres present in the operated roots, and no surviving afferents were encountered in the muscles. Damage to ventral roots is betrayed by lesions in which regenerating fibres, nuclear proliferation, fibre disorientation, and disruption of the perineurium are evident, and also by signs of collateral regeneration in the muscles. As judged by these criteria, all the operated muscles selected for study were supplied by ventral roots that escaped damage during de-afferentation. Some slight damage may have passed undetected, but the amount was certainly not as high as the 10% that Boyd (1962*a*) regards as inevitable. This would involve between 500 and 1000 motor fibres in the L7 ventral root, a proportion that is greater than the total number of myelinated nerve fibres in most of the hindlimb muscle nerves. Boyd's figure of 10% is derived from his finding that if the same muscle nerve is respectively de-afferented and de-efferented in different cats, the sum of the motor and sensory components thus isolated is 15 % less (arbitrarily 10 % motor, 5 % sensory) than the total number of myelinated fibres in the normal nerve. Boyd & Davey (1968) have later amended these proportions to 10 % less, 6 or 7 % motor, 3 or 4 % sensory. In fact, the sum of such counts may be larger or smaller than the normal count; the difference, in our experience, has little to do with root damage and is mainly attributable to the variability of different cats.

(ii) *Sympathectomy*

In order to study a certain aspect of the fusimotor innervation concerned with the non-myelinated preterminal extensions of trail endings, two cats (C 219 and C 232) were sympathectomized 14 days prior to de-afferentation. The sympathetic ganglia L 5 to S 1 were removed from the right side of the animal using a retroperitoneal approach. Removal of the ganglia was checked by dissection performed at the autopsy following the period of sensory degeneration.

(iii) *Nerve section*

The right common peroneal nerve was severed in ten cats at the level where it crosses the lateral head of gastrocnemius. The denervated peroneal muscles were removed for the study of nerve-ending degeneration 24, 30, 48, 54, 60, 66, 72, 78, 84, and 96 h after operation.

(*b*) *Light microscopy*

(i) *Techniques for demonstrating nerve endings*

We have used both silver and histochemical techniques. Experience has shown that teased preparations are essential for the comprehensive analysis of spindle innervation. Reconstruction of spindles from silver-impregnated serial sections, as undertaken by Barker (1948) and Barker & Cope (1962), is extremely laborious, and some of the results are unconvincing since the method involves too great a measure of arbitrary interpretation. Of the methods available that yield teased preparations, we have found the methylene-blue technique to give very variable results and to be restricted in its successful application to thin muscles only. So far as fusimotor innervation is concerned, the results given by the gold chloride technique are altogether too coarse, incomplete, and equivocal for critical analysis. We have therefore used de Castro's silver method as modified by Barker & Ip (1963), which at its best gives remarkably complete and precise impregnations. We have found it an improvement to wash the muscle after fixation in tapwater that has been filtered, and also to mount in glycerine in preference to polyvinyl lactophenol.

Cholinesterase preparations of fusimotor endings were made by adapting the 'direct-colouring' thiocholine method of Karnovsky & Roots (1964) to give results with teased material. Rat muscles were used in trial experiments to adapt the technique. The modification consists of altering the incubation medium so as to include a 50 mM instead of a 5 mM solution of potassium ferricyanide, and to use an acetate buffer at pH 5 instead of pH 6. The muscles are placed in 10 % calcium formol on removal from the animal and immediately squashed between two glass slides and teased with fine needles. Optimum fixation occurs after 9 h at 4 °C. The muscles are then washed in distilled water for 5 min, incubated for 8 min, rinsed in distilled water, and then cleared and stored in glycerine. After a few days spindles are teased out and mounted in glycerine. This method stains fusimotor endings well, though trail endings located within the capsule tend to remain unstained; extrafusal motor end-plates are overstained.

(ii) *Techniques for demonstrating the motor innervation of individual intrafusal muscle fibres*

Most of our information about the motor innervation of individual intrafusal muscle fibres was gained from teased, silver preparations, assisted, in some cases, by drastic squashing, or by squashing after removal of the capsule by dissection. An attempt to verify this information was made by exploring the possibilities of combining our modified Karnovsky cholinesterase method with various histochemical techniques that distinguish between nuclear-bag and nuclear-chain fibres.

A combination of cholinesterase and Sudan black B staining on the lines advocated by Mayr (1969) gave equivocal results. Mayr maintains that in rat spindles treated in this way nuclear-chain fibres have longitudinal striations and stain darkly in contrast to cross-striated nuclear-bag fibres, which are lightly stained. However, in our experience the difference in staining and striation between the two types of fibre, in both rat and cat spindles, is not consistent enough to enable confident correlations to be made between type of motor ending and type of muscle fibre.

A method which proved much more successful combined cholinesterase staining with a modification of a technique devised by Nachlas, Tsou, de Souza, Cheng & Seligman (1957) for the demonstration of succinic dehydrogenase. This combination has been used by Ogata (1965) on frozen sections of mouse skeletal muscle for the demonstration of motor end-plates on extrafusal muscle fibres showing differing succinic dehydrogenase activity. We have modified the technique to obtain teased preparations of spindles in the following way. Muscles are frozen in a mixture of isopentane and liquid nitrogen immediately on removal from the animal, and stored in deep freeze for a minimum period of 4 days. Longer storage improves the quality of the staining, and the best preparations were obtained from muscles that had been stored for 3 weeks. The muscles are then allowed to thaw, and teased in mammalian Ringer (Tyrodes solution) before being placed for 30 min at 37 °C in the succinic dehydrogenase incubation medium, i.e. 0.2 M phosphate buffer (pH 7.6), 25 ml; 0.2 M sodium succinate, 25 ml; 0.1 % nitro-blue tetrazolium, 50 ml. The muscles are then washed in distilled water for 5 min prior to 17 to 20 h fixation in 10 % calcium formol. After a further 5 min wash in distilled water, they are processed according to our modified Karnovsky cholinesterase technique. In adapting this for the combined method it was found necessary to alter the concentration of potassium ferricyanide in the incubation medium to 25 mM, and to increase the incubation time to 15 min. In teased spindles produced by this technique, the rich mitochondrial content and high level of succinic dehydrogenase activity of the nuclear-chain fibres results in them being darkly stained in contrast to the lightly stained nuclear-bag fibres in which the mitochondria are smaller and more sparsely distributed. There is a tendency for the fibres to stain more deeply as they proceed outwards from the equatorial region through the polar regions. The combined cholinesterase staining renders it possible to recognize the three types of fusimotor ending, and identifications of their location on bag or chain fibres in the best preparations can be made with certainty. An advantage of the combined method over the use of the modified Karnovsky method on its own is that both intra- and extracapsular trail endings stain equally well.

(iii) *Muscle-fibre histochemistry*

Peroneal, soleus, and FHL muscles were frozen in a mixture of isopentane and liquid nitrogen, sectioned transversely at 10 μm in a cryostat at −20 °C, and processed according to the method of Eränkö & Palkama (1961) for demonstrating phosphorylase activity. A 1.5 mm^2 area of each muscle, representative of its total cross-section, was sampled for photographing at × 90. Light, grey, and dark colouring of muscle fibres was recognized as indicating respectively low, medium and high phosphorylase activity. Light muscle fibres were classified as type I (slow), grey and dark fibres as type II (fast) (Dubowitz & Pearse 1960). Muscles from the right hindlimbs of two cats were used and the results expressed as average ratios of slow: fast muscle fibres for each of the five muscles.

(iv) *Nerve-fibre measurements*

The total diameters of 100 fusimotor fibres, or fibre branches, supplying each type of ending in 93 peroneal spindles, were measured in teased, silver preparations using a Zeiss micrometer eyepiece and a × 40 objective. The measurements were made up to 0.5 mm from spindle entry as far from the endings as possible, each one being the mean of three internodal readings. Fibres broken off in teasing close to their endings were ignored. One hundred fibres supplying extrafusal plates in peroneal muscles were similarly measured for comparison, the measurements being made where the terminal branches of the fibres fan out from nerve trunks to form end-plates on the muscle fibres. Normal muscles from seven cats were used. Conversions of the micrometer readings into micrometres were such as to enable histograms of total fibre diameter to be constructed in 0.2 μm columns; fibres measuring less than 1.0 μm were classified as such. Stacey (1969) has shown that in order to convert myelinated fibre diameters measured in these silver preparations into their equivalent diameters in fresh material, it is necessary to multiply by a factor of 1.5. This conversion has not been applied in § 3, Results.

In order to construct fibre-diameter histograms of the motor component of muscle nerves, portions of de-afferented nerve were processed as for electron microscopy (see below), and 1 μm sections cut and stained with a 1 % solution of toluidine blue in 1 % borax. Selected sections were photographed at × 1000, and nerve-fibre diameters measured by placing serially graded holes cut in a Perspex sheet over the photograph or photographic montage of the whole nerve, continuous reference being made to the original section under the microscope.

(*c*) *Electron microscopy*

Three methods of fixation were used: (i) immersion for 2 h in 1 % osmium tetroxide buffered with veronal acetate (Palade 1952); (ii) immersion for 2 h in 3 % glutaraldehyde in 0.2 M cacodylate, followed by postfixation for 1 h with 1 % osmium tetroxide in a neutralized 4 % solution of potassium dichromate (Dalton 1955); (iii) perfusion of 3 % glutaraldehyde in 0.1 M cacodylate through the dorsal aorta for 10 min, followed by immersion in this fixative for 2 h and subsequent postfixation in 1 % osmium tetroxide in the same buffer for 1 to 2 h. During fixation the muscles were either cut into small pieces, or teased and spindles removed from them for further processing. The fixed material was dehydrated, embedded in Araldite or Epon, and 100 nm sections cut on an L.K.B. ultrotome. The sections were stained with lead citrate (Reynolds 1963), or double-stained with uranyl acetate followed by lead citrate, and then observed with an A.E.I. EM 6B electron microscope.

3. RESULTS

(*a*) *Diameters of fusimotor fibres close to the spindle*

Before describing the form of the three types of motor ending, it is useful to examine the diameters of the fibres supplying them near spindle entry, particularly in view of the correlation made by Boyd (1962*a*, *b*) between fibre diameter and type of ending in his description of a dual innervation comprising 'γ_1 end-plates' and a 'γ_2 network'. Histograms of the total diameters of fusimotor fibres and fibre branches supplying p_1 and p_2 plates and trail endings in peroneal spindles are shown in figure 1 *a*–*d*, and are compared with a similar histogram of the diameters of the terminal branches of extrafusal motor fibres (figure 1*b*). The diameters of the p_1 fibres (range 1.2 to 3.0 μm, peak 1.6 μm) are seen to be very similar to those of the terminal branches of extrafusal motor fibres (range 1.2 to 2.8 μm, peak 1.6 μm), and to be generally smaller than those of p_2 fibres (range 1.4 to 3.8 μm, peak 2.4 μm). The trail fibres are distinguished by a supply comprised of both myelinated and non-myelinated fibres, the former having a diameter range of 1.0 to 3.8 μm, the latter having diameters of 1.0 μm or less. In electron micrographs the non-myelinated fibres measure 0.2 to 1.6 μm in diameter with a peak at 0.8 μm (Barker & Stacey 1968). Boyd's 'γ_2 fibres' are equivalent to non-myelinated trail fibres and those myelinated trail fibres in the lower part of their diameter range. His 'γ_1 fibres' presumably include a mixture of p_1 and p_2 fibres as well as some thick trail fibres.

Our histograms show that the diameter of a myelinated fusimotor fibre close to spindle entry is no guide as to the type of motor ending it is connected to, though it may be said that the probability of p_1 plates being formed by fibres measuring 3.0 to 4.0 μm thick in teased, silver preparations may virtually be excluded. In tracing the course of 22 γ-stem fibres from muscle nerve to spindle entry in cat deep lumbrical muscles, Adal & Barker (1965) found that seven entered the receptors without prior branching. About one third of the fibres in the p_2 and trail histograms may therefore probably be regarded as γ-stem fibres rather than fibre branches.

(*b*) *The γ innervation: trail endings*

(i) *Light microscopy*

Trail endings are the first motor terminals to be encountered on examining the poles of a spindle on either side of the primary ending, their location being mainly intracapsular and juxta-equatorial. The mean length of the trail-ending area in 30 poles sampled from spindles in various hindlimb muscles was 0.84 mm extending polarwards from a point 0.25 mm distant from the centre of the region of nuclear bags and chains. In terms of Boyd's (1962*b*) areas of secondary sensory innervation, this means that trail endings usually occupy the S_2 region with extensions into the S_1 and S_3 regions. The terminals occasionally encroach on to the P region and may extend over the whole pole as the only type of motor ending present. The length of trail-ending areas in the sample referred to above ranged from 0.44 to 1.58 mm. The general disposition of trail endings is illustrated in figures 2*a*, 3 and 4, and figures 10, 12 and 13, plate 60.

A trail ending supplied by a myelinated fibre is composed of a number of ramifications that arise from the ultimate or 'preterminal' node (Ruffini's 1898 term) and also usually from the two or three nodes immediately preceding this. The naked preterminal axons that lead to these ramifications measure 1 μm or less in diameter, and often course for several hundred micrometres before finally terminating. A trail ending supplied by a non-myelinated fibre consists of a number of ramifications differing only in that they are connected via their preterminal axons to a thin incoming axon devoid of myelin. When present, such fibres almost invariably participate in the trail innervation of a spindle pole together with myelinated trail fibres, and are very seldom the sole contributors. Non-myelinated trail fibres may acquire irregular and intermittent lengths of myelin as they enter the spindle and course over the poles (see figure 37, plate 63); similarly, myelinated trail fibres may have short lengths devoid of myelin. Of 87 peroneal spindle poles, 32 (36.8 %) had a trail innervation in which non-myelinated fibres participated in addition to myelinated ones. In the total sample, the mean number of myelinated trail fibres per pole was 2.6; non-myelinated fibres, when present, contributed a mean number of 1.6 fibres per pole. It is rare to find a trail-ending area supplied by one myelinated fibre only. If intramuscular branching is taken into account (see Adal & Barker 1965), it is evident that there is a

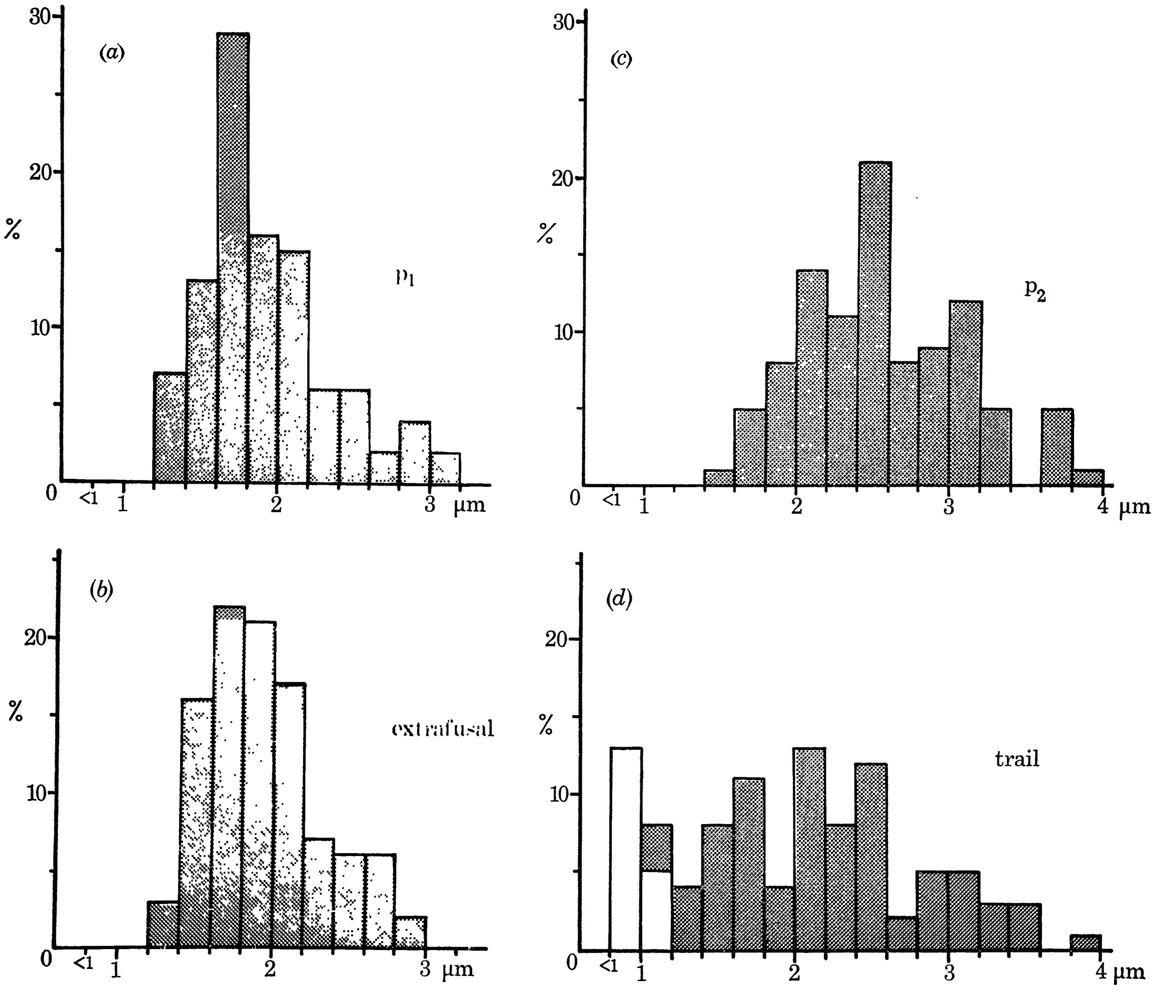

FIGURE 1. (*a*), (*c*), (*d*) Histograms of the total diameters of 100 fusimotor fibres, or fibre branches, supplying each type of ending in peroneal spindles as measured in teased, silver preparations up to 0.5 mm from spindle entry; (*b*), similar histogram of 100 terminal branches of extrafusal motor fibres. Unstippled columns in (*d*) indicate non-myelinated trail fibres.

FIGURE 2. Drawings of trail endings traced from photographs of teased, silver preparations of de-afferented spindles; *b*, nuclear-bag muscle fibre; *c*, nuclear-chain muscle fibre. (*a*) Trail-ending area in one pole of a soleus spindle supplied by two myelinated fibres; bag fibres stippled; *P*, S_1–S_3, Boyd's (1962*b*) areas of primary and secondary sensory innervation; ramifications in asterisked area shown at higher magnification in (*b*). (*c*) to (*e*) Details of trail ramifications (nuclei omitted) in spindles from PDQ, pes interosseous, and PB muscles, respectively.

considerable overlap in trail supply, so that the trail-ending area in most spindles is supplied with ramifications ultimately connected to several different trail stem fibres in the muscle nerve. In the 32 poles that received non-myelinated trail fibres in the above sample, they comprised 28.9 % of the total γ supply. In terms of the total γ supply to all 87 poles, the proportion of non-myelinated trail fibres was 14.1 %. Non-myelinated trail fibres therefore innervate just over one third of the spindles in peroneal muscles, and comprise between one quarter and one third of the total γ supply to these spindles.

It occasionally happens that a preterminal trail axon emerges from the site of a trail-ending area and leaves the spindle, either by way of the spindle nerve trunk or the extreme end of a pole. Examples of such recurrent preterminal trail axons are shown in figure 3 and figure 11, plate 60. In a sample of 227 peroneal spindles, 10 (4.4 %) were detected as having such axons leaving them. It has been suggested by Barker (1968*a*) that they ultimately reach other spindles nearby to contribute to their trail innervation, appearing as non-myelinated axons in their nerve supply. It seems likely that this is so, but the low incidence of recurrent preterminal trail axons renders it improbable that they account for more than a fraction of the non-myelinated trail supply. Most of this probably proliferates from the nodes of myelinated trail fibres some distance from the spindle. Barker & Stacey (1968) have observed non-myelinated fibres in electron micrographs of the main intramuscular nerve trunks of de-afferented and sympathectomized tenuissimus muscles, and are making a further investigation into the origin of these fibres.

The branching of myelinated trail fibres within the spindle is sometimes very profuse. Up to five myelinated branches, or myelinated branches and preterminal axons, may arise from a single node (see figures 33 and 34, plate 63). The majority of trail fibres entering a trail-ending area contribute more than one ramification to it. In a sample of 66 trail fibres, myelinated and non-myelinated, innervating peroneal spindle poles, 23 (34.8 %) ended in one ramification; the rest ended in from two to nine in more or less equal proportions, and there was one myelinated trail fibre that gave rise to twelve ramifications. The mean number of ramifications per fibre was 3.7. Reference to table 3, p. 341, indicates that the mean number of trail fibres supplied per pole varies between 2.7 and 5.0 in spindles sampled from the peroneal, FHL and soleus muscles. From this we can state that the mean number of ramifications in trail-ending areas varies from 10 to 18.5.

Trail ramifications vary from a single, simple brush-like taper to complex configurations that contain a variety of neurofibrillar synaptic contacts in the shape of brushes, hooks, knobs and rings (see figures 2 to 4, and plates 61 and 62). Those nearest to the area occupied by the primary ending are typically simple in form (figure 18, plate 61), while those at the polar end of the trail area tend to have a predominance of knob-like terminals (figure 16, plate 61). Most of the ramifications are elongated configurations, but some are more circular and plate-like (figure 22, plate 61). The length of a complex ramification, including the connecting strands of preterminal axon between the synaptic contacts, may extend up to 210 μm, whereas the simplest type may consist only of a single contact 10 μm long. Nuclei are present among the ramifications but there is no obvious sole plate as in extrafusal or p_1 motor end-plates. In cholinesterase preparations (see figures 38 and 41, plate 63) the trail-ending area appears as a scattered assortment of variously shaped deposits, some linear, others disposed in the form of circular or more or less oval troughs, or as simple spots. Towards the polar end of the trail area the deposits may appear in irregular honeycomb forms.

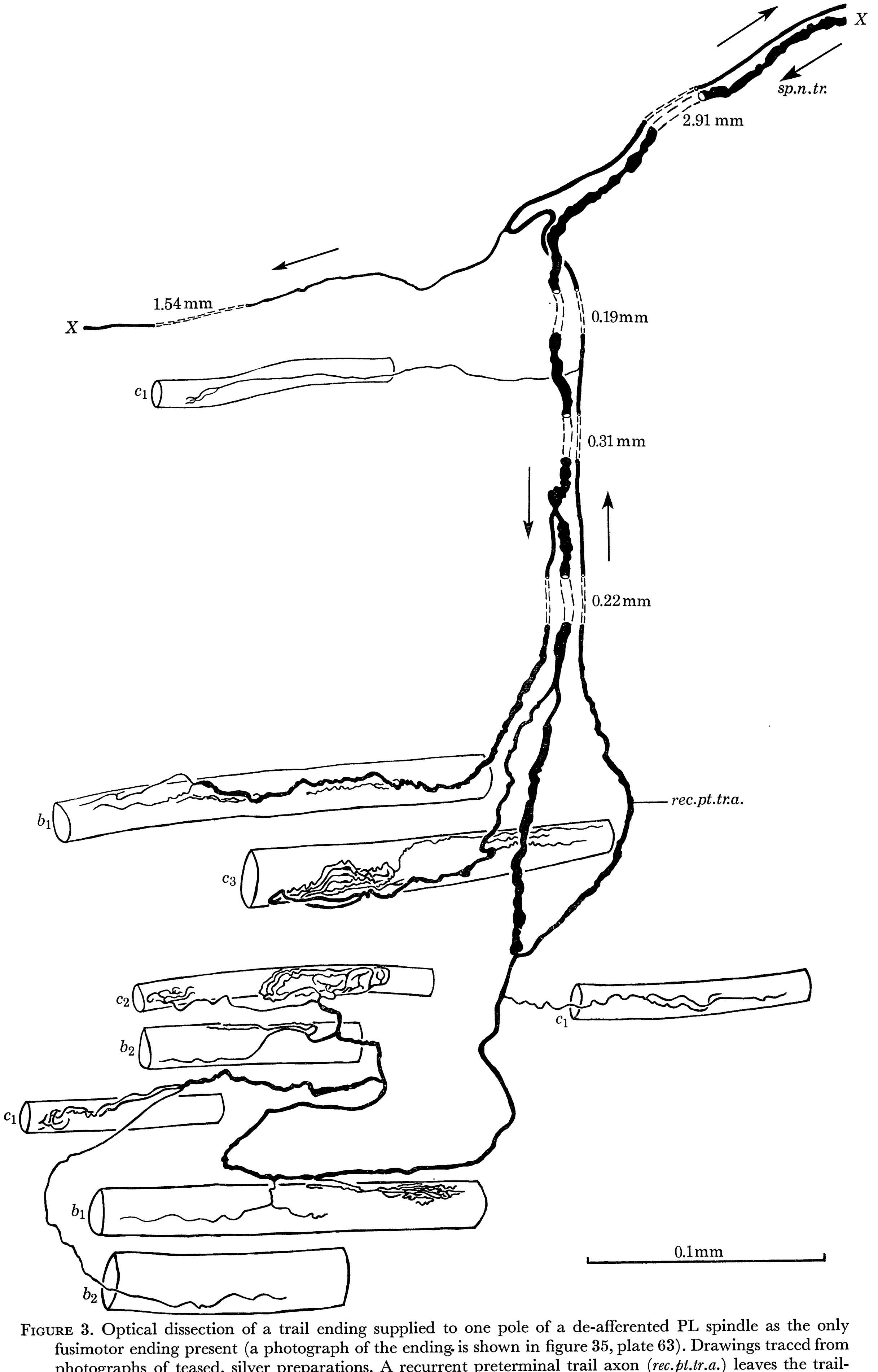

FIGURE 3. Optical dissection of a trail ending supplied to one pole of a de-afferented PL spindle as the only fusimotor ending present (a photograph of the ending is shown in figure 35, plate 63). Drawings traced from photographs of teased, silver preparations. A recurrent preterminal trail axon (*rec.pt.tr.a.*) leaves the trail-ending area and the spindle by way of the spindle nerve trunk (*sp.n.tr.*); a branch travels back alongside the pole outside the spindle. b_1, b_2, nuclear-bag muscle fibres; c_1 to c_3, nuclear-chain muscle fibres; X, fibres cut at these points in teasing.

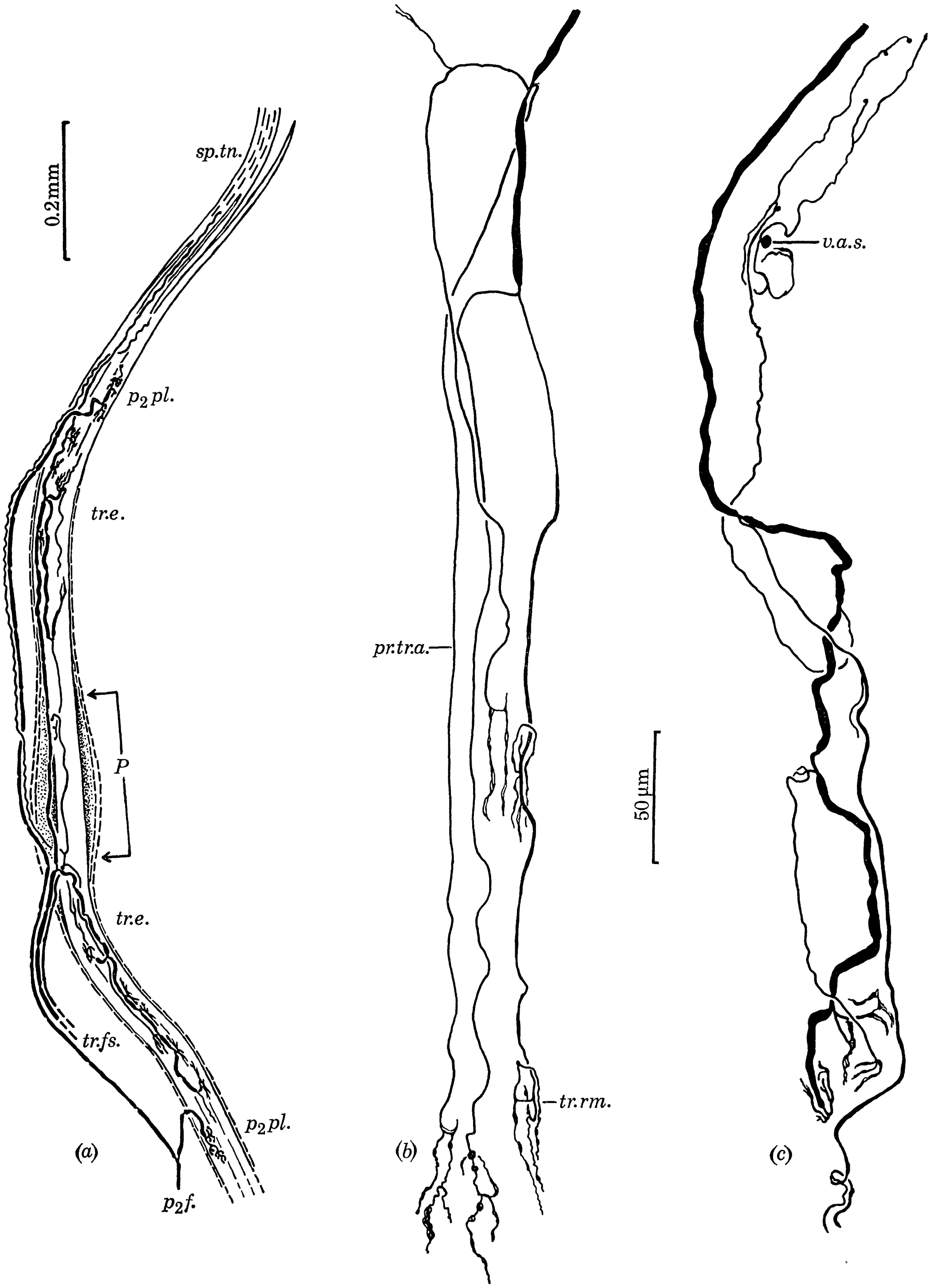

FIGURE 4. Camera lucida drawings of teased, silver preparations of normal spindles. (*a*) Part of a tandem spindle from PDQ innervated by two myelinated trail fibres (*tr.fs.*) and one p_2 fibre (p_2*f.*) which branch so as to supply both poles. Preterminal trail axons from both trail fibres terminate among the primary sensory ending (*P*, omitted) as vesicular axonic swellings. Another preterminal axon runs on to the spindle tendon (*sp.tn.*) to terminate abortively. Fusimotor fibres have been unravelled in their approach to the spindle for clarity. p_2*pl.*, p_2 plate; *tr.e.*, trail endings. (*b*) Trail ending in tibialis anterior spindle consisting of five ramifications (*tr.rm.*) formed by four preterminal axons (*pr.tr.a.*) that arise from the preterminal and penultimate nodes of the supplying myelinated fibre. (*c*) Trail ending in PB spindle. The preterminal axons arise mainly from the last four nodes of the supplying myelinated fibre. The one at the top of the figure ramifies in an area occupied by a secondary ending where it forms a number of vesicular axonic swellings (*v.a.s.*).

Trail endings may intermingle with secondary endings, but their ramifications do not overlap so as to occupy the same area of muscle fibre. However, preterminal axons frequently traverse areas occupied by secondary endings, and may leave one pole and course through the region of the primary ending to terminate in the other. As they make their way among the primary and secondary endings, they often form characteristic vesicular axonic swellings that face outwards away from the surface of the underlying muscle fibre and its sensory terminals (see figures 23 to 25, plate 61). Some preterminal axons may leave one pole and fail to reach the other, ending among the sensory terminals in a single vesicular swelling that has some resemblance to a growth cone (see figure 4*a* and *c*). These swellings are perhaps abortive trail ramifications whose further growth to form synaptic contacts has been inhibited by some influence emanating from the sensory area.

When the trail-ending area extends over the whole polar region in spindles that insert into tendon, some preterminal axons often run on beyond the tips of the intrafusal muscle fibres and ramify over the spindle tendon (see figure 4*a* and figure 15, plate 60). Some of these double back and return to the polar region, but others appear to end in what are presumably abortive contacts.

The ramifications of trail endings are located on both nuclear-bag and nuclear-chain muscle fibres. It is not possible to make any precise statement regarding their proportional distribution to the two kinds of muscle fibre, since it is extremely difficult to trace all the members of the intrafusal bundle through the intracapsular region. Our impression is that the chain fibres, which are from two to three times as numerous as bag fibres in this region, receive the major share of the ramifications, and we have occasionally seen bag fibres running through a trail-ending area in one pole of a spindle without apparently receiving any trail ramifications. On the other hand, we have repeatedly been able to satisfy ourselves, both in silver and in cholinesterase preparations, that bag fibres do receive trail ramifications, and we are confident that the usual condition is for the innervation not to be restricted to chain fibres (see figures 2 and 3; figure 14, plate 60; and figure 41, plate 63). Electron micrographs have confirmed the presence of trail ramifications on both types of muscle fibre (see Adal & Barker 1967, and below).

(ii) *Electron microscopy* (*trail*)

Electron micrographs of a total of 37 trail ramifications have been obtained from sections of 21 spindles from PL (10), PDQ (2), deep lumbrical (4), and tenuissimus (5) muscles. Sixteen of the ramifications were cut in longitudinal section, 21 in transverse. Using the criteria of Landon (1966) for distinguishing between the ultrastructure of nuclear-bag and nuclear-chain muscle fibres, 18 of the ramifications were identified as being located on bag fibres (5 in L.S., 13 in T.S.), and 19 on chain fibres (11 in L.S., 8 in T.S.). Details of their ultrastructure are illustrated in plates 64 and 65.

Typically the axon terminals are applied to the surface contour of the muscle fibre and overlie a thinly spread sole plate that lacks depressions or guttering, In some junctions there is no trace of any sole plate. In a few instances we have observed a break in continuity of the basement membrane in the synaptic cleft resulting in a closer apposition of axon terminal and muscle fibre (see figure 46, plate 65). This feature has been noted by Karlsson, Andersson-Cedergren & Ottoson (1966) in frog fusimotor myoneural junctions, and also by Düring & Andres (1969) in what appear to be trail junctions in spindles of various mammals, including cat. Preterminal axons situated near the terminals running in between the muscle fibres are a characteristic feature of most sections. Junctional folds are either absent or occur sporadically. When present they generally occur at irregularly spaced intervals and are wide and shallow with the basement membrane lining each wall without fusing in the midline (see figure 47, plate 65), as it does in the junctional folds of p_1 and extrafusal plates. The folds are usually U- or V-shaped and seldom have entrances constricted to form a 'neck'. They are unbranched, and two or three neighbouring ones often join together at their bases to isolate small islands of sole plate. As compared with the junctional folds of p_2 plates, those underlying trail terminals are usually shallower, spaced further apart, and have wider entrances.

(*c*) *The γ innervation: p_2 plates*

(i) *Light microscopy*

The p_2 plate is located in the mid-polar region and towards the extreme end of the pole (see figure 4*a* and figures 10 and 13, plate 60). They may thus be situated within the polar end of the trail-ending area, or lie well outside the capsule. Their most characteristic features are the knob-like axon terminals and the diameter of the supplying fibre, which is usually large (see §3 (*a*)). In contrast to trail endings, the preterminal node does not give rise to long non-myelinated stretches of axon before terminating, but ramifies straightaway to form a plate. These occupy an elongated rather than a circular area, and axon terminals are often derived from the last two or three nodes of the supplying fibre as well as from the preterminal one. The average length of 45 p_2 plates in peroneal spindles was 72.9 μm in a range of 27 to 120 μm. The average lengths of extrafusal and p_1 plates are less than half this (see §3 (*d*)).

The general form of the p_2 plate is illustrated in figure 5*c*, *d* and plate 66. Knobs, rings and, less frequently, tapers make up the terminals. Ruffini (1898, p. 203) described them as 'a number of most delicate and elegant knobbed arborescences' and was so impressed by the difference between these plates and extrafusal ones that he concluded they were sensory. Though nuclei are present in the plate there is no obvious Doyère eminence; indeed, a feature of the ending is the manner in which the terminals may be seen in side view to be closely applied to the surface of the muscle fibre (see figures 55 to 57, plate 66). Sometimes a short ultraterminal fibre may run on from the end of a plate and terminate in a ball of axoplasm larger than the knob-like terminals among the ending (see figures 48, 52, plate 66). This may represent the final synaptic contact made by the plate, or it could be interpreted as a growth cone and be the expression of growth among p_2 plates comparable to that described by Barker & Ip (1966) to occur among extrafusal plates. Cholinesterase preparations indicate that each knob-like terminal is associated with a circular subsynaptic ring, the deposits of enzymic activity giving the plate the appearance of an elongated cluster of such rings (see figure 41, plate 63, and figure 54, plate 66).

Some confusion can arise in distinguishing p_2 plates from some of the trail ramifications that occur towards the polar end of the trail-ending area (compare figure 51, plate 66 with figure 22, plate 61, and note the resemblance to p_2 plates of the polar trail ramifications illustrated in figure 16, plate 61). A positive identification of the type of ending involved can usually be made by tracing the supplying fibres concerned, observing the fate of other branches, noting fibre diameter, presence or absence of preterminal axons, and so on.

The majority of p_2 fibres enter a pole to terminate in one plate only; of 100 peroneal p_2 fibres, 70 ended in one plate, 22 in two, 5 in three, and 3 in four. Ninety-one p_2 fibres, or fibre branches, supplied each pole in a sample of 58 peroneal spindle poles, a mean number of 1.6 fibres per pole. This compares with a mean number of 4.2 trail fibres per pole in those

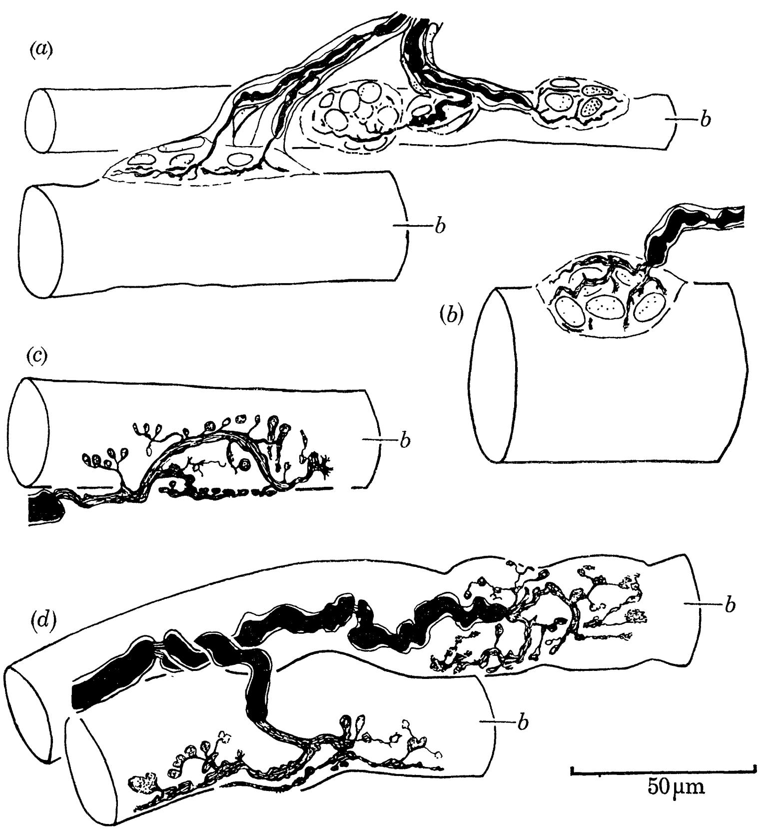

FIGURE 5. Drawings of plate endings traced from photographs of teased, silver preparations of de-afferented spindles. *b*, Nuclear-bag muscle fibre. (*a*) Three p_1 plates supplying a pes interosseous spindle. (*b*) An extrafusal motor endplate from the same muscle as in (*a*) for comparison. (*c*) A p_2 plate supplying a PDQ spindle. Note side-view of knob-like terminals applied to surface of muscle fibre. (*d*) Two p_2 plates supplying a pes interosseous spindle. Nuclei have been omitted from (*c*) and (*d*).

DESCRIPTION OF PLATE 60

Photographs of teased, silver preparations of spindles. The silver technique used in these and all subsequent silver preparations photographed is that of Barker & Ip (1963). *b*, nuclear-bag muscle fibre; *cp.*, capsule; *$p_1$pl.* p_1 plate; *$p_2$pl.*, p_2 plate; *rec.pt.tr.a.*, recurrent preterminal trail axon; *sp.n.t.*, spindle nerve trunk; *sp.tn.*, spindle tendon; *tr.e.a.*, trail-ending area; *tr.f.*, myelinated trail fibre; *tr.rm.*, trail ramification; Ia, primary afferent; II, secondary afferent; *P*, primary ending; S_1, S_2, secondary endings.

FIGURE 10. Pole of a normal PDQ spindle supplied with trail endings and p_2 plates.

FIGURE 11. Recurrent preterminal trail axon (labelled and indicated by arrows) leaves a de-afferented and sympathectomized PB spindle via the spindle nerve trunk.

FIGURE 12. Pole of a normal PDQ spindle supplied with trail endings and p_1 plates.

FIGURE 13. Normal PL spindle photographed at low power to give general impression of sensory and motor innervation. The left-hand wall of the capsule has been torn in teasing. Only the innervated part of each pole, amounting to about one third of the total polar length in each case, is included in the figure. The sensory innervation consists of one primary and three secondary endings, i.e. from bottom upwards, $S_1PS_1S_2$. The upper S_1 ending is of the flower-spray type, the other two are annulo-spiral. Each pole receives trail endings; the lower pole is supplied with a p_2 plate in addition.

FIGURE 14. A nuclear-bag muscle fibre receives a p_2 plate and a trail ramification in a de-afferented and sympathectomized PDQ spindle.

FIGURE 15. Spindle tendon of a de-afferented soleus spindle showing the run-on and abortive termination of a preterminal trail axon.

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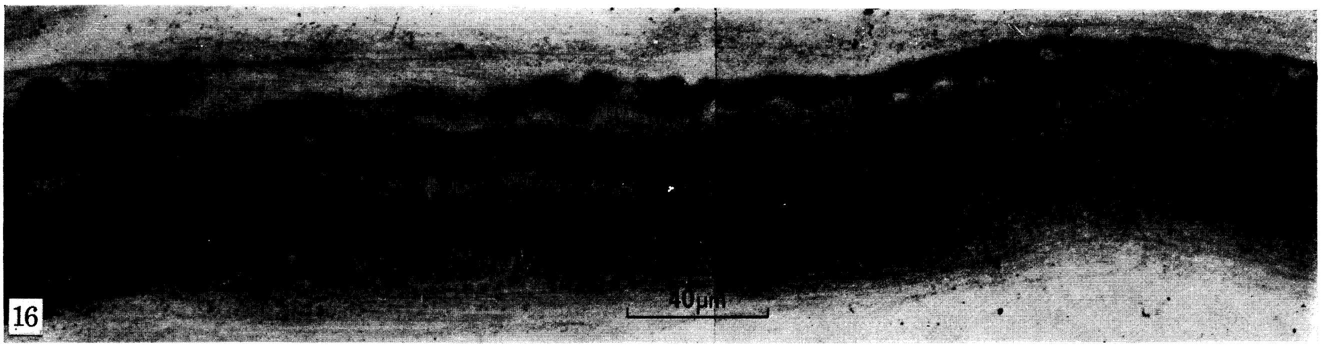
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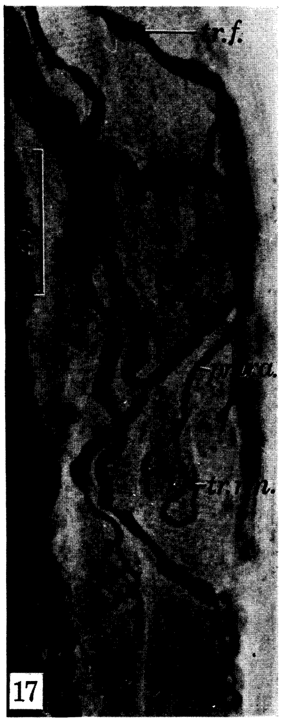
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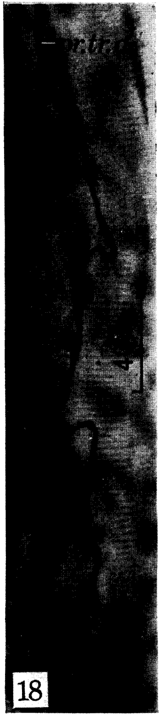
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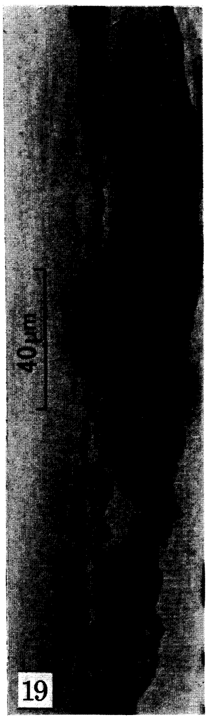
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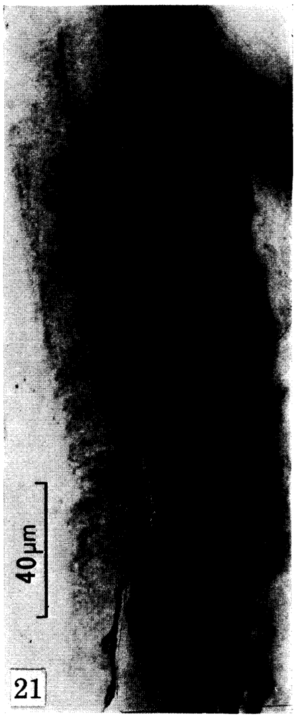
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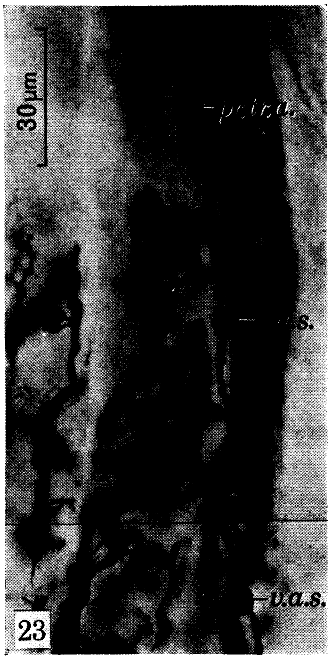
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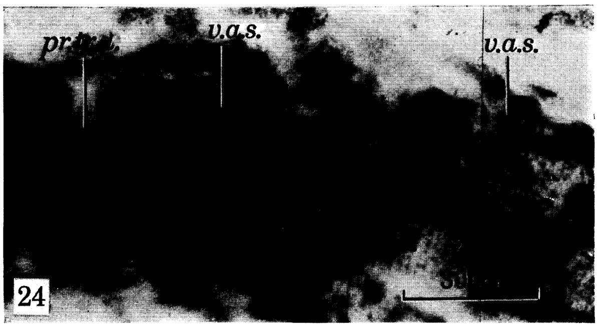
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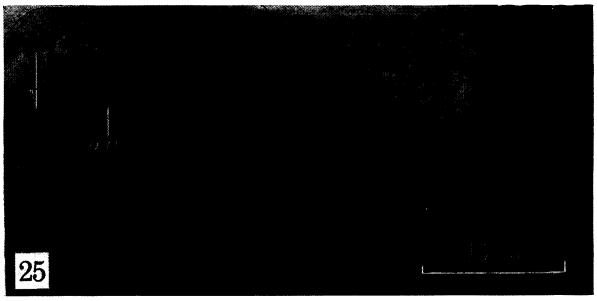
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DESCRIPTION OF PLATE 61

Photographs of teased, silver preparations of spindles illustrating features of trail innervation. *pr.tr.a.*, preterminal trail axon; *tr.f*, myelinated trail fibre; *tr.rm.*, trail ramification; *v.a.s.*, vesicular axonic swelling.

FIGURE 16. Trail ramifications located at the polar end of a trail-ending area belonging to the upper pole of the normal PL spindle illustrated in figure 13, plate 60. Knob-like axon terminals often predominate among ramifications in this location, as in this case.

FIGURE 17. Simple types of trail ramifications. Normal FHL spindle.

FIGURE 18. Simple tapers typical of trail ramifications located nearest to the area occupied by the primary sensory ending. Normal PDQ spindle.

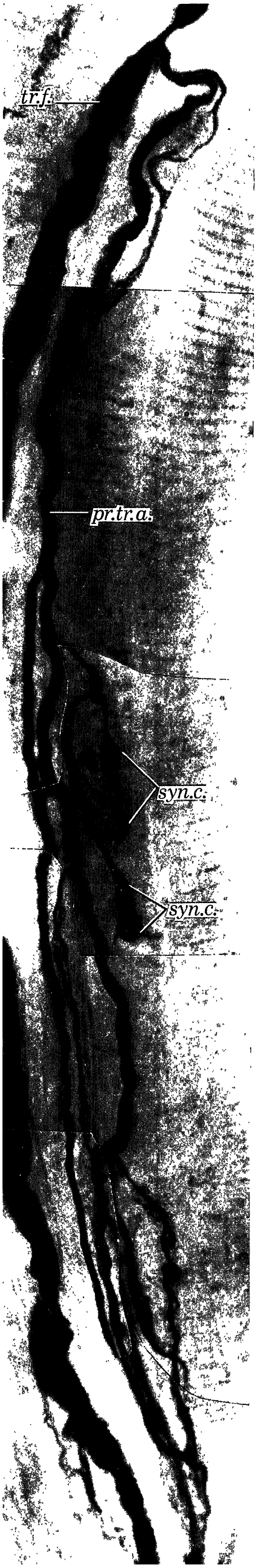
FIGURES 19–21. Complex types of trail ramifications innervating spindles teased from normal PL (figure 19), de-afferented and sympathectomized PB (figure 20), and normal PB (figure 21).

FIGURE 22. Plate-like type of trail ramification. Normal PB spindle.

FIGURES 23–25. Vesicular axonic swellings formed by preterminal trail axons as they course through areas occupied by sensory endings, specifically, the primary ending of a normal PB spindle (figure 23); the primary ending of a normal PDQ spindle (figure 24); and the secondary ending of a normal soleus spindle (figure 25).

Description of plate 62

Figures 26 to 32. Photographs of teased, silver preparations of spindles illustrating details of trail-ending ramifications belonging to the following de-afferented muscles: soleus (figure 26); pes interosseous (figures 27, 28, 30); PDQ (figure 29); and the following normal muscles: FHL (figure 31); and PB (figure 32). Figure 26 shows the nodal origin of a preterminal axon (*pt.tr.a.*) from a myelinated trail fibre (*tr.f.*), and two presumed regions of synaptic contact (*syn.c.*) are indicated. Interpretation of the remaining figures may be made by reference to figure 26.

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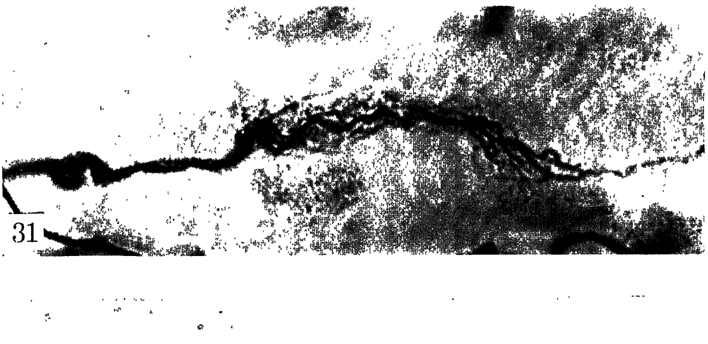
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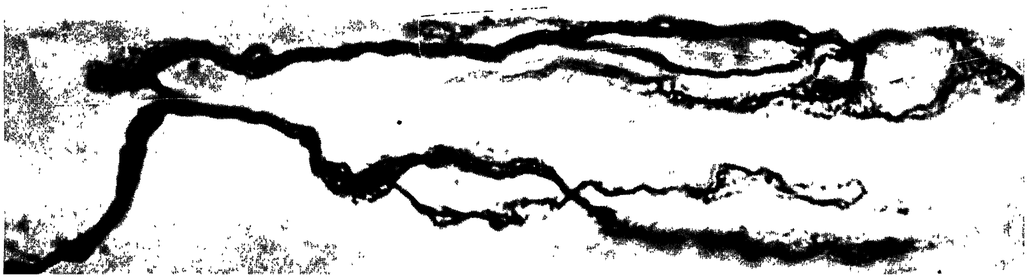

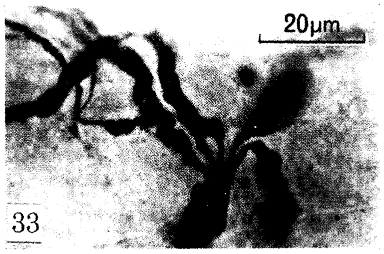

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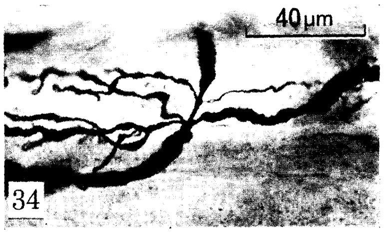

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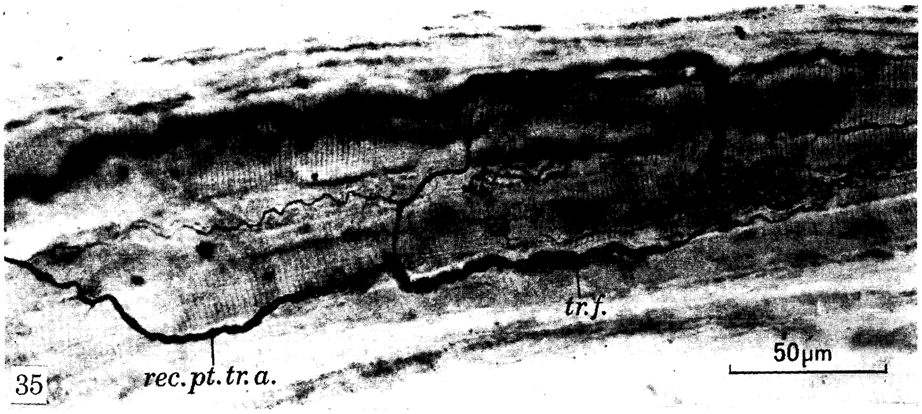

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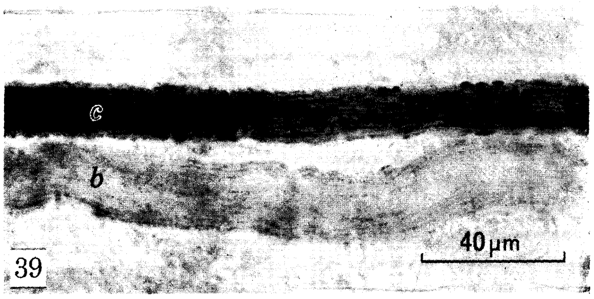

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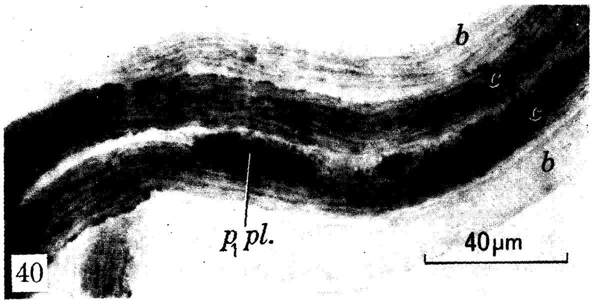

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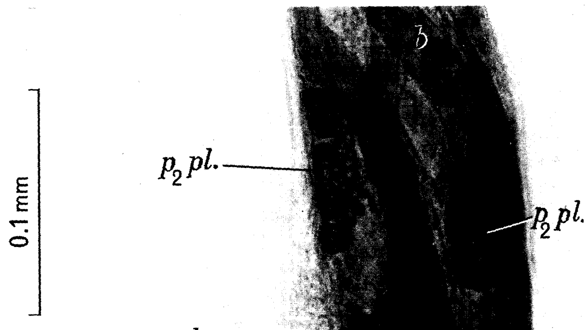
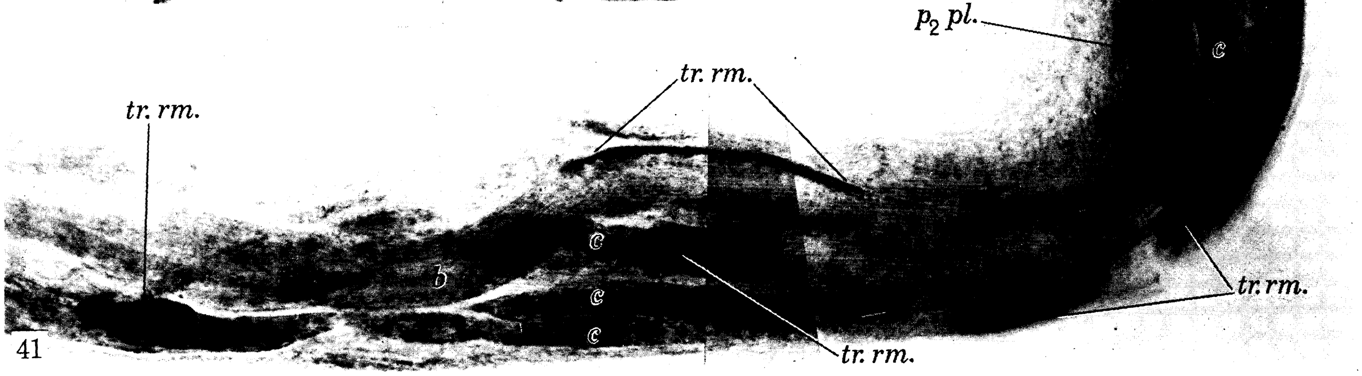

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DESCRIPTION OF PLATE 63

Photographs of teased, silver preparations of spindles illustrating trail innervation. *nm.tr.f.*, non-myelinated trail fibre; *rec.pt.tr.a.*, recurrent preterminal trail axon; *tr.f.*, myelinated trail fibre.

FIGURE 33. Myelinated trail fibre in normal PL spindle giving rise to five branches.

FIGURE 34. A single node of a myelinated trail fibre in a normal PL spindle giving rise to two myelinated branches and four preterminal axons.

FIGURE 35. Trail ending supplied to one pole of a de-afferented PL spindle. An optical dissection of this ending is shown in figure 3, p. 326.

FIGURE 36. Two myelinated and two non-myelinated trail fibres on their way to supplying a trail-ending area in a de-afferented pes interosseous spindle.

FIGURE 37. Two non-myelinated trail fibres each have a short length that is myelinated (indicated by arrows) as both enter a de-afferentated pes interosseous spindle.

Photographs of teased histochemical preparations of normal spindles. Figure 38 is part of a rat spindle (modified Karnovsky & Roots (1964) cholinesterase technique); figures 39 to 41 are parts of cat spindles (combined succinic dehydrogenase/cholinesterase technique). *b*, Nuclear-bag muscle fibre; *c*, nuclear-chain muscle-fibre; *$p_1$pl.*, p_1 plate; *$p_2$pl.*, p_2 plate; *tr.rm.*, trail ramification.

FIGURE 38. Trail-ending area in one pole of a rat peroneal spindle.

FIGURE 39. A nuclear-bag and a nuclear-chain muscle fibre belonging to a deep lumbrical spindle. The chain fibre appears darkly stained in contrast to the lightly stained bag fibre owing to the difference in succinic dehydrogenase activity between the two types of fibre.

FIGURE 40. Differential succinic dehydrogenase activity reveals the presence of two nuclear-bag and two nuclear-chain muscle fibres in one pole of a deep lumbrical spindle. The combined cholinesterase technique shows that one of the chain fibres carries a p_1 plate, the subneural apparatus being seen in side view.

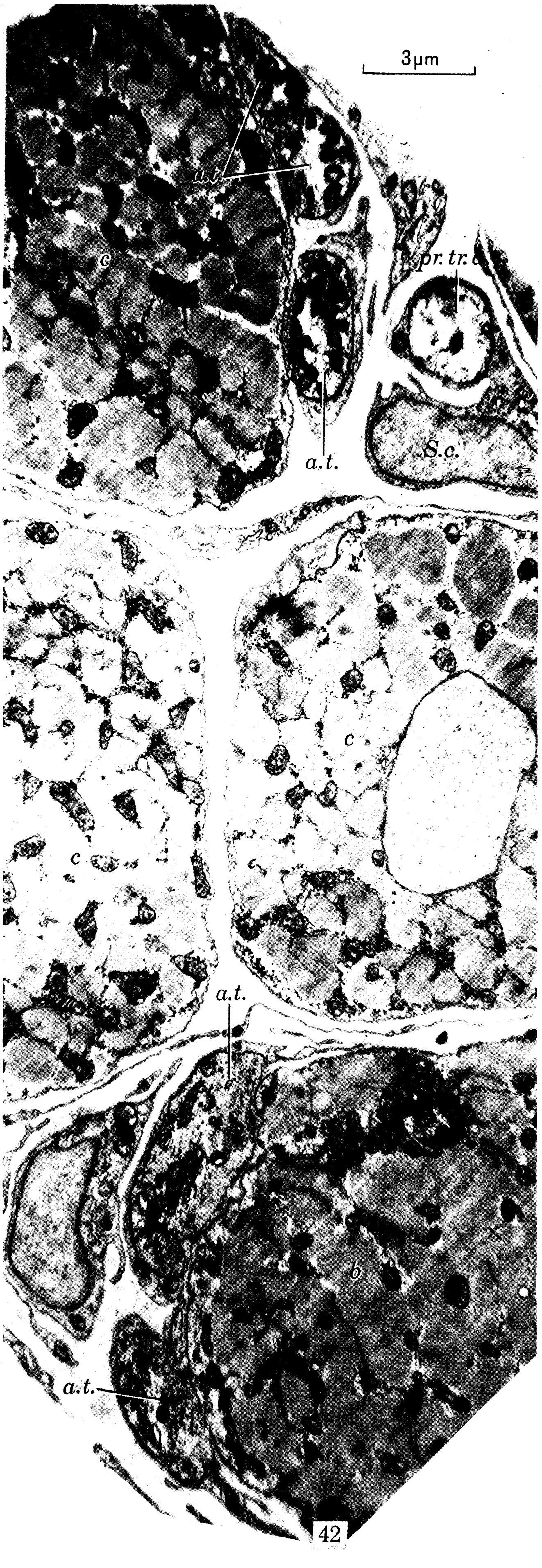
FIGURE 41. Mid-polar region of a tibialis posterior spindle. Differential succinic dehydrogenase activity distinguishes between the nuclear-bag and nuclear-chain muscle fibres, and the combined cholinesterase technique shows that one of the bag fibres receives a trail ramification and two p_2 plates. A third p_2 plate is located on another bag fibre. Other deposits in the figure are those of the subneural apparatuses of trail ramifications on chain fibres.

Description of plate 64

Electron micrographs illustrating the axon terminals of trail endings. *a.t.*, axon terminal; *b*, nuclear-bag muscle fibre; *c*, nuclear-chain muscle fibre; *S.c.*, Schwann cell; *s.p.*, sole plate; *pr.tr.a.*, preterminal trail axon.

Figure 42. Transverse section through the juxta-equatorial region of a spindle located in a normal deep lumbrical muscle. The field of view shows three nuclear-chain muscle fibres and one nuclear-bag fibre. Axon terminals of trail endings form junctions with the bag fibre and with one of the chain fibres; a preterminal trail axon is present near the latter.

Figure 43. Transverse section through the mid-polar region of a de-afferented and sympathectomized PL spindle showing the junction of a trail axon terminal with a nuclear-chain fibre. Note thin sole plate, basement membrane in synaptic gap, and absence of junctional folds. Two preterminal trail axons are included in the section.



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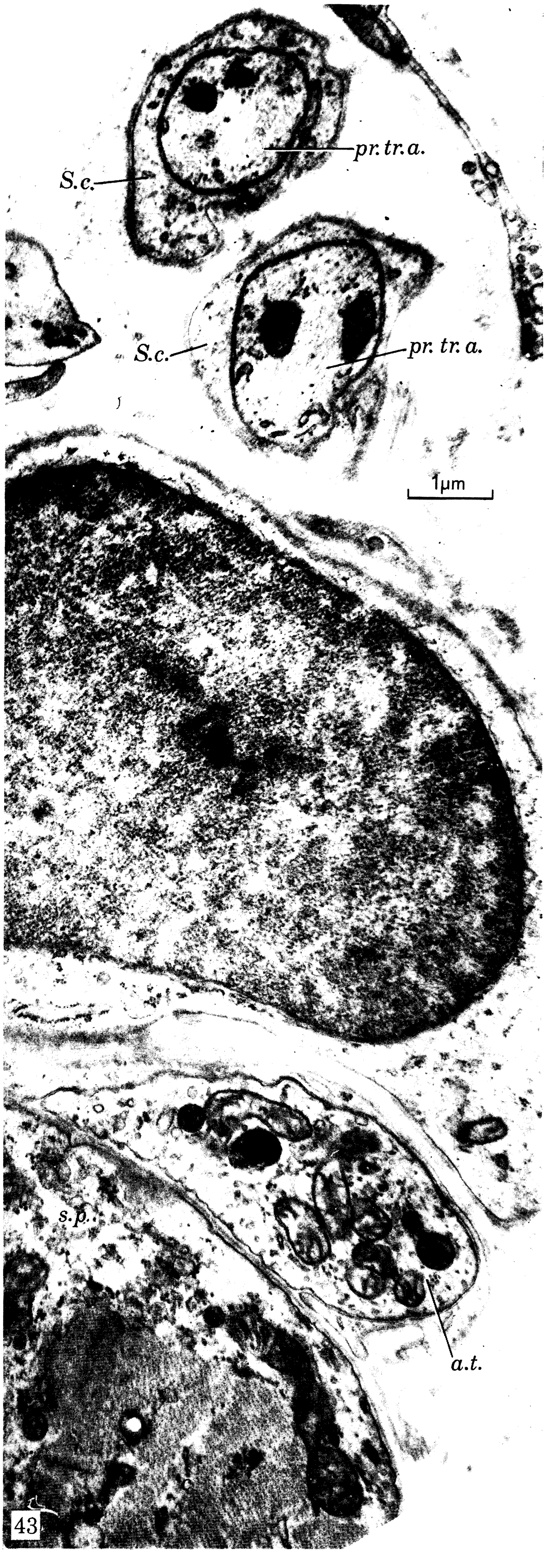

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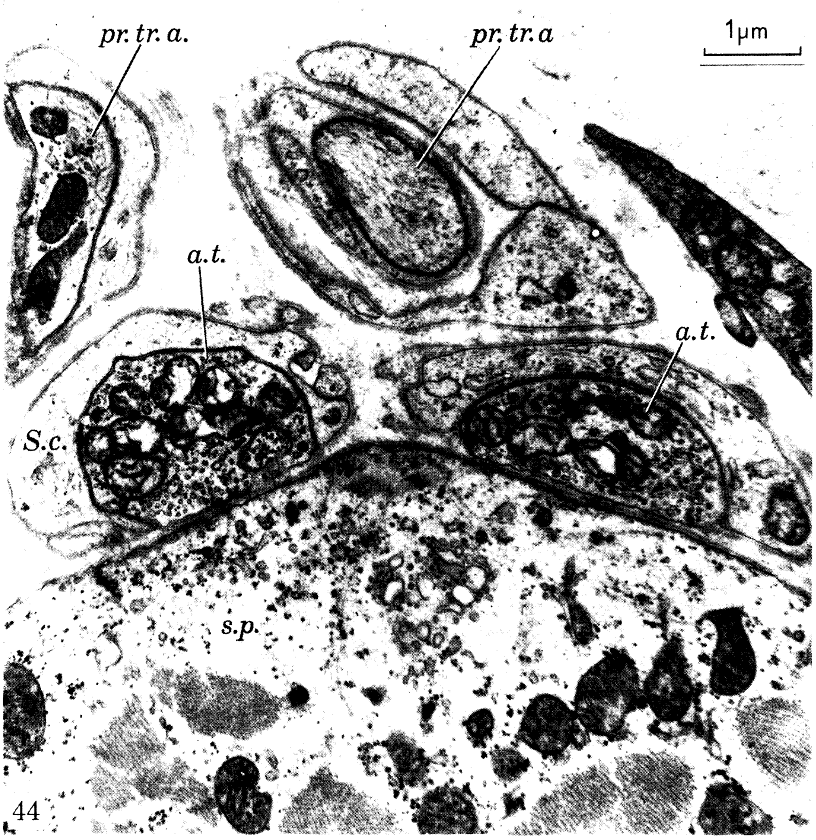
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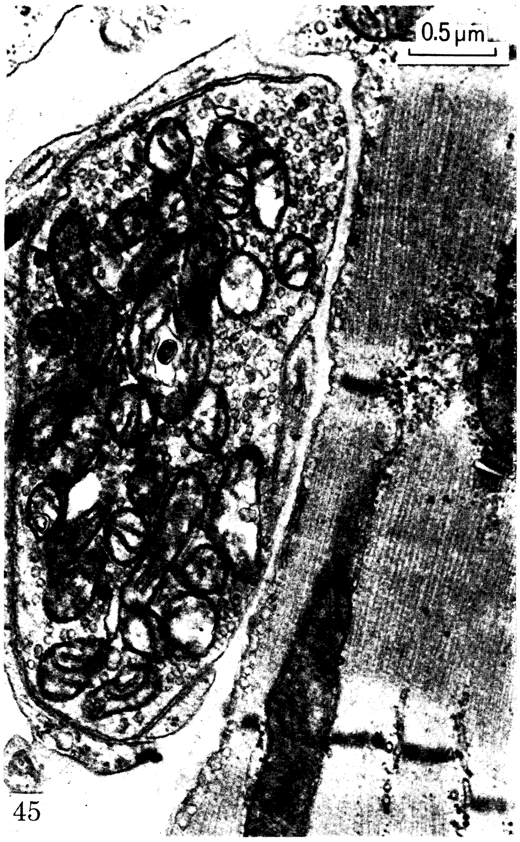
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peroneal spindles that receive both a myelinated and a non-myelinated trail supply (see p. 323). In this sample, p_2 fibres comprised 33.3 % of the total γ supply. Occasionally p_2 fibres, like myelinated trail fibres, may be seen branching outside the spindle so as to supply both poles (see figure 4*a*).

The majority of p_2 plates innervate nuclear-bag muscle fibres. Of 50 peroneal p_2 plates examined in silver preparations, 90 % were located on bag fibres, 10 % on chain. In two instances a p_2 fibre was seen to branch so as to supply one plate to a bag fibre and one to a chain fibre. One p_2 plate was seen to terminate on a bag fibre as well as on an adjacent chain fibre; another spread its terminals over two bag fibres.

(ii) *Electron microscopy* (p_2)

In setting out to examine the ultrastructure of the p_2 plate, one is faced with the situation that their supply to spindle poles is not constant, as it is in the case of trail endings, and that, when present, seven out of ten poles will have one plate only (see previous section and § 3 (*g*) and (*h*)). We finally succeeded in obtaining longitudinal sections of one p_2 plate located on a chain fibre in a PL spindle, and transverse sections of one other p_2 plate located on a bag fibre in another spindle from the same muscle. It is now clear to us that the majority of terminals which we earlier (Adal & Barker 1967) identified as belonging to p_2 plates in fact belong to trail junctions in which irregular folding is present (figure 1B in the earlier communication is nevertheless correctly identified as a longitudinal section of a p_2 plate). As is apparent from light microscopy, there are some similarities between p_2 and trail ramifications that occur towards the polar end of the trail-ending area (see p. 329). The main ultrastructural features of the p_2 plate are the 2 to 3 μm wide knob-like axon terminals, which typically lie in shallow depressions of an extensive, thinly spread sole plate, and are apposed to a folded postsynaptic membrane (see plate 67). These folds are wider and shallower than those of p_1 and extrafusal plates. Associated with their greater width the basement membrane usually lines the sides of the folds without fusing together in the middle. Branching of the folds rarely occurs. Occasionally the bases of two or three neighbouring folds may become confluent so as to isolate small islands

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DESCRIPTION OF PLATE 65

Electron micrographs illustrating the axon terminals of trail endings. *j.f.*, junctional fold. Other lettering as in plate 64.

FIGURE 44. Transverse section through the mid-polar region of a de-afferented and sympathectomized PL spindle showing the junctions of two trail axon terminals with a nuclear-chain fibre. Note relatively thick sole plate, and presence of two preterminal trail axons.

FIGURE 45. Longitudinal section through the juxta-equatorial region of a normal PL spindle (teased out from the muscle prior to preparation for sectioning) showing the junction of a trail axon terminal with a nuclear-bag fibre. Note absence of sole plate, and absence of M line from the H band of the sarcomeres.

FIGURE 46. Longitudinal section through the juxta-equatorial region of a normal tenuissimus spindle (teased out from the muscle prior to preparation for sectioning) showing the junction of a trail axon terminal with a nuclear-chain fibre. At the level of the asterisk there is a break in the continuity of the basement membrane and a decrease in the width of the synaptic gap. Note thin sole plate, presence of occasional shallow junctional folds, and presence of an M line in the H band of the sarcomeres.

FIGURE 47. Similar section to that shown in figure 46 cut from the mid-polar region of another spindle teased out from the same muscle. It illustrates the maximum development that the irregular folding of the postsynaptic membrane achieves in some trail junctions. Note the sole plate, which is relatively thick as in figure 44.

of sole plate (see figure 59, plate 67). The size range of the presynaptic vesicles does not differ significantly from that of similar vesicles in other fusimotor endings, and the width of the synaptic cleft is the same.

(*d*) *The α innervation*: p_1 *plates*

(i) *Light microscopy*

The location of the p_1 innervation is the same as that of p_2 plates (see figure 12, plate 60). In poles where both types of plates are present, the p_2 plates may be mid-polar and the p_1 plates located towards the extreme end of the pole, or the locations may be reversed. In its general appearance the p_1 plate resembles an extrafusal motor end-plate consisting of axon terminals in the form of tapers and, less frequently, knobs or rings, ramifying upon a nucleated sole plate that has a Doyère eminence (see figures 5 and 6, and plate 68). There is a wide range of form from simple plates consisting of only two or three axon terminals (figure 67, plate 68), to complex ones in which over a dozen axon terminals are derived from two branches of the supplying nerve fibre (figure 5*a*, and figure 63, plate 68). Alternatively, such branching may give rise to two or three separate but adjacent plates on the same muscle fibre (figure 5*a*, and figure 70, plate 68). Occasionally a non-myelinated axon may arise from the penultimate node of the supplying fibre to contribute terminals to the plate, or to form a small plate of its own alongside as an 'accessory ending' as illustrated in Barker & Ip (1966, figure 3*a*). In cholinesterase preparations p_1 plates appear as compact deposits in which the synaptic guttering lies in a tight coil instead of being loosely spread out as in extrafusal end-plates (figures 71, 72, plate 68). In side view the deposit appears as a single dark line (figure 40, plate 63).

The plates are supplied to both bag and chain muscle fibres. In a sample of 100 p_1 plates in peroneal and FHL spindles, 75 were located on bag fibres, and 25 on chain fibres. Two instances were observed in which a p_1 fibre branched within the spindle so as to innervate both bag and chain fibres. The mean length of the plates on the bag fibres was 31.6 μm in a range of 13 to 62 μm; the plates on the chain fibres had a mean length of 27.8 μm in a range of 14 to 53 μm. These figures compare with the following lengths of samples of 50 extrafusal end-plates measured

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DESCRIPTION OF PLATE 66

Photographs of teased preparations of spindles illustrating p_2 innervation. All are silver preparations except figure 54, which is a cholinesterase preparation (modified Karnovsky & Roots (1964) technique). All the plates shown are located on nuclear-bag fibres. The scale shown in figure 48 also applies to figures 49 to 54 and 56.

FIGURE 48. A p_2 plate supplying a normal PL spindle. Note ultraterminal fibre with terminal bulb (the apparent bulb immediately preceding this is a kink in the ultraterminal fibre).

FIGURE 49. A p_2 plate supplying the same pole of the normal PDQ spindle shown in figure 10, plate 60.

FIGURE 50. A p_2 plate supplying a de-afferented PL spindle. Note contributions of axon terminals from the two nodes that precede the preterminal one.

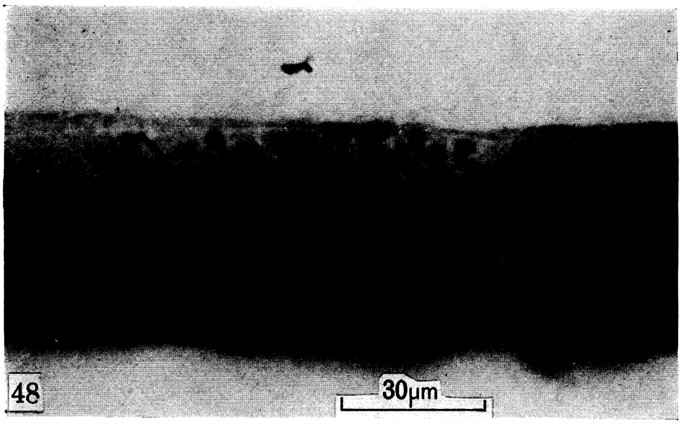
FIGURE 51. A p_2 plate supplying a normal PB spindle. This is circular rather than elongated in form and bears some resemblance to certain trail ramifications (cf. figures 16 and 22, plate 61).

FIGURE 52. A p_2 plate supplying a de-afferented PL spindle. Note ultraterminal fibre with terminal bulb.

FIGURE 53. A p_2 plate supplying a normal PDQ spindle.

FIGURE 54. Cholinesterase preparation of a p_2 plate supplying a normal PL spindle.

FIGURES 55–57. Side view of p_2 plates showing close application of knob-like axon terminals to muscle-fibre surface. Plates located in spindles teased from normal PL (figure 55), PB (figure 56), and tenuissimus (figure 57) muscles.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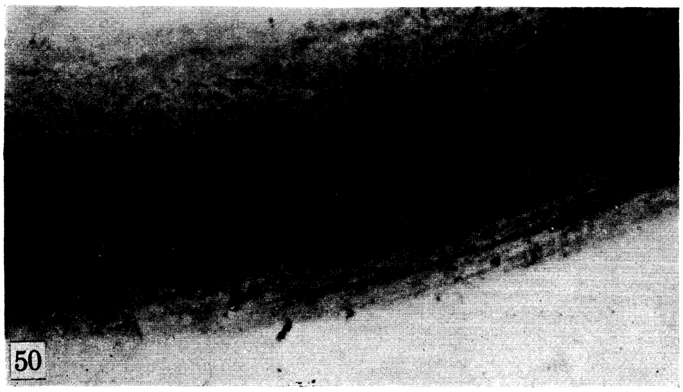
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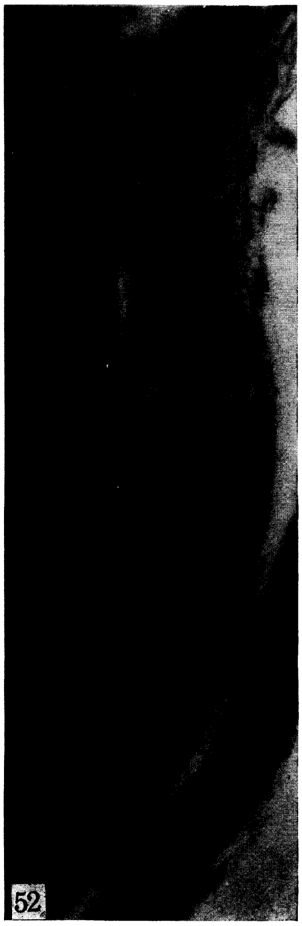
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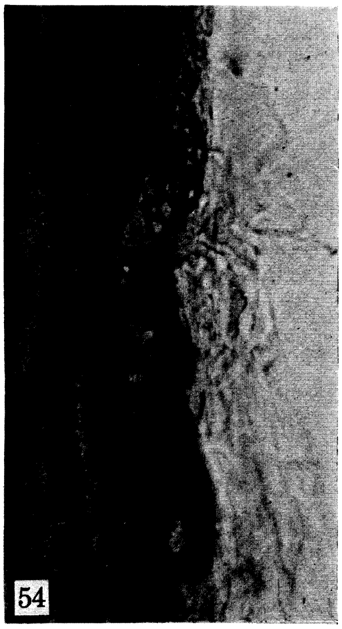
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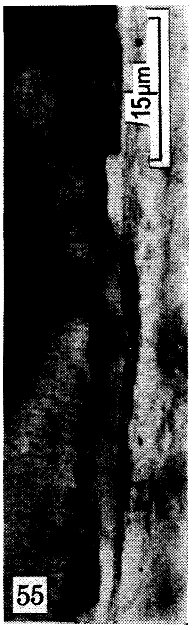
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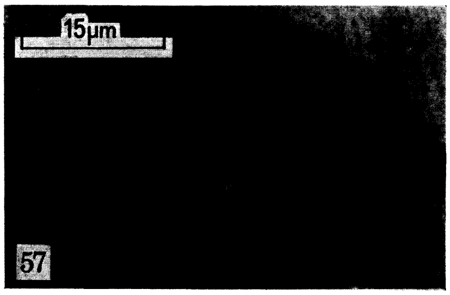
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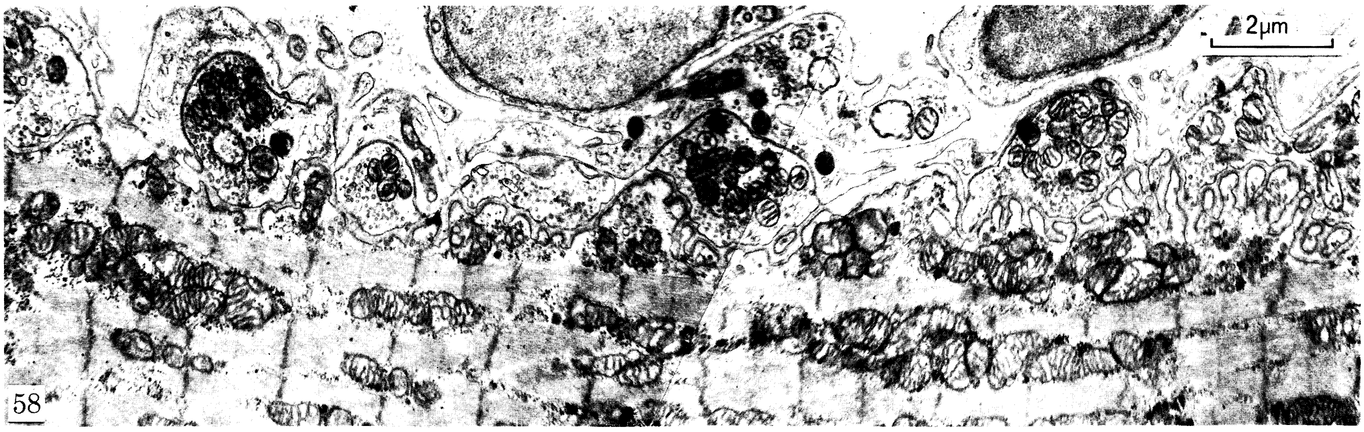
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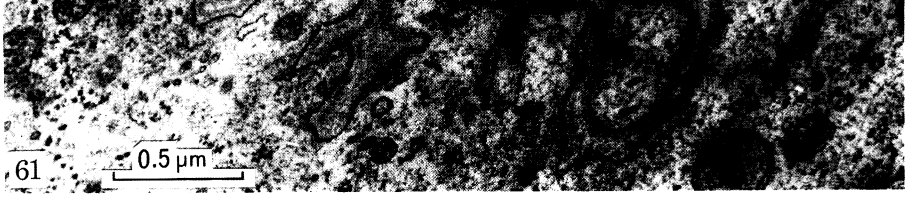
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along the long axis of the muscle fibres in similar silver preparations: PDQ; mean length 30.3 μm, range 19.7 to 46.0 μm; soleus; mean length 25.9 μm, range 9.5 to 40.7 μm (A. R. Tuffery, personal communication). The greater range of length among p_1 plates may perhaps be attributed to the considerably smaller diameters of intrafusal as compared with extrafusal muscle fibres.

As in the case of p_2 innervation, the majority of p_1 fibres enter a pole to terminate in one plate only; of 100 peroneal p_1 fibres, 77 ended in one plate, 18 in two, 4 in three, and 1 in six. Fifty-seven p_1 fibres supplied each pole in a sample of 23 peroneal spindle poles, a mean number of 2.5 fibres per pole. They comprised 40.7 % of the total fusimotor supply. In contrast to the p_2 innervation, in which a pole of a single intrafusal muscle fibre receives at most two plates from one p_2 fibre, two p_1 fibres may be involved in supplying up to five plates to a pole of a single intrafusal muscle fibre (see figure 6*c*). In some cases two p_1 fibres of apparently separate origin will form adjacent plates on the same muscle fibre (see figure 6*a*), and on occasion may contribute to one and the same plate, as has been described in rabbit spindles by Barker (1948).

A feature of the p_1 innervation is its frequent derivation from relatively large intramuscular nerve trunks passing close by the spindle, rather than from spindle nerve trunks, though this also occurs. Typically, single p_1 fibres will leave such trunks to innervate a spindle pole, while the rest of the fibres continue on their course, many of them destined to innervate extrafusal muscle fibres. In a number of instances it has been possible to locate the origin of p_1 fibres as branches of fibres within these trunks (see figure 6). In three preparations the other branch was seen to form an end-plate on an extrafusal muscle fibre (see figure 6*d*, and figure 64, plate 68). In these cases the two branches can positively be identified as taking part in skeleto-fusimotor innervation and may be said to be connected to 'mixed' or β fibres (see Bessou *et al.* 1965; Adal & Barker 1965). In other cases, however, there is no histological criterion by which one can determine whether the p_1 fibres are connected to β fibres, or to α fibres that have an exclusively fusimotor distribution.

(ii) *Electron microscopy* (p_1)

As in the case of the p_2 plate, and for similar reasons, the location of p_1 plates in ultra-thin sections proved very elusive. We finally succeeded in locating two plates, one in the pole of a spindle teased from tenuissimus, the other in one teased from tibialis posterior. Both, as it happens, were located on nuclear-chain fibres (see plate 69). As was to be expected from observations with the light microscope, the ultrastructure of the p_1 plate closely resembles that of

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Description of plate 67

Electron micrographs illustrating the ultrastructure of the p_2 plate (figures 58, 59 and 61). A micrograph of part of an extrafusal plate is included for comparison (figure 60).

Figure 58. Longitudinal section through the mid-polar region of a normal PL spindle (teased out from the muscle prior to preparation for sectioning) showing part of a p_2 plate located on a nuclear-chain fibre. A thin sole plate with folded postsynaptic membrane underlies a succession of knob-like axon terminals.

Figure 59. Detail of figure 58. Note the wide, shallow, and unbranched junctional folds, which are occasionally confluent at their bases, and compare them with those of the extrafusal plate shown in figure 60.

Figure 60. Longitudinal section through part of an extrafusal end-plate supplying a deep lumbrical muscle fibre showing an axon terminal and junctional folds.

Figure 61. Part of a p_2 axon terminal with associated junctional folds located on a nuclear-bag fibre in the mid-polar region of a de-afferented and sympathectomized PL spindle. Transverse section.

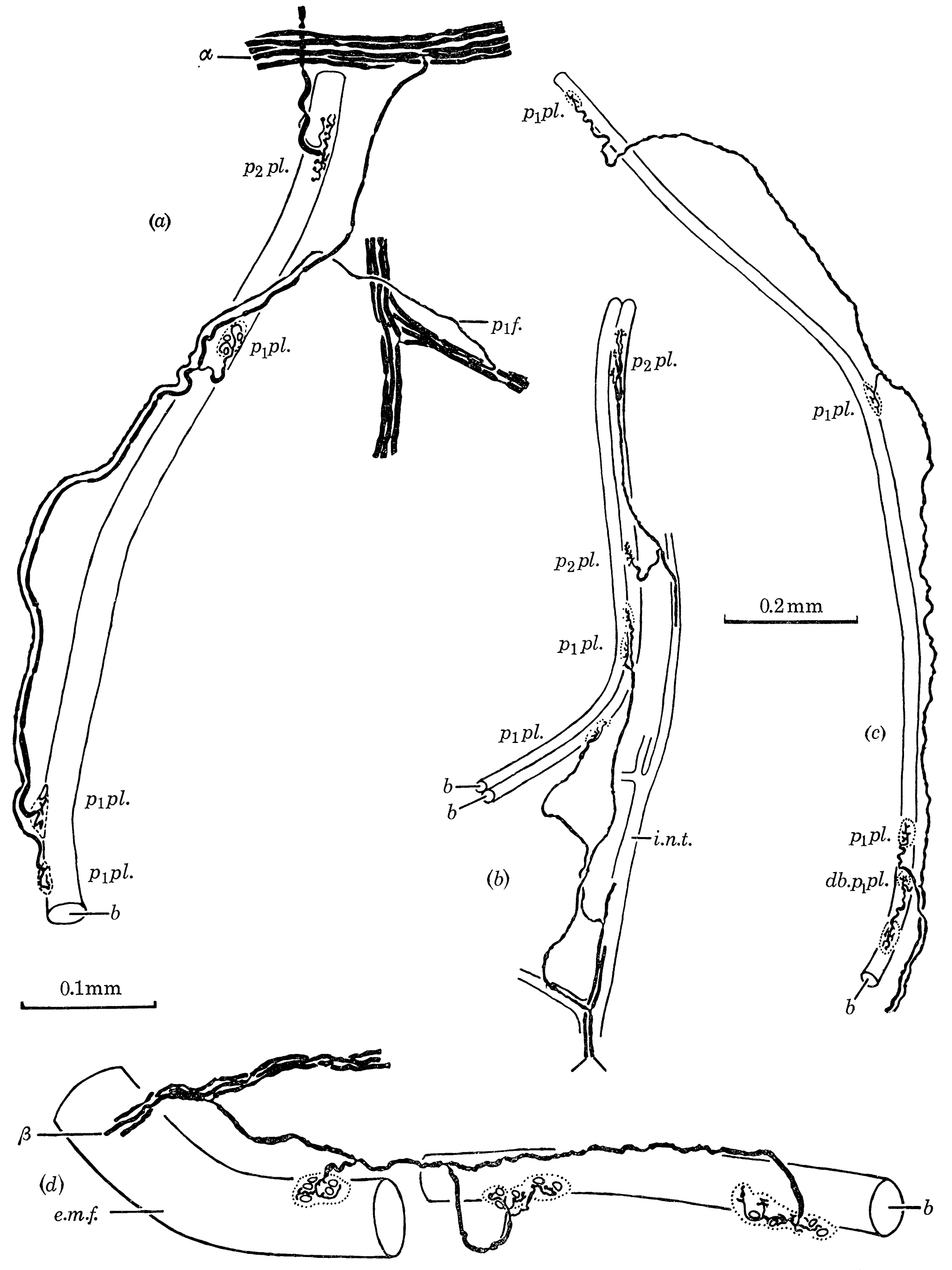

FIGURE 6. Drawings of teased, silver preparations of fusimotor plate innervation (by camera lucida in (*a*) to (*c*); traced from photographs in (*d*)). (*a*) A nuclear-bag muscle fibre *b*, belonging to a de-afferented and sympathectomized PB spindle, receives two p_1 plates (p_1*pl.*), spaced 400 μm apart, from the collateral of an α fibre (α) located in the nerve trunk crossing the top of the figure. A p_1 fibre (p_1*f.*) leaves another nerve trunk to form a third plate adjacent to the lower one formed by the α collateral, whose course together with that of the p_1 fibre has been unravelled for clarity. The p_2 plate at the top of the figure is located 200 μm distant from the nearest p_1 plate. The fibre supplying it also forms another p_2 plate 300 μm further along the bag fibre beyond the upper limit of the figure. (*b*) α-Collateral and p_2 innervation of two bag fibres belonging to one pole of a normal PDQ spindle. The α collaterals originate from an intramuscular nerve trunk (*i.n.t.*) from which the main nerve supply of the spindle is derived. One of the bag fibres receives one p_1 plate and two p_2 plates spaced 300 μm apart. (*c*) Two p_1 fibres supply five plates to one pole of a bag fibre located in a de-afferented and sympathectomized PL spindle. *db.*p_1*pl.*, double p_1 plate. (*d*) A β fibre innervates an extrafusal muscle fibre (*e.m.f.*) and supplies two p_1 plates to a bag fibre located in a de-afferented pes interosseous spindle.

extrafusal motor end-plates. The axon terminals lie in synaptic gutters on a substantial sole plate. The postsynaptic membrane is folded, the spacing and dimensions of the junctional folds being very similar to those of extrafusal plates. As in extrafusal plates, p_1 junctional folds may branch, and they may also occur as infoldings of the sole-plate surface membrane in between the synaptic gutters.

(*e*) *The motor innervation of individual intrafusal muscle fibres*

In teased, silver preparations intrafusal muscle fibres are much more difficult to trace than the nerve fibres supplying them. It is seldom possible to trace a nuclear-chain fibre with confidence from one end of the spindle to the other, but nuclear-bag fibres present less difficulty mainly owing to their greater length and diameter. The extracapsular regions are the most favourable for fibre identification for here they are no longer restricted by the sleeve of lamellated connective tissue, and if not naturally spread out can often be made to by squashing. In spindles prepared by the combined succinic dehydrogenase/cholinesterase technique, the muscle fibres can more easily be traced owing to their differential staining (see figure 39, plate 63), and to their appearance in the equatorial region, where, for example, bag fibres can easily be detected by the staining of the aggregations of mitochondria that occur in between the nuclei of the myotube regions. The following observations are based on teased, silver preparations, modified Karnovsky cholinesterase preparations, and succinic dehydrogenase/cholinesterase preparations.

(i) *Nuclear-bag fibres*

We have frequently observed instances of one pole of a bag fibre receiving a supply of both p_1 and p_2 plates, usually spaced a few hundred micrometres apart. Examples are illustrated in figure 6*a* and *b*. The maximum number of plates seen in such instances was five, three p_1 and two p_2. We have also seen poles of single bag fibres receiving both a plate (either p_1 or p_2) and a trail innervation. A silver preparation showing a bag fibre receiving a p_2 plate and a trail ramification is shown in figure 14, plate 60, and a similar innervation in a succinic dehydrogenase/cholinesterase preparation is shown in figure 41, plate 63. Bag fibres thus receive both types of plate ending as well as trail endings, and if complete fibres are considered over their total course through both poles it seems almost certain that instances occur in which they are supplied with all three types of ending.

(ii) *Nuclear-chain fibres*

The majority of chain fibres receive trail endings only. It has proved impossible to check whether each chain fibre in a spindle pole receives trail innervation, but in the average situation of from 10 to 18.5 ramifications (see p. 325) distributed among two bag and four to five chain fibres (Barker & Gidumal 1961), few are likely to remain non-innervated. Tracing some of those chain fibres that receive p_1 (25 %) or p_2 (10 %) plates has shown that this is additional to a trail innervation. No chain fibre has been observed to receive both a p_1 and a p_2 innervation.

The motor innervation of the bag and chain fibres of a cat spindle is shown schematically in figure 7.

(*f*) *Degeneration of fusimotor endings after nerve section*

When a muscle nerve is severed, the nerve endings degenerate at rates that differ according to the diameter of the supplying nerve fibre, those supplied by the largest fibres being the first to disappear. This affords an opportunity for distinguishing between the α and γ sources of

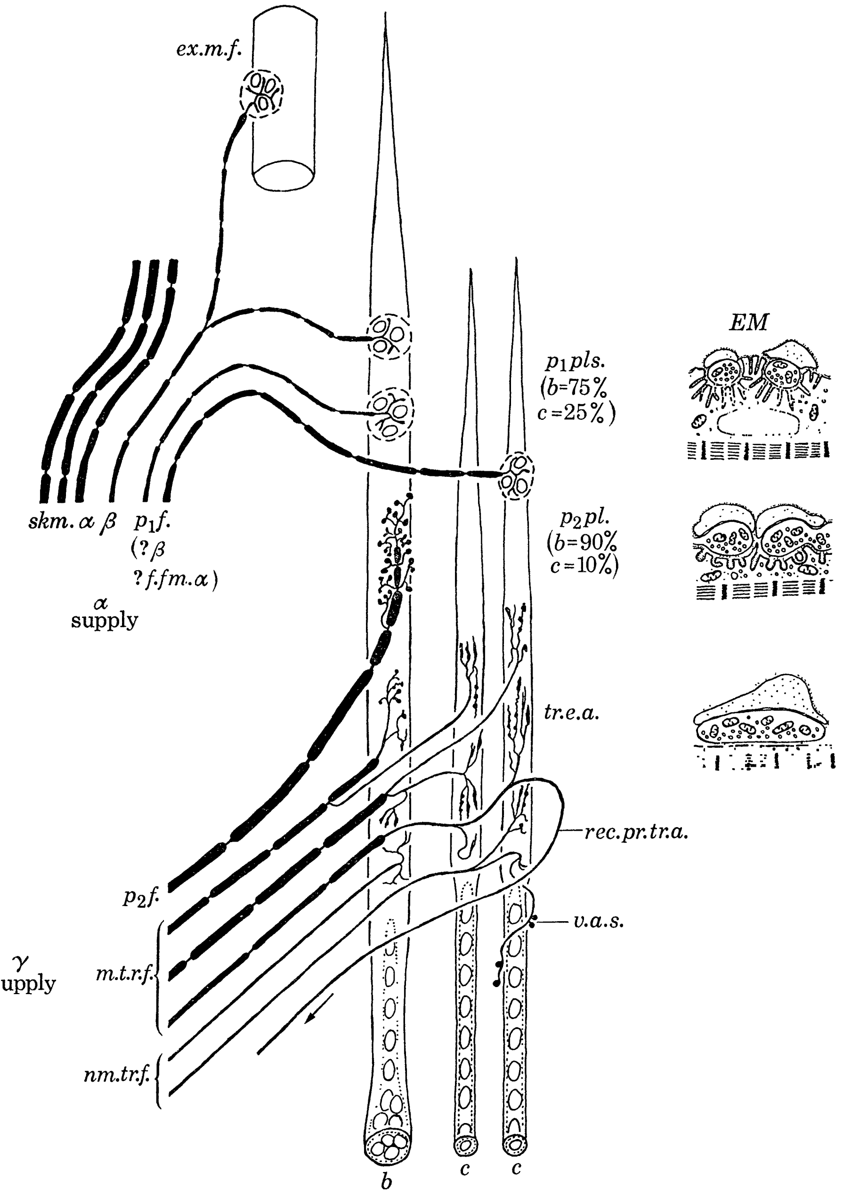

FIGURE 7. Schematic diagram of the motor innervation of nuclear-bag (*b*) and nuclear-chain (*c*) muscle fibres in the cat, based on observations, described in the text, made on spindles from the peroneal, FHL, and soleus muscles. Non-myelinated trail fibres (*nm.tr.f.*) do not always participate in the innervation: in the peroneals they are present in addition to myelinated trail fibres (*m.tr.f.*) in just over one third of the spindles. Less than 5% of spindles have recurrent preterminal trail axons (*rec.pr.tr.a.*) leaving them. Some p_1 fibres ($p_1f.$) can be identified as collaterals of fibres (β) that also innervate extrafusal muscle fibres (*ex.m.f.*); this is not possible for the majority, which may either be connected to β fibres or to fast fusimotor α fibres (*f.fm.*α). Sketches of the ultrastructure of the myoneural junctions of the three types of fusimotor ending are shown on the right of the figure under *EM*. Other lettering: $p_1 pls.$, p_1 plates; $p_2 pl.$, p_2 plate; $p_2 f.$, p_2 fibre; *skm.*α, skeletomotor α fibres; *tr.e.a.*, trail-ending area; *v.a.s.*, vesicular axonic swelling.

fusimotor supply. The progress of nerve-ending degeneration in peroneal muscles during a period of 96 h following nerve section is summarized in figure 8, and the fusimotor effects are illustrated in plate 70.

The sequence of degenerative changes that occurs in extrafusal motor end-plates after nerve section is for the axon terminals to inflate, retract, and finally disappear. This is accompanied by an increase in diameter of the terminal branches of the supplying fibres, followed by their fragmentation, and, finally, disappearance, leaving behind empty endoneurial tubes. Fragmentation and disappearance of the end-plates and their terminal branches occurs between 48 and 60 h after nerve section. The p_1 plates and their fibres follow the same time course (see figures 76 to 79, plate 70). The γ innervation, however, persists intact throughout this period (see figure 80, plate 70) and is still present 72 h after nerve section, though by this time most of the axon terminals show signs of inflation (see figure 82, plate 70). The p_2 plates disappear between the 72 and 78 h intervals, followed by the trail endings 78 to 84 h after nerve section. At 84 h all that is left of the trail innervation is a few preterminal axons ending in inflated ramifications (see figures 83, 84, plate 70), and by 96 h after section all trace of fusimotor innervation has disappeared.

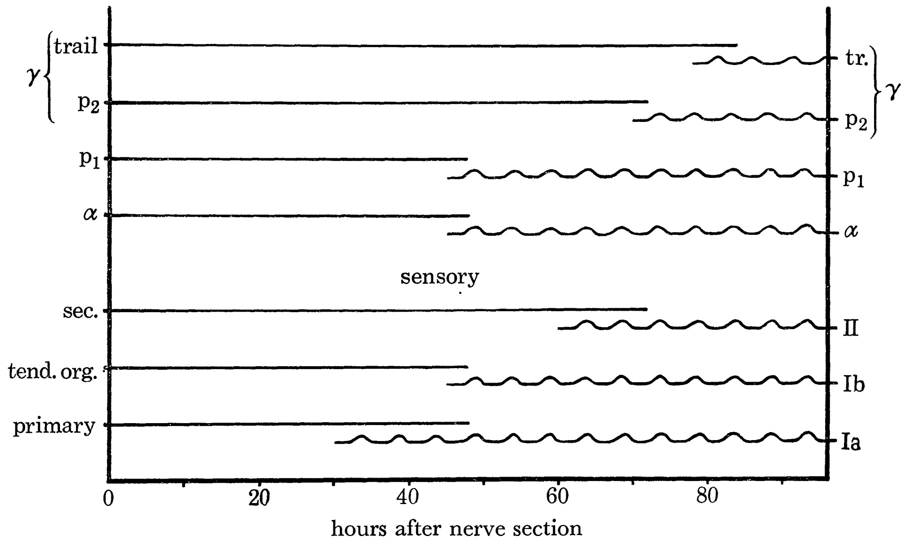

FIGURE 8. Degeneration of nerve endings in peroneal muscles over a period of 96 h after cutting the common peroneal nerve. —, Endings present; ⁓, endings absent.

The different rates of degeneration of the p_1 and p_2 plates thus confirms their derivation from stem motor fibres that are respectively large and small in diameter. If γ fibres participate in p_1 plate innervation we should expect to find some p_1 plates intact 60 to 84 h after nerve section when the γ innervation still survives, but none was observed. The persistence of trail endings for the longest period after nerve section indicates that the thinnest γ fibres are included in providing the trail innervation.

(*g*) *Types of motor innervation received by spindle poles*

Trail endings are almost invariably present. Of 345 spindle poles sampled from peroneal, FHL and soleus muscles only one lacked trail innervation. In 54 (15.6 %) of these poles it was the only type of fusimotor ending present; 32 (59.3 %) of these inserted into tendon, usually as very short poles. The 'trail only' pole, and combinations of trail and plate innervation, result in four possible polar patterns of motor innervation, which when combined yield ten possible patterns for the motor innervation of complete spindles. If the trail innervation is symbolized as 'tr', the plate innervation as 'p_1' and 'p_2', and a colon is used to represent the equatorial region, the ten variants may be expressed as follows:

$$\begin{array}{cccccccccc}
 & & & & & & & p_1 & p_1 & \\
p_1 & p_1 & p_1 & p_1 & p_2 & p_2 & p_2 & p_2 & p_2 & \\
tr & tr & tr & tr & tr & tr & tr & tr & tr & tr \\
: & : & : & : & : & : & : & : & : & : \\
tr & tr & tr & tr & tr & tr & tr & tr & tr & tr \\
p_1 & p_2 & p_2 & & p_2 & p_2 & & p_2 & & \\
 & & p_1 & & & p_1 & & p_1 & &
\end{array}$$

Examples of all ten variants have been observed. On rare occasions spindles may possess one pole devoid of any motor innervation (cf. Jones 1966), but none has been encountered in this study.

Table 1A shows the percentage distribution of the different polar patterns of fusimotor innervation occurring in spindles sampled from the peroneal, FHL and soleus muscles. Features worthy of note are the high frequency of the $p_1$tr pole in the FHL (57.1 %) and soleus (39.0 %) muscles, and the high frequency of the p_2 tr pole in PL (63.4 %) and PB (52.4 %). The p_1 innervation is more prominent in PDQ than in the other peroneal muscles by virtue of a greater provision of poles receiving a p_1 tr and a p_1p_2 tr innervation. Table 1B shows the frequency distribution of the different polar combinations that occurred in spindles sampled from the same five muscles. The samples are perhaps too small for many features to emerge, but the absence of the p_1 tr pole in PL is significant, as is the frequent occurrence of the p_2 tr pole in this muscle in various combinations. The low frequency of spindles in which both poles

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DESCRIPTION OF PLATE 68

Photographs of teased preparations of spindles illustrating p_1 innervation. All are silver preparations except figures 71 and 72, which are cholinesterase preparations (modified Karnovsky & Roots (1964) technique). The scale shown in figure 63 also applies to figures 65 to 68. β, beta fibre; *ex.m.ep.*, extrafusal motor end-plate; *$p_1$pl.b.*, p_1 plate on a nuclear-bag fibre; *$p_1$pl.c.*, p_1 plate on a nuclear-chain fibre; *$p_2$pl.c.*, p_2 plate on a nuclear-chain fibre.

FIGURE 62. Two nuclear-bag fibres receive p_1 plates in a normal FHL spindle.

FIGURE 63. A nuclear-chain fibre receives a p_1 plate in a normal PDQ spindle.

FIGURE 64. A β fibre in normal soleus branches to supply two p_1 plates to a nuclear-bag fibre in a spindle, and also terminates in an extrafusal motor end-plate nearby.

FIGURES 65 to 67. Side views of p_1 plates supplied to a de-afferented PB spindle (figure 65), and to normal PDQ (figure 66) and FHL (figure 67) spindles. All are located on nuclear-bag fibres. That in figure 67 is typical of the simplest type of plate; note sole-plate nuclei.

FIGURE 68. Surface view of a p_1 plate supplied to a nuclear-bag fibre in a normal FHL spindle.

FIGURE 69. Four p_1 fibres enter a pole of a normal FHL spindle. Each forms one plate; as indicated, two are located on nuclear-bag fibres, and two on nuclear-chain fibres. Also included is a p_2 plate located on a nuclear-chain fibre.

FIGURE 70. A p_1 fibre branches to form three adjacent plates on a nuclear-bag fibre in a normal FDL spindle.

FIGURE 71. Cholinesterase preparation of a p_1 plate in a peroneal spindle. Note tightly coiled synaptic guttering as compared with that shown in figure 72.

FIGURE 72. Cholinesterase preparation of a peroneal extrafusal motor end-plate.

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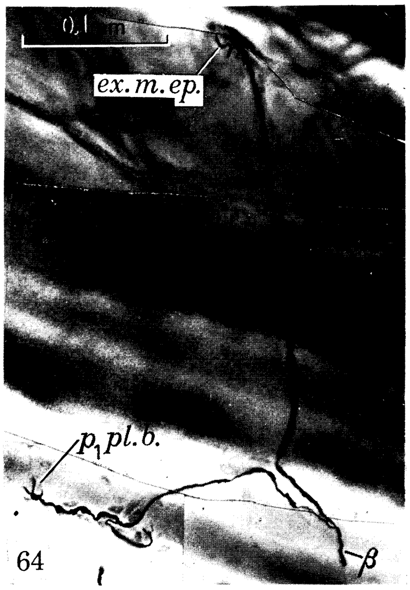

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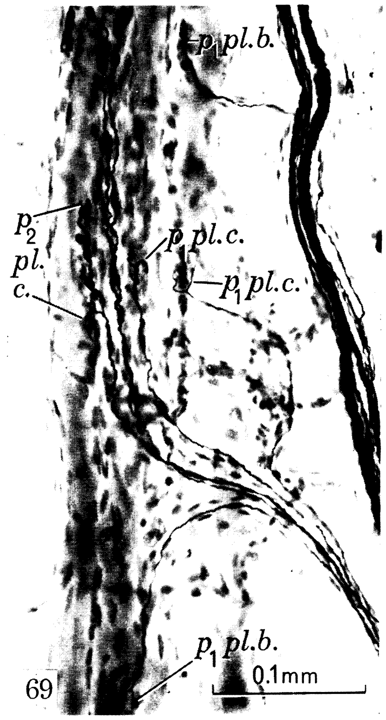

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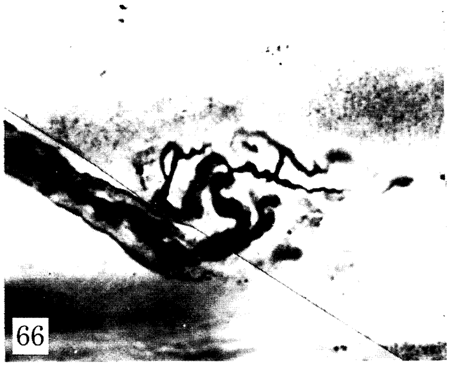

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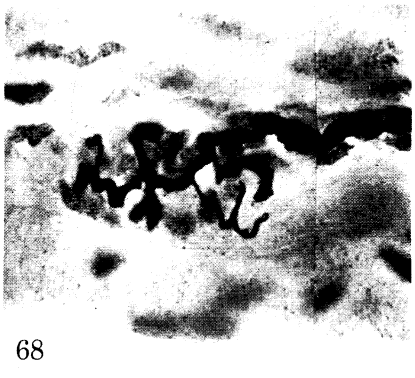

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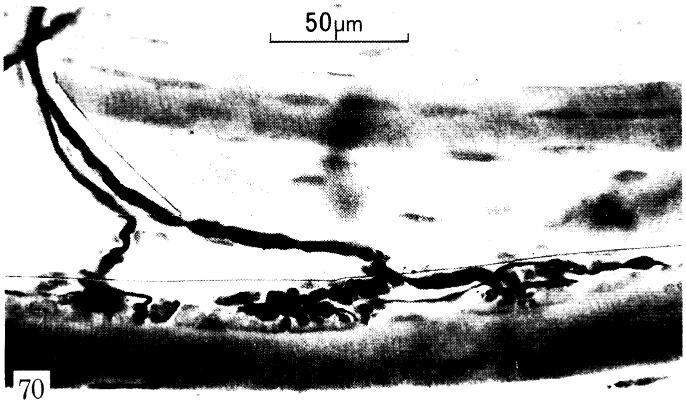

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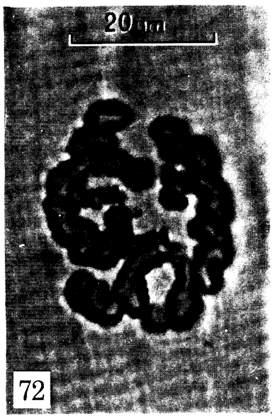

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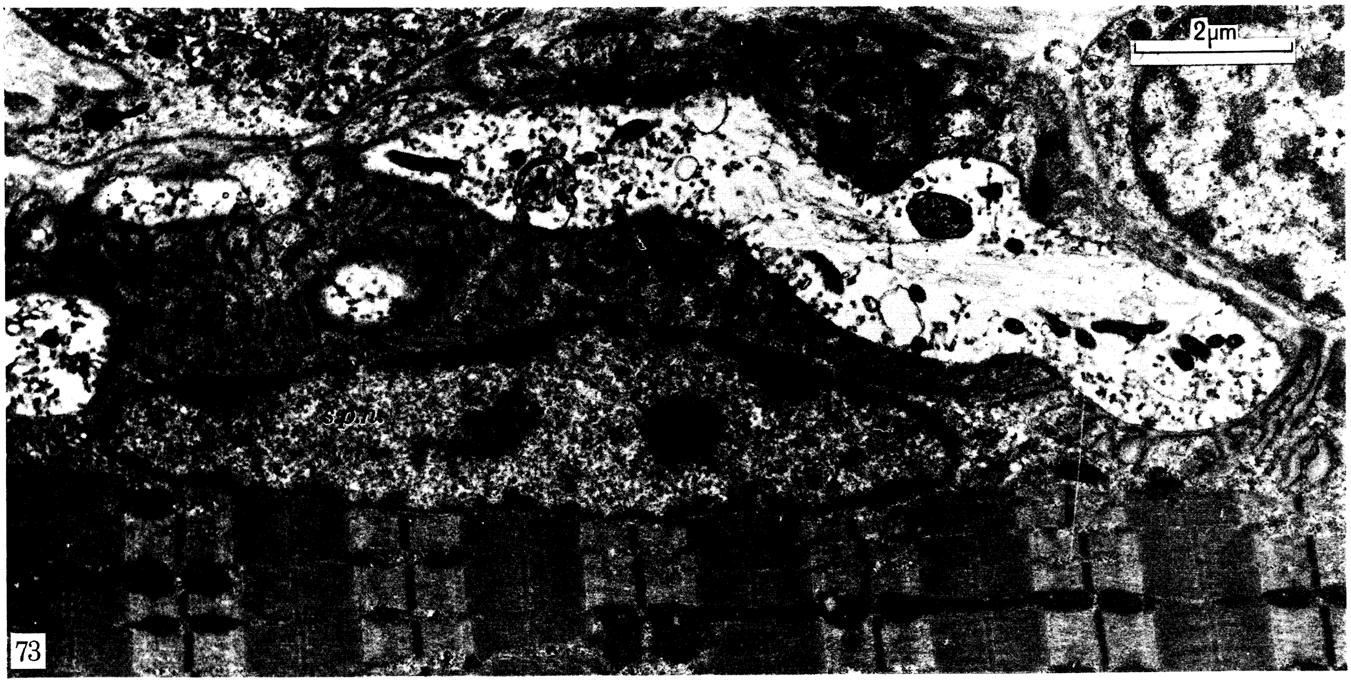

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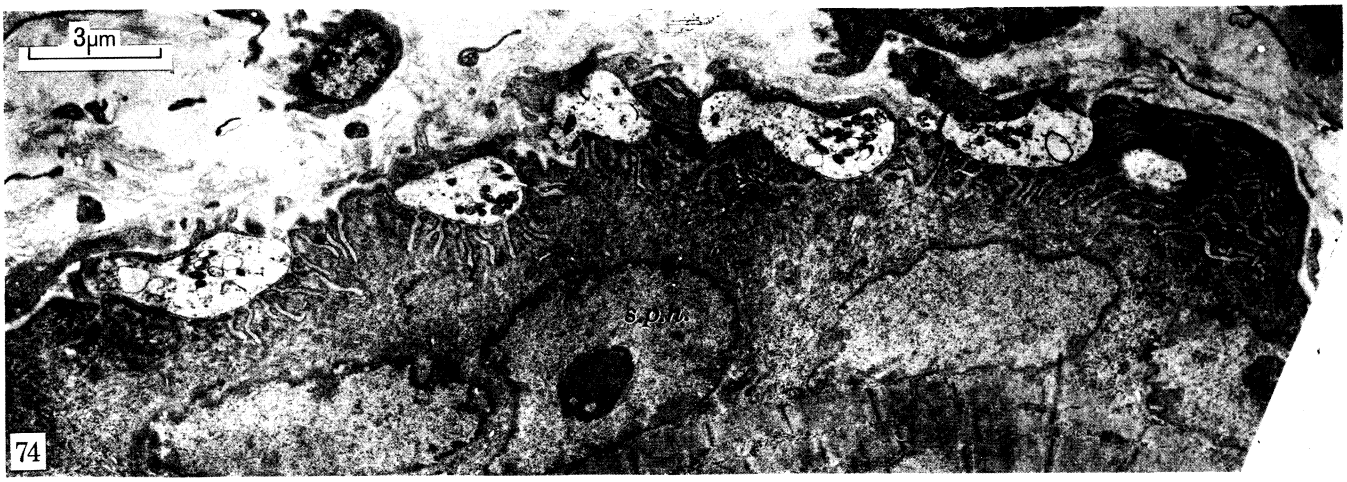

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receive only trail endings is evident, only 8 (7.5 %) occurring in the combined total of 107 spindles analysed in all five muscles.

(*h*) *The nature of the fusimotor innervation in fast and slow muscles*

The assumptions made in the Introduction (p. 317) were that if p_1 plates are supplied exclusively by collaterals of slow α fibres they should occur more frequently among spindles located in slow muscles than in fast; and that fast muscles should have a plate innervation that is predominantly p_2. Of the five muscles studied, FHL and soleus are the standard fast and slow muscles used in cross-innervation experiments with twitch times of 40 and 157 ms, respectively (Buller & Lewis 1965). Tests of phosphorylase activity in the two muscles show that in FHL the average ratio of slow to fast muscle fibres is 1:5.6 as compared with 1:0.1 in soleus. Similar tests for the three peroneal muscles give average ratios of slow to fast fibres of 1:4.6 in PL, 1:2.8 in PB, and 1:8.55 in PDQ. PDQ, which flexes the foot and extends and abducts the fifth digit, is thus a fast muscle, while PL (foot flexor) presumably has a medium speed of contraction somewhat faster than PB (foot extensor). If the assumption made in the Introduction is correct, the plate

TABLE 1. A. PERCENTAGE DISTRIBUTION OF DIFFERENT POLAR PATTERNS OF FUSIMOTOR INNERVATION IN SPINDLES FROM PERONEAL, FHL AND SOLEUS MUSCLES

	PL, 63 poles	PB, 84 poles	PDQ, 85 poles	FHL, 70 poles	SOL, 64 poles
p_1 tr:	3.2	10.7	24.7	57.1	39.0
p_2 tr:	63.4	52.4	22.4	14.3	25.0
p_1p_2 tr:	15.9	23.8	34.1	15.7	17.2
tr:	15.9	13.1	18.8	12.9	18.8
p_1 p_2:	1.6	—	—	—	—

B. FREQUENCY DISTRIBUTION OF DIFFERENT POLAR COMBINATIONS IN SPINDLES (sp.) FROM PERONEAL, FHL AND SOLEUS MUSCLES

	PL, 19 sp.	PB, 17 sp.	PDQ, 29 sp.	FHL, 22 sp.	SOL, 20 sp.
p_1 tr:tr p_1	—	—	2	—	3
p_1 tr:tr p_2	—	4	6	13	3
p_1 tr:tr p_2p_1	—	1	3	1	3
p_1 tr:tr	—	—	3	—	1
p_2 tr:tr p_2	7	2	1	1	1
p_2 tr:tr p_2p_1	4	6	5	3	2
p_2 tr:tr	7	2	3	—	3
p_1p_2 tr:tr p_2p_1	1	—	4	—	1
p_1p_2 tr:tr	—	—	1	2	—
tr:tr	—	2	1	2	3

DESCRIPTION OF PLATE 69

Electron micrographs illustrating the ultrastructure of the p_1 plate. *s.p.n.*, sole-plate nucleus.

FIGURE 73. Longitudinal section of part of a p_1 plate located on a nuclear-chain fibre in the polar region of a normal tenuissimus spindle that was teased out from the muscle prior to preparation for sectioning. The ultrastructure is very similar to that of extrafusal plates.

FIGURE 74. Longitudinal section of a p_1 plate located on a nuclear-chain fibre in the polar region of a normal spindle that was teased out from tibialis posterior prior to preparation for sectioning. The axon terminals are situated in synaptic gutters located on the surface of a thick, nucleated sole plate that forms a Doyère eminence on the surface of the muscle fibre.

FIGURE 75. Detail of the same p_1 plate illustrated in figure 74 showing two axon terminals with associated junctional folds. Compare with detail of extrafusal plate shown in figure 60.

innervation in the fast FHL and PDQ should therefore be predominantly p_2. In fact, as the results have so far shown, it is predominantly p_1.

The situation may be analysed further by examining the relative proportions in which spindle poles sampled from the peroneal, FHL, and soleus muscles receive a p_1 and p_2 plate innervation in addition to trail endings. Table 2 shows the results of such an analysis. In the FHL sample, the proportion of poles receiving a p_1 innervation is the highest (73.3 %) among the five muscles studied. In the peroneals, PDQ again emerges as the odd member of the group, there being approximately the same percentage of poles in the sample receiving p_1 innervation as p_2, whereas in PL and PB the percentage of poles receiving p_2 is high and the percentage receiving p_1 is low. Soleus is similar to PDQ, though with a lower proportion of poles receiving a p_2 innervation.

In some of the poles in the five samples it was possible to analyse the total fusimotor supply in terms of the numbers of fibres contributing the three types of motor ending, and from this to work out the mean number of fibres per pole of each of the three kinds of fusimotor fibre. The results are shown in table 3. Trail fibres comprise about two thirds (64.6 to 67.1 %) of the total fusimotor supply to each sample of spindle poles, except that of soleus in which the proportion is slightly higher (74.8 %). The relative proportions of the p_1 and p_2 supplies in each sample confirm the tendencies previously noted. In the peroneal group, the p_1 supply in PDQ is high (22.1 % of the total fusimotor supply), while the p_2 supply predominates in PL (28.0 %) and PB (20.3 %). In FHL the p_1 supply is markedly high (28.8 %) and the p_2 very low (4.1 %). In soleus there is little difference in the proportion of the two types of plate supply (14.2 % p_1, 11.0 % p_2). The data for the mean number of fibres, or fibre branches, per pole of the three types of fusimotor fibre provide a further expression of the same features.

These analyses led us to inquire whether the supply of γ-stem fibres to a muscle such as PL, whose spindles receive very little p_1 innervation, is significantly greater than the γ supply to muscles such as FHL and PDQ, whose spindle p_1 innervation is high. We accordingly obtained fibre-size histograms for the motor component of the nerves supplying FHL, soleus, and each of the three peroneal muscles in a de-afferented cat, removing these muscles from the animal

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DESCRIPTION OF PLATE 70

Photographs of teased, silver preparations of spindles illustrating the progressive degeneration of fusimotor endings after nerve section, with extrafusal motor end-plates included for comparison. The number of hours after nerve section is indicated in the top right-hand corner of each figure for ready reference. All the preparations are from PB. The scale shown in figure 76 also applies to figures 77, 80 and 82. *cp.*, capsule; *$p_1pl.$*, p_1 plate; *$p_2pl.$*, p_2 plate; *tr.rm.*, trail ramification.

FIGURE 76. Two p_1 plates with inflated axon terminals and the beginnings of retraction 48 h after nerve section.

FIGURE 77. An extrafusal motor end-plate at a similar stage to the p_1 plates in the previous figure.

FIGURE 78. A p_1 plate 60 h after nerve section. The supplying fibre has fragmented and the axon terminals have disappeared.

FIGURE 79. Two extrafusal motor end-plates at a similar stage to the p_1 plate in the previous figure.

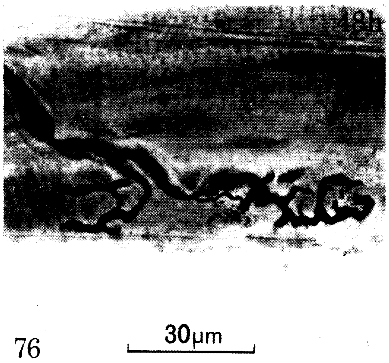
FIGURE 80. A p_2 plate 72 h after nerve section showing no obvious signs of change from normal, though others at this stage have inflated axon terminals.

FIGURE 81. A trail-ending area 72 h after nerve section. Some of the ramifications show signs of terminal inflation.

FIGURE 82. Two trail ramifications with inflated axon terminals 72 h after nerve section.

FIGURES 83, 84. Remnants of preterminal trail axons with inflated ramifications 84 h after nerve section.

FIGURE 85. At 60 h after nerve section the fibre supplying a p_1 plate has fragmented and the plate terminals have almost disappeared, while the trail and p_2 innervation remains intact.



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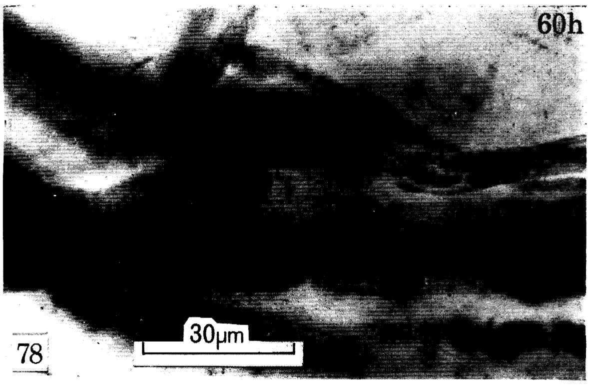

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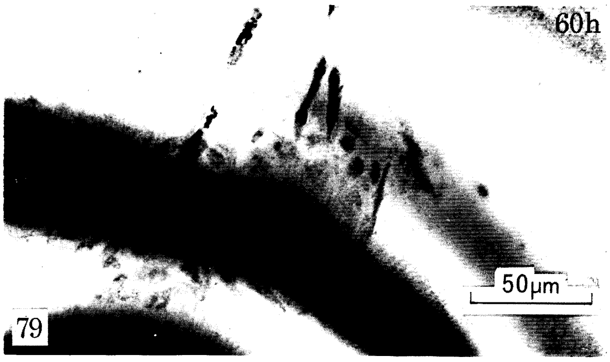

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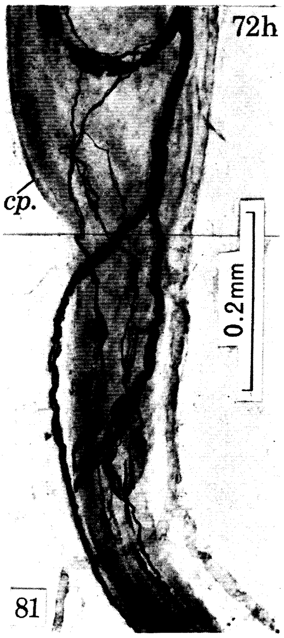

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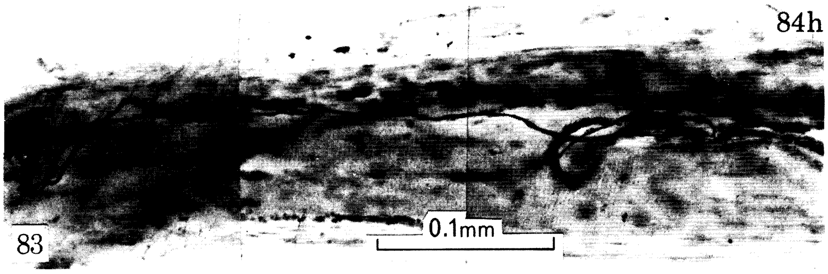

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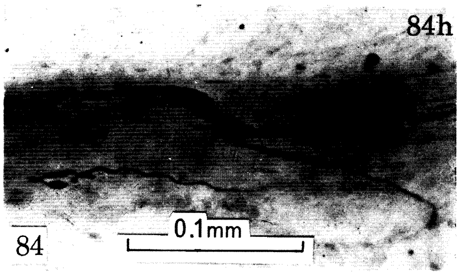

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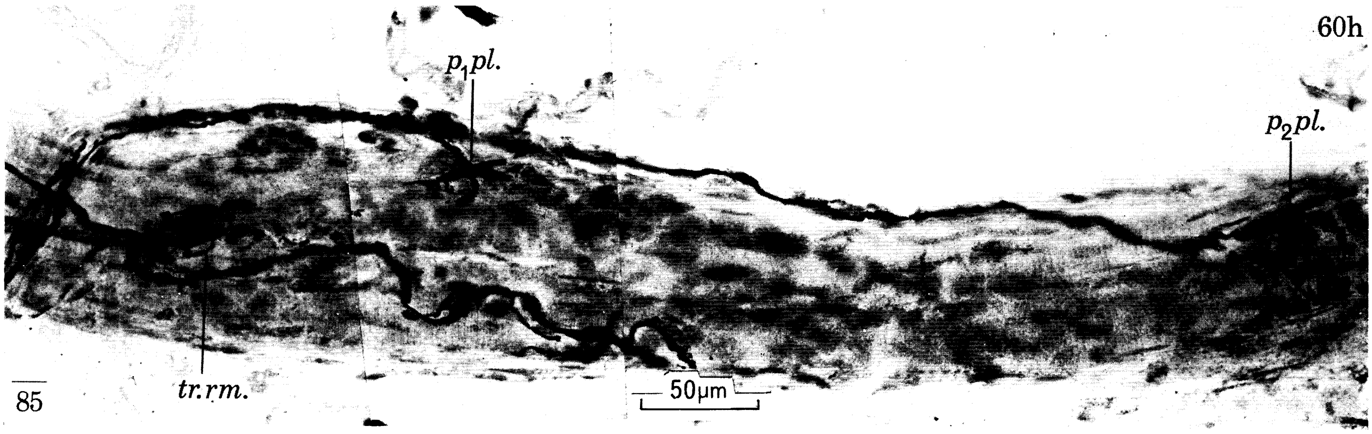

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and teasing them in order to determine their spindle content. The results are shown in figure 9 and table 4.

The number of γ-stem fibres per spindle in PL is seen to work out at 4.09 and is in fact considerably higher than in the other four muscles in the series, in which the provisions range from 1.42 to 2.69. The converse relationship, in which a high p_1 innervation associated with a relatively low γ innervation is reflected by a low input of γ-stem fibres, does not consistently apply in this series. The number of γ-stem fibres supplying a spindle population evidently only fluctuates significantly when there is a marked discrepancy between the levels of γ and p_1 innervation, as occurs in PL where 94 % of the total fusimotor supply is γ as against 6 % p_1 (table 3).

TABLE 2. PROPORTIONS OF SPINDLE POLES RECEIVING TRAIL, p_1 AND p_2 INNERVATION IN PERONEAL, FHL AND SOLEUS MUSCLES

muscle	no. spindle poles	percentage of poles receiving		
		trail fibres	p_2 fibres	p_1 fibres
PL	63	98.4	81.0	20.6
PB	84	100.0	76.2	34.5
PDQ	85	100.0	56.5	58.8
FHL	70	100.0	30.0	73.3
SOL	64	100.0	42.2	56.2

TABLE 3. SUPPLY OF FUSIMOTOR FIBRES TO SPINDLE POLES IN PERONEAL, FHL AND SOLEUS MUSCLES

muscle	no. spindle poles	no. fibres in total fusimotor supply (% in brackets)			mean no. fibres per pole in total sample			
		trail	p_2	p_1	trail	p_2	total γ	p_1
PL	36	97 (66.0)	41 (28.0)	9 (6.0)	2.7	1.1	3.8	0.25
PB	32	124 (64.6)	39 (20.3)	29 (15.1)	3.9	1.2	5.1	0.9
PDQ	19	56 (65.1)	11 (12.8)	19 (22.1)	2.95	0.6	3.55	1.0
FHL	23	114 (67.1)	7 (4.1)	49 (28.8)	5.0	0.3	5.3	2.1
SOL	23	95 (74.8)	14 (11.0)	18 (14.2)	4.1	0.6	4.7	0.8

TABLE 4. SPINDLE CONTENT CORRELATED WITH γ FIBRE SUPPLY TO PERONEAL, FHL, AND SOLEUS MUSCLES IN A DE-AFFERENTED CAT

muscle	total no. motor fibres ($\alpha+\gamma$)	γ	$\frac{\gamma}{\alpha+\gamma}$ %	no. spindle capsules (s)	$\frac{\gamma}{s}$
PL	168	90	53.6	22	4.09
PB	172	97	56.4	36	2.69
PDQ	54	27	50.0	19	1.42
FHL	321	123	38.3	51	2.41
SOL	200	82	41.0	47	1.75

The α fibres in the PL muscle nerve are mostly larger than those in the PB and PDQ muscle nerves, so that the α component in the PL fibre-size histogram is more widely separated from the γ component (see figure 9). If all p_1 fibres are branches of slow α fibres, this could be interpreted as another expression of the low p_1 innervation of the spindles in this muscle. On the other hand, the PL muscle nerve is the first to branch off the common peroneal, and it seems more probable that the smaller size of the α fibres in PB and PDQ is due to the fibres branching during the further subdivision of the parent nerve.

These results indicate that the α innervation of spindles via p_1 plates is not characteristic of slow muscles. In this series such an innervation is more evident in the fast FHL than in the slow soleus. In the peroneals it is also more evident in PDQ, the fastest member of the group. The nature of the plate innervation in PL spindles indicates that a low incidence of p_1 innervation is offset by a high provision of p_2 plates, and this is reflected in the γ supply to the muscle by the provision of a relatively high number of γ-stem fibres per spindle.

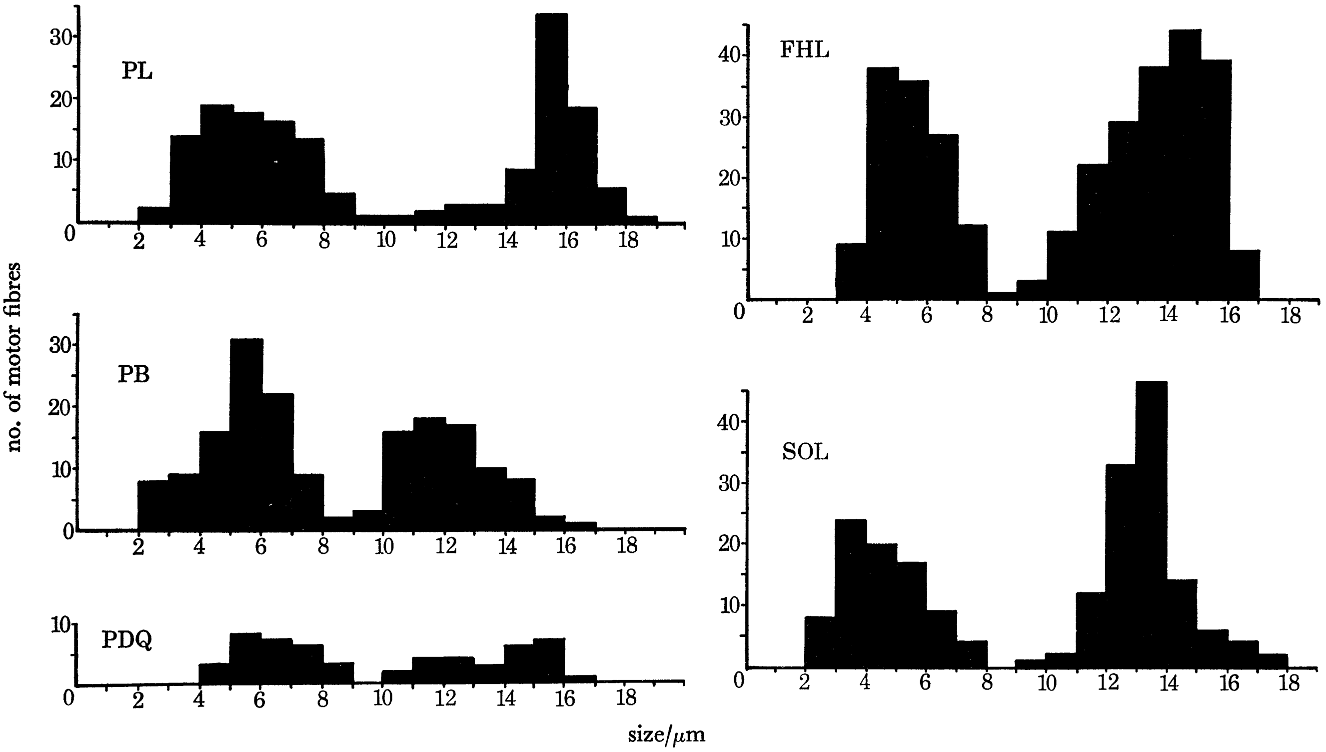

FIGURE 9. Motor fibre-size histograms of the muscle nerves supplying the peroneal, FHL and soleus muscles in the right hindlimb of a de-afferented cat.

4. DISCUSSION

The two results that emerge clearly from this study are that the plate and trail innervation is not selectively distributed to the bag and chain muscle fibres; and that p_1 plates feature in the fusimotor innervation of both fast and slow muscles.

So far as selective innervation is concerned, it is true that the majority of chain fibres receive trail endings only, and that the plate innervation is distributed mainly to the bag fibres. Nevertheless, we have conclusively demonstrated that a proportion of the plate innervation ($25\ \%\ p_1$, $10\ \%\ p_2$) is received by the chain fibres, and that bag fibres are included in the trail innervation. The implications of non-selective innervation are either that different types of fusimotor ending supplied to the same intrafusal muscle fibre can cause it to contract in different ways (recalling the situation that obtains in arthropod muscle); or that each type of intrafusal muscle fibre gives only one kind of contraction regardless of the type of ending initiating it. The fact that fusimotor fibres may branch so as to innervate both bag and chain fibres renders the latter condition less likely. By obtaining frequencygrams of primary ending discharge during stimulation of single fusimotor fibres, Bessou, Laporte & Pagès (1968) have shown that the contractions elicited by static fibres are fast and strong, and that those elicited by dynamic fibres are slow and weak. If these types of contraction are the separate properties of the two types of intrafusal muscle fibre, the stimulation of a fusimotor fibre innervating both bag and chain fibres would elicit both contractions so as to produce a compound response. The frequencygrams of Bessou *et al.* provide no evidence that such responses occur. Moreover, the stimulation of static fibres distributed in this way would result in an increase of the dynamic index, but this does not happen. It appears, therefore, that whether the contraction is fast and strong or slow and weak depends upon the type of fusimotor ending initiating it rather than upon the type of muscle fibre executing it. If this is so, static and dynamic γ fibres may be equated with homogeneous categories of fusimotor fibres and endings, and trail fibres may be identified as static and p_2 fibres as dynamic on the following grounds. (i) The high proportion of trail fibres participating in spindle innervation, as compared with p_2 fibres (see table 3, p. 341), corresponds with the numerical preponderance of static as opposed to dynamic γ fibres isolated in single-fibre experiments, e.g. 47 static fibres as opposed to 17 (26.6 %) dynamic fibres in a sample of tibialis posterior γ fibres (Brown, Crowe & Matthews 1965); 37 static fibres as opposed to 13 (26.0 %) dynamic in a tenuissimus sample (Bessou *et al.* 1968). (ii) The fact that trail endings are the most resistant to degeneration after nerve section (see §3 (*f*)) suggests that the γ-stem fibres supplying them include those of the smallest diameter in the γ range, and this agrees with the finding that the slowest γ fibres are invariably static (Brown *et al.* 1965). (iii) Static fibres have a strong excitatory effect upon secondary endings (Appelberg, Bessou & Laporte 1966; Bessou & Pagès 1969), the majority of which, like the trail endings, terminate mainly on the chain fibres. Since stimulating the fusimotor collaterals of β fibres produces a dynamic effect (Bessou *et al.* 1963, 1965; Brown *et al.* 1965), we may conclude that the dynamic response is controlled via the p_1 and p_2 plates, and that the static response is controlled by the trail endings.

Turning now to the p_1 innervation, our findings show that it is a widespread feature of spindle innervation, and that it can occur either in addition to, or instead of, the p_2 innervation. In connexion with the former condition it is relevant to note that both Bessou *et al.* (1965) and Brown *et al.* (1965) have recorded from spindles in which they were able to produce dynamic effects by stimulating either β or γ fibres. There is some evidence that when there is little p_1 innervation among a spindle population, as in PL, there is a compensatory increase of the p_2 innervation and a relatively more liberal supply of γ-stem fibres per spindle. We had expected such a condition to be characteristic of fast muscles, but the contrary proved to be the case. The incidence of p_1 innervation in FHL was the highest in the series of muscles studied, with a mean number of 2.1 p_1 fibres supplying each pole (see table 3, p. 341). The FHL muscle providing the data shown in table 4 and figure 9 contained 51 spindles and would thus require 204 p_1 fibres to supply its p_1 innervation. It received a total of 198 α fibres ranging in diameter from 9.0 to 16.9 μm, and we may arbitrarily regard 65 of these as slow, i.e. those that fall within the lower half (9.0 to 12.9 μm) of this range. Assuming that these are all β fibres, we may note that Adal & Barker (1965) have shown that 9.0 to 12.5 μm β fibres are predominantly skeletomotor in their distribution and supply only one or two collaterals to spindles. The 65 β fibres could thus at the most provide only 130 p_1 fibres, which falls short of the estimated total p_1 fibre supply. The balance could be found if we assume that some fast α fibres supply p_1 collaterals in addition to those supplied by the slow fibres. Some recent findings by Yellin

(1969) point in this direction. He has shown that fast hindlimb muscles in the rat are composed of superficial fascicles of fast type A muscle fibres surrounding a central core of deep fascicles that consist of a mixture of slow type B and fast type C fibres. Spindles are located among the deep fascicles only, and could thus receive a collateral innervation not only from the slow α fibres supplying the B fibres, but also from the fast α fibres supplying the C fibres. Alternatively, it may be that there are some motor fibres of α conduction velocity (over 45 m/s) that have an exclusively fusimotor distribution, as suggested by the work of Haase and Pompeiano and their colleagues (for references, see Introduction, p. 317). The origin of the p_1 innervation clearly requires further investigation, especially by neurophysiologists.

Precision of movement is developed to a greater degree among mammals than other tetrapods, and it is significant in this context that the mammalian spindle has acquired an independent (γ) fusimotor innervation instead of one that is purely collateral, as occurs in the spindles of lower forms. This permits finer adjustment of muscle contraction. Moreover if, as A. Crowe (personal communication) suggests, precision of movement, rather than the exertion of strong contraction, is controlled via the dynamic fusimotor fibres and the primary ending, this would account for the provision of an independent supply of dynamic fibres (p_2) in addition to a collateral supply (p_1). Although purely speculative, this at least provides a clue in correlating the functions of specific muscles with the nature of their fusimotor innervation. On this basis there is perhaps some significance in the fact that among the peroneal muscles, PDQ, which carries out the precise movements of abducting and extending the fifth digit, has the highest frequency of spindle poles that receive both a p_1 and a p_2 innervation (see table 1A, p. 339).

A number of papers have recently appeared on the fusimotor innervation of rat spindles. Using a combination of cholinesterase and Sudan black B staining on lumbrical muscles, Mayr (1969) distinguishes between multiterminal (trail) endings supplying the juxta-equatorial regions, and two types of plate innervating the extracapsular polar regions, which are similar in appearance to cholinesterase preparations of p_1 and p_2 plates in the cat. According to his analysis, the p_1 plates are located on the nuclear-chain fibres, and the p_2 plates innervate the bag fibres, which also receive most of the trail endings. It remains to be seen whether other techniques will confirm this distribution. In our opinion the cholinesterase/Sudan black B technique does not give consistent enough results (see Methods, p. 320) to accept it without reservation. Moreover, Karlsen (1965) has observed extrafusal-type plates (equivalent to cat p_1 plates) in silver-stained sections of spindles from jaw muscles, and states that they are most frequently located on bag fibres. Finally, Hennig (1969) has recently published a study of the ultrastructure of rat fusimotor endings in lumbrical muscles. We agree with his identification of juxta-equatorial trail axon terminals, but there are discrepancies between his description of the plate ultrastructure and our own. An electron micrograph identified as being a section through a p_1 plate (his figure 9) shows an axon terminal overlying a thin sole plate evenly apposed to the postsynaptic membrane except in one area where there are a few wide and shallow junctional folds. We recognize this as a trail myoneural junction; its polar location does not exclude this identification in cat spindles. Moreover, its structure bears no resemblance to that of extrafusal end-plate junctions as we have demonstrated does occur in cat p_1 plates. Hennig's p_2 plate has an intracapsular location at the beginning of the polar region. He describes it as consisting of a row of end-bulbs enclosed in deep inlets of sarcoplasm and covered by tongues of sarcoplasm and Schwann cytoplasm. Junctional folds are absent. In his micrographs (figure 8*a*, *b*), however, neither Schwann cells nor basement membrane are obviously present, and the suspicion that the end-bulbs belong to a secondary ending cannot easily be dismissed. Moreover, it is difficult to equate this ultrastructure with the form of the ending that Gladden (1969) identifies as a p_2 plate in teased, silver preparations of spindles from intertransverse caudal muscles. When histochemical and ultrastructural studies of fusimotor innervation are made without knowledge of the form and disposition of the endings such as may be obtained from teased, silver preparations, they lack a firm basis for correlation and their full value cannot be realized.

Some of the silver preparations studied in this investigation were prepared by Dr M. C. Ip in connexion with earlier work. We gratefully acknowledge his help in this respect. We also wish to thank Miss J. B. Elliott and Miss G. M. Geller for their invaluable assistance with histochemical techniques; David Hutchinson for his excellent photographic assistance; and Professor J. Z. Young, F.R.S., Professor Y. Laporte, Dr S. Cooper, and Dr A. Crowe for their helpful comments on the first draft. We thank the Medical Research Council for financial support.

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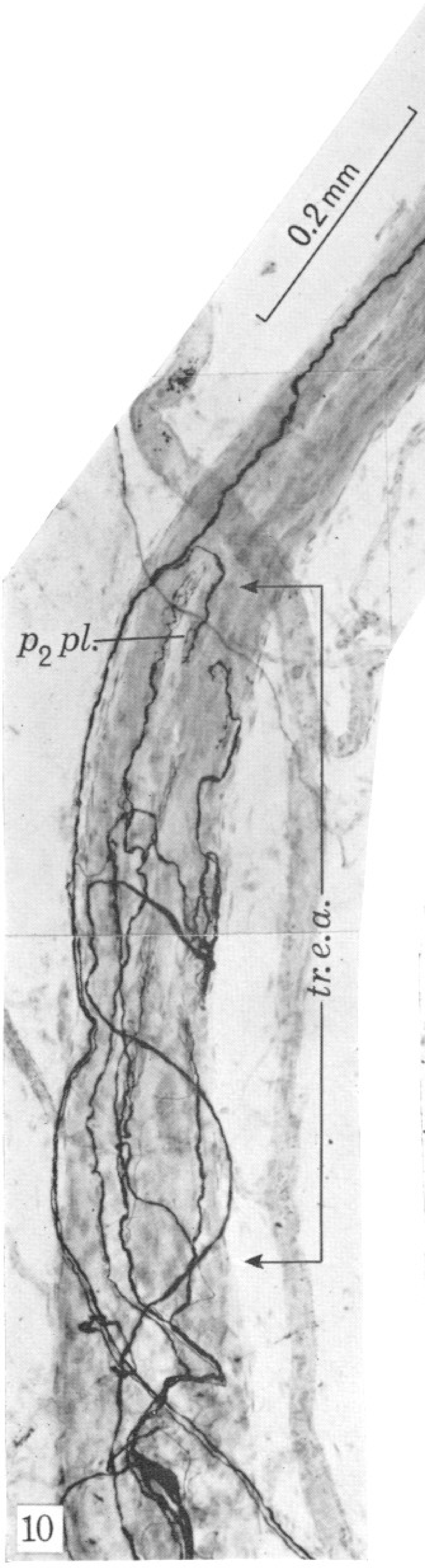
Note added in proof, 4 *March* 1970

Since this paper went to press, N. Corvaja, V. Marinozzi & O. Pompeiano have published an electron microscopic study of cat muscle spindles (*Arch. ital. Biol.*, 1969, **107**, 365–543). Their finding of trail endings located on both nuclear-bag and nuclear-chain fibres agrees with our observations and those of Adal & Barker (1967). They observed a plate ending in one instance only located on a bag fibre. They describe its axon terminals as penetrating deeply into the sarcoplasm, note the presence of rudimentary junctional folds, and doubt whether this type of ending corresponds to the p_1 or p_2 plate. We are not convinced of this interpretation of what appear to be micrographs of oblique sections (figures 60 and 61), and suggest that, taking obliquity and preterminal axons into account, they can more probably be interpreted in terms of the structure of a p_1 plate.



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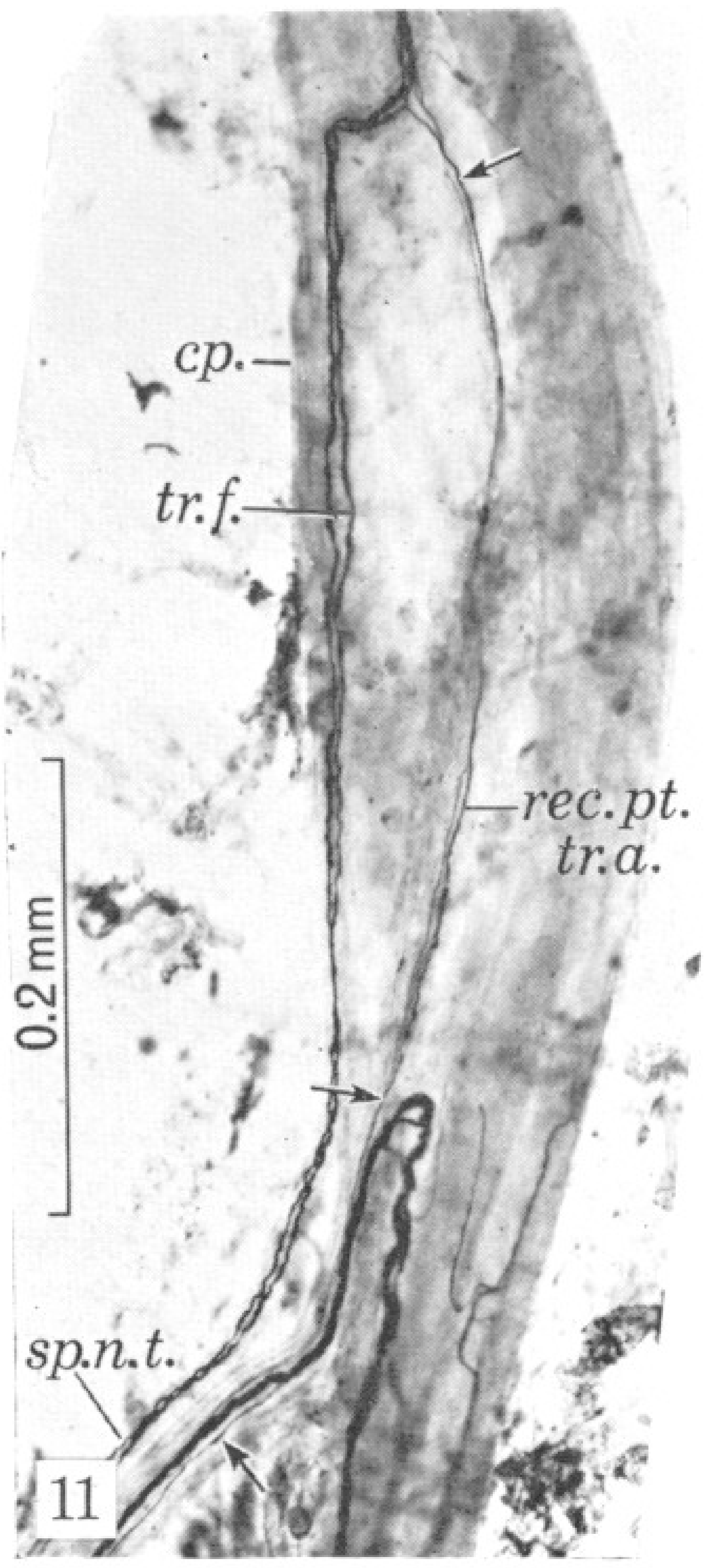

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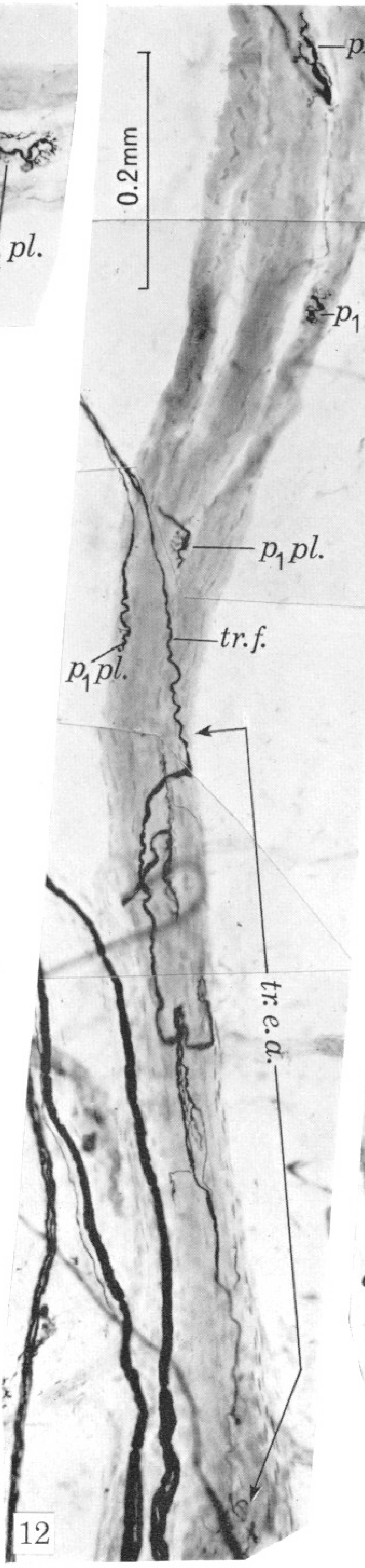

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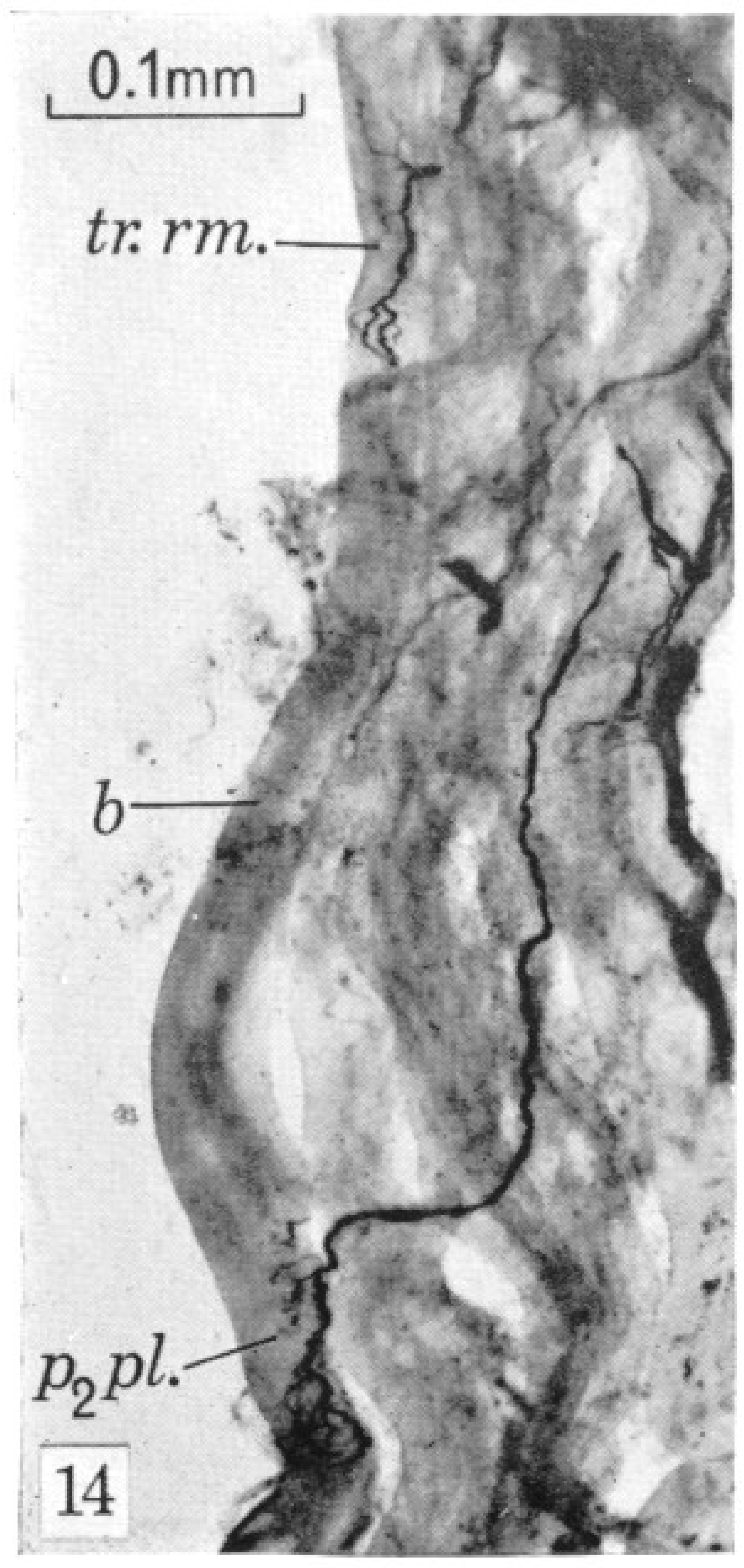

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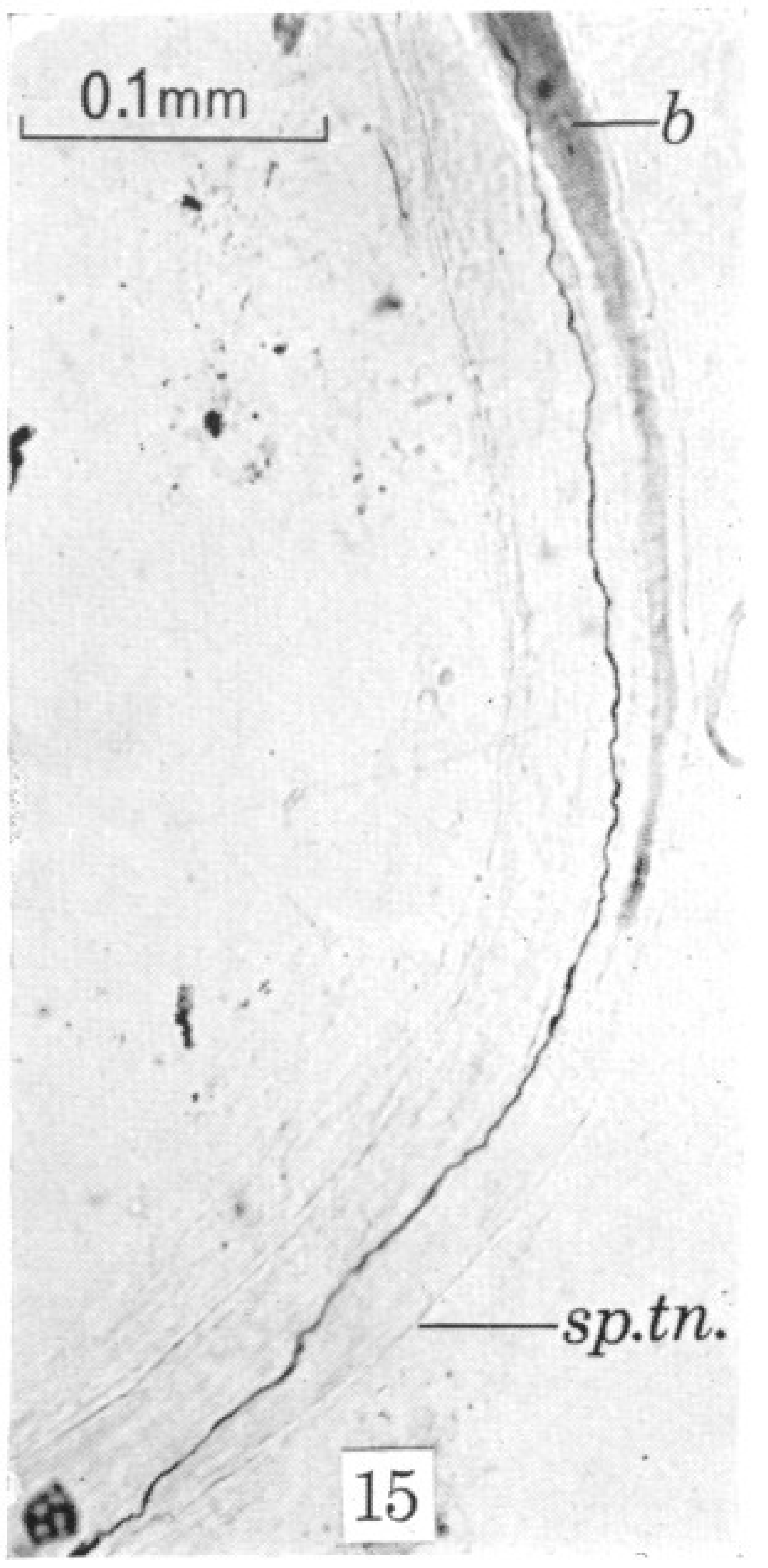

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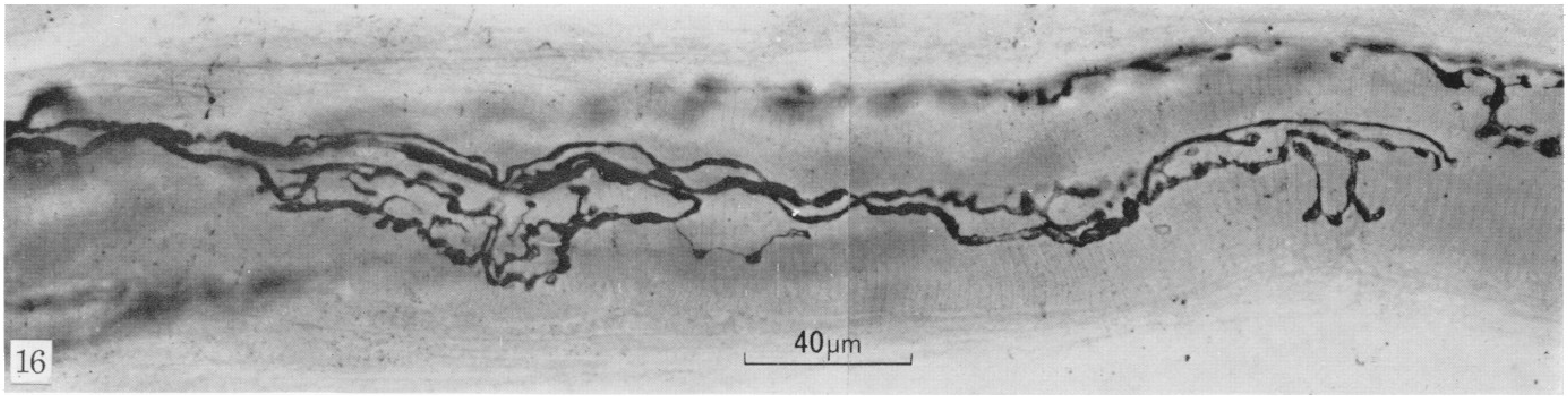

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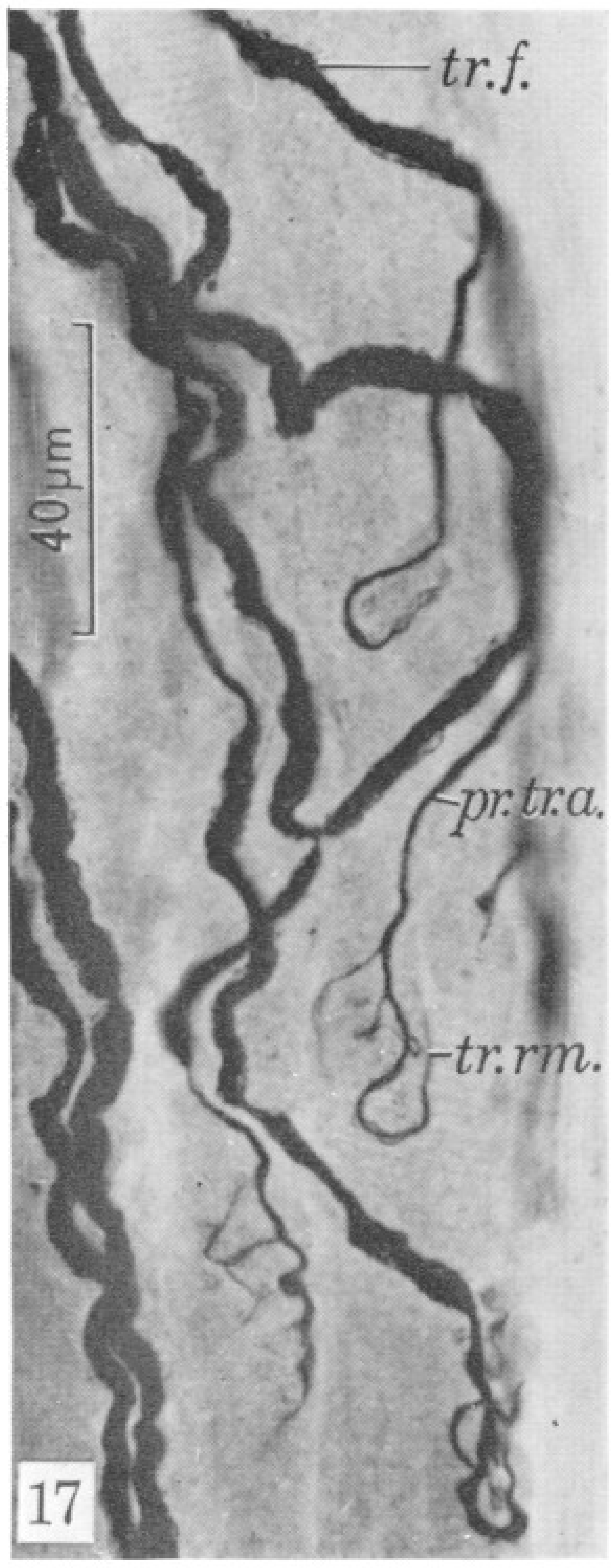

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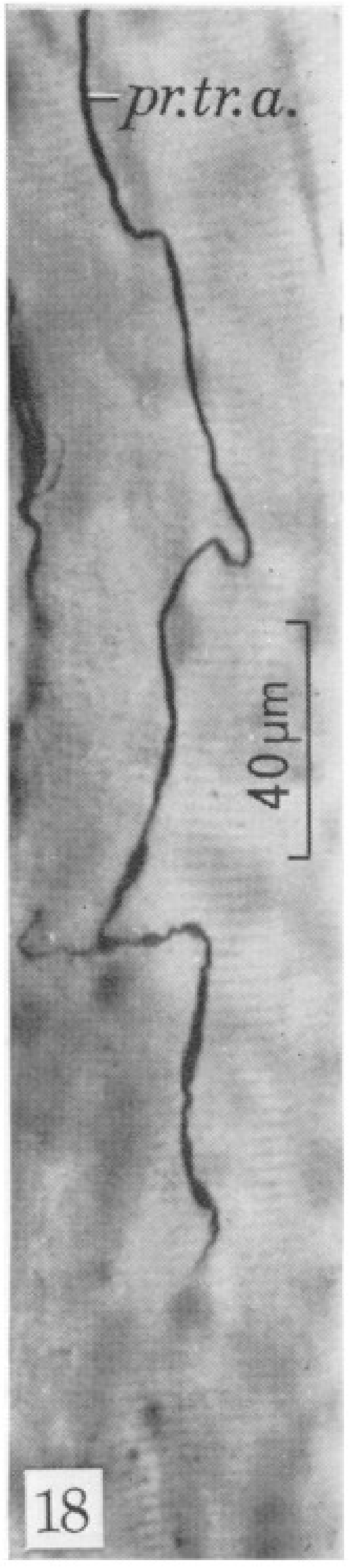

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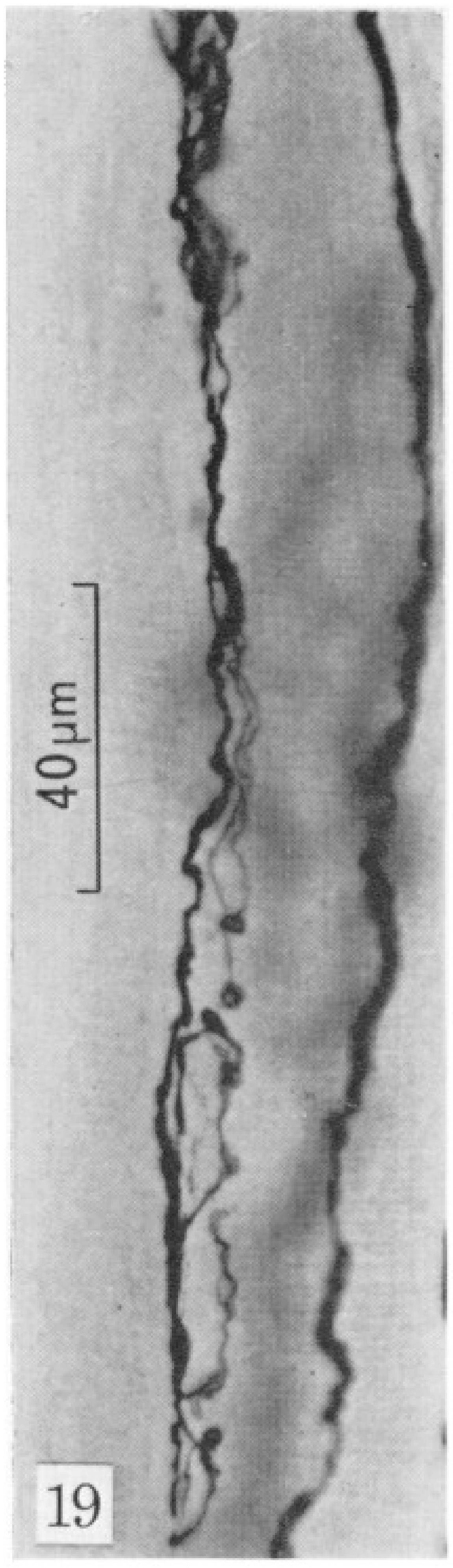

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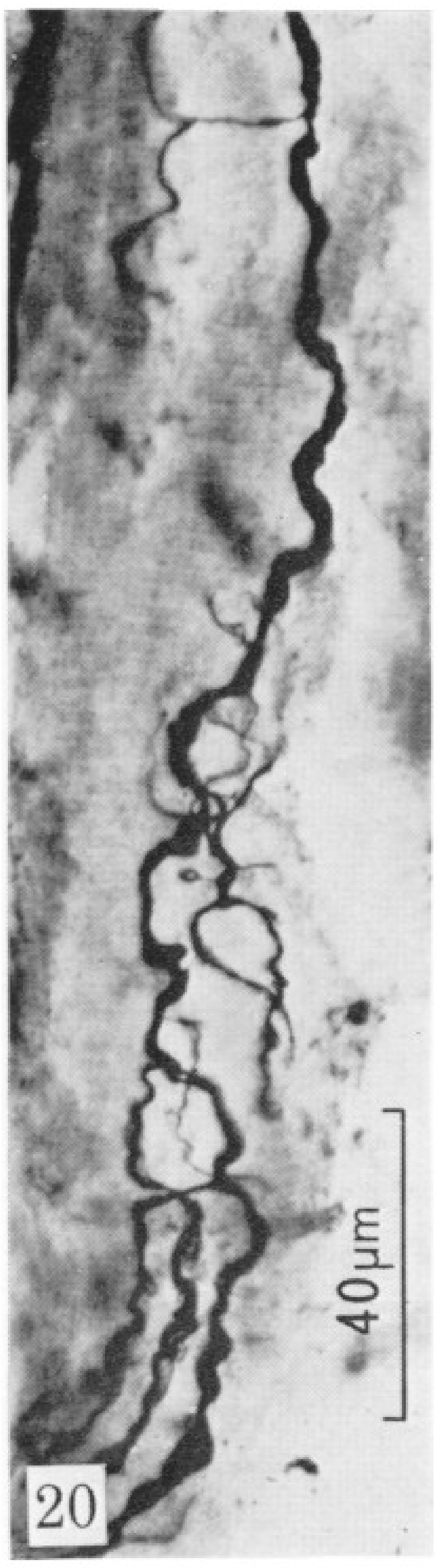

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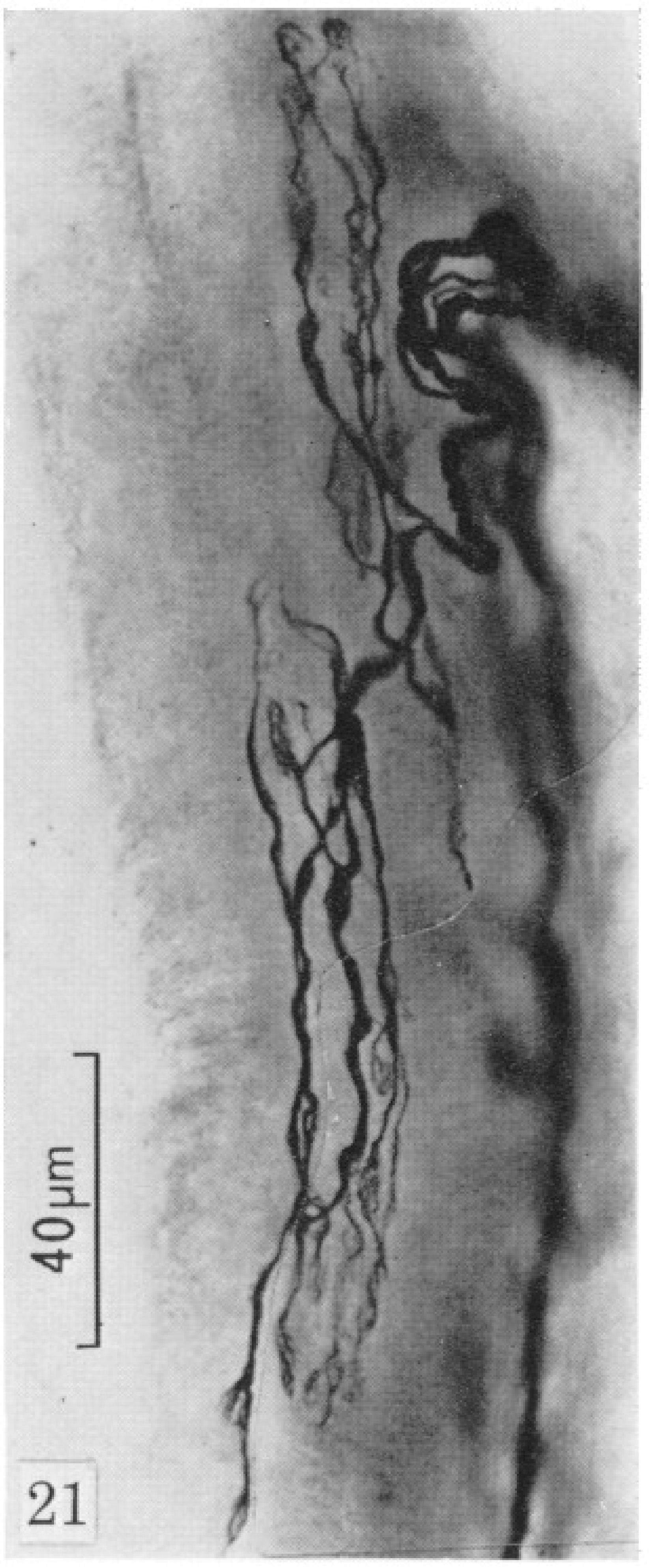

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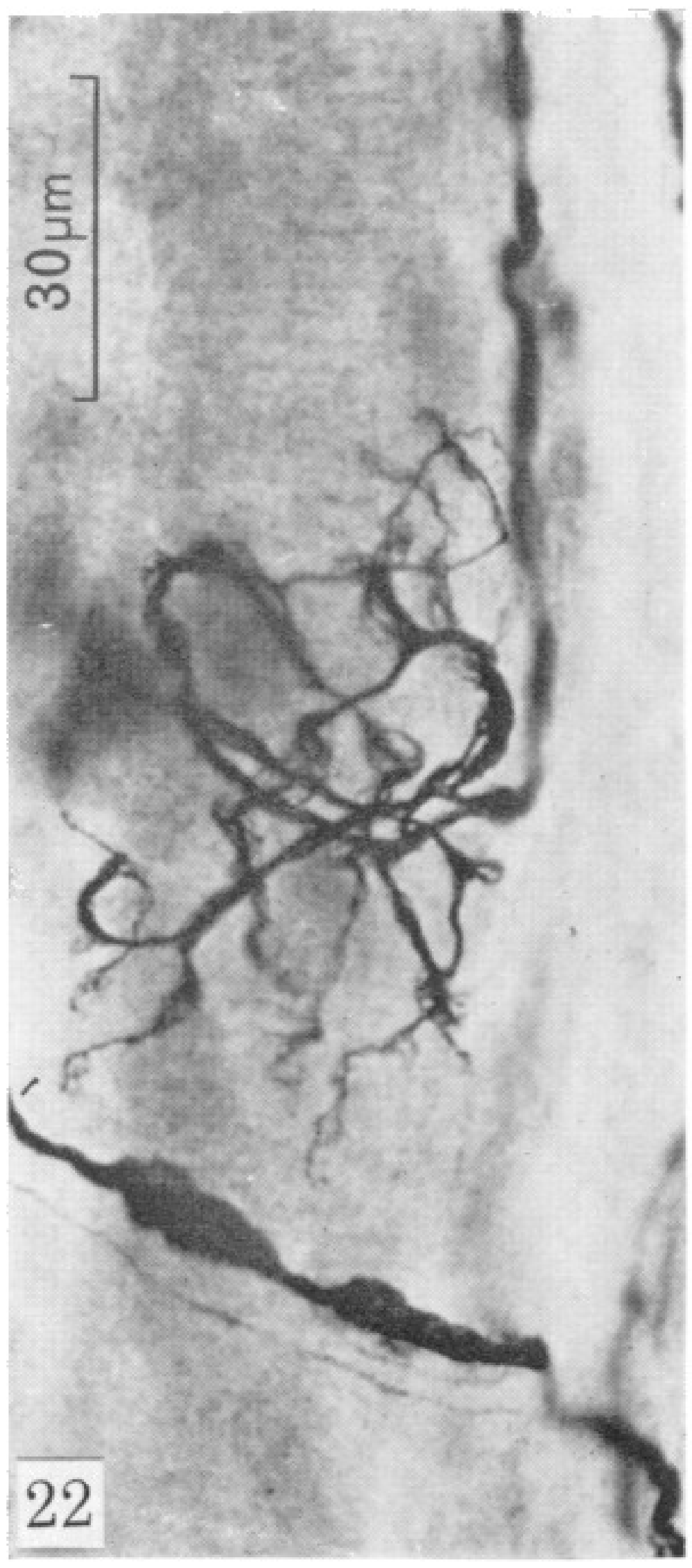

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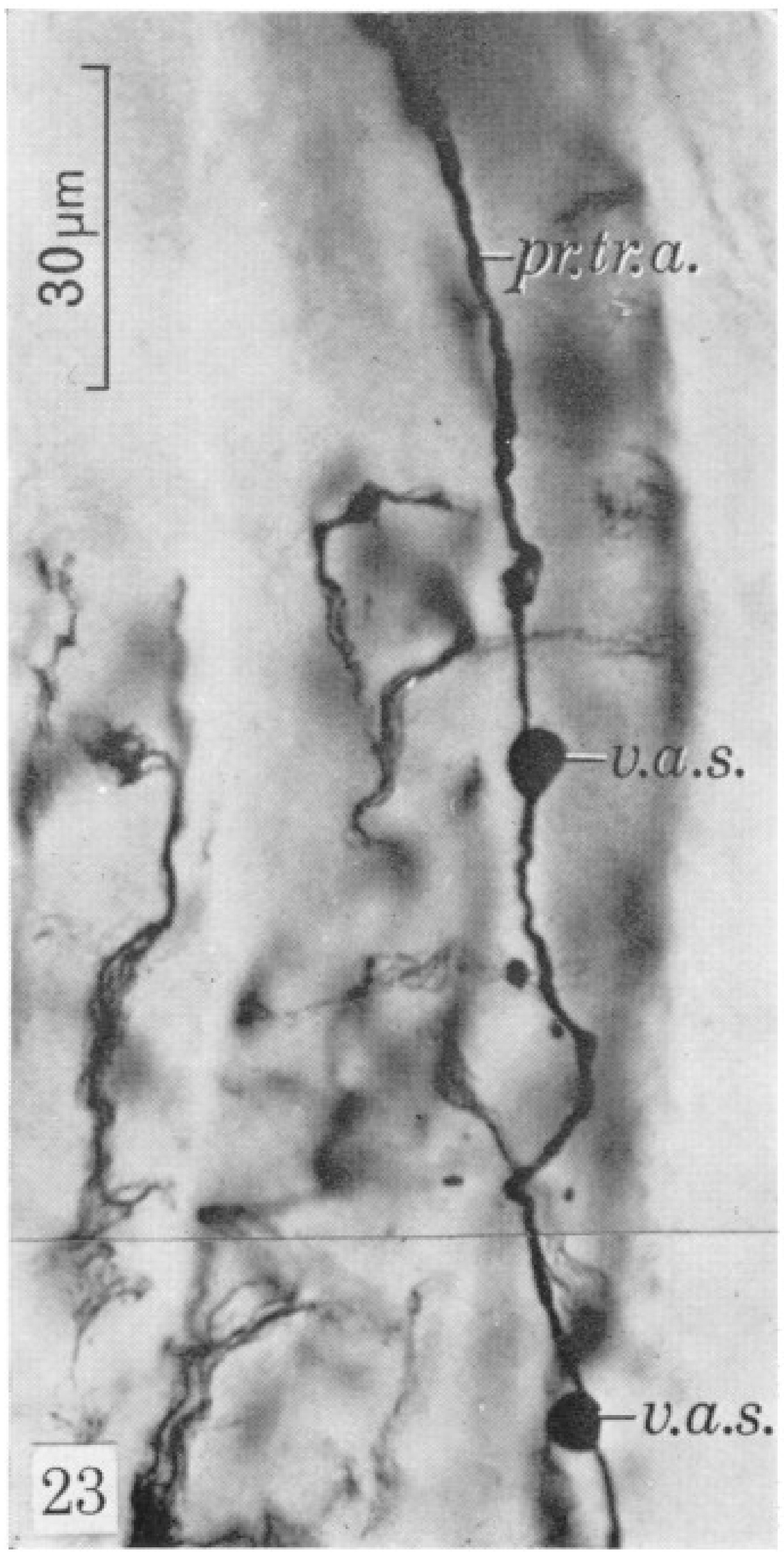

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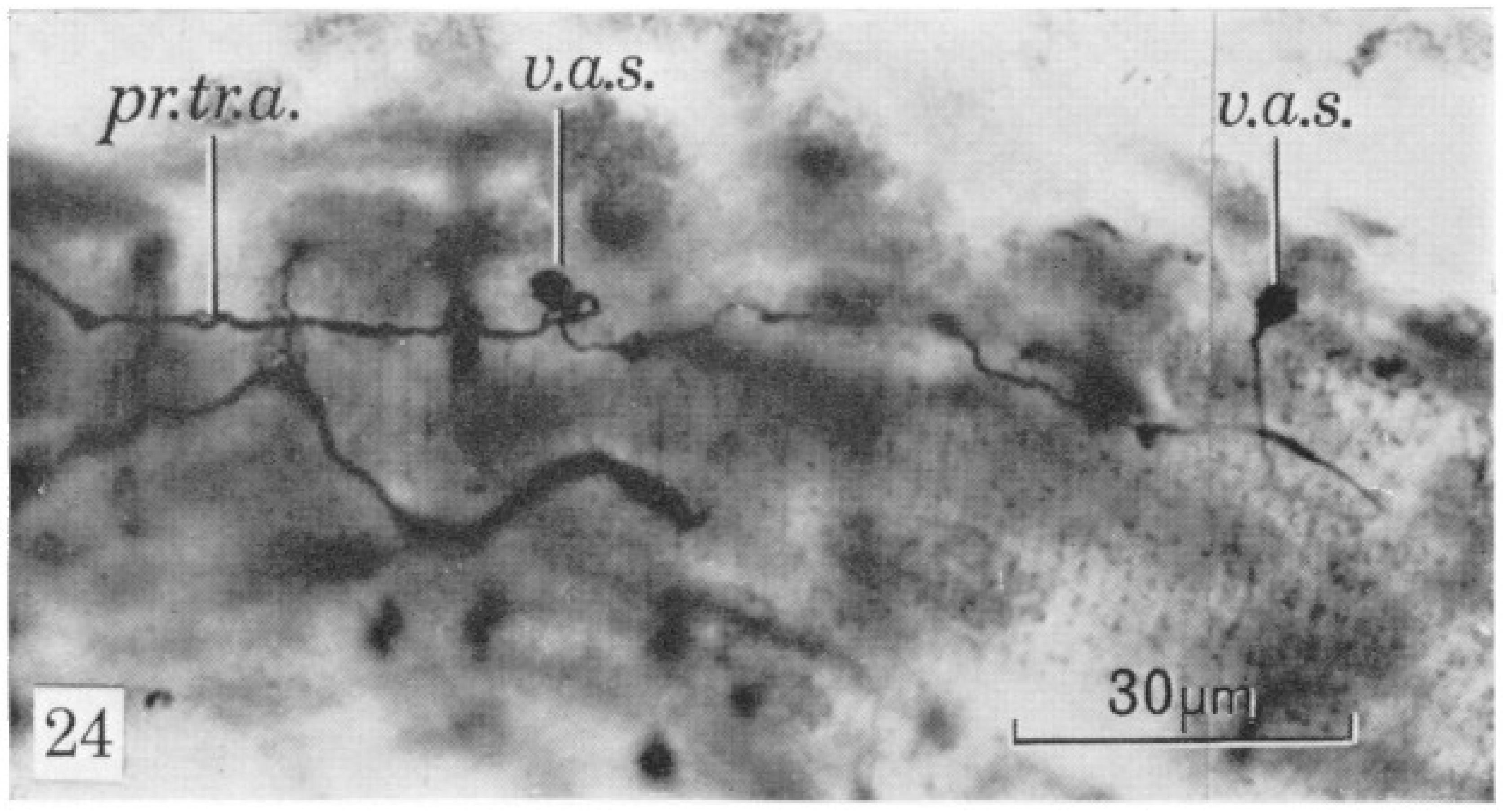

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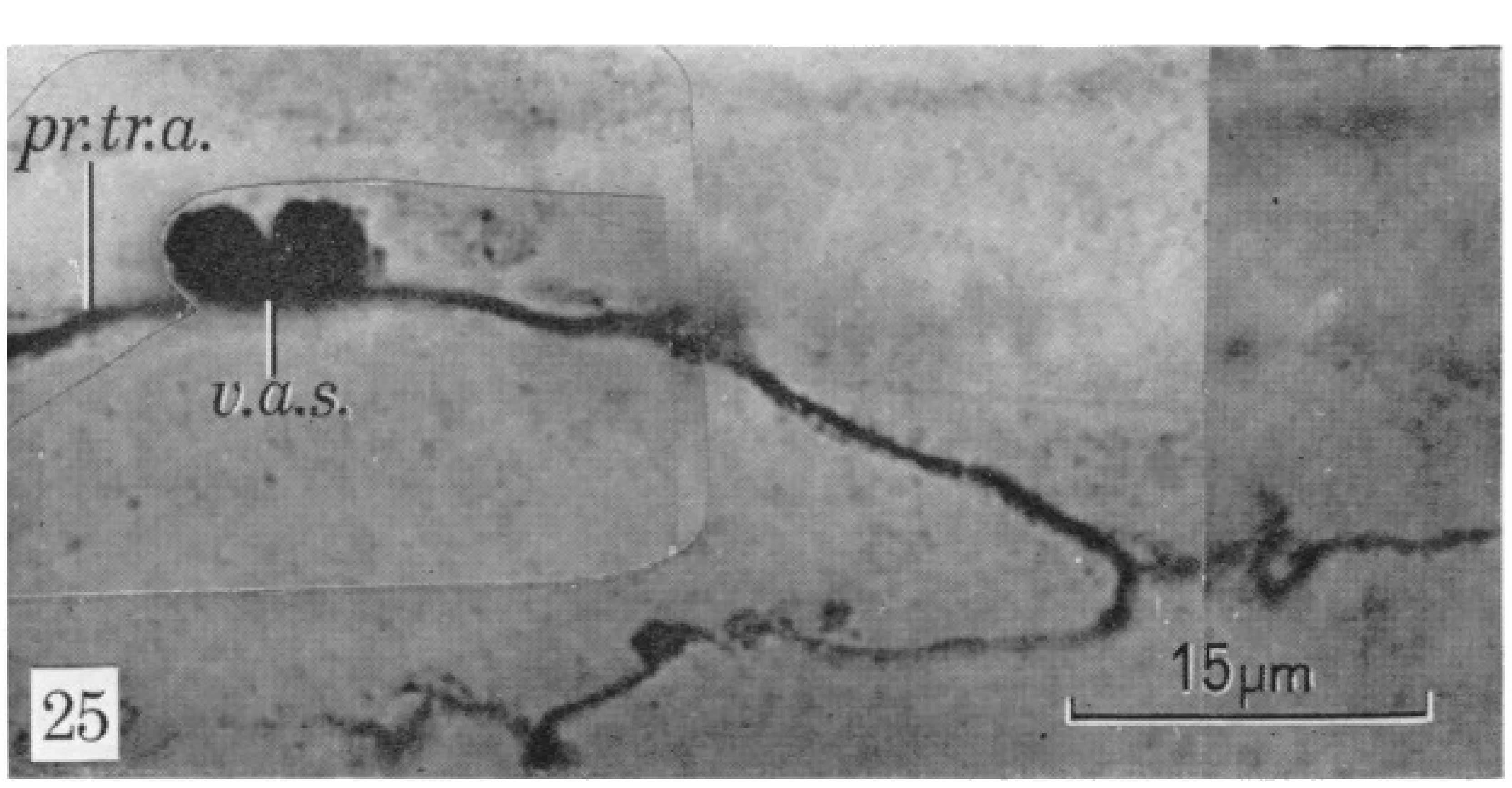

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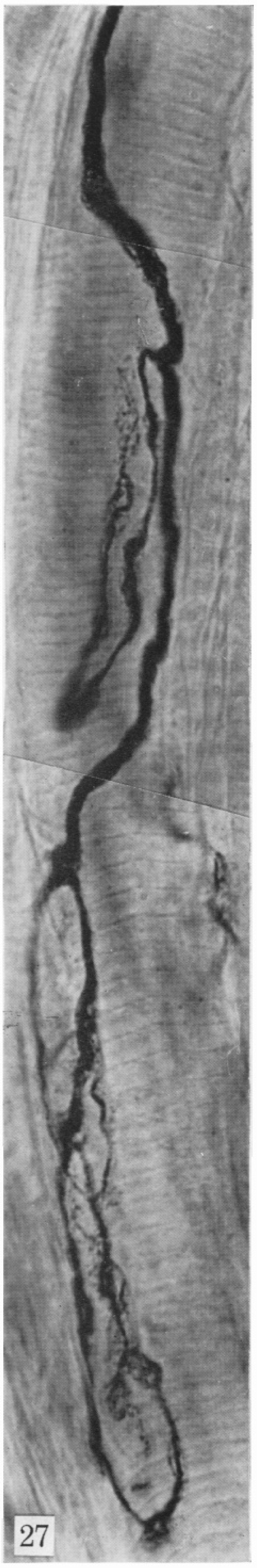

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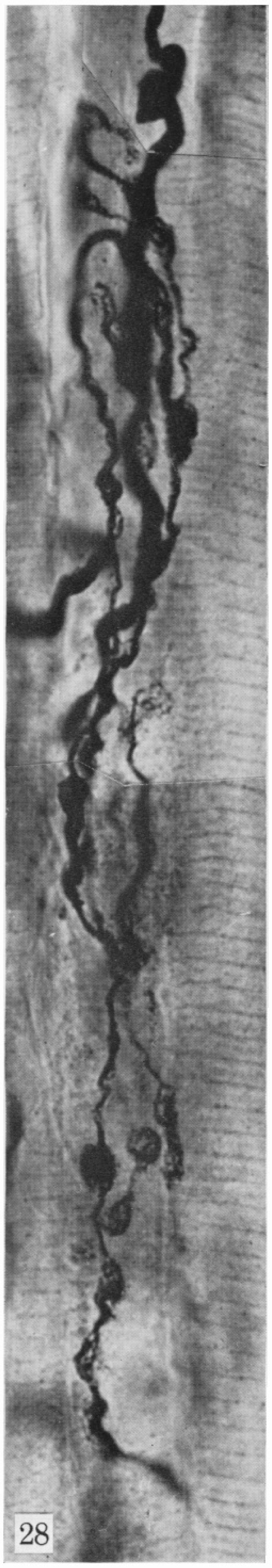

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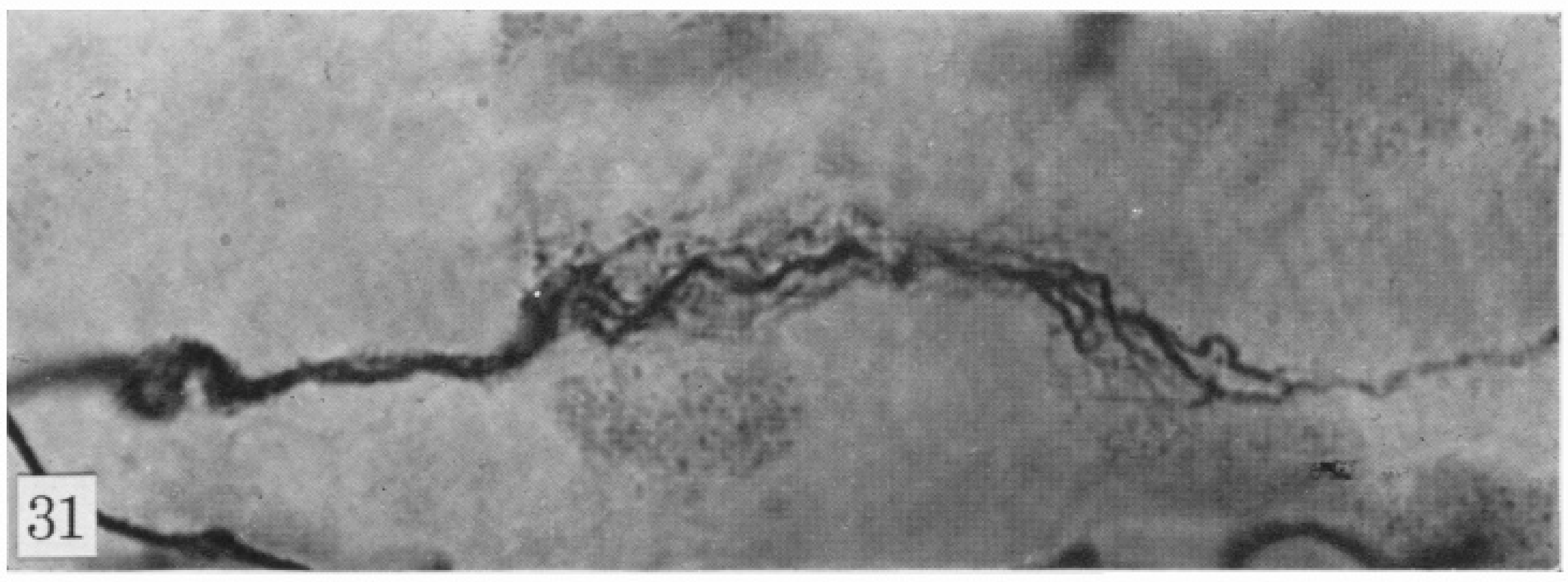

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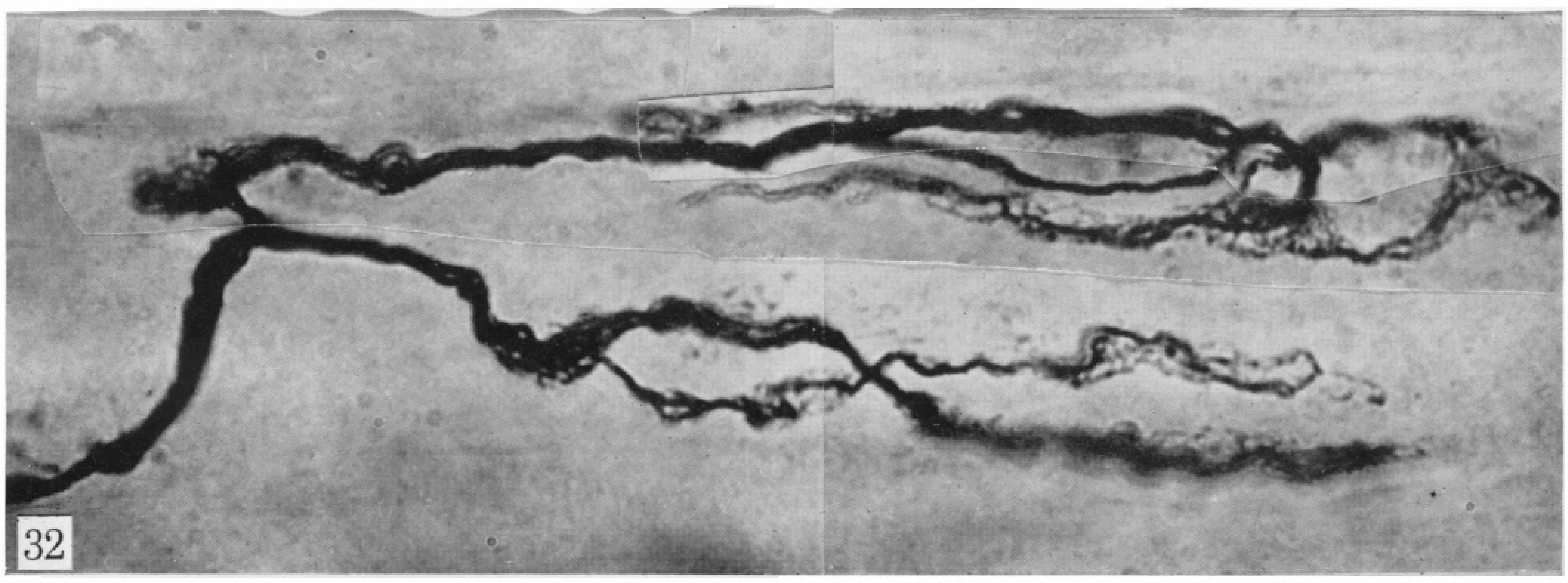

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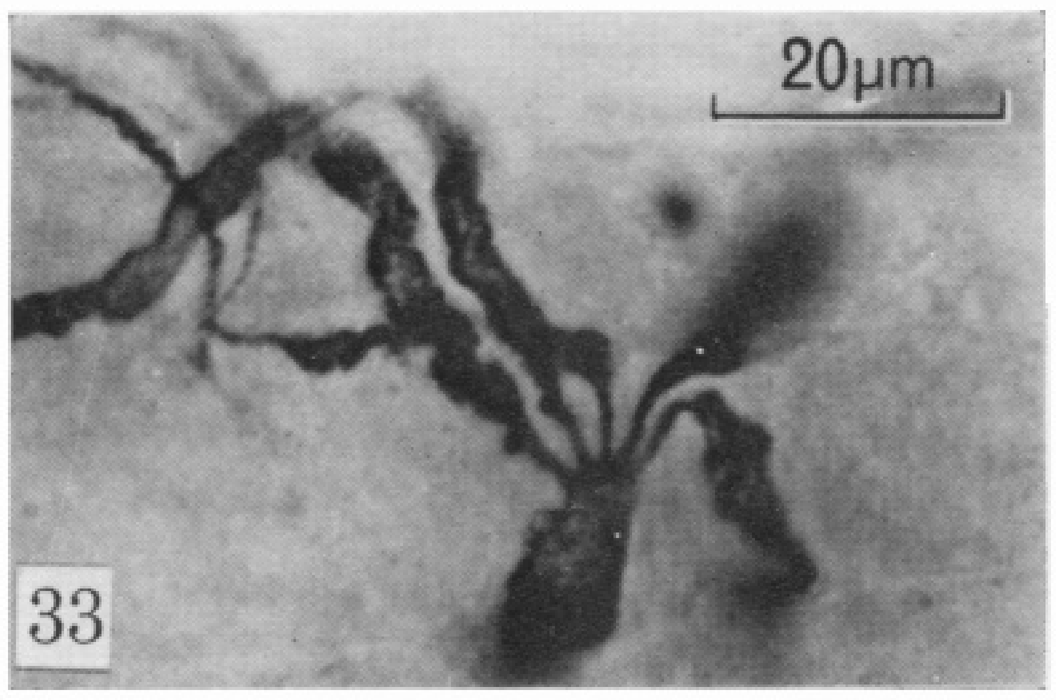
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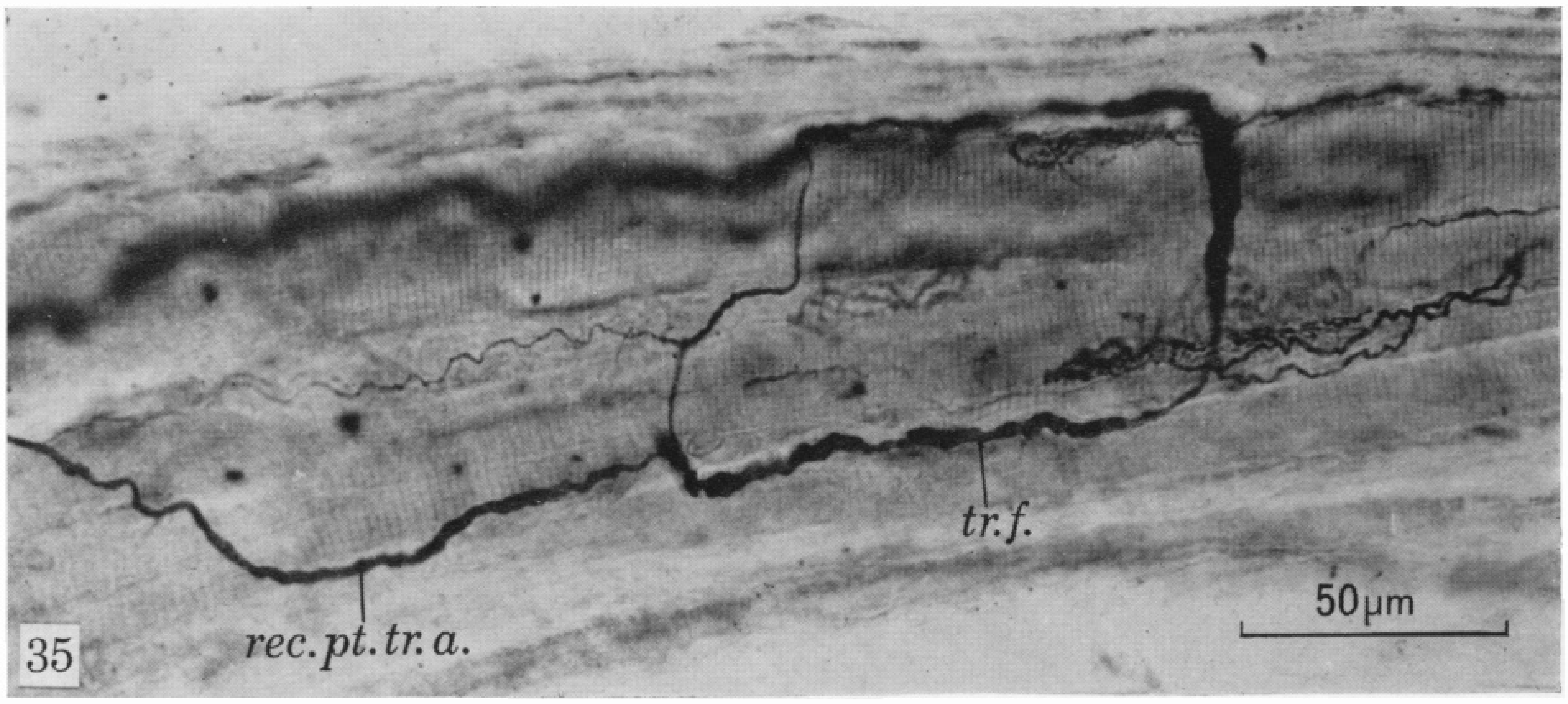
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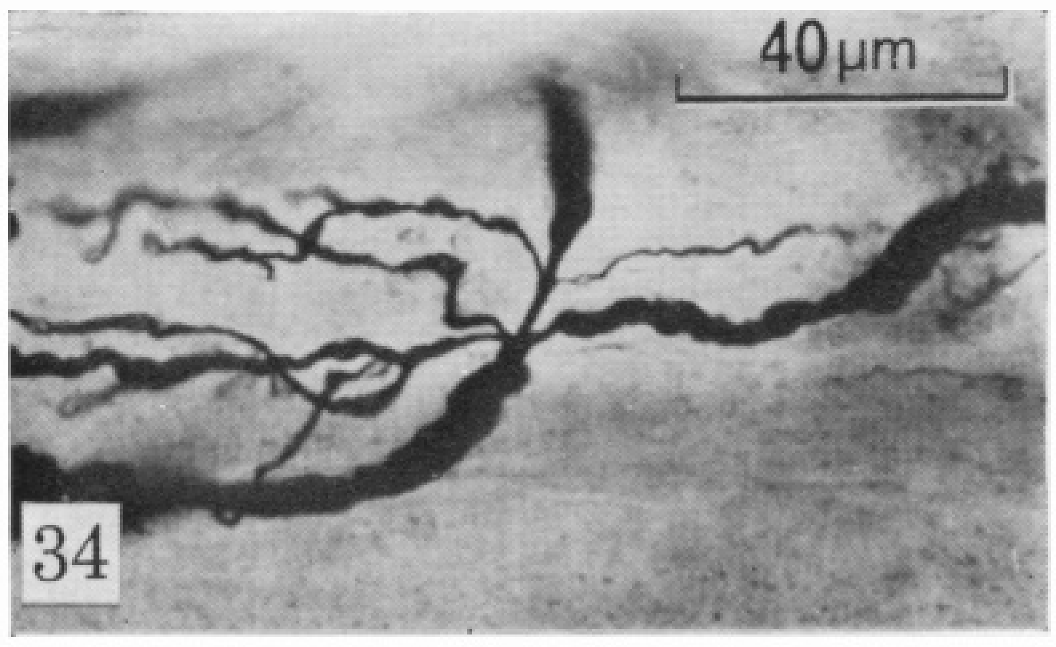
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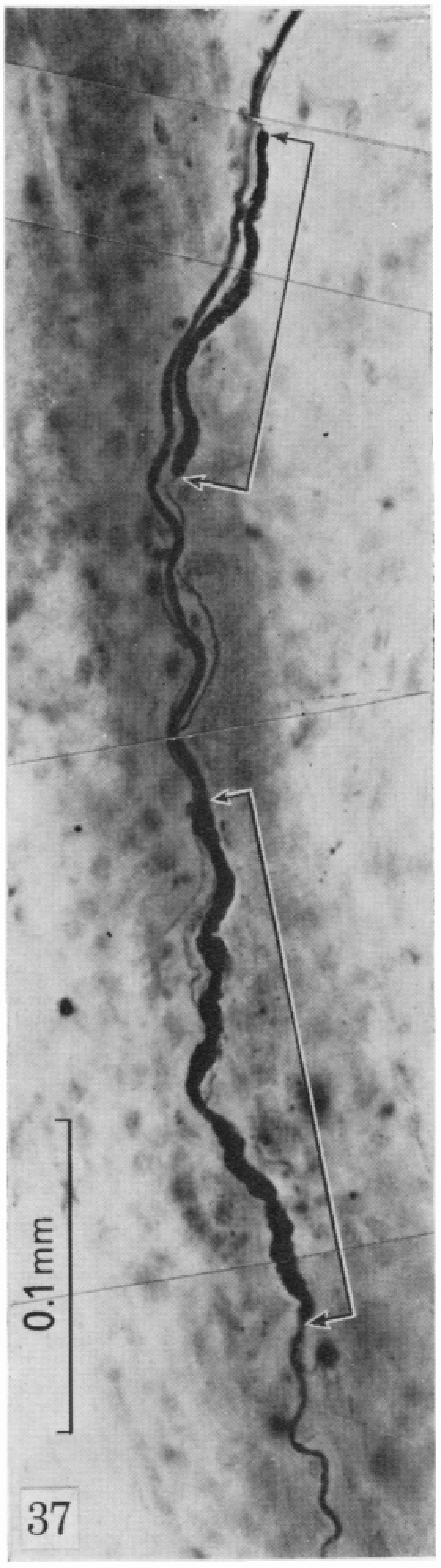
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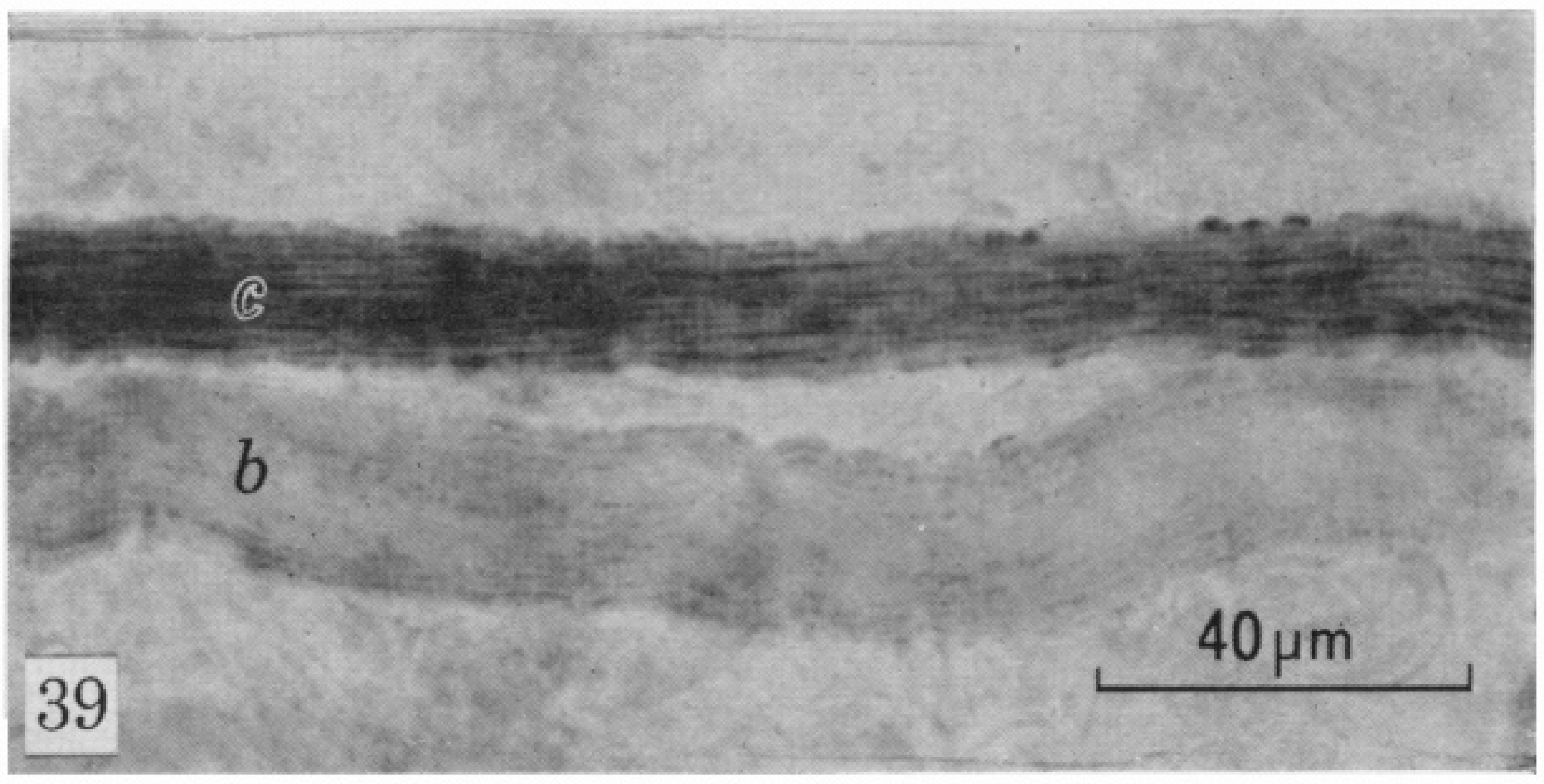
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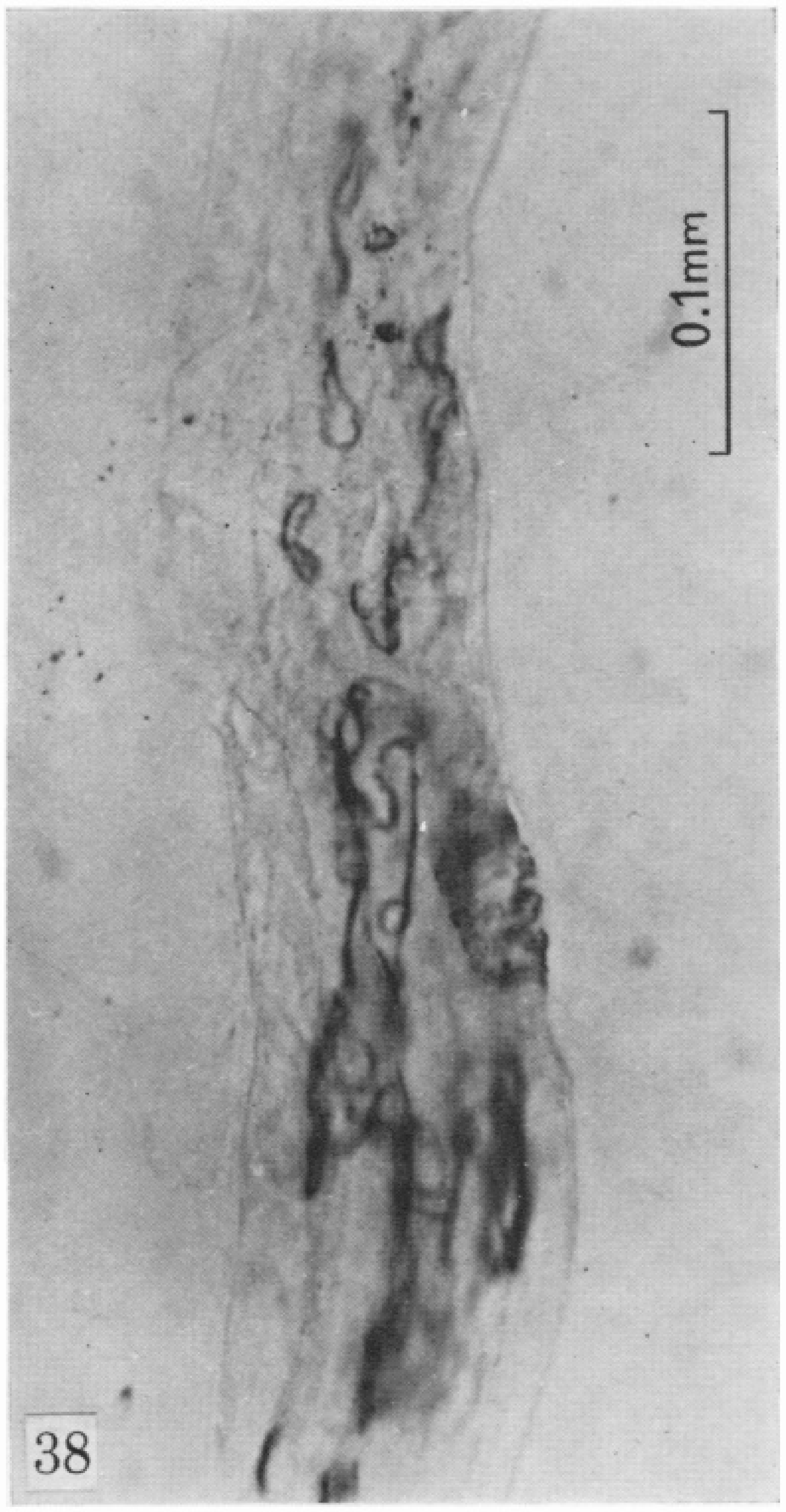
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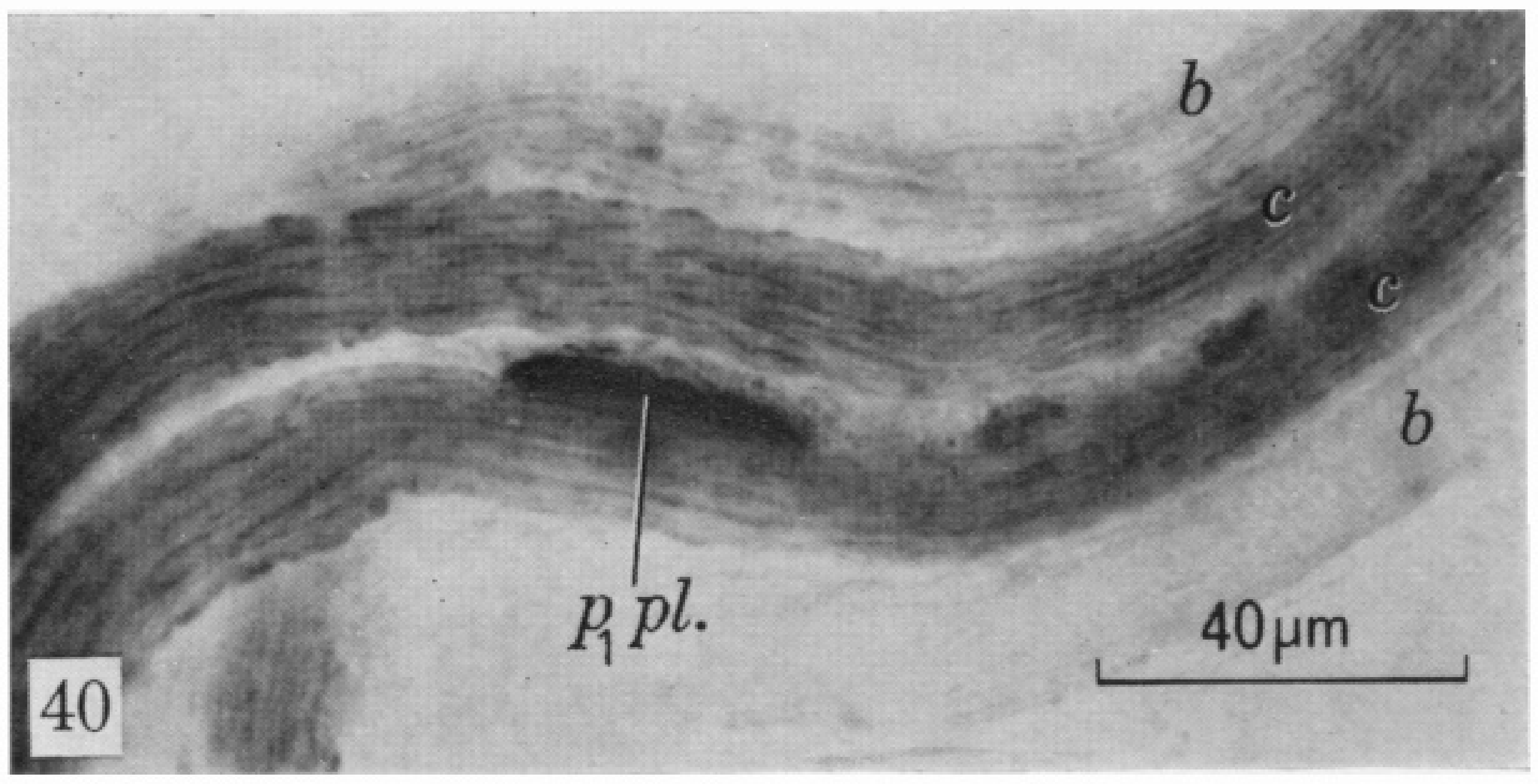
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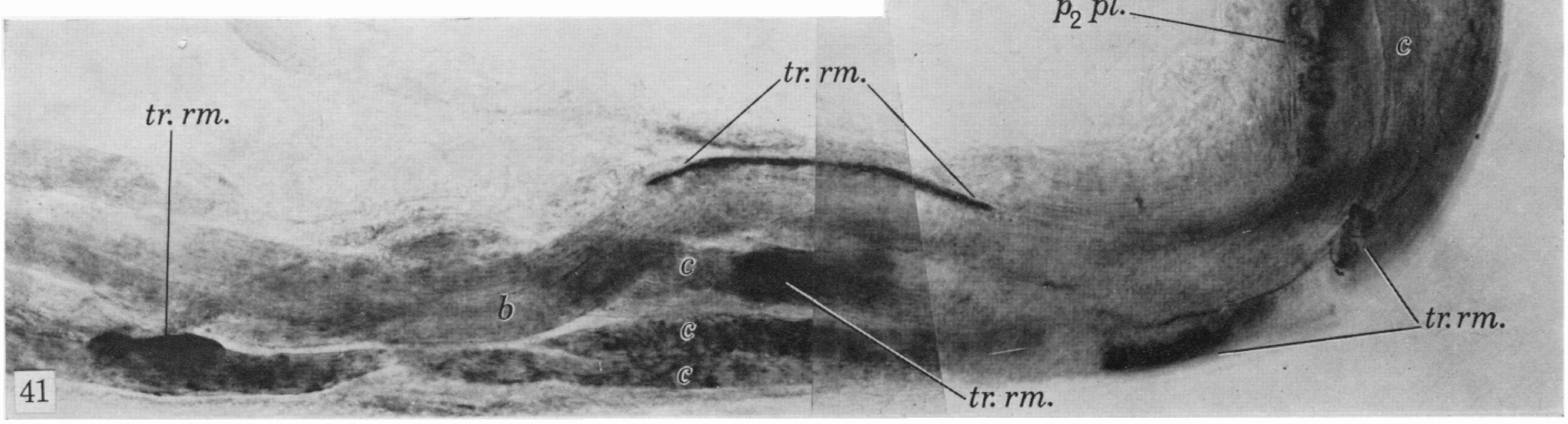
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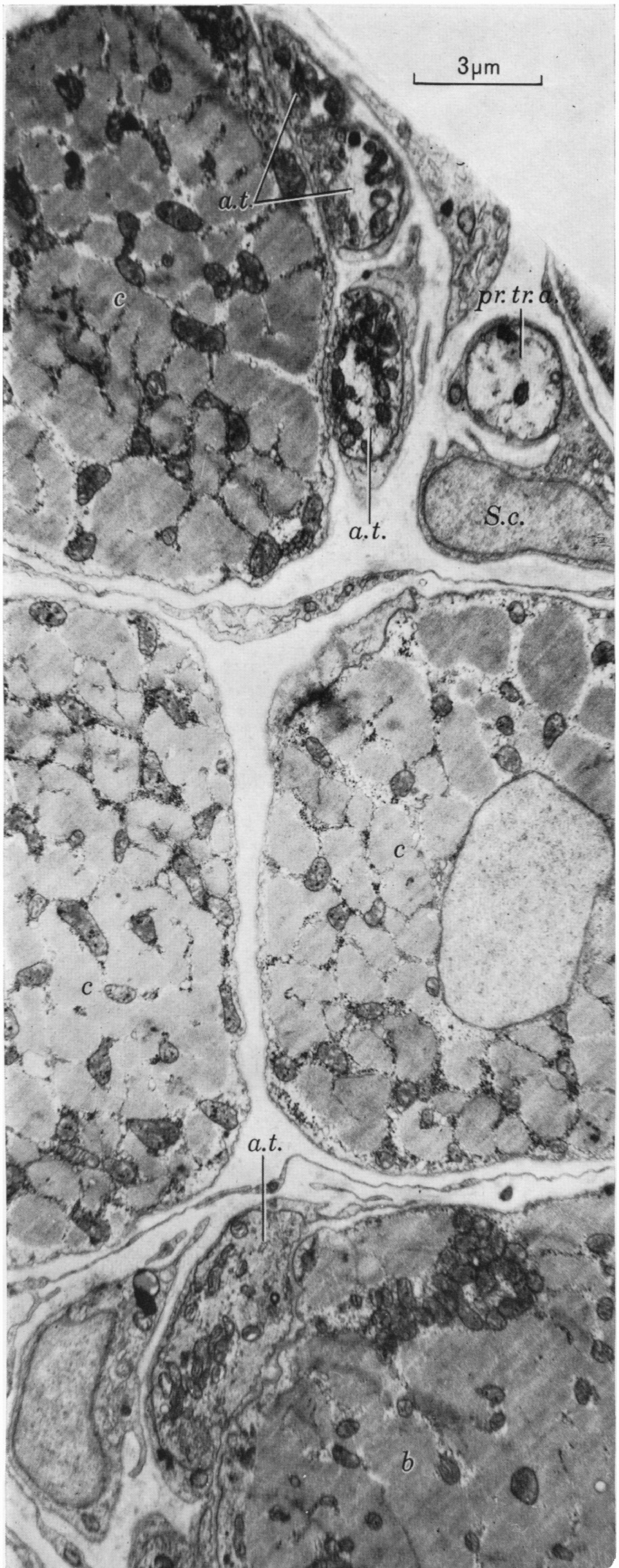

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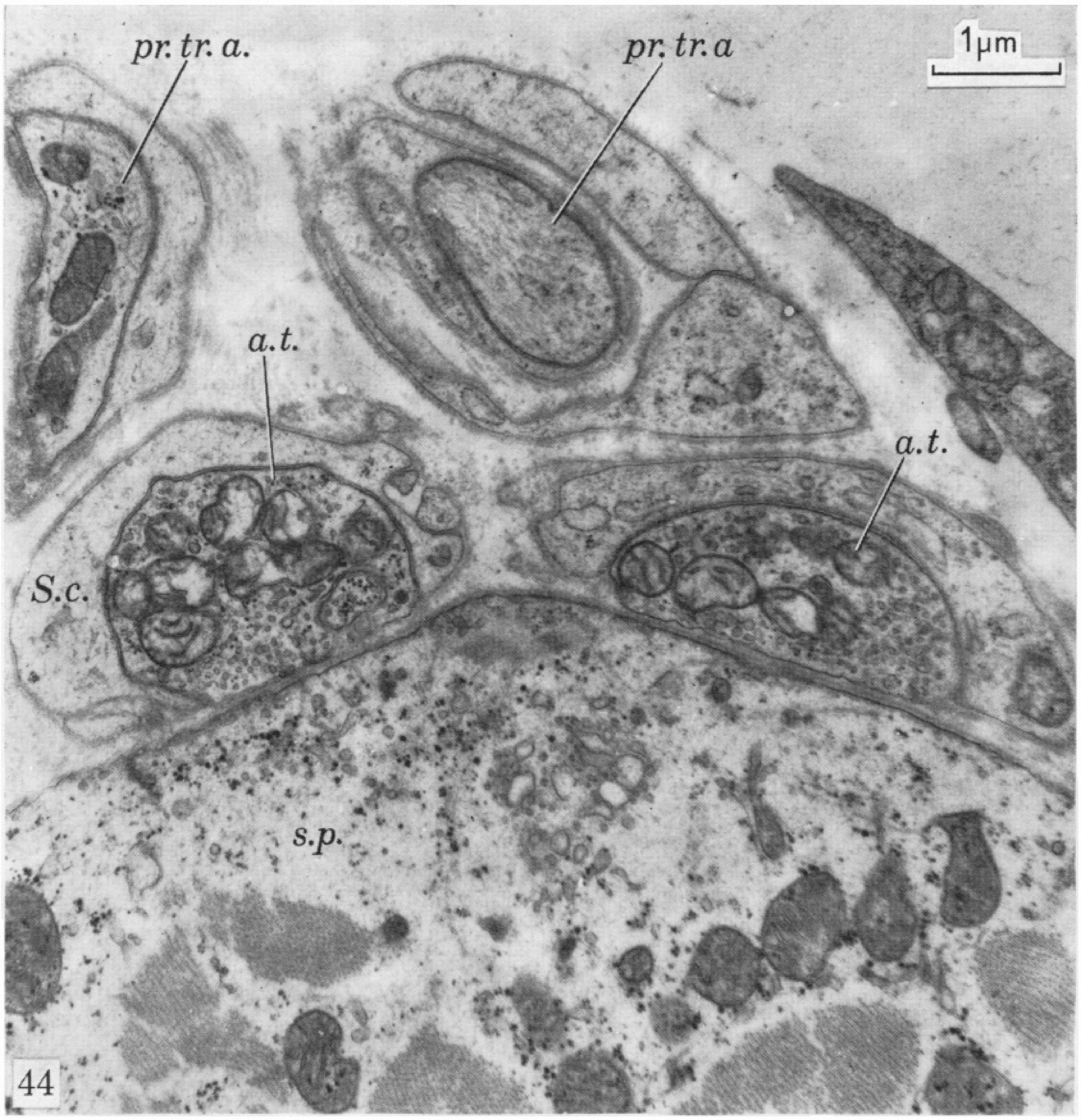

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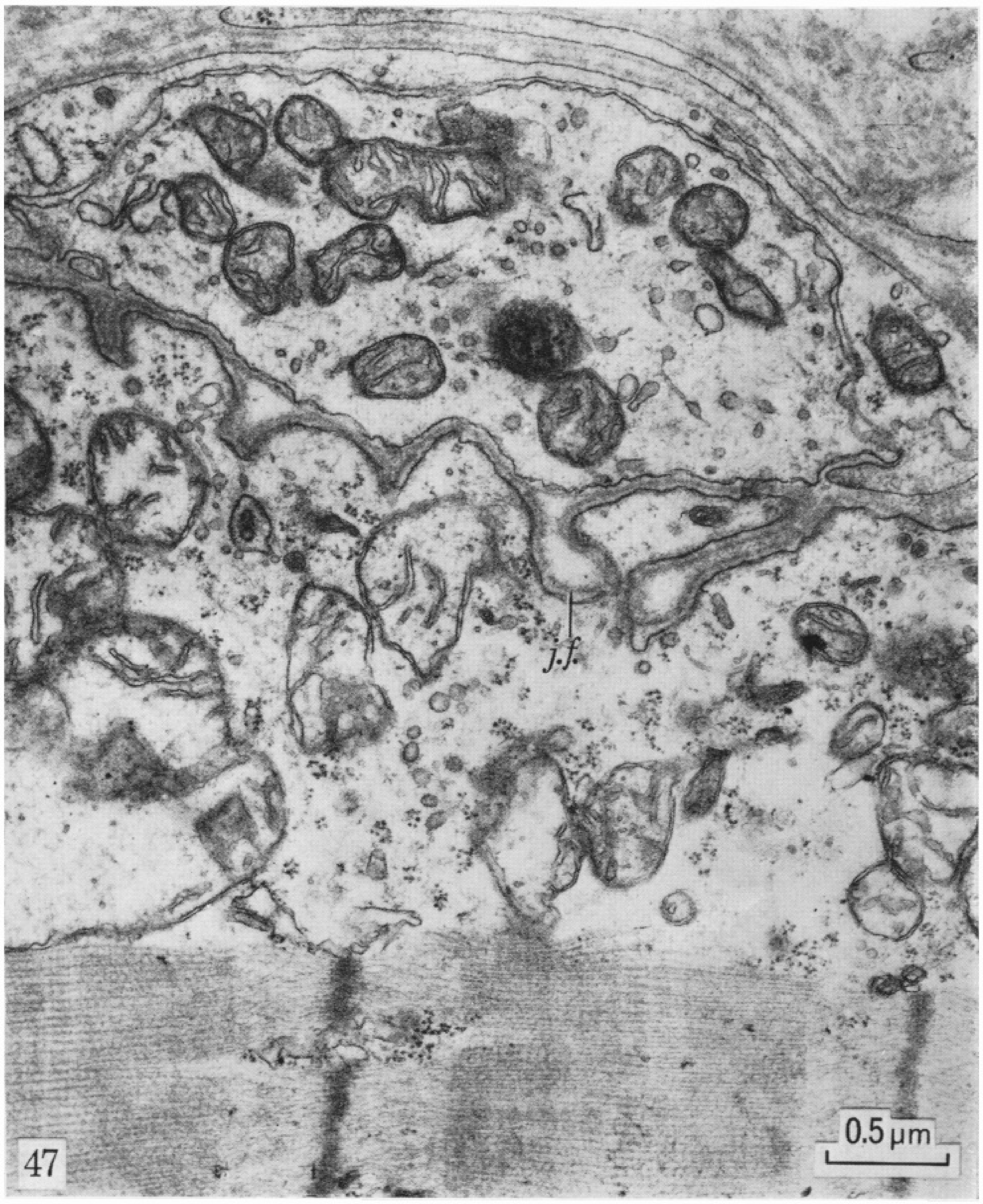

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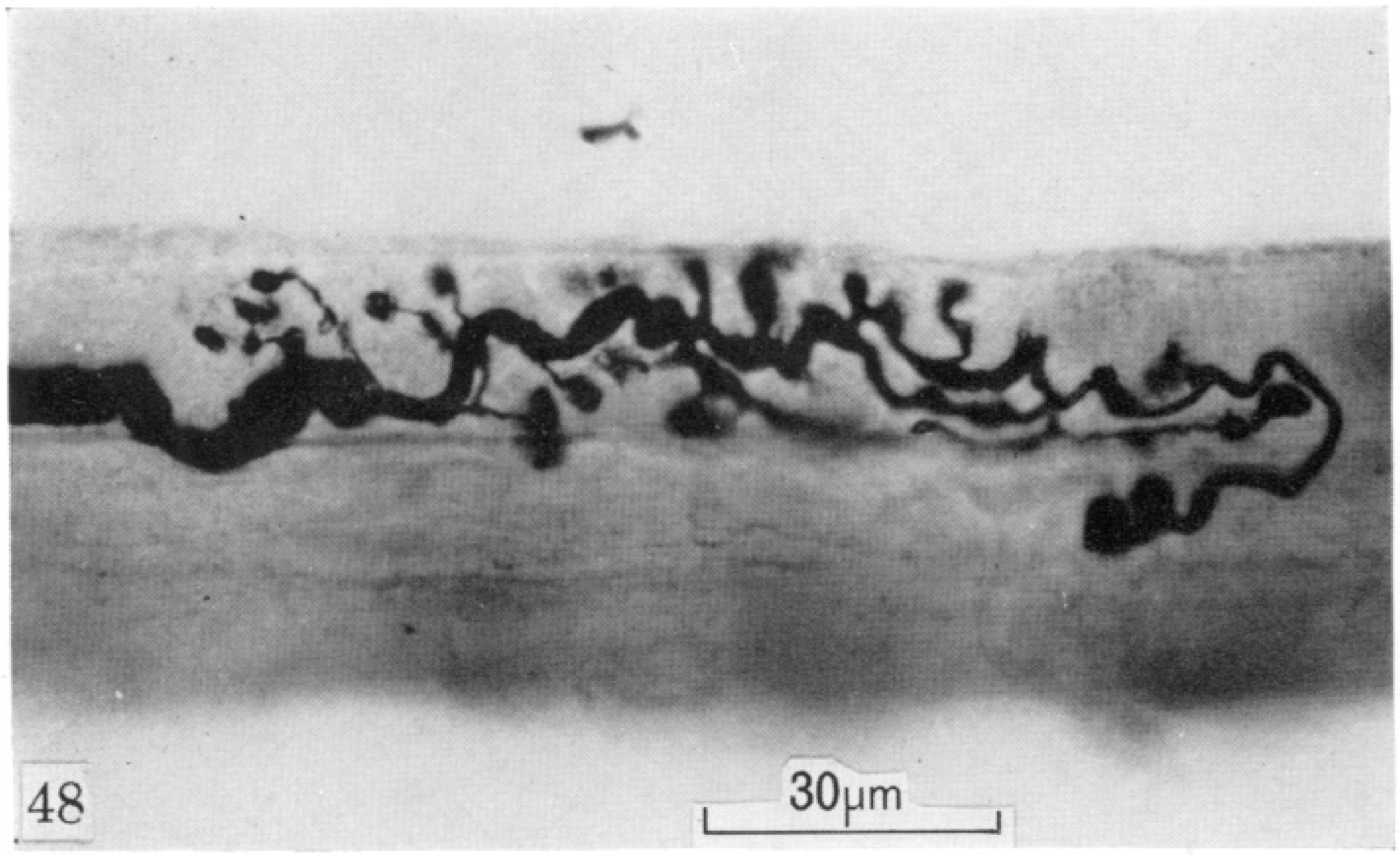
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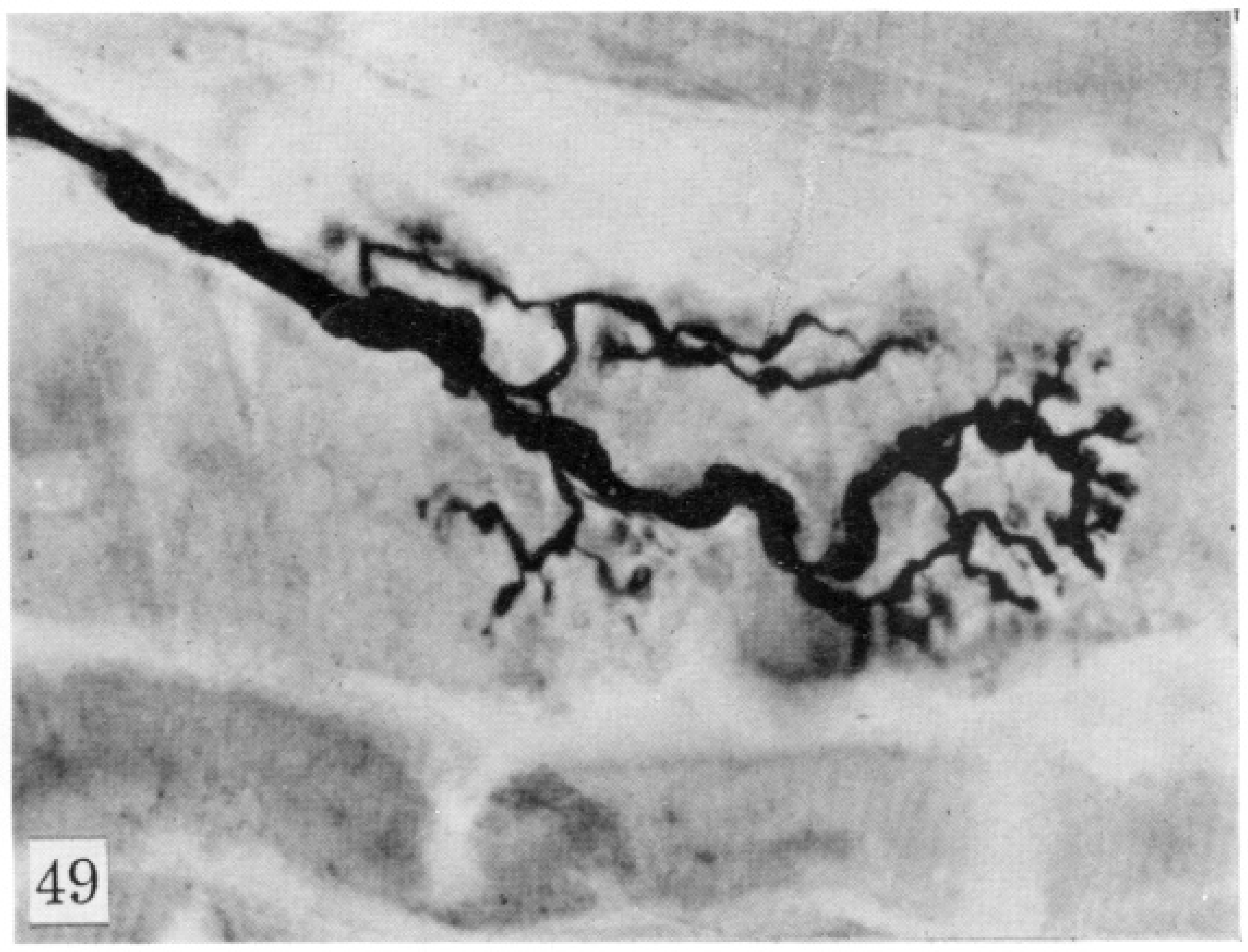
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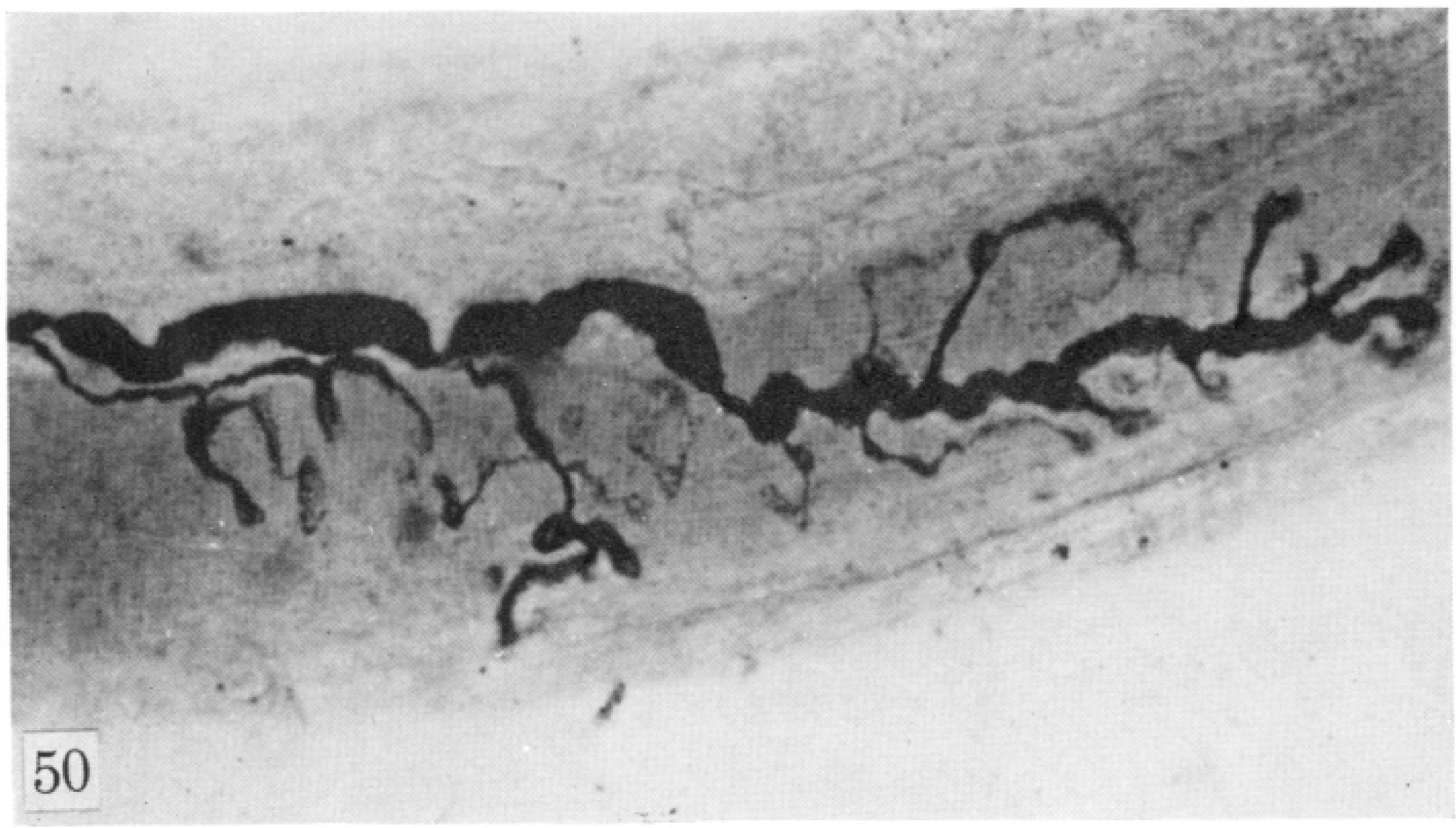
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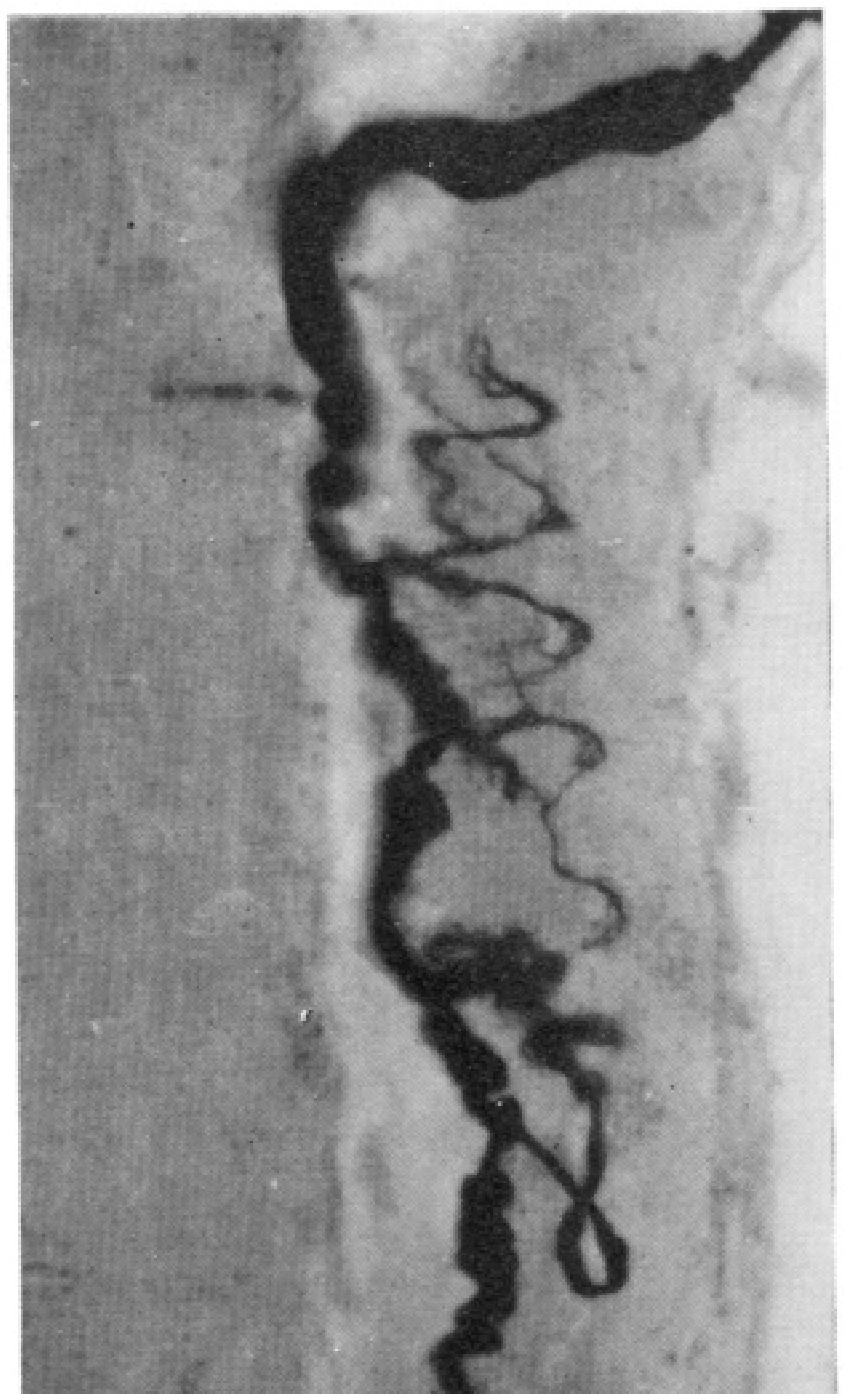
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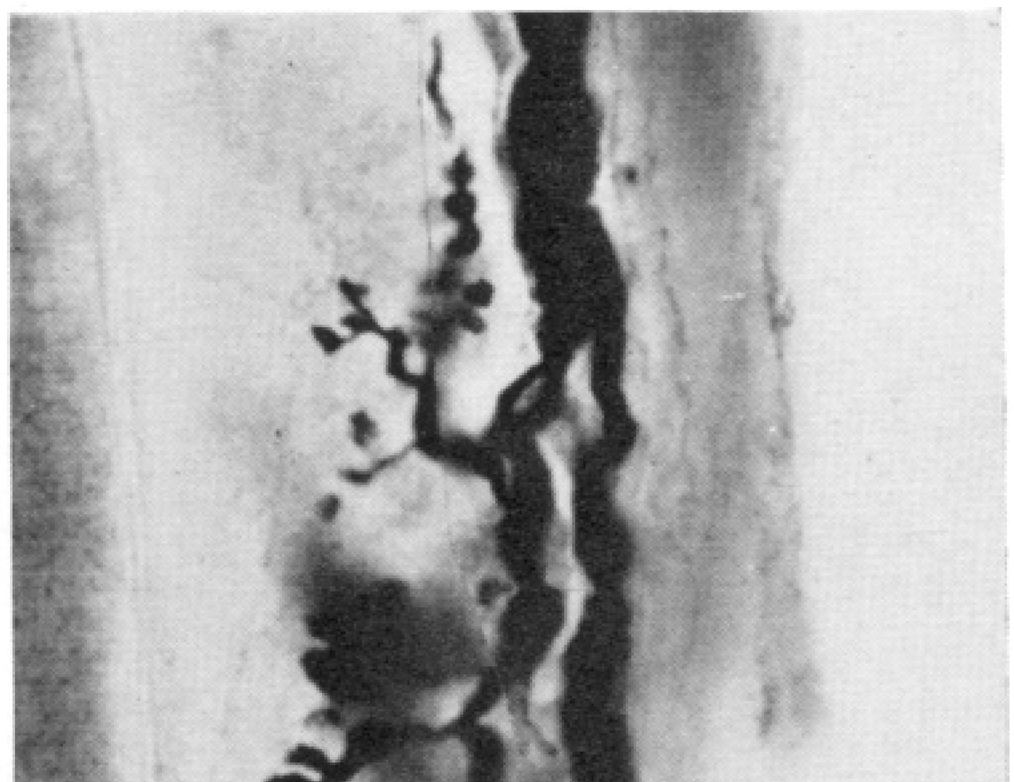
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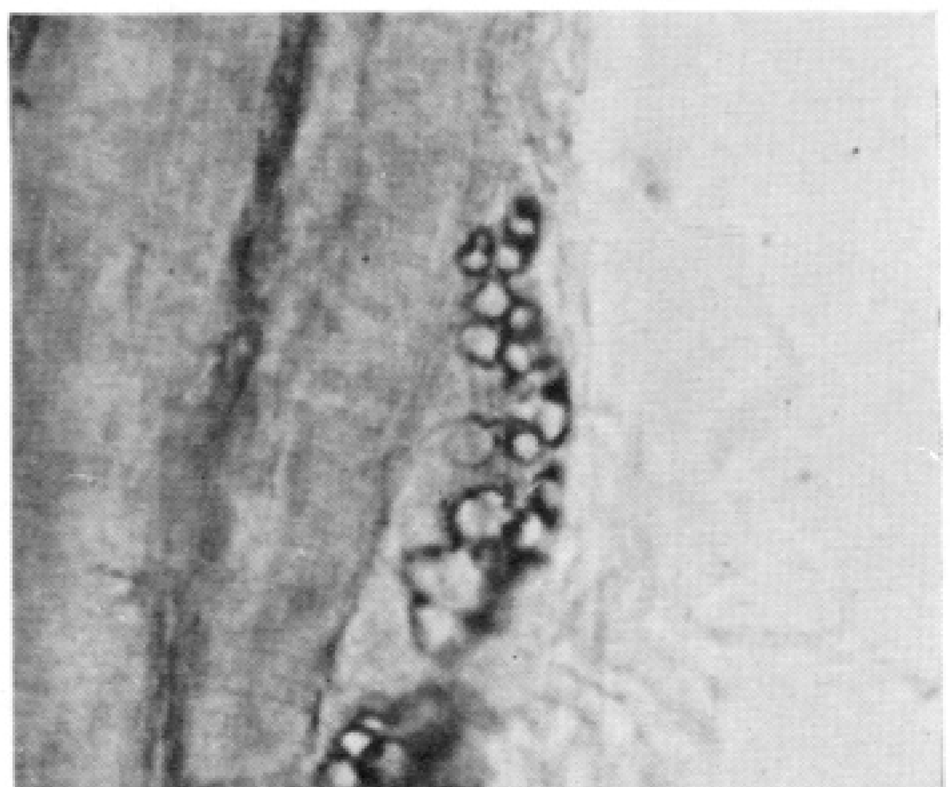
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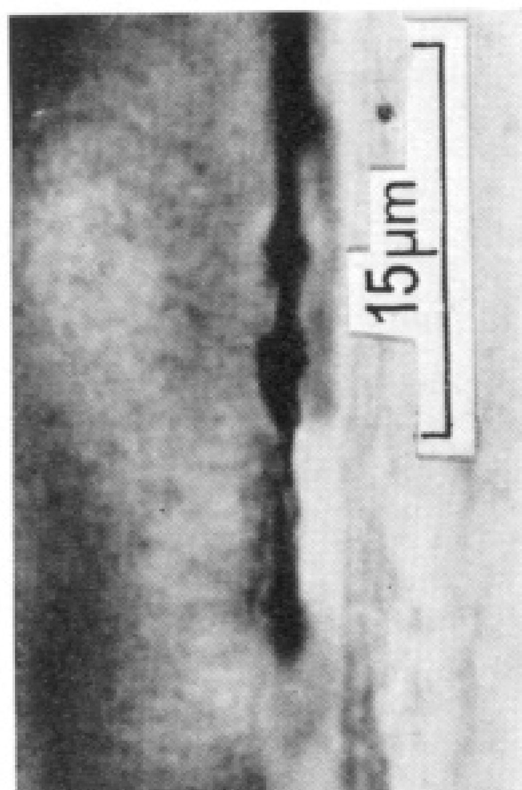
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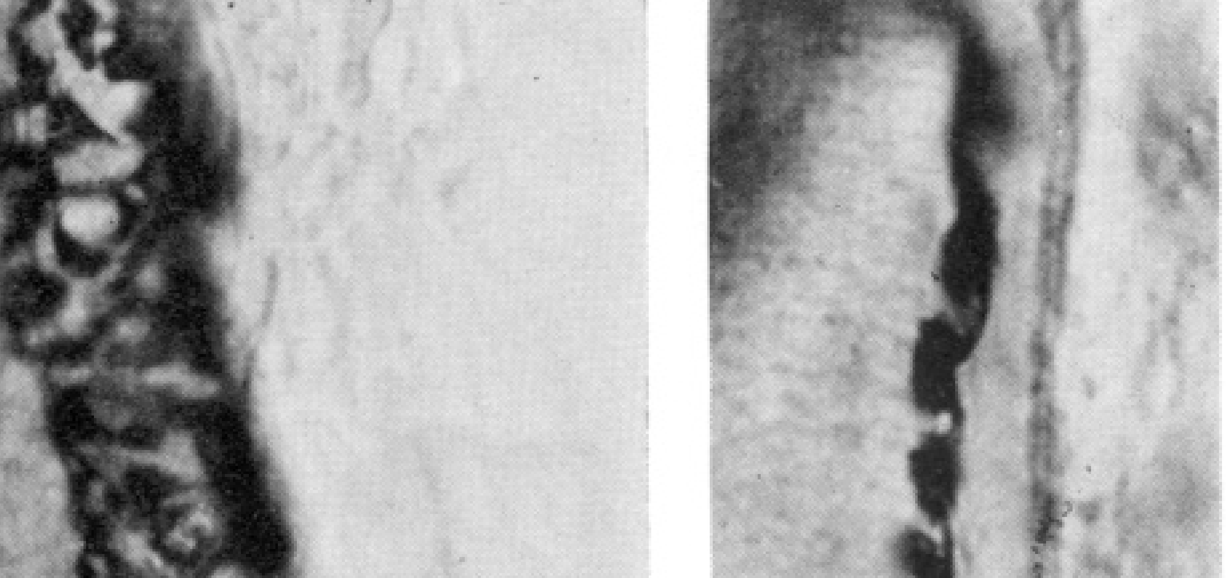
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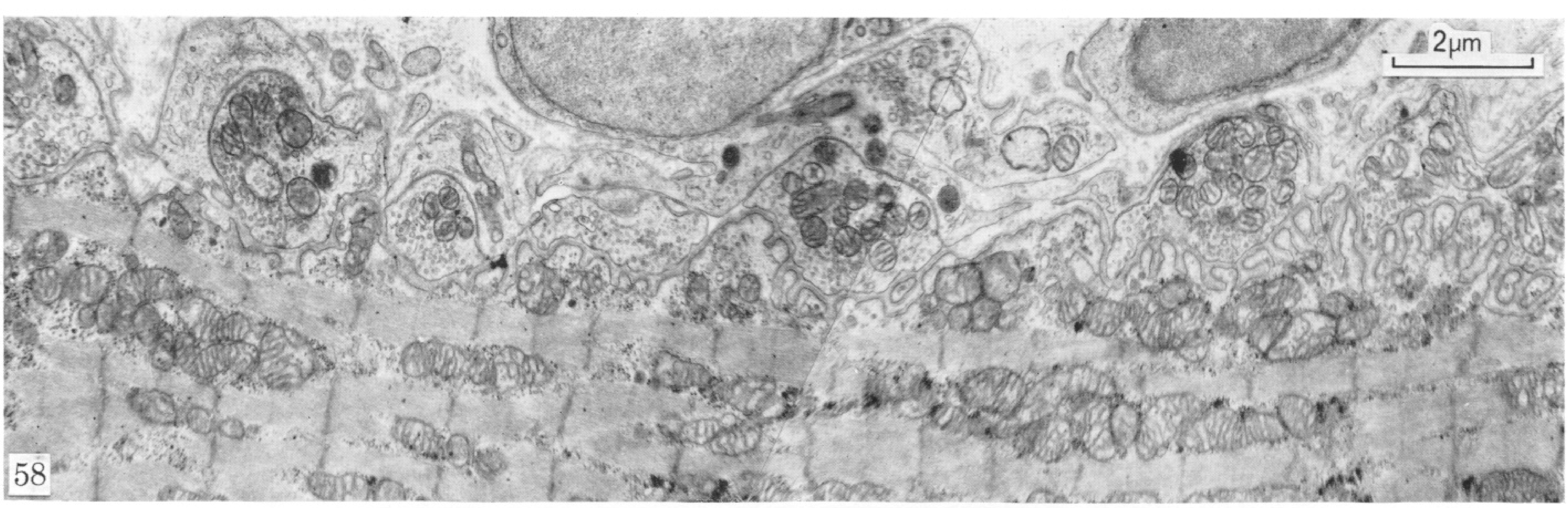

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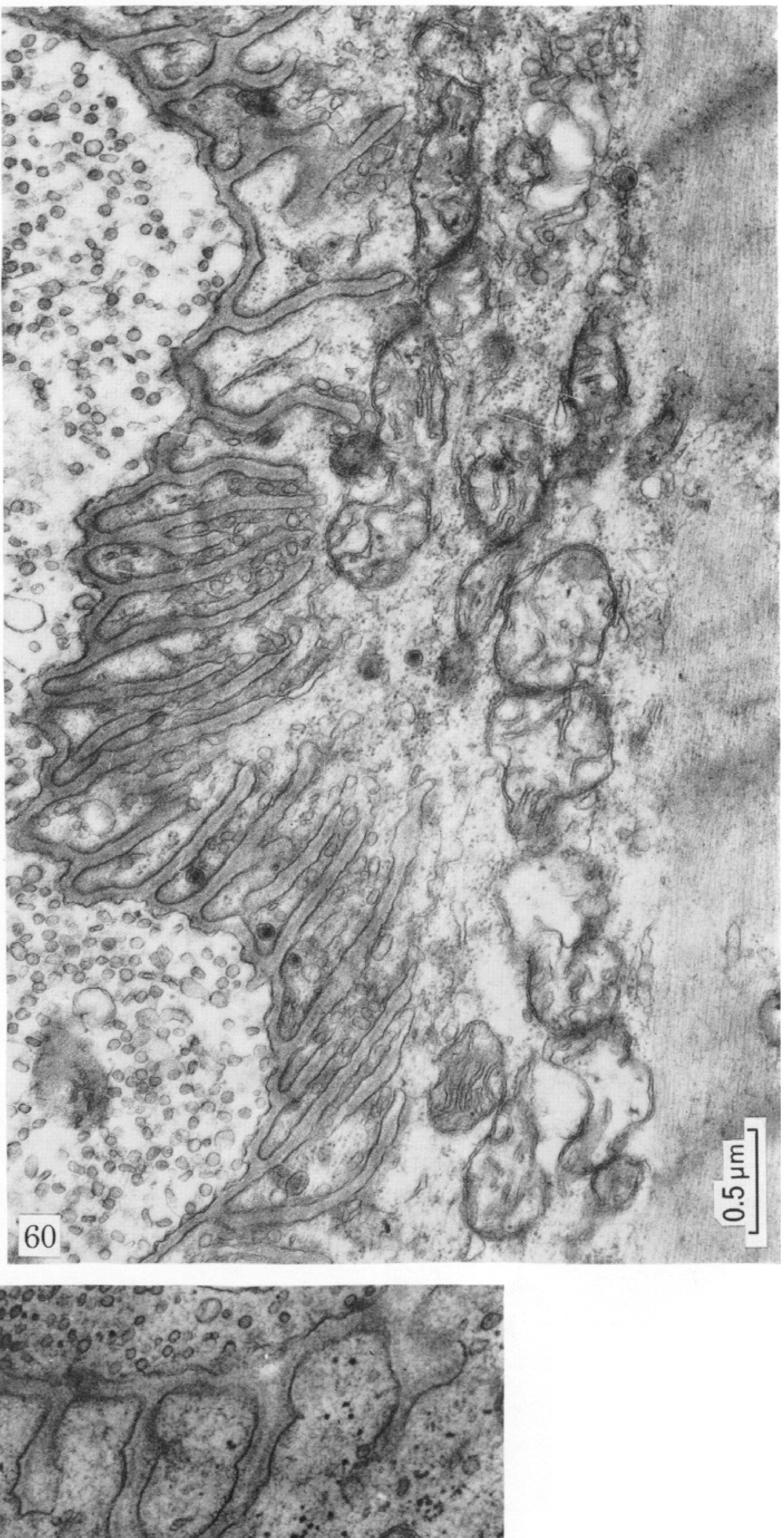

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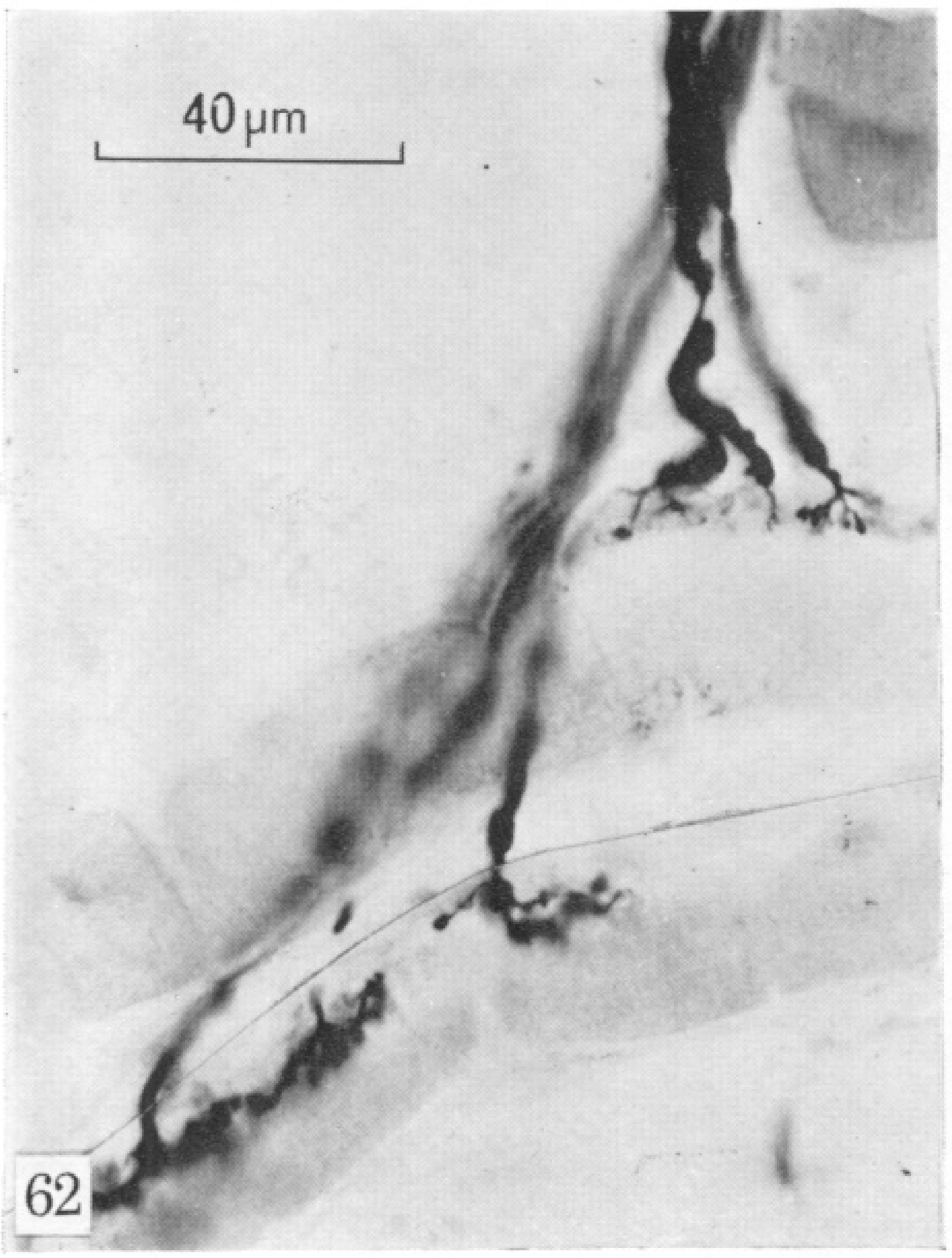

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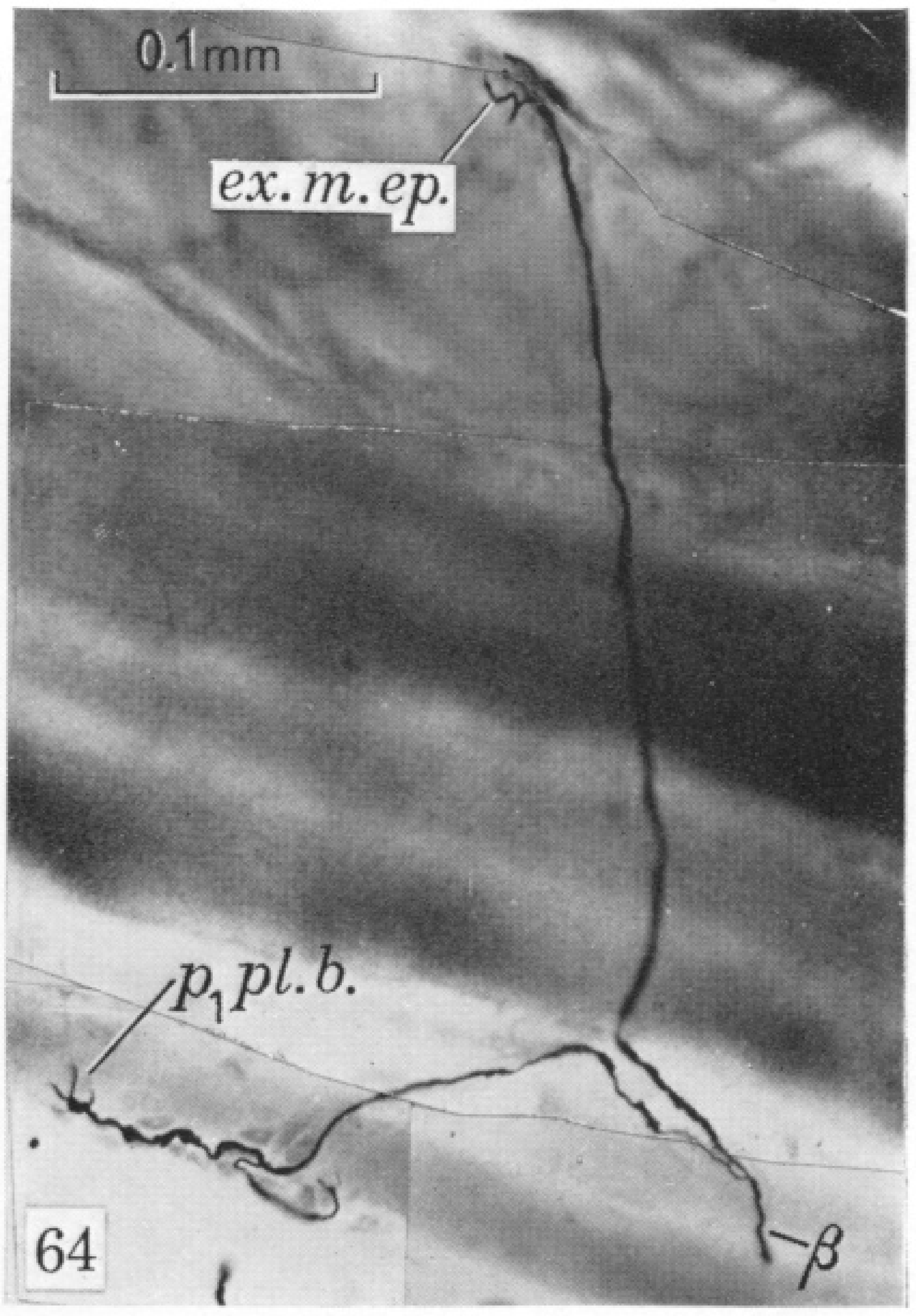

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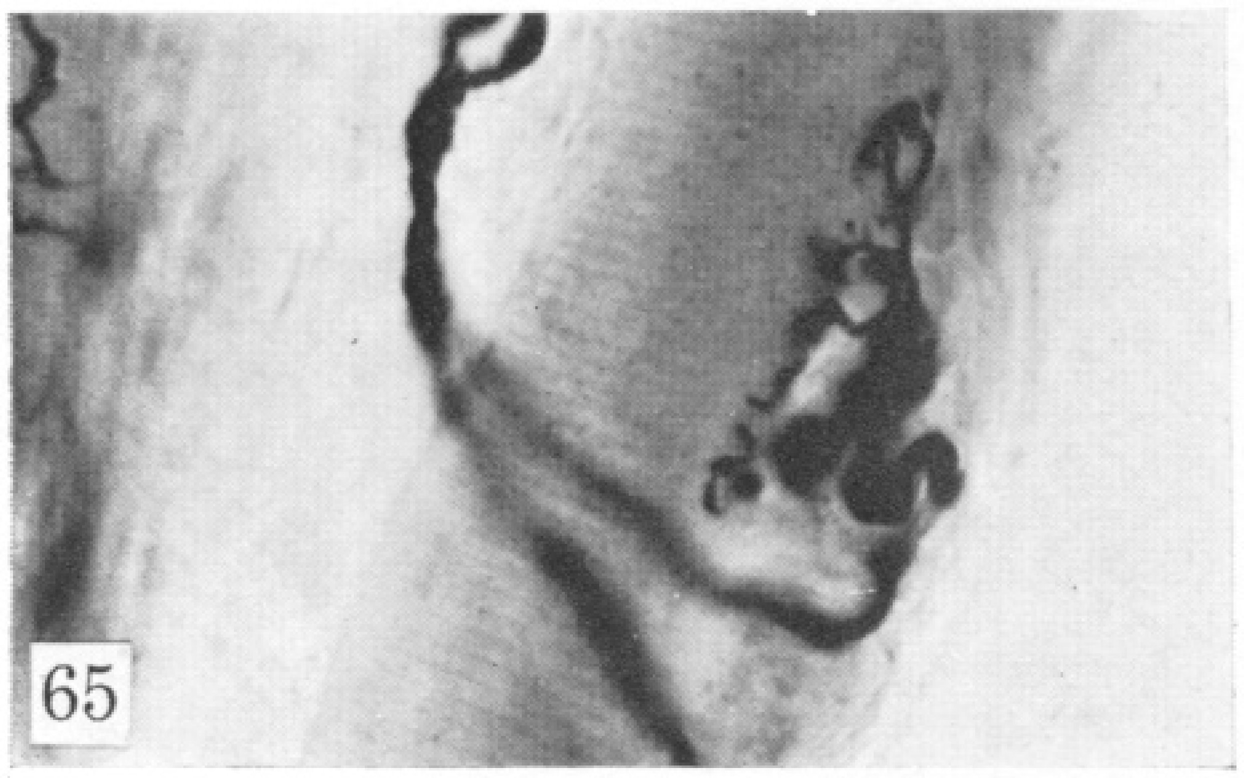

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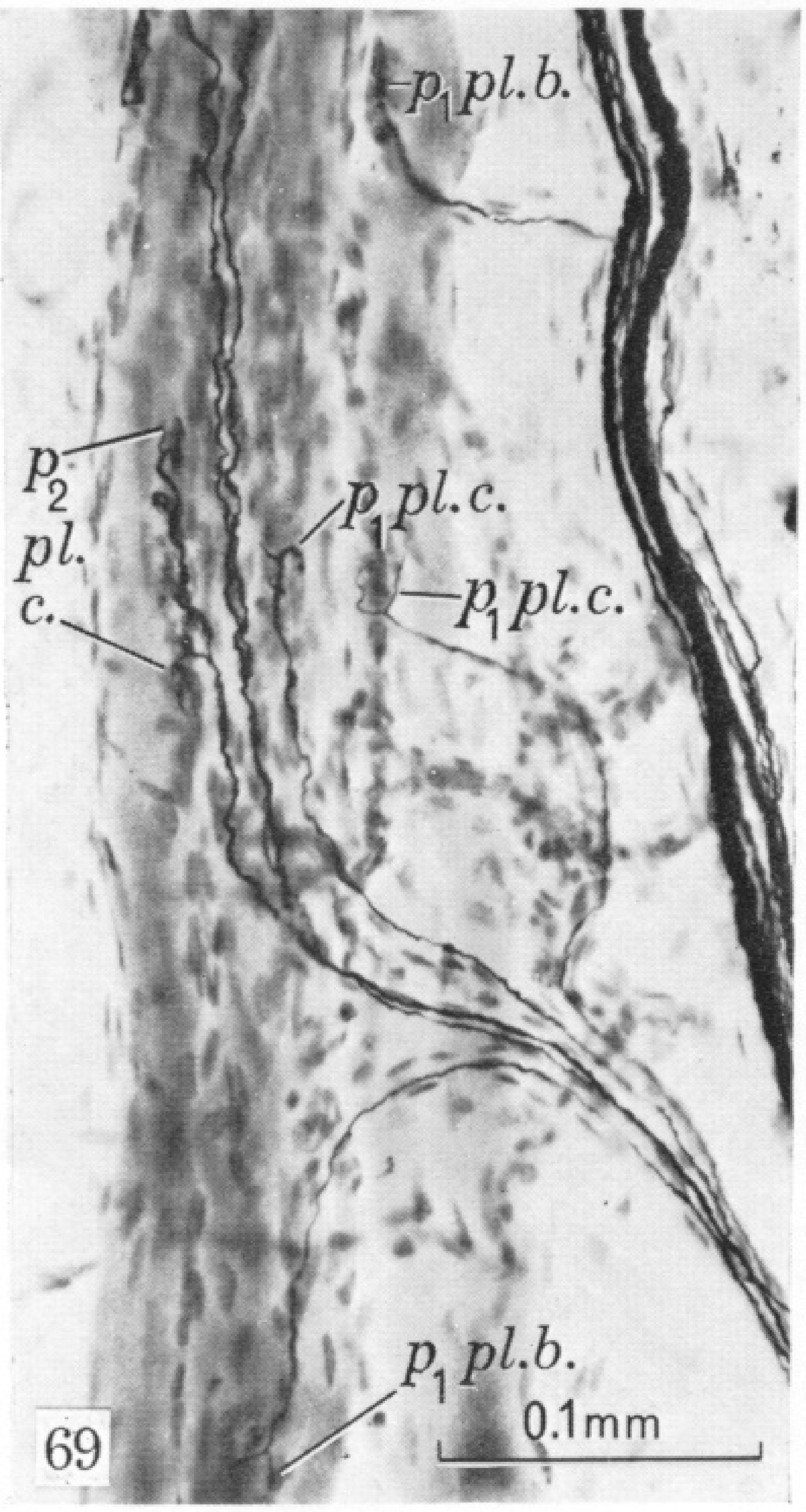

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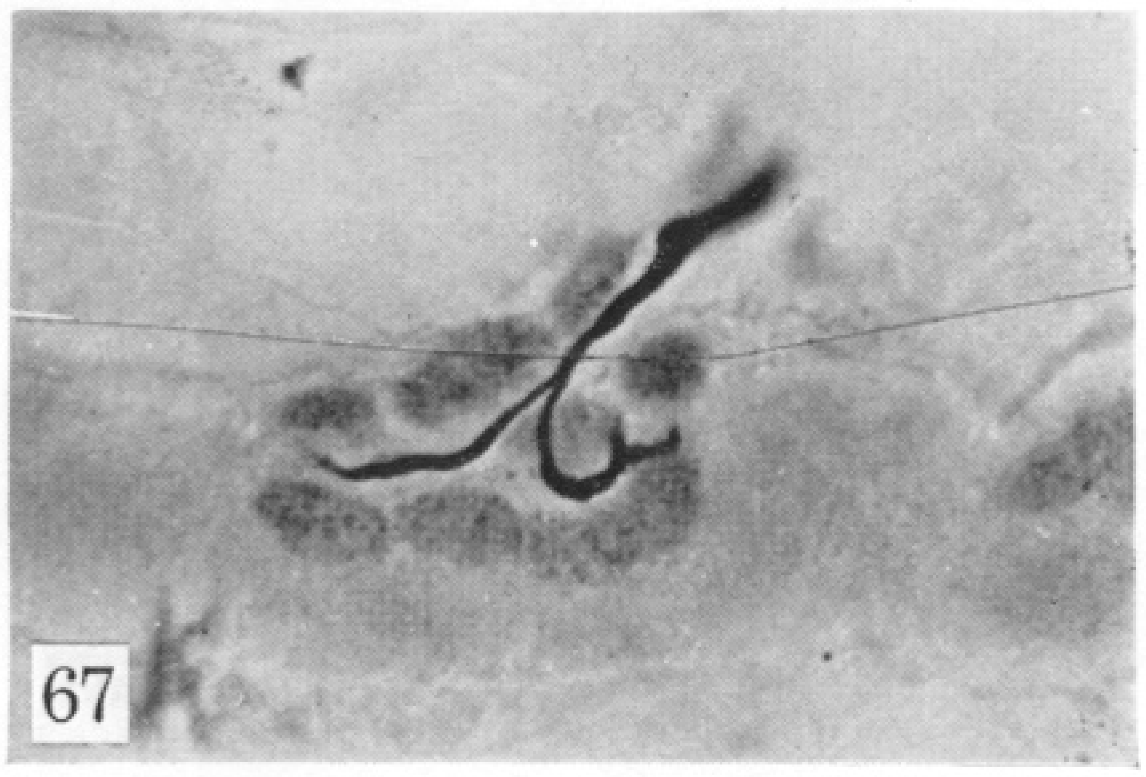

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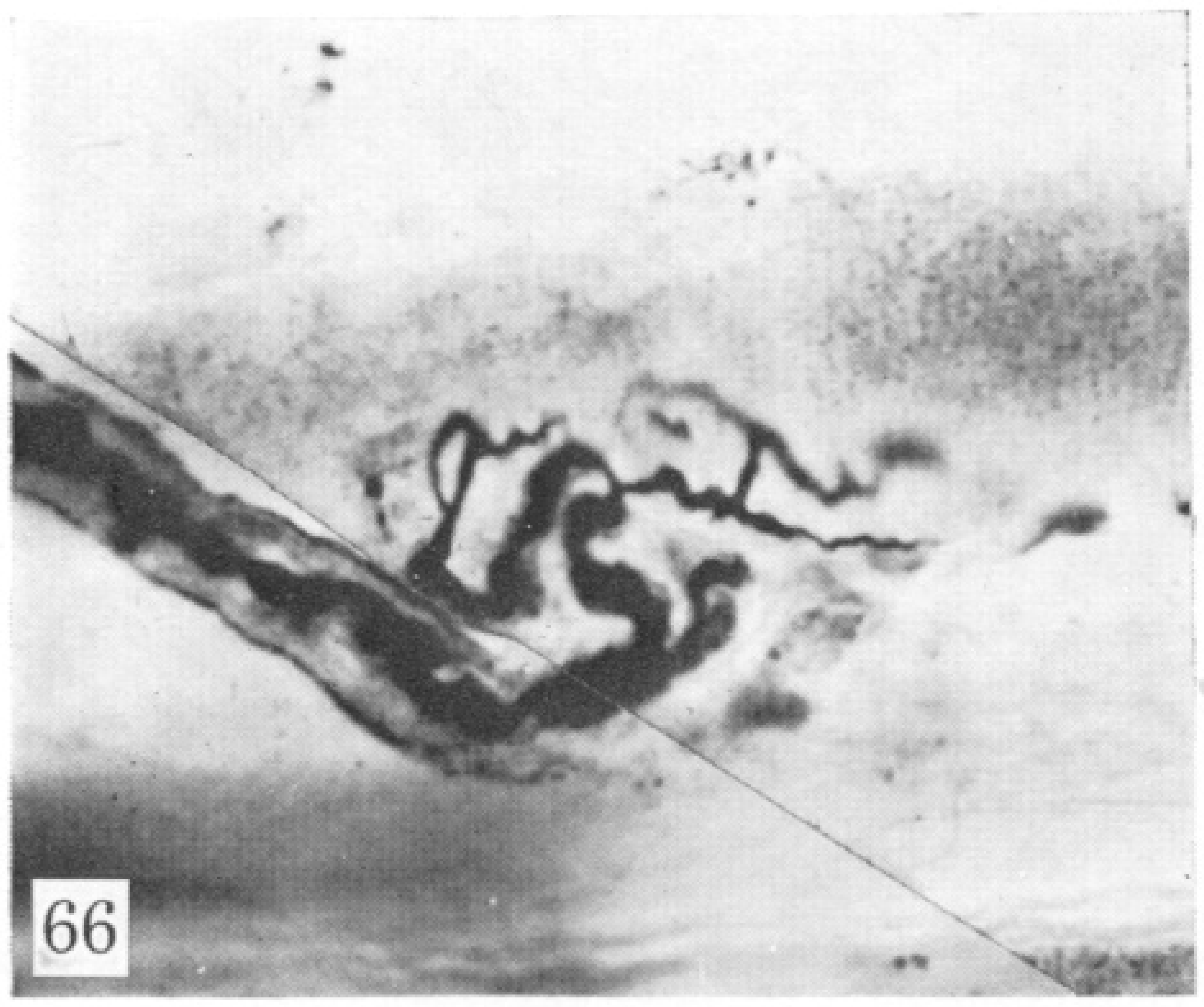

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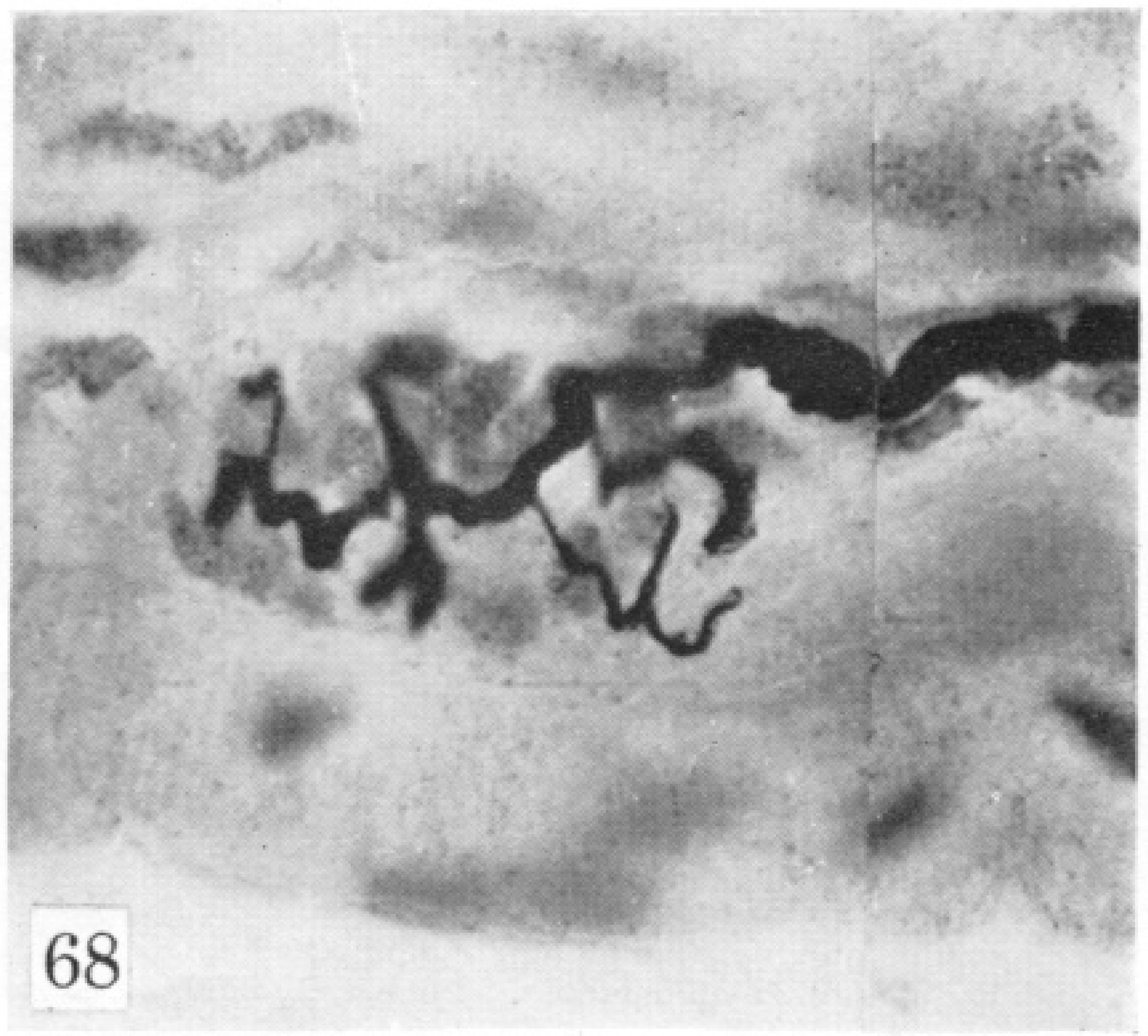

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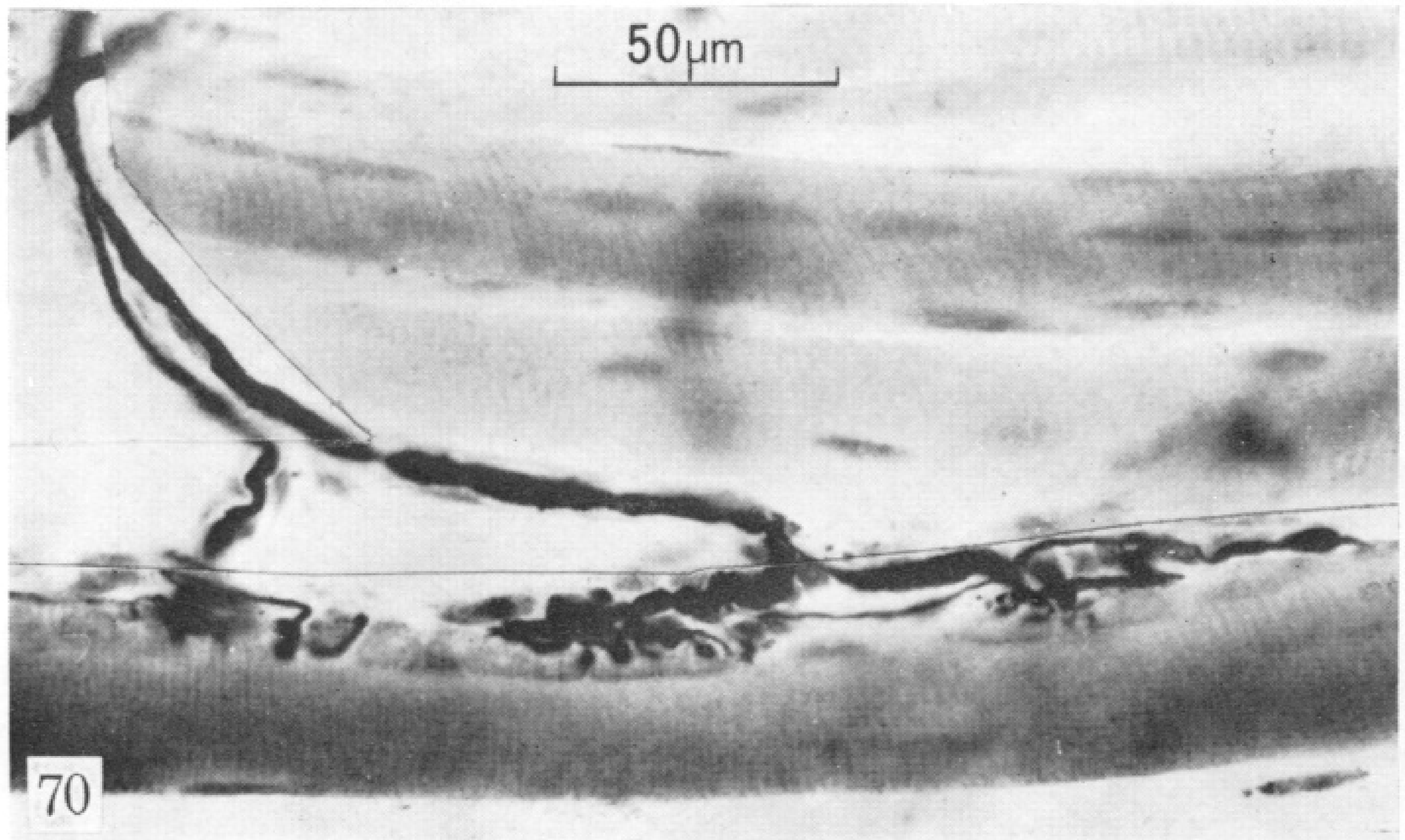

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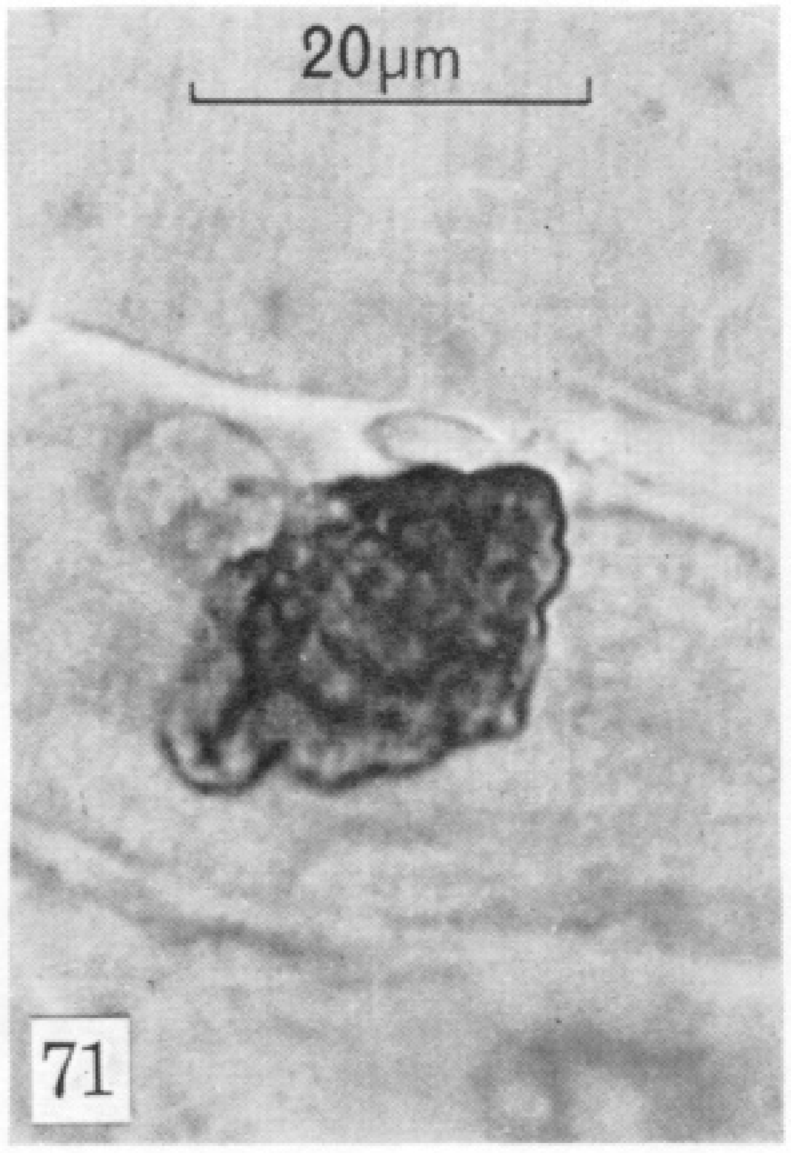

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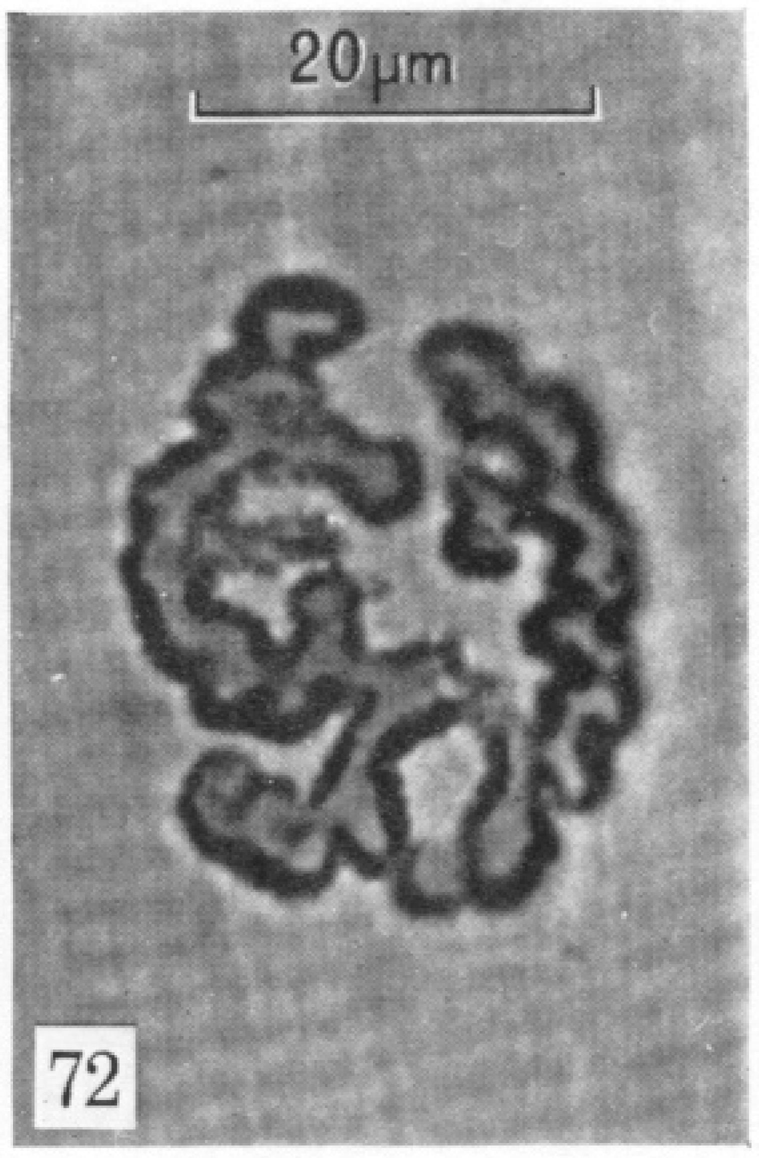

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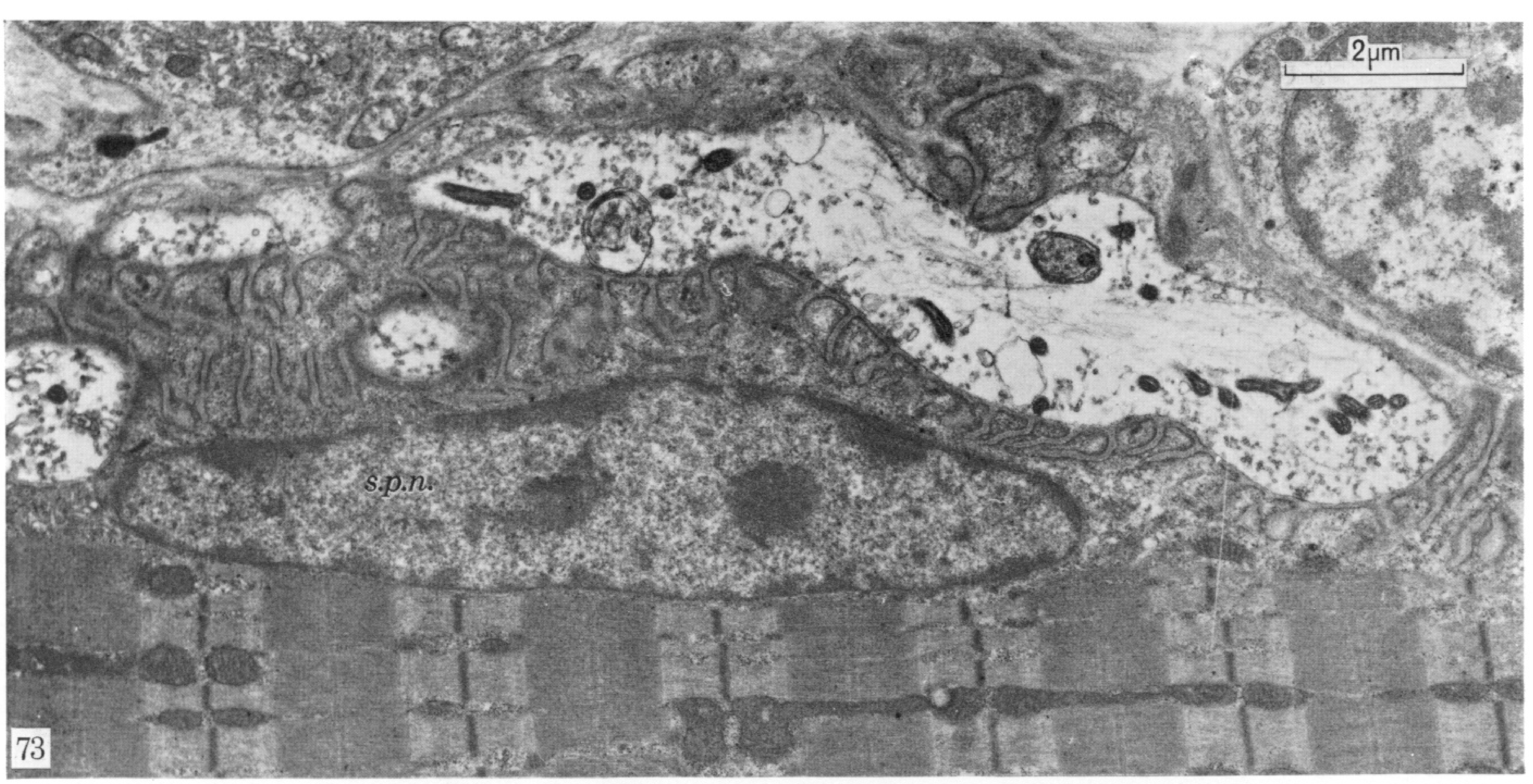

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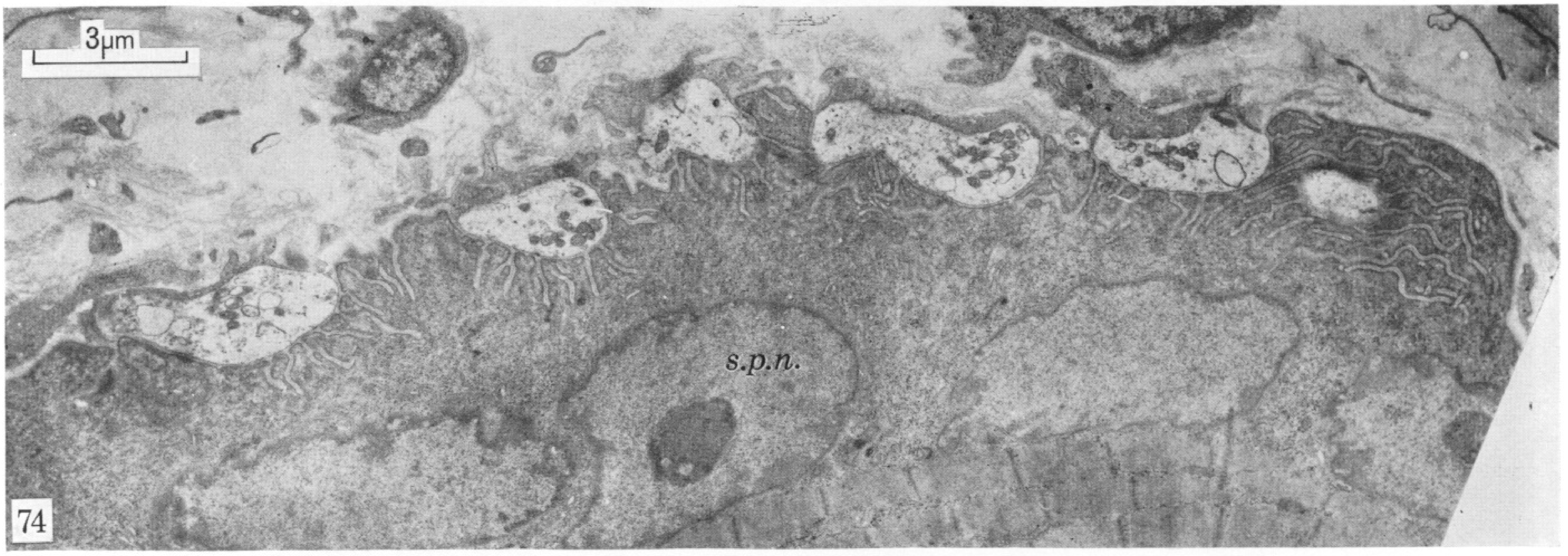

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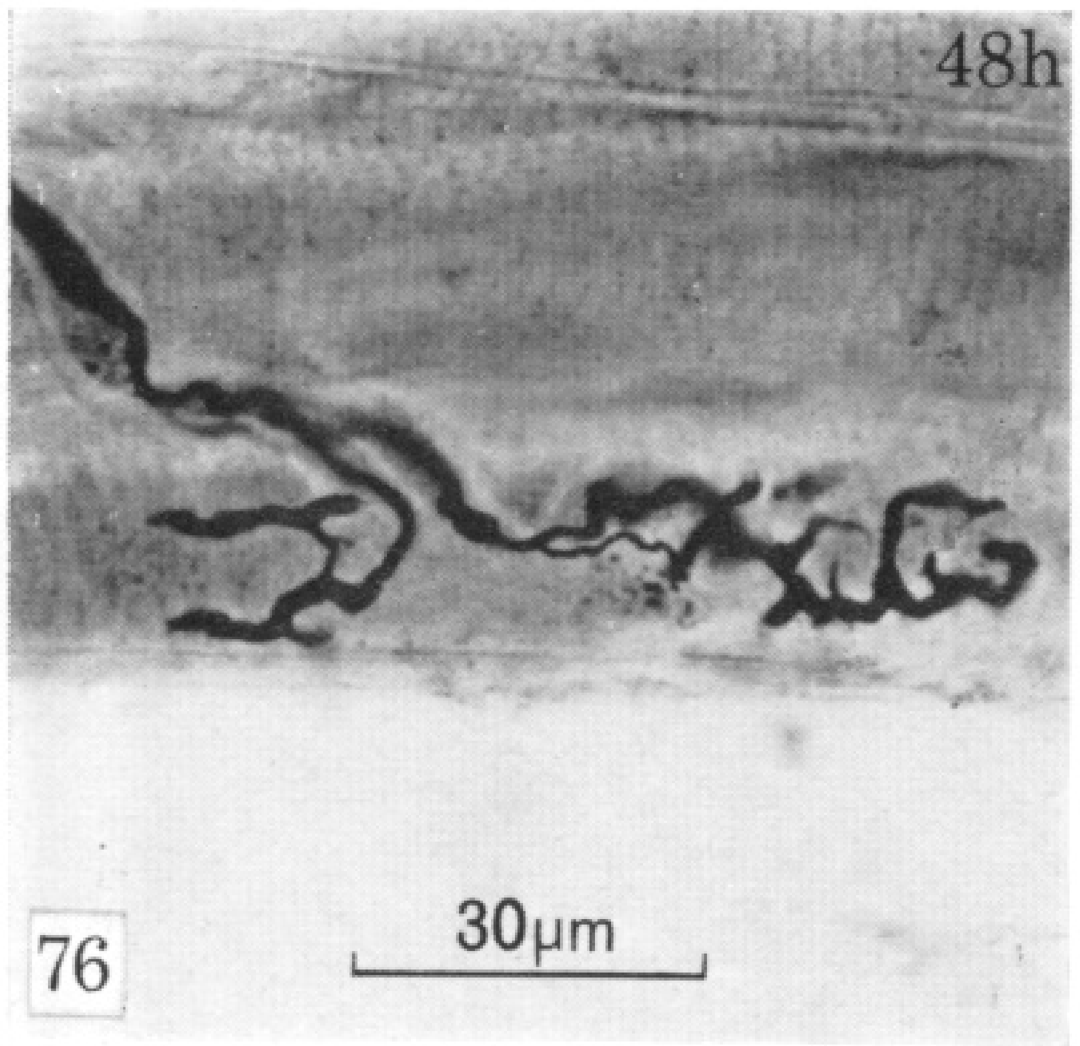
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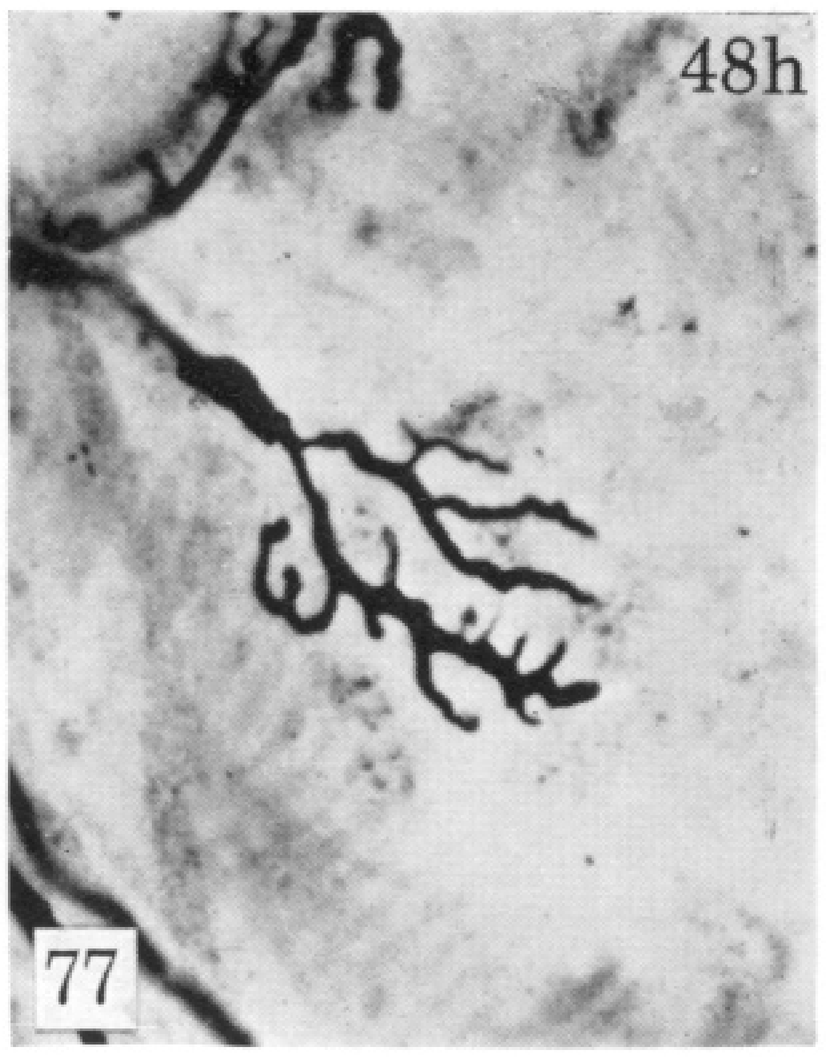
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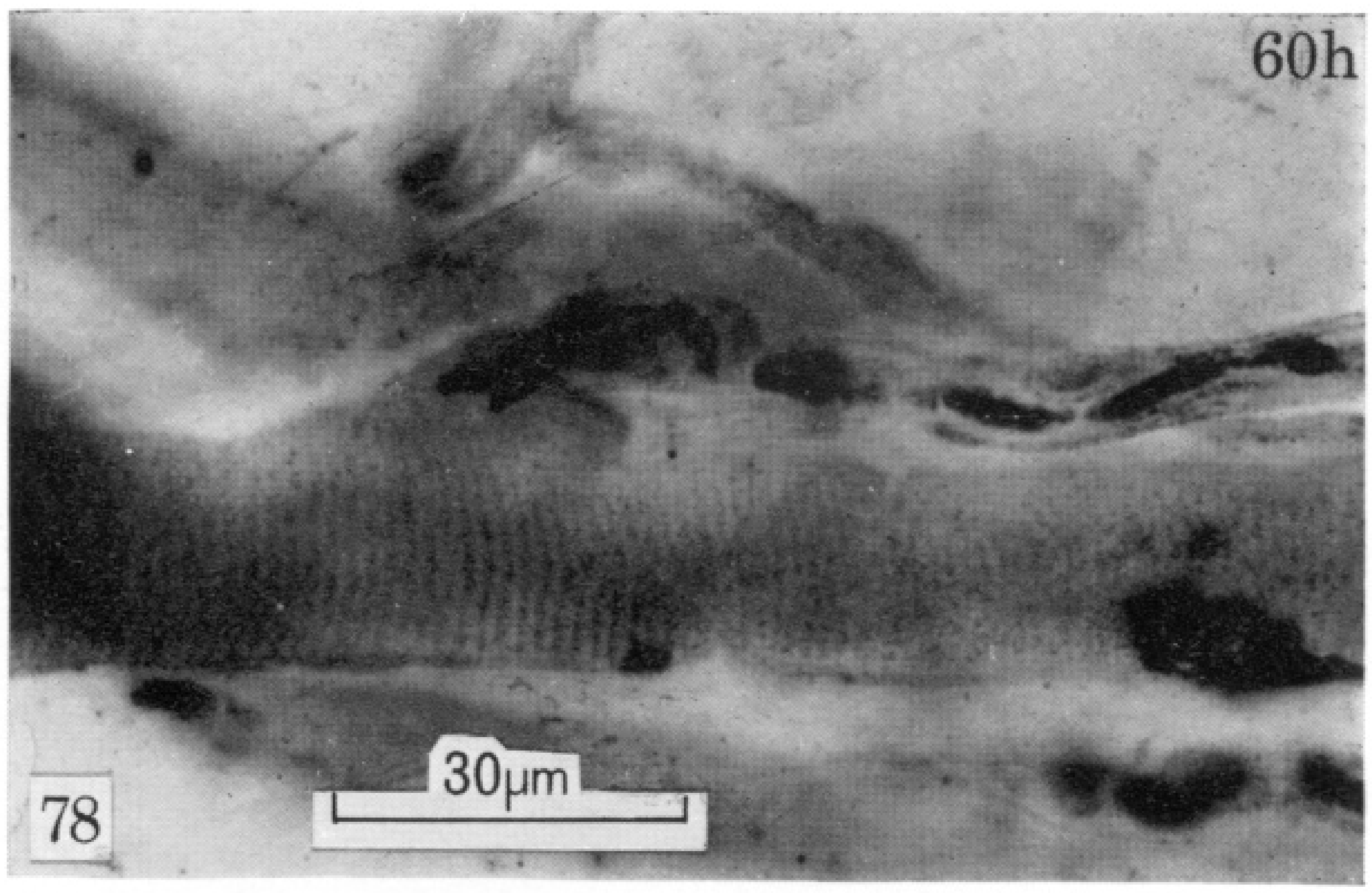
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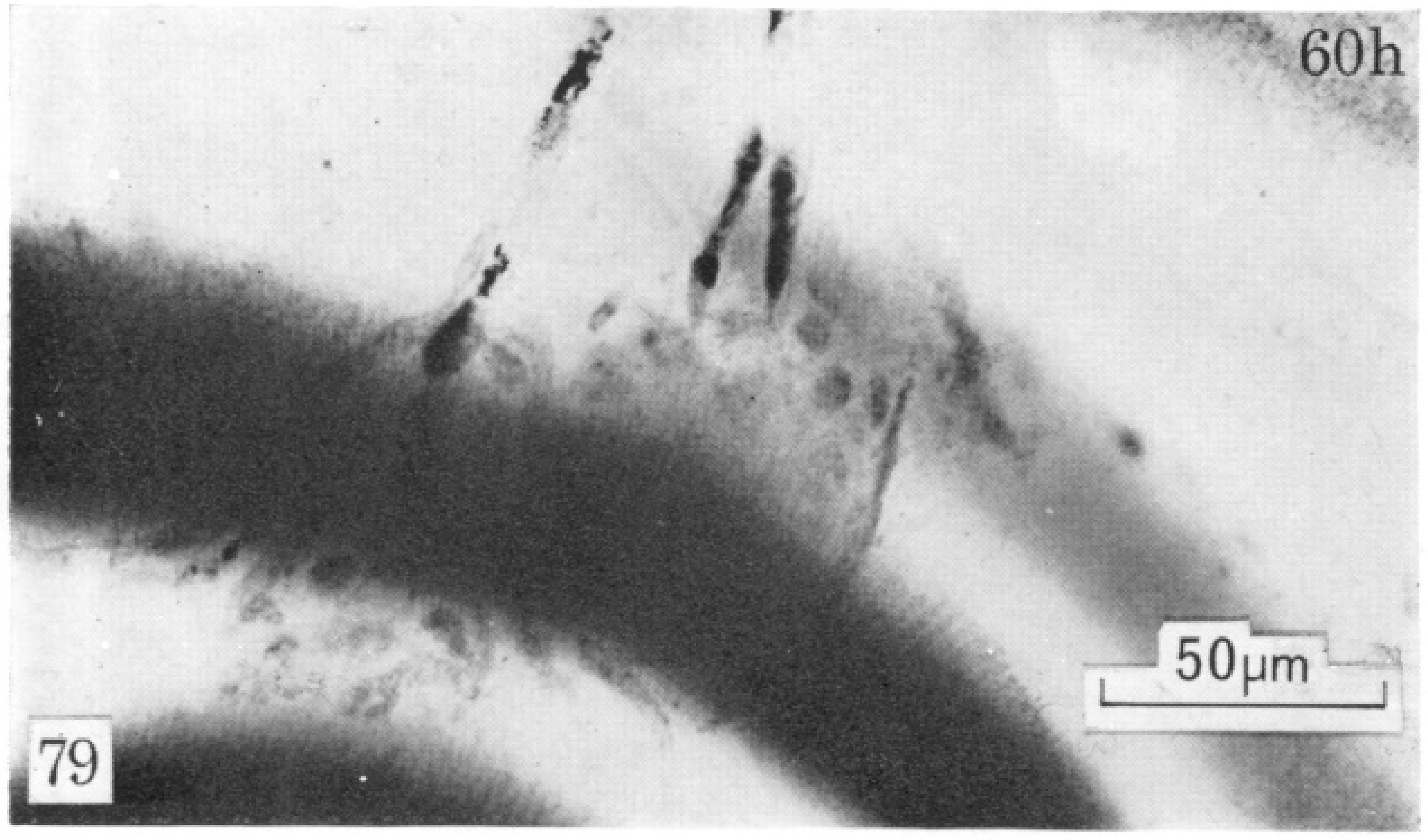
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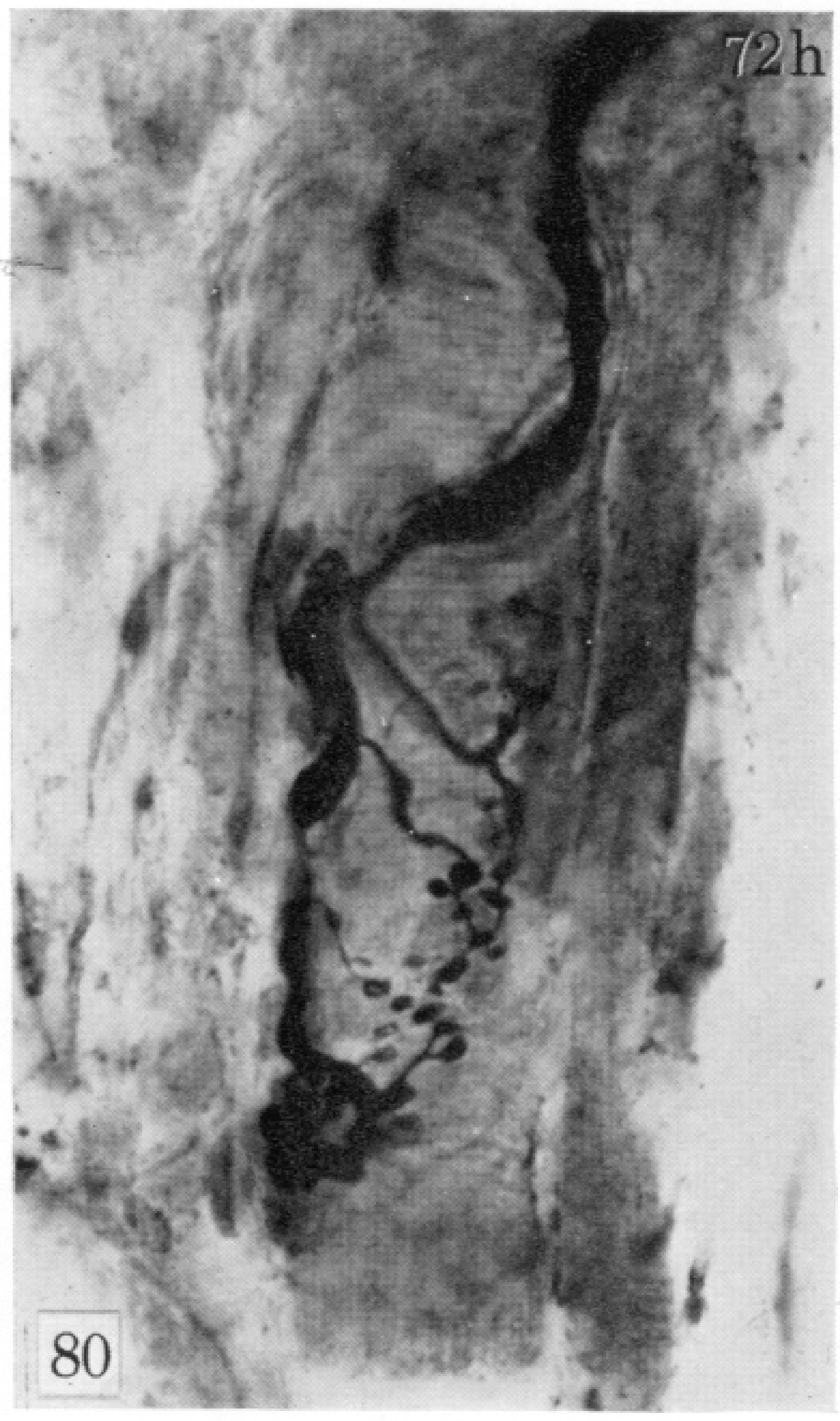
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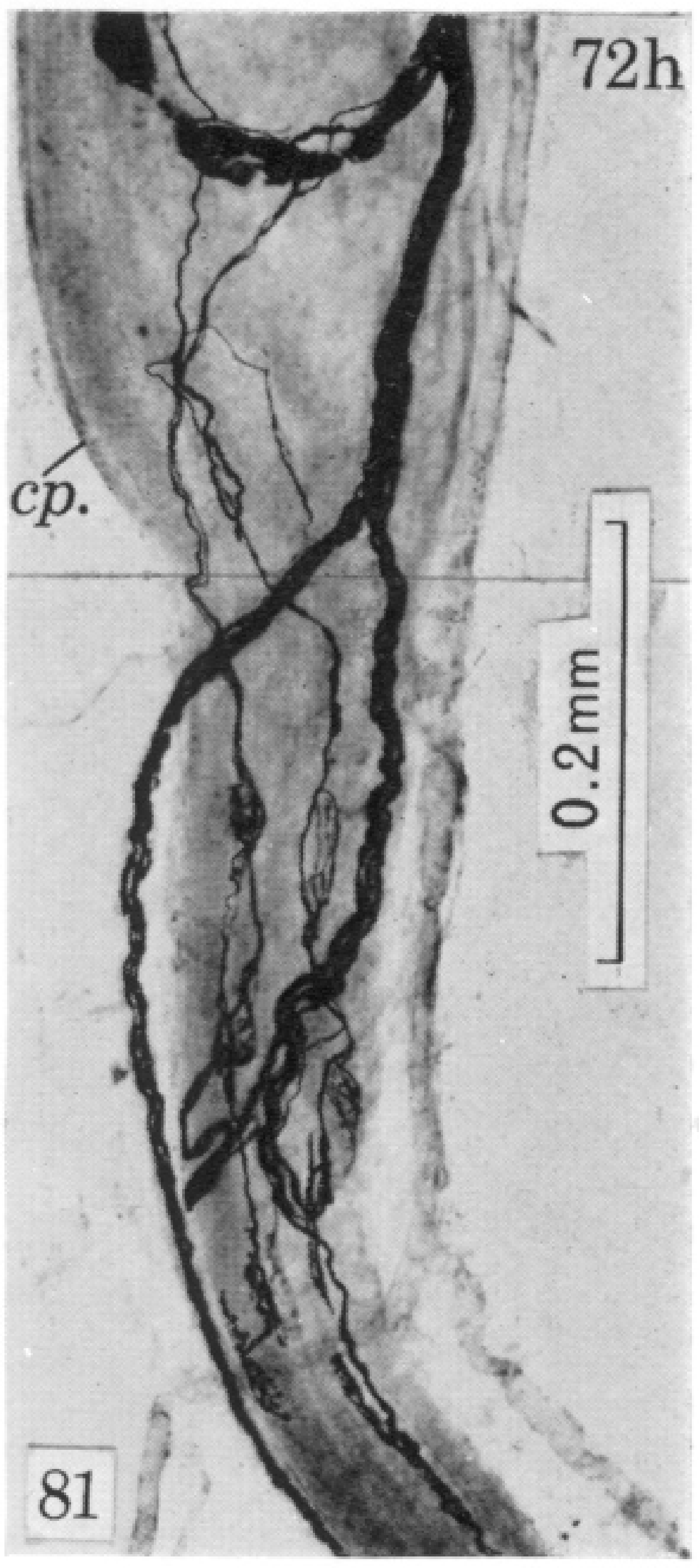
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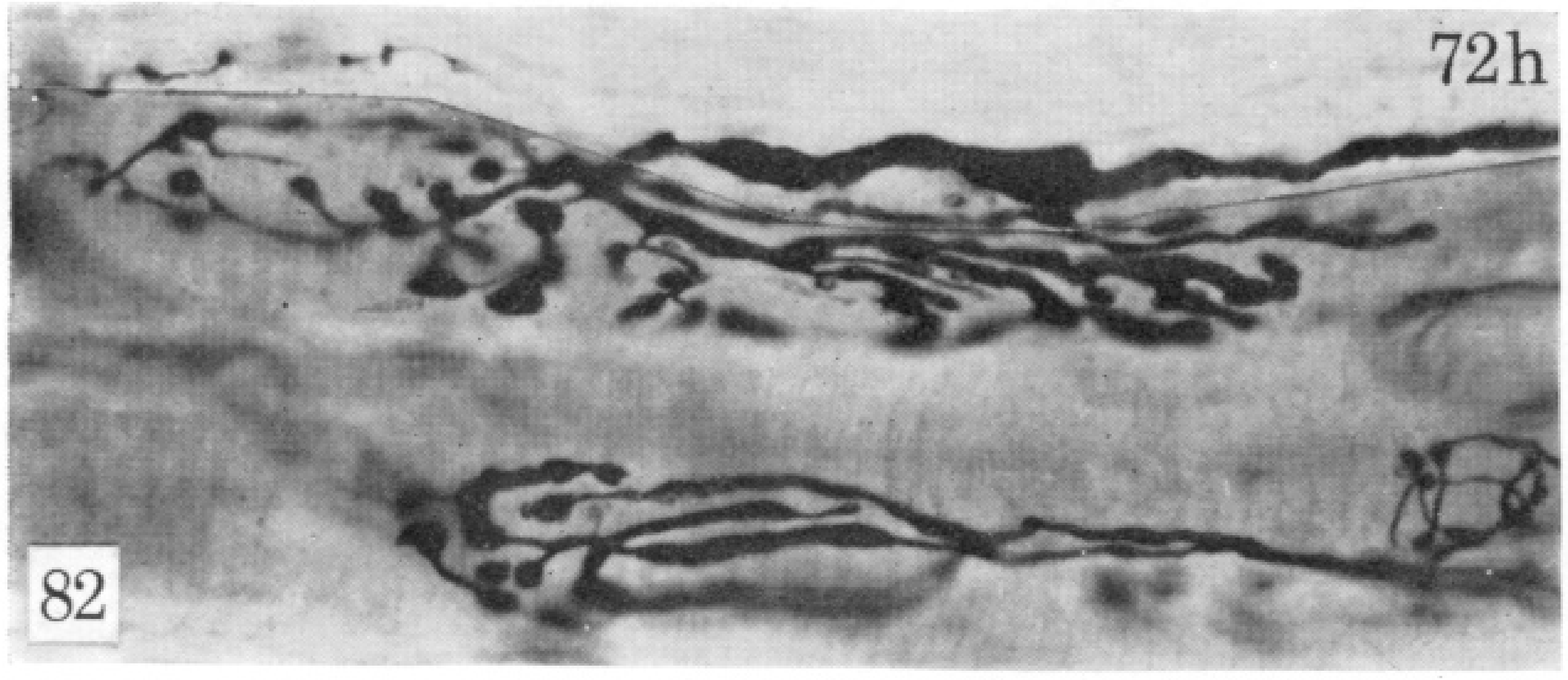
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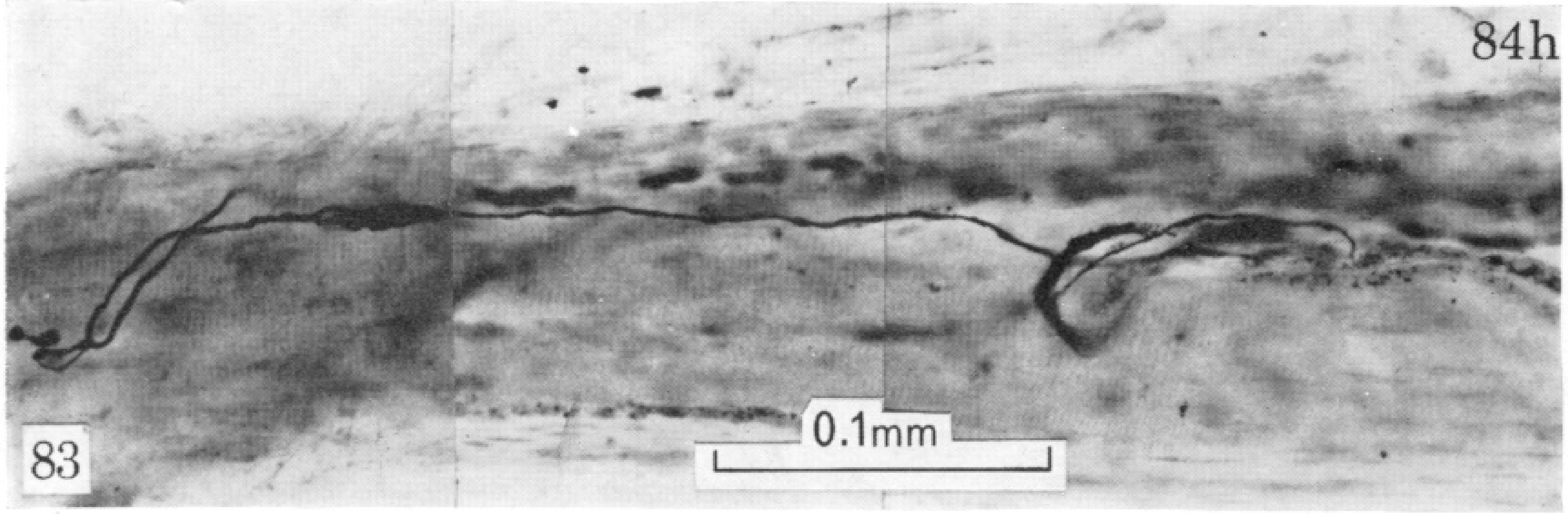
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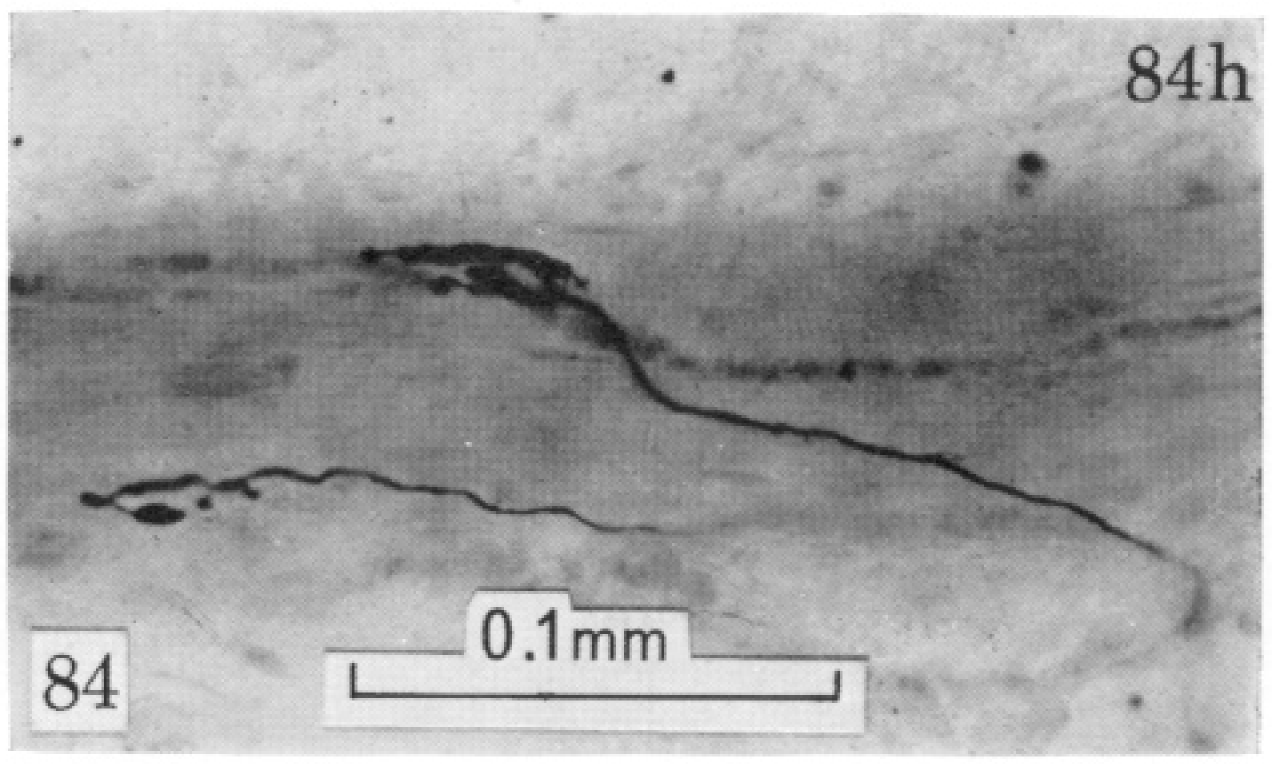
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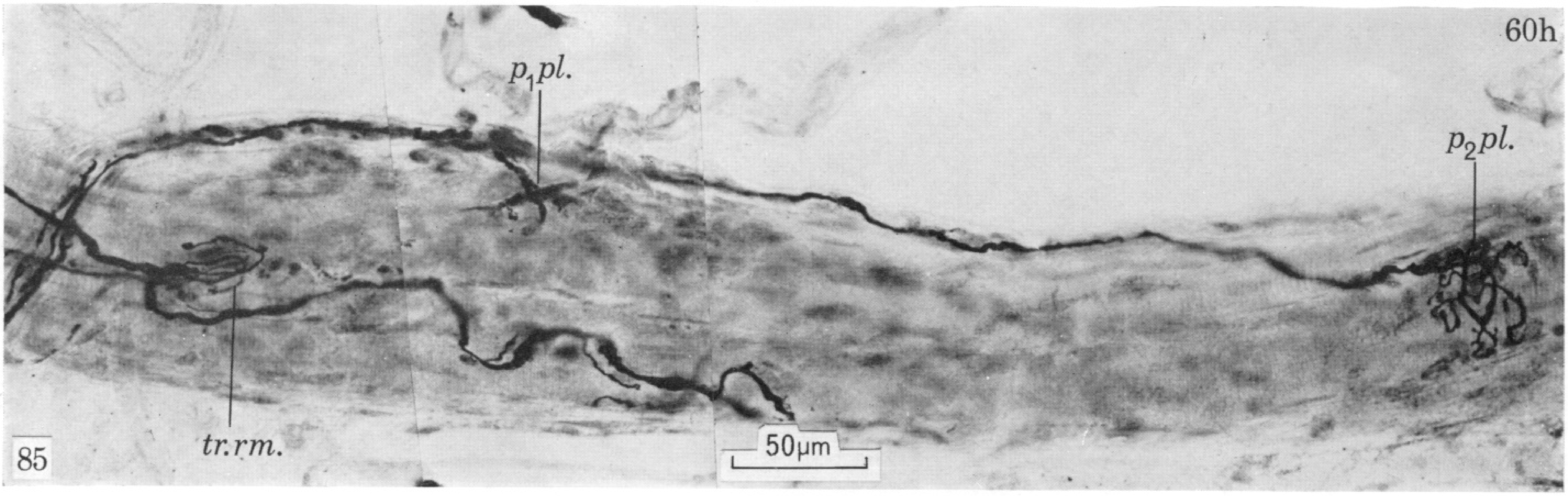
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